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The Behavioral and Neurochemical Profile of the Spontaneously Diabetic Wistar B.B. Rat

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Thesis Presented to the Faculty of Graduate Studies of the University of Ottawa in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Psychology.

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I would like to dedicate this thesis to my Father, Mother and Brother. Without their love and support this project would not have been achieved.

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ABSTRACT

The Spontaneously Diabetic Wistar B.B. Rat (SDR) is considered to be a genetically determined animal model of human Type-1 diabetes. The overall objective of this thesis was to elucidate the behavioral and neurochemical profile of the SDR.

This objective was attained using various pharmacological, behavioral and neurochemical approaches. The course of the changes was followed sequentially, at discretely defined time frames (0-2, 2-8 and 8-12 months duration of diabetes), to explore and characterize the contended dysfunctions.

Overall, it was found that the insulin treated SDR exhibited a significantly attenuated locomotor and rearing response to the systemically administered dopamine agonists d-amphetamine and amfonelic acid. In the case of d-amphetamine, it was found that the attenuated response was robust and chronic as it persisted across all three time frames. The attenuated response of the insulin treated SDR (5-6 months diabetic) to amfonelic acid demonstrated that the behavioral deficit could also be elicited by a dopamine agonist with a different mechanism of action from d-amphetamine.

In a nonpharmacological experiment, it was found that the insulin treated SDR manifested a significantly attenuated nocturnal locomotor and rearing response, particularly to transitional photoperiodic cues (i.e. lights on and off). This deficit in responding was chronic and robust as it was observed across all three time frames.

The possible neurochemical substrates of the aforementioned effects were investigated. A post-mortem neurochemical analysis of the region specific basal levels of CNS catecholamines and metabolites, in the insulin maintained and deprived SDR, was undertaken. There were no significant differences between the insulin maintained SDR and non-diabetic littermates or genetically distinct controls. The cessation of insulin administration to the SDR for four consecutive days resulted in significant increases in the levels of norepinephrine in the cortex and hypothalamus, dopamine in the hippocampus, and homovanillic acid in the striatum.

The neurochemical response of the insulin treated SDR was assessed following a pharmacological challenge. The SDR was exposed to a single dose of (1.0 mg/kg, i.p.) amfonelic acid. The SDR exhibited a significantly greater reduction in the post-mortem levels of dopamine in the striatum, midbrain, and olfactory bulbs as well as striatal norepinephrine.

The behavioral effects elicited by d-amphetamine and amfonelic acid are believed to be dopamine mediated. Thus, it was hypothesized that one source of the observed neurochemical and behavioral deficits may be related to an impairment of dopaminergic neurotransmission. Therefore, the concomitant measurement of spontaneous nocturnal locomotor activity and levels of interstitial dopamine from the ventral striatum was measured using *in vivo* microdialysis. No significant differences between the insulin treated SDR and controls were found. The SDR

did exhibit significantly lower levels of locomotor activity. There was no significant correlation between interstitial dopamine levels and locomotion in both groups.

In a different vein, the behavioral response of the insulin treated SDR was assessed following exposure to environments varying in degree of novelty. It was found that the SDR exhibited a heightened behavioral response to novelty-stress. This heightened response was chronic as it was observed across all three time frames.

The insulin maintained SDR manifested a greater aversion to the anxiogenic regions of the open field and elevated plus maze whilst being treated with chlordiazepoxide. The anxiolytic effects of this drug were significantly attenuated in the SDR when compared to controls.

In essence, it would appear that the SDR when treated with insulin and unchallenged by: 1) withdrawal of insulin treatment, 2) pharmacological stimulation or, 3) environmental stimulation, is able to maintain relatively stable baseline levels of brain catecholamines and behavior. It is only under challenging conditions that the SDR manifests abnormal behavioral and neurochemical responses. These results may have clinical implications, in the realm of neuroendocrinological and/or behavioral complications associated with insulin dependent diabetes mellitus.

INTRODUCTION

Diabetes

Diabetes Mellitus is a diagnostic term that embraces a variety of disorders and syndromes that have one common feature: chronic elevation of blood glucose concentration (hyperglycemia). Diabetes is often defined in terms of insulin deficiency. Although, insulin does act to lower blood glucose concentration, the insulin deficiency hypothesis may be misleading. Namely, because insulin replacement does not necessarily reinstate normal blood glucose levels. Regulation of blood glucose is a complex process involving numerous hormones and metabolic processes in addition to insulin secretion. Thus, it is best to describe diabetes as chronic hyperglycemia and realize that the causes are complex and multifactorial.

Diabetes is often categorized according to a number of clinical criteria (National Diabetes Data Group, 1979). Diabetes mellitus can be of primary (that is of unknown cause) or secondary (a feature of some other known disease process) cause. Primary diabetes mellitus represents a heterogenous group of disorders that are difficult to classify on the basis of etiology. The present classification system (National Diabetes Data Group, 1979) distinguishes between Type-I (insulin dependent) and Type-II (non-insulin dependent) diabetes mellitus.

Type-I diabetics usually have an onset of diabetes before the age of 30 and are at or below normal body weight and are prone to develop diabetic coma (ketoacidosis). Type-II diabetics usually have an onset after the age of 40, are overweight to obese, and develop ketoacidosis only under states of extreme physical stress. This thesis will focus on an animal model of Type-I diabetes.

The etiology of Type-I diabetes mellitus clearly involves genetic, immunologic, and infectious factors (Atkinson & McLaren, 1991; Craighead, 1978). One of the better accepted formulations at present for the pathogenesis of Type-I diabetes is that a person with genetic susceptibility acquires a non-specific viral infection. As a part of the course of the infection, the pancreas becomes inflamed, resulting in damage to the beta cells. The body's immune system then generates specific antibodies that are directed against components of the beta cell, which eventually destroy most or all of these cells (Atkinson & McLaren, 1991; Craighead, 1978). The result is a termination of the production of insulin, which ultimately may have dire consequences. Insulin aids most cells of the body in utilizing biological fuels, of which one is glucose. In the absence of insulin, an excess of glucose becomes evident within the body. In an attempt to compensate and regulate this excess, the body becomes dehydrated as the kidneys are overworked trying to filter the excess glucose and excrete it through the urine. Unable to use glucose, the cells of the body begin to breakdown their stores of fat and

protein in an effort to supply more fuel to the body. In the extreme, the breakdown of fat may be so severe that acidic by-products, ketones, accumulate. These ketones, combined with a dehydrated condition, can result in a coma and possibly death. Treatment with exogenously administered insulin can prevent the aforementioned sequence of events. Insulin treatment alone, however, cannot halt the progressive long-term deterioration caused by the many complications associated with the diabetic condition.

Research in the field of diabetes is diverse and as a consequence, multifaceted. Two areas of diabetes research which are particularly germane are; 1) the status of central nervous system (CNS) functioning in the diabetic (DeJong, 1977; Bischoff & Zimmerman, 1979; Pozzessere, 1988) and 2) the psychological and behavioral status of the diabetic (Surwit et al, 1983; Wilkinson, 1981; Fisher et al, 1982).

In recent years, growing attention has focussed on the role of the CNS in diabetes (Pozzessere et al, 1988). This trend has not always been a central factor in studies concerning diabetes. In fact, it had been suggested that diabetes was a disease primarily of the peripheral nervous system and thus, the CNS had little role, if any, in the pathophysiology of diabetes (Warren & LeCompte, 1952; Williams, 1960). The former view reflects a perspective on CNS-diabetes research which has been discounted by both human and animal research in the last two decades.

A growing body of research in recent years has provided

evidence linking uncontrolled diabetes (Type-1 and Type-II) with behavioral deficits (Lozovsky et al, 1981; Trulson & Himmel, 1985; Rowland et al, 1985; Rowland & Bellush, 1989). Moreover, this research has indicated that a possible source of many of these deficits may be due to a dysfunction in CNS neurotransmission (Rowland & Bellush, 1989; McCall, 1992). With a few exceptions this research literature has almost exclusively been drawn from animal models of diabetes.

Animal models provide us with a viable option in the investigation of possible diabetes related CNS alterations. Animal models are important simply because they provide for a greater degree and range of experimental manipulation which is not always possible in humans. In fact, the majority of information obtained on CNS alterations in the human have been derived from either, post-mortem measures (Lakovic et al, 1991), or the use of non-invasive techniques such as; neuro-psychological evaluations (Holmes et al, 1983), evoked potentials (Cirillo et al, 1984) or electro-encephalographic (EEG) measurements (Haumont et al, 1977).

Animal Models of Diabetes

The use of animal models in the study of diabetes is well documented (Shafrir & Reynolds (eds), 1984). Initial studies involved the use of techniques such as pancreotectomy to induce diabetes in dogs and also rats. Later studies employed chemicals

such as alloxan or streptozotocin (STZ) (Rerup, 1970). These pancreotoxins were found to selectively damage the insulin producing pancreatic β-cells (Rerup, 1970). STZ gained extensive use when it was found that multiple low doses of STZ is accompanied by insulitis and also immune system alterations, two important characteristics of Type-1 insulin dependent diabetes mellitus (IDDM) in humans (Kolb, 1987; Rossini et al, 1978). STZ was originally isolated as an antibiotic, and its toxic effects on the β-cells was first recognized in 1963 (Rakieten et al, 1963). As a pancreotoxin, it is primarily administered systemically as a single, high dose causing complete β-cell necrosis and consequently hyperglycemia twenty-four hours following administration. The toxicity of STZ to β-cells is thought to stem from its capacity to accumulate specifically and rapidly in these cells (Kolb, 1987).

Hyperglycemia can also occur spontaneously in animals. The majority of these species and strains exhibit a syndrome which more closely resembles Type-II diabetes. There are several models of IDDM which manifest varying degrees of similarity to the human condition, the Chinese Hamster (Gerritsen, 1982), the Non Obese Diabetic (NOD) mouse (Toyota et al, 1984) and the Spontaneously Diabetic Wistar BB rat (SDR) (Nakhooda et al, 1977). The Chinese Hamster although being insulopenic and ketosis prone, develops a syndrome different in several respects from that of man. The NOD mouse demonstrates many symptoms homologous to the human IDDM; however, there are some discrepancies. For example, there is a

much higher incidence of IDDM in females (85%) as compared to males (15%) and a late onset of diabetes (approximately 6-7 months of age). The SDR has been recognized as an animal model of Type-1 diabetes most closely resembling the human condition (Marliss et al, 1982).

The Spontaneously Diabetic Wistar BB Rat

The Spontaneously Diabetic Wistar B.B. Rat (SDR) was discovered serendipitously in 1974, amongst outbred, non-obese, Wistar-Furth rats at a commercial breeding facility (BioBreeding Laboratories, Ottawa, Ontario) (Chappel & Chappel, 1974). The overt clinical syndrome occurs abruptly and manifests itself usually between 60 to 140 days of age (Marliss et al, 1982). The initial symptoms include rapid loss of weight, hyperglycemia, glycosuria, polyuria, and polydipsia. Within 3-5 days of the overt syndrome, hyperglycemic values of 400 to 500 mg/dL are evident. The normal blood glucose range in the rat is 80-150 mg/dL. Twenty-four hour urine volume increases dramatically from 15 to greater than 100 mL, and extraordinarily high urine glucose values (10 g/dL) arise (Sima, 1985). The hyperglycemia is also associated with hypoinsulinemia and hyperglucagonemia. The pancreas of such rats usually shows less than 0.1% of normal insulin content and 2% of normal, in insulin-treated rats (Nakhooda et al, 1978). Marliss et al, (1982) reported that the pancreatic morphological features of the SDR diabetic syndrome,

are "strikingly" similar to those found in the pancreases of Type-1 diabetic patients early after diagnosis. Specifically, the pancreatic β-cells have disappeared, the islets of Langerhans are at an end stage, consisting only of glucagon, somatostatin, and pancreatic polypeptide producing cells (Nakhooda et al, 1978).

It is not clear how the SDR inherits Type-1 diabetes. However, there are diabetes susceptibility genes associated with the major histocompatibility complex in the SDR, similar to that observed in man (Crisa et al, 1992). The exact nature of the inheritability is controversial and still under intensive investigation (Crisa et al, 1992). There is no doubt that a critical component in the development of diabetes in the SDR is autoimmune (Crisa et al, 1992). This hypothesis has received support from the finding that immune-interventions can ameliorate and even prevent the onset of the disease (Crisa et al, 1992). Naji et al, (1981) demonstrated that neonatal bone marrow from normal Wistar rats, implanted into diabetes susceptible SDR, prevented the development of diabetes in the SDR. Like et al, (1979), demonstrated that the incidence of Type-1 diabetes decreased in the SDR injected with high doses of a rabbit anti-rat-lymphocyte.

Despite the clear similarities between the form of diabetes exhibited by the SDR and that exhibited by humans, there are also some differences. The SDR does not exhibit islet cytoplasmic antibodies, is inbred, and has a much higher incidence of diabetes (30-90%) within its population. Overall, however, there

are many affinities between human Type-1 diabetes and the diabetes in the SDR.

Despite the existence of the SDR since 1974, the majority of studies investigating the CNS and behavior have been undertaken using the chemically diabetic rat. The relative paucity of research investigating the CNS and behavior in the SDR may be due to a number of factors such as: 1) limited availability of the SDR, 2) lack of appropriate facilities for the care and maintenance of the SDR, 3) lack of a research history on the SDR, brain and behavior.

In the context of this thesis, it is important to review the literature concerned with the CNS and/or behavior in the chemically diabetic rat, as it has yielded some important research findings and trends. Thus, the following discourse will focus on the chemically diabetic rat.

Central Nervous System Neurotransmission and Diabetes

A number of researchers in recent years have been exploring region specific alterations in catecholamine levels and their receptor status in the diabetic rat brain. The majority of these studies have focussed on the post-mortem CNS analysis of the acute chemically diabetic condition, typically untreated with insulin.

The chemically-induced state of diabetes mellitus has been reported to be accompanied by a number of dysfunctions in CNS

neurotransmission. Bitar et al, (1985) reported that uncontrolled STZ induced diabetes in male Sprague-Dawley rats (10-90 days diabetic) was associated with significant disturbances in brain monoamine metabolism. Specifically, the activity of tyrosine hydroxylase (TH) was decreased and the concentration of norepinephrine (NE) was increased in several brain regions, including the thalamus, hypothalamus, medulla, and midbrain. These observations are concordant with those of Trulson and Himmel (1985), who further reported that a single dose of insulin reversed the manifested alterations. In the Bitar study (1985), concentrations of dopamine (DA) and serotonin (5-HT) remained unaltered, but the levels of their metabolites 3,4-dihydroxy-phenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5HIAA) were lower in the diabetic animals. Trulson et al, (1986), also reported a decrease in the synthesis and turnover of 5-HT as measured by 5HIAA activity in STZ treated rats 4-6 weeks diabetic.

Chu et al, (1986), reported increased levels of DA in the corpus striatum of STZ male Sprague-Dawley rats (2 weeks diabetic). These researchers also reported significantly lowered levels of catecholamines (epinephrine (E), NE, and DA) and 5-HT in the hypothalamus. Again, the observed deficits were reversed by acute insulin administration. Kulikov et al, (1986), found a significant reduction in NE in the neocortex and caudal portion of the brainstem in alloxan diabetic male Wistar rats, 1-2 weeks diabetic. In contrast, Kolta et al, (1986), found no significant

alterations in the levels of DA in the whole brain of alloxan diabetic rats (0-2 weeks diabetic). In 1987, Heric-Oliver et al, reported alterations in the turnover rate of E, DA, L-DOPA and Vanilmandelic acid in discrete hypothalamic regions of the insulin treated female STZ diabetic rat. In that study, it is interesting to note that alterations occurred while the diabetic animals were maintained on insulin replacement therapy. Wesselman et al, (1988), described the early effects of alloxan-induced diabetes (6 days of duration) on central catecholamine concentrations in the male Lewis rat. These researchers reported a significant increase in cerebellar NE in comparison to non-diabetic controls and insulin treated diabetic controls. No alterations were found concerning DA, DOPAC or NE in the striatum, pons-medulla, or the remaining brain. In contrast, Shimomura et al, (1988), found a significantly lower turnover rate of DA in the striatum of STZ treated rats, 3 weeks diabetic.

In an attempt to assess the effects of insulin, Bellush and Reid (1991) measured DA, DOPAC, 5-HT and 5HIAA in the CNS of insulin treated, insulin withdrawn and insulin naive STZ diabetic rats. It was found that DA turnover was significantly lower in the striatum and hypothalamus of all diabetic groups. 5-HT turnover was significantly reduced in the chronically hyperglycemic group in the frontal cortex, hypothalamus, striatum and brainstem. 5-HT turnover was also found to be reduced in the frontal cortex of the insulin withdrawn group. Insulin treated STZ rats had normal 5-HT turnover rate. Insulin treatment

however, did not normalize the observed DA alterations.

Lakovic et al, (1990), undertook an investigation of the duration of diabetes and CNS alterations in the STZ and alloxan diabetic rats. In essence, it was found that in the whole brains of the non-insulin treated, chemically diabetic rats there was an increase in the levels of NE, DA,and 5-HT at 12 and 40 weeks duration of diabetes. Whereas, there was a gradual decrease in the metabolites 5HIAA and HVA with the duration of diabetes. Furthermore, an analysis of discrete brain structures revealed a decrease in hypothalamic NE content in the alloxan diabetic rat with 1 week duration of diabetes followed by an increase in NE and DA levels 13 weeks later. In an important extension of this study, Lakovic and colleagues (1990) measured post-mortem monoamine concentrations in 11 brain regions in chronically diabetic insulin dependent humans, with no record of chronic drug use, psychiatric, neurologic, or endocrine disease. It was found that there was an increase in the content of 5-HT in the medial and lateral hypothalamus, of DA in the medial hypothalamus, putamen, and medial and lateral pallidus and of NE in the lateral pallidus. There was a decrease of the content of NE in the nucleus accumbens and claustrum.

In a different vein, Forman et al, (1986), reported that STZ induced diabetes (8 weeks in duration) resulted in a significant reduction in the levels of the endogenous opioid peptide β-Endorphin. These reductions were found in the hypothalamus, pituitary and plasma. Also, Williams et al, (1988) found

increased hypothalamic neuropeptide Y concentrations in the STZ treated Wistar rat (1-14 weeks diabetic). No changes in six other peptides (bombesin, galanin, neuromedin B, substance P, somatostatin, and vasoactive intestinal peptide) were found.

In summary, there exists an accumulation of data derived from a diversity of sources: diversity in terms of duration of diabetes, strain of rat used, CNS site(s) studied, and pancreotoxin used. There exist some commonalities in the research literature. The majority of the studies are concerned with an acute duration of uncontrolled diabetes (i.e. 0-3 weeks) with the exception of Bitar et al, (1985) and Lakovic et al, (1990). Also, dynamic alterations appear to be occurring with NE levels and metabolism in the hypothalamus, between 0-13 weeks duration of diabetes. The status of CNS DA is less clear. Alterations in DA metabolism appear to be occurring between a period of 0-13 weeks in a variety of CNS regions. In terms of levels of DA, it is difficult to ascertain any consistent trend(s). Lakovic et al, (1990) have suggested that varying degrees in the duration of diabetes may be an important determinant in accounting for some of the discrepancies in the literature. It is also important to note that alterations are occurring in 5-HT metabolism, and β-Endorphin levels over a duration of 4-8 weeks of diabetes. In conclusion, there are widespread alterations in terms of CNS neurotransmission in the chemically diabetic brain.

Metabolic Factors and Receptor Alterations

Although it is difficult to ascertain the precise causal factors regarding CNS changes in the diabetic brain, one might hypothesize that alterations in enzymatic and metabolic activity may be consequential in altering the availability of the neurotransmitter(s). In fact, studies have shown that the inhibition of TH by pharmacological agents (i.e. a-methyl-p-tyrosine) or physiological states (i.e. age) can result in a marked decrease in the levels of NE and/or DA (Brodie et al, 1966). Bitar et al, (1985) reported that during uncontrolled diabetes, the activity of TH was decreased. This finding was further supported by Kwok and Juorio (1986). These researchers reported significant reductions in the striatal concentration of p-tyrosine, DOPAC and HVA, in rats treated with STZ, with a duration of diabetes of 1-2 weeks. Kwok and Juorio (1986) reported that the acute administration of insulin ameliorated the deficits. It has also been found by Mayanil et al, (1982) that there was a reduction in the activity of monoamine oxidase (MAO) activity in the non-insulin treated alloxan diabetic rat. The activity of MAO is an important enzymatic factor in the metabolism and hence the availability of catecholamines.

In light of these findings, it might be hypothesized that the aforementioned alterations in neurotransmitter levels could result in a decreased presynaptic release of the neurochemicals (i.e. catecholamines, indoleamines) at the respective terminals.

Such a change could be expected to be accompanied by a compensatory change at the level of the receptor(s). In this context, Lozovsky et al, (1981) reported a significant increase in the binding of [^{3}H] spiperone to the striatal membranes in 6 week STZ and alloxan diabetic rats. These researchers reported that the acute administration of insulin "normalized" the DA receptor sensitivity. Concordant with the findings of Lozovsky et al, (1981), Trulson and Himmel (1983), reported a significant increase in DA receptor binding in the striatum and limbic forebrain in the STZ diabetic rat (4-6 weeks diabetic) untreated with insulin. Trulson and Himmel (1983) also found that the acute administration of exogenous insulin reversed the observed deficits. In addition to the above findings, Serri et al, (1985), reported that in the alloxan diabetic rat (4 weeks diabetic) there was a significant increase in the number of DA binding sites in the striatum, but not in the anterior pituitary, as measured by [^{3}H] spiroperidol binding. Serri et al, (1985), also found that although, there was increase in receptor number in the striatum, the affinity of the DA receptor itself appeared to be unaltered when challenged *in vivo* with DA, bromocriptine and haloperidol.

In contrast to the studies outlined above, Rowland et al, (1985), reported that in the STZ male Sprague-Dawley rat (3 weeks diabetic) no significant increase in the [^{3}H] spiroperidol binding in the striatum and other brain regions was found. In fact, Rowland et al, suggested that there was a strong trend

towards decreased specific binding. The findings of Rowland et al, have yet to be explained in light of other research reports.

In summary, it would appear that dynamic alterations are occurring at the level of the DA receptor, specifically between a 3-6 week duration of diabetes. Three out of four of the studies outlined above report a significant increase in DA receptor binding in the striatum. Furthermore, it appears that the exogenous administration of insulin can reverse the alterations observed at the level of the DA receptor.

Given the mulitude of neurochemical and receptor changes observed in the diabetic brain, it is not surprising that behavioral alterations have also been observed in the chemically diabetic rat. In the following section, the recent literature concerning diabetes and behavior in the chemically diabetic rat will be reviewed.

Diabetes and Behavior

There are relatively few reported studies focussing on the issue of diabetes and behavior. Despite this, there are a number of research trends which have developed in the last two decades.

Pharmacological Interventions: Dopamine System

Given the reported alterations observed in DA turnover, and at the level of the DA receptor, it is not surprising that

behavioral alterations have also been observed in diabetic rats following pharmacological challenge with DA agonists.

In 1976 and 1978 Marshall et al, reported that rats with alloxan-induced diabetes manifested a "greatly diminished" behavioral response to the DA agonist d-amphetamine. Specifically, they reported a significant reduction in locomotor activity, stereotyped behavior and anorexia following a wide range of doses of d-amphetamine, in the diabetic rat (5-6 weeks duration). The alterations in stereotyped and anorectic behavior were restored to control levels following 10 days of insulin treatment. Marshall and colleagues hypothesized that the observed alterations could be related to a dysfunction(s) in CNS DA functioning. Furthermore, Marshall et al, drew a parallel between the behavior of the diabetic animals and those with pharmacologically or lesion-induced depletions of brain catecholamines.

Expanding upon the findings of Marshall et al, Rowland et al, (1985), reported that rats made diabetic with STZ manifested decreased stereotyped behavior following the administration of d-amphetamine or apomorphine. Again the administration of insulin resulted in an amelioration of the observed deficits. Importantly, these researchers reported that reduced tissue access by d-amphetamine or apomorphine was not evident, giving further credence to other diabetes mediated factors. In a recent investigation, Bellush and Reid (1991) assessed the effects of insulin on d-amphetamine-induced stereotyped behavior in the STZ

diabetic rat. It was found that stereotyped behavior was attenuated in insulin naive diabetic rats but normalized in insulin treated diabetic rats. In rats acutely withdrawn from insulin, an attenuated stereotyped response was also observed, but not to as great an extent as that observed in the insulin naive diabetic group.

In contrast to these studies, Chu et al, (1986) reported an increased sensitivity of the STZ-diabetic rat to the locomotor stimulant effects of a single dose (15 mg/kg) of d-amphetamine. These researchers suggested that the enhanced d-amphetamine induced hyperactivity in the diabetic condition is due to an enhancement of brain DA receptor activity. Considering the extremely high dose of d-amphetamine used in the Chu et al, (1986) study, it is interesting that the occurrence of stereotyped behavior was not reported.

Finally, Bjorenson and Quock (1988), investigated the responsiveness of alloxan-treated male Sprague-Dawley rats (approximately 3 weeks diabetic) to apomorphine induced hypothermia. They found that alloxan diabetic rats experienced a significantly greater hypothermic response to a single dose of apomorphine (0.5 mg/kg) than did controls. It was hypothesized by Bjorenson and Quock, that the enhanced hypothermic response in the diabetics, could be linked to a decrease in DA activity and an increase in DA receptor binding, inherent to the diabetic condition.

In summary, it appears that pharmacological interventions at both the level of the pre- and post-synaptic DA receptor(s), results in an altered behavioral responsiveness in the non-insulin treated diabetic rat. Furthermore, it appears that at least some of these alterations may be centrally mediated. Support for this view comes from the aforementioned observations of altered behavioral activation following the administration of either d-amphetamine or apomorphine. Atleast in the case of d-amphetamine, the decreased behavioral activation may be the result of an inability of the non-insulin treated diabetic rat to sustain high levels of DA release. Evidence for this hypothesis is tentatively supported by an *in vitro* study in which synaptosomal release of DA was reduced by the addition of glucose to the medium (Dorris, 1978). It has also been demonstrated that glucose loads, in normal rats, reduce the intensity of amphetamine induced stereotyped behaviors (White & Blackburn, 1986).

The behavioral results concerning apomorphine are enigmatic when compared to the receptor binding studies in the untreated diabetic rat. Given the reliable increases in DA receptor binding in the diabetic brain, a concomitant enhanced behavioral responsiveness to apomorphine would be expected. However, this does not seem to be the case. Rowland and Bellush (1990), suggest that perhaps deficits exist at the level of postreceptor events. Indeed, a reduced sensitivity of adenylate cyclase to NE stimulation of the cerebrum and to both NE and DA in the retina,

was demonstrated in rats 8 weeks diabetic after STZ treatment (Palmer et al, 1983). Also, Gawler et al (1987) demonstrated that the insulin treated STZ rat manifested an overall reduction in adenylate cyclase activity in hepatocytes.

Finally, when drugs are administered systemically one must always consider whether adequate penetration across the blood brain barrier has occurred. Knudsen et al, (1986) have demonstrated alterations in the permeability of the blood brain barrier in the STZ rat untreated with insulin. As mentioned earlier, Rowland et al, (1985) demonstrated that the tritiated uptake of d-amphetamine was not compromised by the diabetic condition. An alternative approach would be to employ pharmacodynamic techniques such as *in vivo* microdialysis to assess both behavioral and direct CNS neurochemical alterations in synchrony. This approach would circumvent possible confounds associated with blood brain barrier deficits.

Another factor that must be considered is the possibility that the diabetic condition alters drug metabolism. In this regard, Ackerman et al, (1975) and Ackerman (1976), demonstrated that altered drug metabolism often observed in conjunction with chemically induced diabetes is due to a lack of insulin. These researchers concluded that a lack of insulin results in a rise in hepatic cyclic AMP which in turn causes the release of an inhibitor of drug metabolism in the liver cytosol. Such a result might account for the amelioration of the altered behavioral response to drugs such as d-amphetamine, following insulin

administration. This finding stresses the importance of behavioral measurements in the insulin treated diabetic rat. Such an approach would aid in differentiating between the effects of hyperglycemia versus the insulin treated state of diabetes.

Stress and Diabetes in the Rodent

Despite continuous referrals in the human literature to a possible relationship between diabetes and stress (Danowski 1963; Linn et al, 1983), there exists relatively few animal studies investigating the interaction between stress, diabetes and CNS functioning.

As suggested by Bellush et al, (1991), many of the hormonal and neurochemical alterations exhibited by the diabetic rodent are consistent with the state of chronic stress. Thus, it is not surprising that the diabetic rodent exhibits an altered biochemical and behavioral response to various stressors.

Bellush and Rowand (1989) reported that memory for a passive avoidance task (i.e. footshock) was enhanced in diabetic rats. A similar finding was reported in STZ-diabetic mice by Leedom et al, (1987) and Meehan and Leedom, (1986). Moreover, these researchers reported a significant increase in the submissive behavior of the diabetic mice (Meehan et al, 1986). Flood et al, (1987) reported that active avoidance was impaired in STZ diabetic mice. In light of the aforementioned studies, the findings of Flood et al, (1987) are puzzling. However, they do

indicate that the stress response of the diabetic rat may be subject to a complex interaction between response and activity (Bellush & Rowland, 1989).

In terms of plasma catecholamines, Bellush and Rowland (1989) reported elevated levels of plasma NE and E in diabetic rats subjected to a passive avoidance task. Lee et al, (1988), documented that both alloxan and STZ diabetic rats (2 weeks diabetic) manifested a significant elevation in plasma catecholamine levels following footshock, when compared to controls. It has also been found that STZ treated mice (Kamei et al, 1991) manifested a lower analgesic threshold as induced by the stress of footshock.

It is important to note, that physiological alterations in corticosterone have been reported in the diabetic rodent. Tornello et al, (1981) reported that increased levels of plasma corticosterone in STZ diabetic rats were accompanied by a down regulation of corticosterone receptors within the hippocampus. Leedom et al, (1987) reported elevated levels of plasma corticosterone in diabetic mice in response to a moderate intensity footshock. Bellush et al, (1991) demonstrated that the STZ diabetic rat exhibited elevated corticosterone responses when subjected to restraint stress. These findings have important implications given the known role of the corticosteroids in mediating the stress response.

In a different vein, Bellush and Henley (1990), measured the biochemical response of the non-insulin treated STZ rat to 24 hrs

of cold or hypobarbic hypoxia. It was found that the STZ rat when exposed to cold exhibited a significantly reduced level of adrenal NE and significantly elevated levels of urinary NE and E. The STZ-rat response to hypoxia yielded elevated urinary levels of NE as compared to controls. There was also a significant attenuation in the response of 5-HT in the brainstem. In an assessment of the functional significance of biochemical alterations, in the non-insulin treated-STZ diabetic rat (3 weeks duration of diabetes), Bellush et al, (1991) measured the neurochemical and behavioral response following restraint stress. It was found that the diabetics exhibited a smaller (but statistically non-significant) increment in 5-HT turnover following restraint. No alterations were observed in DA turnover following restraint. In terms of behavior, it was found that restraint stress suppressed exploratory activity to a greater extent in the diabetics. Behavioral suppression was also observed in the diabetics following exposure to a novel environment. In this regard, Levine et al, (1984) also demonstrated an altered behavioral response of the STZ diabetic mouse to the stress of a novel environment. Specifically, novelty-stress modulated the suppressive effect of naloxone on feeding in the STZ diabetic rat.

In summary, the available literature indicates that the chemically diabetic rat manifests an altered behavioral and/or biochemical responses to various stressors. Moreover, there

exists the possibility that the behavioral dysfunctions may be mediated by alterations in CNS functioning.

Rationale

As can be ascertained, the use of pancreotoxins to produce an animal model of IDDM has enabled diabetologists to investigate a variety of hypotheses concerning the interaction between the diabetes, CNS and behavior. There are however, some considerations which must be attended to when using pancreotoxin-based diabetic animals. The primary concern, is the inability of these models to mimic the genetic and salient immunological facets of Type-1 diabetes. As referred to earlier, STZ when administered repeatedly and in low doses, can produce immunological changes in the rodent; however this has not been the methodology of choice in the literature reviewed. Other concerns include, variations in the dose of the pancreotoxin used and the route of administration. These factors may affect the severity of the diabetes produced and also the degree and nonspecificity of the lesion, perhaps contributing to some of the variability observed between studies in the literature. Indeed, Rowland and Bellush, (1990) have suggested that the nonspecific effects of the pancreotoxins (particularly high doses) may influence outcomes.

The use of insulin replacement therapy must be addressed when discussing the pancreotoxin-based models. As alluded to

earlier, the use of exogenous insulin replacement in human Type-1 diabetes is standard; however in the case of the STZ or alloxan diabetic animals, insulin replacement is often not considered in the daily maintenance routine of the animal. Moreover, when insulin is used, it is done on an acute basis, often without a procedure for titrating the dose according to changes in the diabetic condition. The rationale concerning the absence of insulin therapy may stem from an attempt to maximize the hyperglycemia-related changes or to avoid an interaction between insulin and drugs administered. Moreover, it is clear that the majority of studies do have as their main thrust the investigation of hyperglycemia per se.

Another issue which needs to be addressed is the relative lack of information on the effects of duration of diabetes on behavior. Most studies have focussed simply on the acute hyperglycemic condition. It is important to address this issue due to the possibility that observed effects may be related to a particular time frame in the progression of the diabetic condition.

Nevertheless, use of the SDR, offers a unique perspective on investigations of brain and behavior. As outlined earlier, there exists a paucity of research on the SDR's behavior and neurochemistry. Due to its inherent uniqueness and its homology to the human diabetic condition, research on the SDR, CNS and behavior, needs little justification.

Research Objectives

The main hypothesis addressed by this thesis is that the state of diabetes in the SDR, may be associated with altered CNS functioning, which may be reflected in an altered behavioral and/or neurochemical profile.

The overall objective of this thesis was to test the above hypothesis using a multifaceted approach. The course of the contended changes was followed sequentially, at discretely defined time frames (0-2, 2-8 and 8-12 months duration of diabetes), to explore and characterize possible dysfunctions.

EXPERIMENT 1: Amphetamine-induced behavioral changes: effects of duration of diabetes.

Introduction

It has been suggested that central DA systems may play a role in the behavioral and neurochemical dysfunctions observed in the SDR (Merali et al, 1988). One of the indications of a possible DA dysfunction is an altered behavioral responsiveness of the SDR to systemically administered d-amphetamine an indirect DA agonist (Merali, et al, 1988). It was found that the insulin treated SDR (2-4 months duration of diabetes) demonstrated a shift to the right in their behavioral dose-response curve, indicating a decreased sensitivity of the SDR to the behavioral (locomotor, rearing) effects of d-amphetamine (Merali et al, 1988). In this context, animals made hyperglycemic with the pancreotoxins alloxan or STZ also display an altered behavioral response to the systemic administration of d-amphetamine (Marshall, 1976, 1978; Rowland et al, 1985).

It is clear that whether chemically or genetically diabetic, both conditions are associated with a reduced potency of behavioral activation. Although, the aforementioned studies have established an altered response, they did not consider as a factor the time course of the disorder. Despite treatment, diabetes is a progressive disease, therefore it is possible that

the altered responsiveness to d-amphetamine may vary as a function of the progression of the diabetic condition.

The purpose of the present study was to examine the behavioral responsiveness of the insulin treated male SDR and matched controls to systemically administered d-amphetamine, across the following time frames; acute (0-2 months), intermediate (2-8 months), and long-term (8-12 months) durations of diabetes. In all experiments, we investigated the effects of d-amphetamine on the locomotor and rearing components of behavior in an attempt to gain a more comprehensive behavioral profile of the SDR response to d-amphetamine.

General Methods

Animals

All experiments were conducted on male Wistar rats from the following three groups: 1) SDR; 2) Non-Diabetic Wistar BB-Rats which are the genetic littermates of group 1 but have failed to develop diabetes (non-diabetic controls); 3) genetically distinct Wistar rats (controls). The SDR were obtained from the Health Protection Branch, Ministry of Health and Welfare, Ottawa, Ontario and/or bred at the University of Ottawa. A special note of consideration must be given to the use of the non-diabetic littermates. These animals were used as controls in some experiments but not others. The reasons were: 1) availability and 2) susceptibility of these animals to developing diabetes.

Marliss et al, (1983), reported that non-diabetic littermates may exhibit a subclinical form of diabetes and therefore, may not always be appropriate as controls. In this study, the non-diabetics used were continuously tested for urine glucose, weight loss and water consumption to ensure that the integrity of the non-diabetic status was maintained.

All animals were housed individually, with free access to food and water. The environment was maintained at a consistent temperature of 24° C, a relative humidity of 60% and a 12/12 hr light/dark cycle (lights on from 6:00 AM to 6:00 PM). Unless otherwise stipulated all diabetic rats were maintained on insulin therapy in accordance with the guidelines outlined by Health and Welfare Canada, Animal Resources Division (1985).

Maintenance of Diabetic Animals

As the SDR neared the onset of sexual maturity, they were closely monitored for symptoms of diabetes (glucosuria, polydipsia, polyurea, and loss of 10 g of body weight). Upon detection of one or more of these symptoms, animals were immediately tested for glucosuria using the Beckman Glucose Analyzer II. If the values exceeded 25 mg/DL, a regimen of daily insulin replacement therapy immediately ensued. The administration of protamine zinc insulin (P.Z.I.) (Connaught Laboratories, Toronto), in 1.0 I.U. increments began until euglycemia (7-25 mg/DL, urine glucose) was achieved. The dose of P.Z.I. was titrated according to alterations in glucosuria and

body weight. Urine glucose values were obtained daily and maintained at a level of 2% urine/glucose content, using Tes Tape (Eli Lilly). Weekly urine glucose values were also assessed using the Beckman Glucose Analyzer II.

Behavioral Monitoring

All animals were assessed on the basis of three behavioral parameters; locomotion, rearing duration and rearing frequency. Unless otherwise specified, all analyses were based on 1 hr of behavioral monitoring, which was conducted using a modification of the procedure described by Merali et al, (1985). Each behavioral observation chamber consisted of an inner clear polycarbonate cage identical to the home cage, and an outer frame that projected an array of 10 strategically placed infrared light beams through the inner cage. A grid of 5 beams located 1 cm above the floor was utilized to time the locomotor activity of the animal (see Table 1 for definitions). A curtain of four beams, located 14 cm above the floor detected the frequency and duration of rearing activity for each animal (see Table 1). A Z-80 microprocessor interfaced with custom designed software performed the timing and scoring functions. The system consisted of 12 chambers, with all beams being sampled once every second. Simultaneous observations by human raters were conducted from an adjacent room through a one-way mirror.

Table 1. Operational definitions of behaviors

Behaviors	Definitions
Locomotion	Number of quadrants entered and/or number of cm traversed.
Rearing Duration	The time spent rearing by the animal.
Rearing Frequency	The number of times the animal reared.

Statistics

The data were analyzed using the statistical package GBSTAT. A two-way repeated measures analysis of variance ANOVA (Groups x Dose) was used and post hoc analysis was undertaken using Tukey tests when necessary.

Results

Experiment 1.1

The behavioral effects of d-amphetamine on the diabetic rat with 0-2 months duration of diabetes.

Procedure

A group of SDR (n=8) and controls (n=8) were used in this study. Every third day, both groups received one of the following doses (0.0, 0.3, 0.5, and 1.0 mg/kg i.p.) in a randomized order. Behavioral monitoring ensued 10 min later and lasted for 60 min.

Analysis

Statistical analyses revealed a significant group effect (SDR vs control) for each behavioral parameter: locomotion, $F(1,14)=160.17$, $p<.01$; rearing duration $F(1,14)=22.57$, $p<.01$; rearing frequency $F(1,14)=34.99$, $p<.01$. A significant dose effect was also obtained for each behavioral parameter: locomotion $F(3,42)=89.3$ $p<.01$; rearing duration; $F(3,42)=9.15$; rearing frequency $F(3,42)=45.20$, $p<.01$. A significant interaction of experimental condition (SDR vs control) and dose of d-amphetamine was obtained for each behavioral parameter: locomotion $F(3,42)=48.40$, $p<.01$; rearing duration $F(3,42)=6.21$, $p<.01$; rearing frequency $F(3,42)=59.05$, $p<.01$. In each case, the interaction appeared to be largely due to the reduced responsiveness of the SDR to d-amphetamine. This pattern of responding is illustrated by Fig. 1 (uppermost panel). The controls displayed a dose-dependent increase in locomotor activity, the stimulation being statistically significant at doses of 0.3 mg/kg and higher. The SDR animals also responded to the stimulatory effects of d-amphetamine however; they displayed significantly lower locomotor activity, particularly at the doses of 0.5 and 1.0 mg/kg doses, when compared to controls (Tukey, $p<.01$). In the case of rearing frequency and rearing duration, the stimulatory effects of d-amphetamine did not induce a dose dependent increase in behavior in the SDR (see Fig. 2). There was

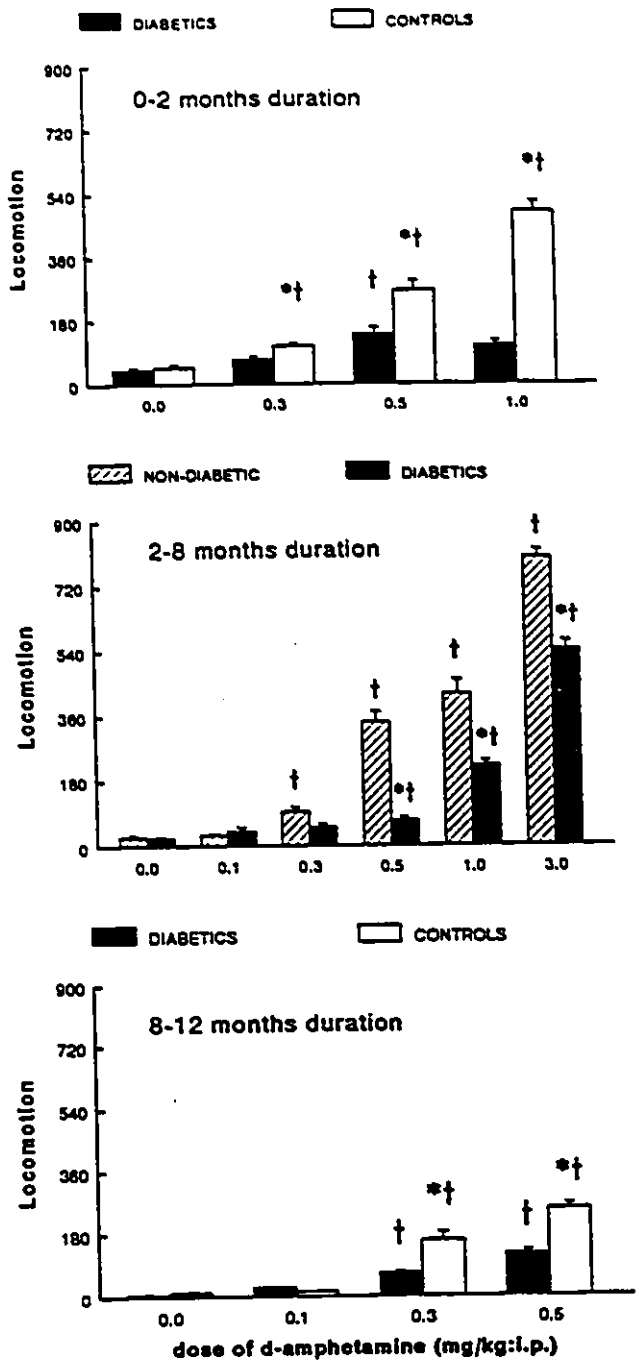

Fig. 1. The effects of d-amphetamine on locomotor activity of the diabetic rat. The locomotor activity by the rats over 1 hr following various doses of d-amphetamine is shown. Each column represents the mean ± S.E.M. † Significantly different from respective baseline (0.0) at $p<.05$. * Significantly different from matched control group value at $p<.05$.

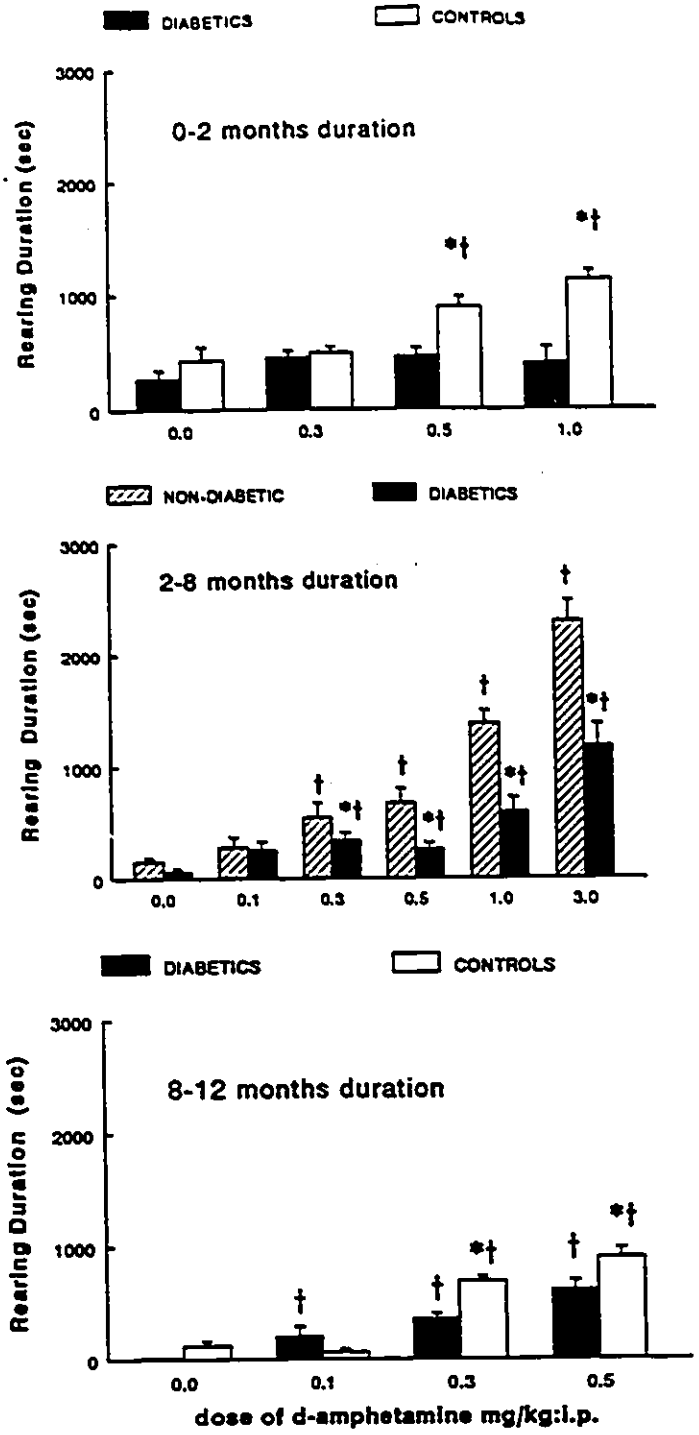

Fig. 2. The effects of d-amphetamine on rearing duration of the diabetic rat. The time spent rearing (sec) by the rats over 1 hr following various doses of d-amphetamine is shown. Each column represents the mean ± S.E.M. † Significantly different from respective baseline (0.0) at $p<.05$. * Significantly different from matched control group value at $p<.05$.

however, a dose dependent increase in the behavior of the control animals. Furthermore, in both rearing duration and frequency, at the doses of 0.5 and 1.0 mg/kg, there was a significantly diminished response by the SDR when compared to controls. These patterns of responding are depicted in Fig. 2 and 3 (uppermost panels), respectively. The SDR rearing frequency at the 0.3 mg dose was significantly greater than the controls (Fig 3, uppermost panel). It is difficult to explain the enhanced SDR rearing frequency response in light of the diminished response of the SDR (relative to controls) at the higher dosages.

Experiment 1.2

The behavioral effects of d-amphetamine on the diabetic rat with 2-8 months duration of diabetes.

Procedure

A group of SDR (n=12) and non-diabetics (NDR) (n=12) were used in this study. Every third day both groups received one of the following doses of d-amphetamine (0.0, 0.1, 0.3, 0.5, 1.0, 3.0 mg/kg i.p.) in a randomized order and behavioral monitoring ensued as described in Experiment 1.

Analysis

A two-way ANOVA repeated over the factor dose revealed a significant group (SDR vs NDR) effect for each behavioral parameters: locomotion $F(1,22)=174.23$, $p<.0001$; rearing frequency

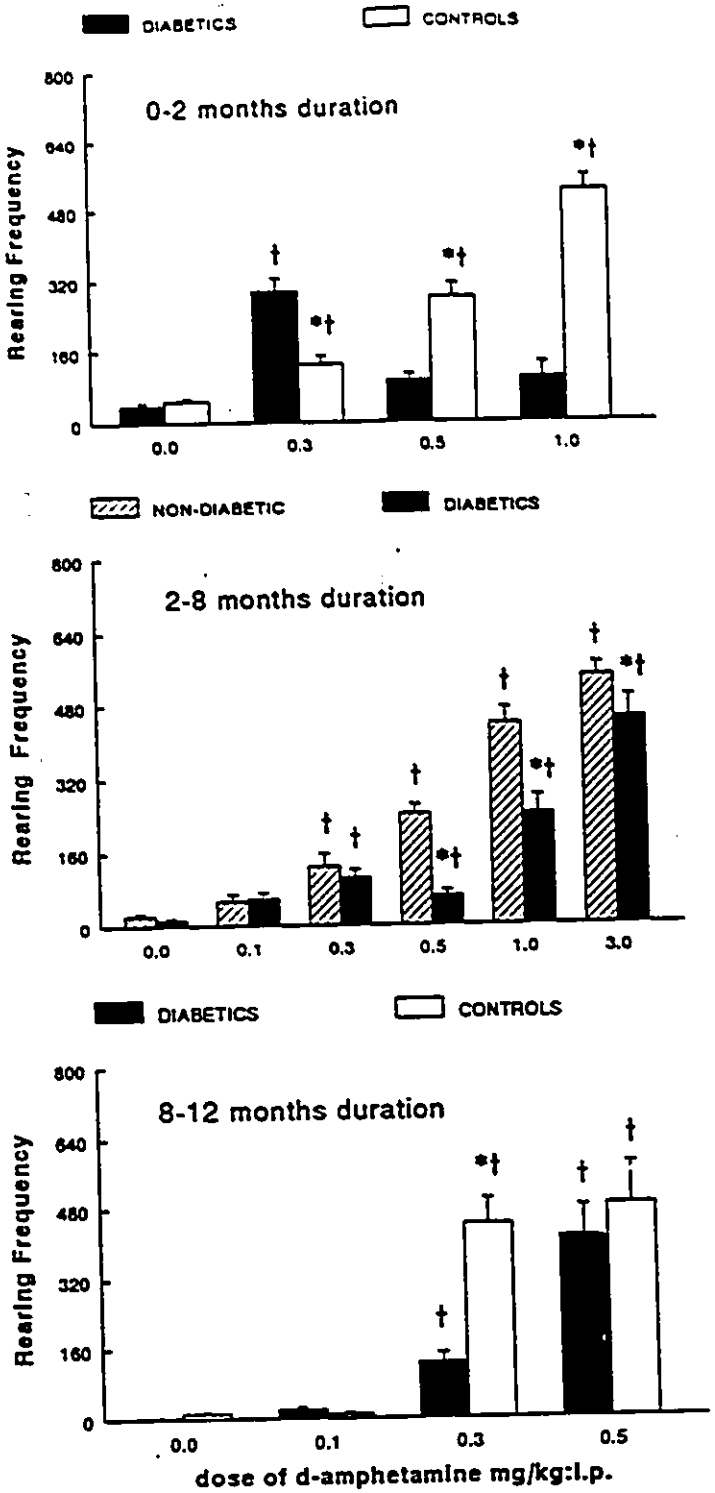

Fig. 3. The effects of d-amphetamine on rearing frequency of the diabetic rat. The number of rears by the rats over 1 hr following various doses of d-amphetamine is shown. Each column represents the mean ± S.E.M. † Significantly different from respective baseline (0.0) at $p<.05$. * Significantly different from matched control group value at $p<.05$.

$F(1,22)=20.51$, $p<.0001$; rearing duration $F(1,22)=30.72$, $p<.0001$. A significant dose effect was also obtained for each behavioral parameter: locomotion $F(5,110)=371.66$, $p<.0001$; rearing frequency $F(5,110)=110.65$, $p<.0001$; rearing duration $F(5,110)=58.85$, $p<.01$.

In all three behavioral parameters a significant interaction of experimental condition (SDR vs NDR) and dose of d-amphetamine was obtained: locomotion $F(5,110)=25.45$, $p<.0001$; rearing frequency $F(5,110)=6.25$, $p<.0001$; rearing duration $F(5,110)=7.51$, $p<.0001$. In each case, the interaction was largely an aspect of the decreased response of the SDR group at the moderate (0.5 and 1.0 mg/kg) and/or high (3 mg/kg) but not at the lower (0.1-0.3 mg/kg) range of d-amphetamine doses. This pattern of responding is illustrated by Fig. 1 (middle panel). The NDR exhibited a dose-dependent augmentation in locomotor activity, which was statistically significant at doses of 0.5 mg/kg or greater. Although, the SDR also responded to locomotor stimulatory effects of d-amphetamine, there was a shift to the right of the dose response curve. The SDR displayed a significant stimulation only at doses of 1.0 mg/kg or greater. At the doses of 0.5 and 1.0 mg/kg the SDR group displayed a significantly lower level of locomotor activity in comparison to the NDR group (Tukey, $p<.01$).

An analogous pattern of responding was obtained for rearing frequency. Again the greatest differential response ocurred at the doses of 0.5 and 1.0 mg/kg (Tukey, $p<.01$). However, at the highest dose (3 mg/kg), a near normal response occurred. In the

case of rearing duration, significant differences were not obtained at the doses up to 0.3 mg/kg. As illustrated in Fig. 2 (middle panel), the SDR spent significantly less time rearing than the NDR, at the doses 0.5, 1.0 and 3.0 mg/kg (Tukey, $p<.05$).

Experiment 1.3

The behavioral effects of d-amphetamine on the diabetic rat with 8-12 months duration of diabetes.

Procedure

A group of SDR (n=8) and control rats (n=8) was used in this study. Every third day each group received one of the following doses (0.0, 0.1, 0.3, 0.5 mg/kg;i.p.) of d-amphetamine in a randomized order. Behavioral monitoring ensued for a duration of 1 hr as described in Experiment 1.

Analysis

The statistical analyses revealed a significant group effect for each behavioral parameter: locomotion $F(1,14)=41.70$, $p<.0001$; rearing duration $F(1,14)=16.44$, $p<.001$; rearing frequency $F(1,14)=6.24$, $p<.05$. A significant effect of dose was also obtained for each behavioral parameter; locomotion $F(3,42)=153.62$, $p<.0001$; rearing duration $F(3,42)=59.60$, $P<.0001$; rearing frequency $F(3,42)=45.19$, $p<.0001$.

A statistically significant interaction between group and dose was obtained for each behavioral parameter: locomotion

$F(3,42)=26.02$, $p<.0001$; rearing duration $F(3,42)=6.22$, $p<.001$; rearing frequency $F(3,42)=5.45$, $p<.005$. In each case, the source of the interaction was derived from the differential response between the SDR and controls. Multiple post hoc comparisons revealed that in two of the three behaviors assessed, the SDR response was significantly attenuated at the doses of 0.3 and 0.5 mg/kg, when compared to the controls (Tukey, $p<.01$). This pattern of responding is illustrated in Figs. 1 and 2 (bottom panels). The exception to this pattern of responding was rearing frequency (see Fig.3, bottom panel) where the SDR achieved a similar level of responding to the controls at the 0.5 dose of d-amphetamine.

Summary

This study examined the effects of duration of insulin treated diabetes on the behavioral effects of systemically administered d-amphetamine, a drug which is reputed to cause the release of stored DA and retard its uptake (Beninger, 1983). Specifically, three duration time frames were examined, acute (0-2 months), intermediate (2-8 months) and long-term (8-12 months) diabetes. Overall, the SDR demonstrated a significant attenuation in d-amphetamine stimulated behavior across all three time frames, when compared to controls.

In the first experiment the behavioral responsiveness of the acutely diabetic, insulin treated SDR, was examined. It was found

that the SDR displayed a significantly attenuated behavioral response (locomotor and rearing) to specific doses of d-amphetamine (0.5 and 1.0 mg/kg). The results of Experiment 1.1, are concordant with those of Merali et al, 1988. Moreover, the findings in Experiment 1.1 demonstrated that the altered behavioral response of the SDR to d-amphetamine occurs proximal to the development of the diabetic syndrome in the SDR.

In Experiment 1.2, we examined the behavioral responsiveness of the SDR during the intermediate duration of diabetes. Given the results obtained from Experiment 1.1, the dose range was expanded in this experiment. The results of this experiment, confirmed and replicated those reported by Merali et al, 1988. In essence, the SDR manifested a shift to the right in their behavioral dose-response curve, indicating a reduced sensitivity to the stimulatory effects of d-amphetamine. This deficit in responding endured from a period of 2-8 months, a time period not previously examined in the response pattern of the SDR to d-amphetamine. For the most part, the pattern of response by the SDR was qualitatively similar to that observed during the acute time frame. In both experiments, the SDR manifested a diminished behavioral response between the doses of 0.5 and 1.0 mg/kg. In Experiment 1.2, the addition of the 3.0 mg dose demonstrated that the SDR could be sufficiently stimulated to higher levels of locomotor and rearing behavior, and in the case of rearing frequency and total activity, achieve near normal levels of responding. In this context, it appears that with sufficient

pharmacological stimulation, the SDR may be able to overcome the deficiency experienced when being stimulated at a specific dose range. It should also be noted that the non-diabetic littermates of the SDR were used as controls in Experiment 1.2. The behavioral response of the non-diabetic control group was essentially similar to the genetically distinct control group used in Experiment 1.1.

In Experiment 1.3, we examined the response of the SDR with long-term (8-12 months duration) diabetes. Again, the SDR manifested an attenuated response to d-amphetamine when compared to controls, particularly the 0.3 and 0.5 mg doses. Interestingly, in Experiments 1.1 and 1.2 there were no statistically significant differences observed between the SDR and controls at the 0.3 mg dose. However, there was a significant attenuation in the SDR response to the 0.3 mg dose at the 8-12 month time frame.

There are few studies in the literature which examine the effects of long-term diabetes on behavior. This is perhaps, in part due to the difficulty with chronic maintenance of and increased mortality amongst the diabetic animals with age (Schmidt et al, 1980). It is for this reason, that the dose range used in Experiment 1.3 was purposely truncated in order to decrease the probability of subject mortality in the SDR. It was observed that the LD 50 for d-amphetamine for the SDR occurred at 1.5 mg/kg (Ahmad & Merali, unpublished observations). Caution must be undertaken when interpreting the results of the long-term

time frame, as the diabetic condition even when treated with insulin, is often accompanied by other complications in both the peripheral nervous system and CNS (Rossi & Bestetti, 1981; Schmidt et al, 1980).

In general, a qualitative comparison of the behavioral output in both groups, indicates some differences in the magnitudes of response between the time frames within the various behaviors measured. In terms of all behaviors, there was a general decline in baseline behaviors with an increase in age. An exemplary behavior was locomotion, across all time frames d-amphetamine elicited a dose-dependent monotonic augmentation in behavior. There were fluctuations in the magnitude of the behavioral response of both the controls and SDR, when comparing the same dose, across different time frames. These fluctuations may be due to a number of factors inherent in the experimental regimen and genetic predispositions of the different sets of animals used. There was not always a decline in stimulated behavior with age. For example, the SDR often manifested a higher frequency of behavior at 8-12 months compared to 0-2 months at the 0.3 and 0.5 mg/kg doses. In general, the same trend occurred in rearing duration and rearing frequency.

Factors such as altered pharmacokinetics and blood brain barrier penetration may have also, in part, accounted for the some of the observed differences between the SDR and controls. This study does not include measures which can directly address the aforementioned. However, studies by Marshall et al, (1976)

and Rowland et al, (1985) in the alloxan and STZ hyperglycemic diabetic rat, demonstrated that reduced access of d-amphetamine to the brain tissue was not significantly different in the diabetics. Also, altered drug metabolism may have been a factor, however, a study by Ackerman (1976), demonstrated that insulin treatment can correct the alterations in drug metabolism associated with diabetes.

As previously outlined, the SDR must be maintained on insulin in order to ensure their survival. With the daily administration of insulin, it is possible that an interaction between insulin and d-amphetamine may have occurred and thus, influenced the results. In this regard, insulin receptors are known to exist in the CNS, with the highest concentration of receptors in the hypothalamic-limbic system (Unger et al, 1991). Moreover, serum insulin is known to directly influence insulin levels in the CNS (Unger et al, 1991). Thus, there exists the possibility that exogenously administered insulin can affect CNS functioning. In fact, Lozovsky et al, (1985) showed that rats made hypoglycemic with the chronic administration of insulin demonstrated alterations in striatal DA receptor sensitivity. These researchers concluded that insulin has a modulatory effect on DA in the striatum, an area where d-amphetamine is known to act. Insulin administration in this study was specifically aimed at maintaining the SDR in a normoglycemic range. In studies where insulin has been found to affect CNS functioning, the animals were severely hypoglycemic. When the diabetic rat is maintained

in normoglycemia, levels of CNS monoamines have been found to be in the normal range (Lozovsky et al, 1981; Trulson & Himmel, 1983).

Taken together, the results from all three experiments demonstrate a chronic and enduring deficit in behavioral responsiveness of the SDR to d-amphetamine.

EXPERIMENT 2: The behavioral effects of amfonelic acid.

Introduction

The objective of this study was to determine whether results of Experiment 1 were associated with the state of diabetes rather than a pharmacokinetic or idiosyncratic interaction between d-amphetamine and the diabetic condition. In this context, an acute sampling of behavior was taken from the SDR with 3-4 months duration of diabetes. Amfonelic acid, an indirect DA agonist was selected. Amfonelic acid (although biochemically dissimilar in structure to d-amphetamine) produces a similar behavioral profile to that of d-amphetamine in both animals and humans (Shore, 1976; Schechter, 1980; Robinson, Koe and Seymour. 1987).

The contended difference between amfonelic acid and d-amphetamine lies in amfonelic acid's apparent ability to access "older stored" pools of DA whereas d-amphetamine acts to preferentially release newly synthesized pools of DA (Shore, 1976; Miller & Shore,1982). Evidence for this difference comes from the finding that the acute blockade of tyrosine hydroxylase (TH) with alpha-methylparatyrosine does not prevent amfonelic acid's central actions while reserpine treatment does (Aceto et al, 1970). In the present study, the behavioral responsiveness of the insulin treated male SDR to amfonelic acid was assessed to engender further support for the contention that the state of

diabetes may be associated with an alteration in CNS DA neurotransmission.

Methods

Animals

All experiments were conducted on male Wistar rats (400-500g) from the following two groups: 1) SDR with a duration of diabetes 3-4 months; 2) NDR, which are genetic littermates of group 1 that have failed to develop diabetes.

Housing and environmental conditions were as described in Experiment 1 (Methods). All diabetic animals were maintained in a normoglycemic state with protamine zinc insulin as outlined in Experiment 1 (Methods).

Procedure

Amfonelic acid doses were administered in a randomized order (0.0, 0.3, 1.0 mg/kg; i.p.) and the locomotor and rearing components of behavior (as described in Table 1, Experiment 1.1) of the SDR and NDR (n=6 each) were assessed. Specifically, on the test day, each animal was habituated to the testing apparatus for 1 hr, then administered amfonelic acid, and monitored for 30 min. The behavioral monitoring was undertaken as described under Methods, Experiment 1.

Results

A two-way ANOVA repeated over the factor dose revealed a significant group effect (SDR vs NDR): locomotion $F(1,10)=52.8$, $p<.0001$; rearing frequency $F(1,10)=14.4$, $p<.01$; rearing duration $F(1,10)=5.9$, $p<.05$. A significant dose effect was also obtained for each behavioral parameter: locomotion $F(2,20)=58.4$, $p<.0001$; rearing frequency $F(2,20)=58$, $p<.0001$. In all 3 of the behavioral parameters, a significant interaction of experimental condition (SDR vs NDR) and dose of amfonelic acid was obtained: locomotion $F(2,20)=13.7$, $p<.001$; rearing frequency $F(2,20)=5.7$, $p<.01$. In each case, the interaction was largely attributable to the diminished response of the SDR with respect to all behaviors at the 0.3 mg/kg dose and locomotor activity at the 1.0 mg/kg dose. This pattern of responding is illustrated by Fig. 4. In the top panel of Fig. 4, it is evident that both groups displayed a dose-dependent increase in locomotion however, the level of augmentation in the SDR was significantly lower than that of the NDR (Tukey, $p<.01$). Thus, there appears to be a shift to the right in the dose-response curve in the SDR. A similar pattern of responding was obtained for the rearing behaviors. Interestingly however, at the 1.0 mg/kg dose a near normal response occurred in the SDR. This is also illustrated by Fig. 4 (lower panel) with the SDR spending significantly less time rearing at the 0.3 mg/kg dose (Tukey, $p<.01$) but not at the 1.0 mg/kg dose.

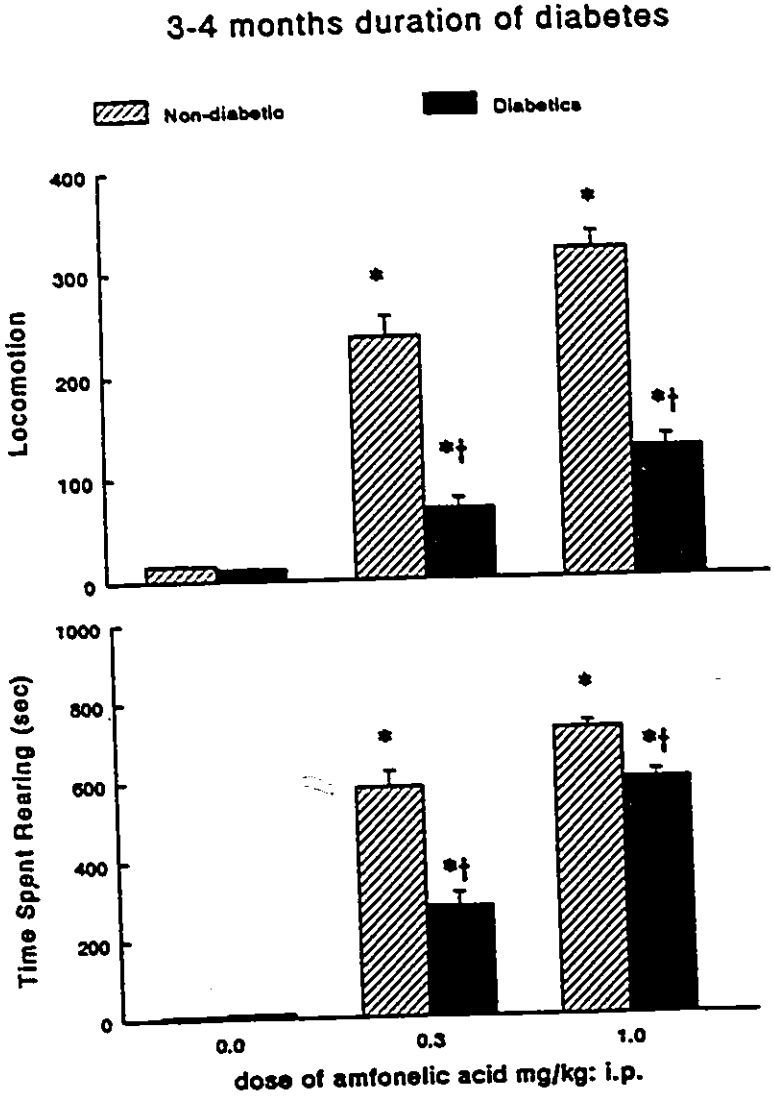

Fig. 4. The effects of amfonelic acid on locomotion (top) and rearing duration (bottom) of the diabetic rat. Each column represents the mean ± S.E.M. * Significantly different from respective baseline (0.0) at p<.05. † Significantly different from matched control group value at p<.05.

Summary

In essence, it was found that the SDR manifested an attenuated response to the behavioral effects of amfonelic acid particularly at the 0.3 mg/kg dose. It is interesting to note, that, although biochemically and structurally dissimilar, both amfonelic acid and d-amphetamine produced a similar behavioral profile in the SDR. For example, in Experiment 1.2, the SDR displayed a reduced sensitivity to the behavioral effects of d-amphetamine. However, if the dose d-amphetamine was sufficiently high (i.e 3.0 mg/kg), a recovery in responsiveness could be elicited in some behaviors. A similar finding was obtained from the rearing duration response with 1.0 mg/kg of amfonelic acid.

The results of this study indicate that the behavioral deficits in the SDR following d-amphetamine stimulation are not idiosyncratic to d-amphetamine alone. Amfonelic acid is also known to cause the release of DA (Miller & Shore, 1982). The mechanisms underlying the inability of the SDR to respond to amfonelic acid-induced stimulation may be multifold. However, if amfonelic acid does access a more permanent pool of DA, then the results of this experiment in combination with Experiment 1, would seem to indicate an altered functional capacity of the SDR, to drugs known to stimulate behavior via the release of DA.

EXPERIMENT 3: Novelty stress response: effects of duration of diabetes.

Introduction

One area receiving increasing consideration is the relationship between stress and diabetes. Studies comparing rodent models of pancreotoxin-induced diabetes to non-diabetic controls have demonstrated differences in the behavioral, biochemical, and neurochemical responses of the diabetics to various stressors (Bellush et al. 1991; Kamei et al. 1992; Bellush & Rowland, 1989; Lee et al. 1988).

Several studies have examined the response of the SDR to various types of stressors. Carter et al, (1987) reported that a variety of environmental stressors may hasten the age of onset of overt diabetes in the diabetic prone SDR. Nakhooda et al, (1978) demonstrated a similar trend. Also, Nakhooda et al, (1981), found that immobilization stress resulted in specific changes in the adrenergic regulation of glucagon and insulin secretion in the hyperglycemic SDR. Ahmad and Merali (1988) reported that the SDR displayed an increased behavioral sensitivity to the stress of a novel environment. Overall, the few studies available in the literature indicate that the SDR when compared to non-diabetic controls displays a behavioral and biochemical hypersensitivity to various stressors. Given the progressive nature of the diabetic condition, it would be important to determine whether

some of the reported findings are a consequence of a specific time frame. Also, the majority of studies to date have not employed insulin replacement as a daily treatment regimen. This is an important factor considering that few individuals afflicted with IDDM can exist without insulin replacement therapy.

In an effort to investigate the previous findings, this study examined the behavioral response of the acute, intermediate, and long-term diabetic, male SDR treated with insulin, to the stress of novel environments. Specifically, separate groups of SDR and matched controls were compared at 0-2, 2-8 and 8-12 months duration of diabetes. Animals were exposed to environments of increasing novelty and their grooming and exploratory responses were measured (Hennessy & Levine, 1978).

General Methods

Animals

All experiments were conducted on male Wistar rats rom the following two groups: 1) SDR; 2) genetically distinct Wistar rats (controls). Housing, environmental conditions, and the maintenance of the diabetic animals were the same as described in Experiment 1 (Methods).

Behavioral Monitoring

The monitoring of behaviors is based on a variation of the time sampling method described by Gispen et al, (1975). In this

case the animal was monitored for 2 sec every 16 sec for a total of 1 hr using a datalogger developed at the University of Ottawa. The Datalogger was designed to interface with an IBM PC, equipped with custom software. This software was designed to analyze and score behavioral events which occurred during the monitoring period. The advantage of this technique is that up to 12 animals can be scored simultaneously. The experimenter was placed in a position unobtrusive to the animals, usually behind a one-way mirror. Table 2 gives the operational definitions of the behaviors monitored.

The Novelty Environments

In this experiment the same groups of animals was exposed to one of four environments. Exposure to the environments were separated by one week periods. The environments were designed to deliver varying levels of novelty, with the most novel environment being the most stressful condition. For a description of the environments please refer to Table 3. On the day of experimentation the SDR and matched controls were placed into the environment such that the experimenter was blind to the position of the animals. Behavioral monitoring then ensued for a period of 1 hr.

Table 3. Description of Novelty Environments

Environments	Description
Same Cage /Same Room	This environment consisted of the animals home cage (43 x 23 x 7 cm) made of clear polyethylene with a metal grid top and wood chips on the floor, situated in the animals home (colony) room.
Different Cage /Same Room	In this condition the animals were placed in an unfamiliar polyethylene cage with the same dimensions and internal milieu as described in the above and left in their home room.
Different Room /Same Cage	In this condition, the animals home cage was transported a short distance (10 feet) to a room unfamiliar to them.
Different Room /Different Cage	In this environment, the animals were transported (20 feet) to a room unfamiliar to them (smaller with bright lighting) and placed into polyethylene cages devoid of bedding with polyethylene tops (43 x 23 x 15 cm).

Table 2. Behaviors Monitored for Novelty-Induced Grooming

Behavioral Measure	Symbol	Definition
Head Washing	HW	The forepaws are wiped over the face and crown.
Head Scratching	HS	The hindpaws are brought into contact with the side of the head and a scratching motion occurs.
Body Washing	BW	The ventral surface of the thorax and abdomen are licked.
Body Scratching	BS	The body flanks are contracted by the hindpaws and a scratching motion ensues.
Exploring	EXP	The animal moves around the cage, rearing, sniffing and orienting itself to its environment.
Resting	REST	The animal is either sleeping or in a stationary pose.

Statistics

The data were analyzed using the statistical package GBSTAT. A two-way repeated measures ANOVA (Groups x Environment) was used and post hoc analysis using Tukey tests were employed when necessary. Significance levels of $p<.05$ were used throughout.

Results

Experiment 3.1

The effects of novelty stress on the diabetic rat with 0-2 months duration of diabetes.

This experiment employed two groups with an n=8 per group. An analysis of the combined washing scores (headwash + body wash) and of the combined scratching scores (head scratch + body scratch) revealed a significant group effect (SDR vs control) in both cases $F(1,14)=112.6$, $p<.05$; $F(1,14)=42.4$, $p<.05$ respectively. There was also a significant effect for environment in each case, $F(3,42)=166.1$, $p<.05$ (washing); $F(3,42)=17.4$, $p<.05$ (scratching). A significant interaction was obtained in both cases, $F(3,42)=28.6$, $p<.05$ (washing); $F(3,42)=7.0$, $P<.05$. Post hoc analysis revealed that exposure to novelty-induced stress elicited a significant increase in washing behaviors in both the SDR and controls when compared to the baseline condition of same cage/same room (Fig. 5, upper most panel). It was also evident that the SDR washed at significantly higher frequencies than the controls at each of the three novel environments. The same trend was not entirely evident in scratching behaviors (as illustrated

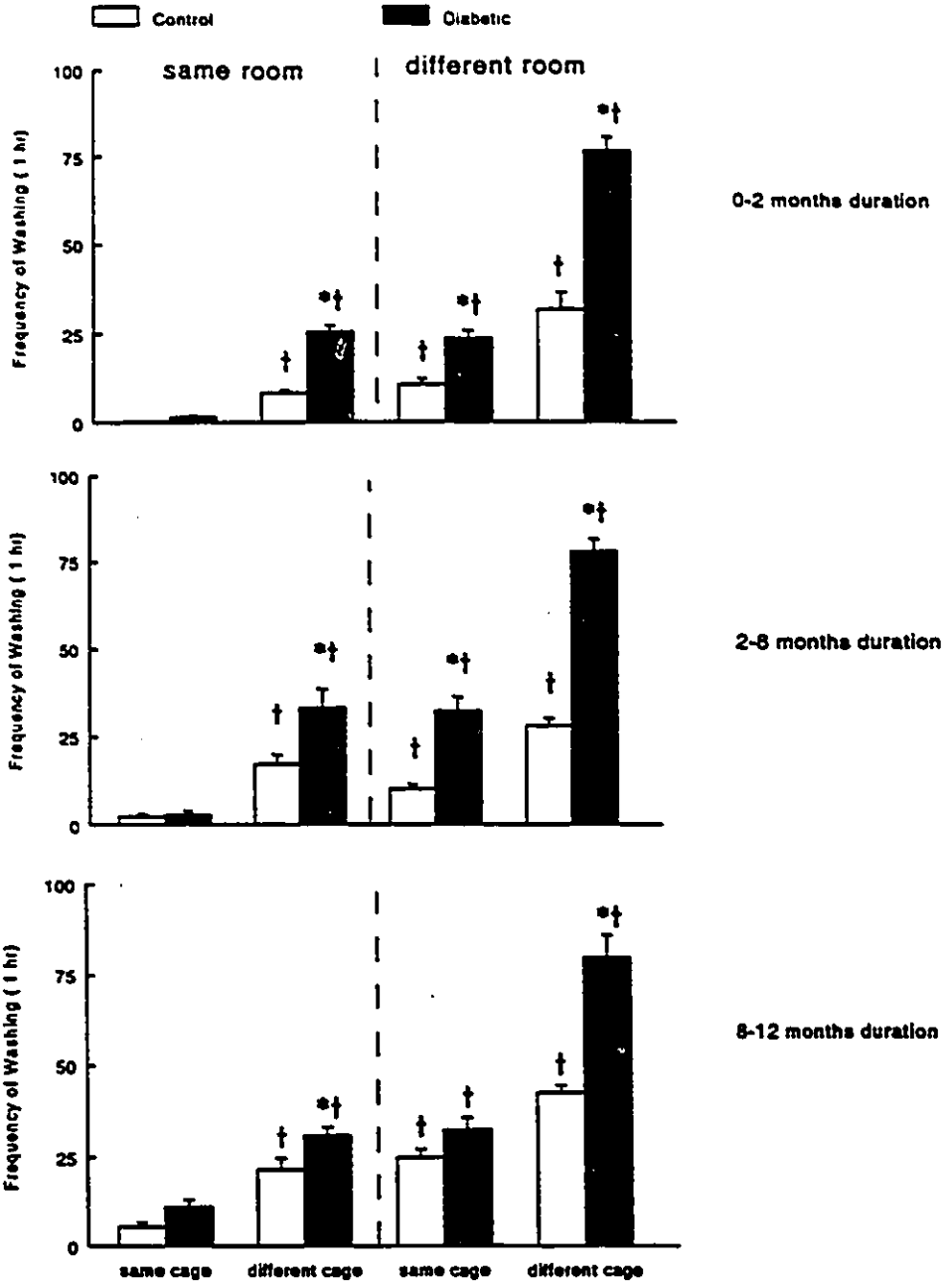

Fig. 5. The novelty-stress induced washing behavior response of the diabetic rat. Each column represents the mean ± S.E.M. † Significantly different from respective baseline (same cage/same room) at p<.05. * Significantly different from respective control group value at p<.05.

by Fig. 6, uppermost panel). In this case, only the SDR displayed a significant increase in their behavioral response to the novel conditions. The SDR when compared to controls displayed significantly higher levels of scratching at each of the three novel conditions.

An analysis of the individual grooming elements revealed a significant group effect (SDR vs control) for each of the the grooming elements: head washing $F(1,14)=85.0$, $p<.05$; body washing $F(1,14)=85.2$; head scratching $F(1,14)=29.9$, $p<.05$; body scratching $F(1,14)=36.6$. Significant group effects were also obtained with exploration $F(1,14)=73.4$, $p<.05$ and resting $F(1,14)=28.6$. A significant effect for environment was also obtained for each behavioral parameter: head washing $F(3,42)=165.6$, $p<.05$; body washing $F(3,42)=103.5$, $p<.05$; head scratching $F(3,42)=5.9$, $p<.05$; body scratching $F(3,42)=34.6$, $p<.05$; exploration $F(3,42)=146.6$, $p<.05$; resting $F(3,42)=181.5$, $p<.05$. A significant interaction between group (SDR vs control) and environment (novelty condition) was obtained for each behavioral parameter: head washing $F(3,42)=32.3$, $p<.05$; body washing $F(3,42)=16.9$, $p<.05$; head scratching $F(3,42)=3.1$, $p<.05$; body scratching $F(3,42)=11.7$, $p<.05$; exploration $F(3,42)=33.8$, $p<.05$; resting $F(3,42)=34.3$, $p<.05$.

As displayed in Table 4, The data indicate that the SDR manifested a significant increase in their grooming and exploratory response at each of the three novelty environments

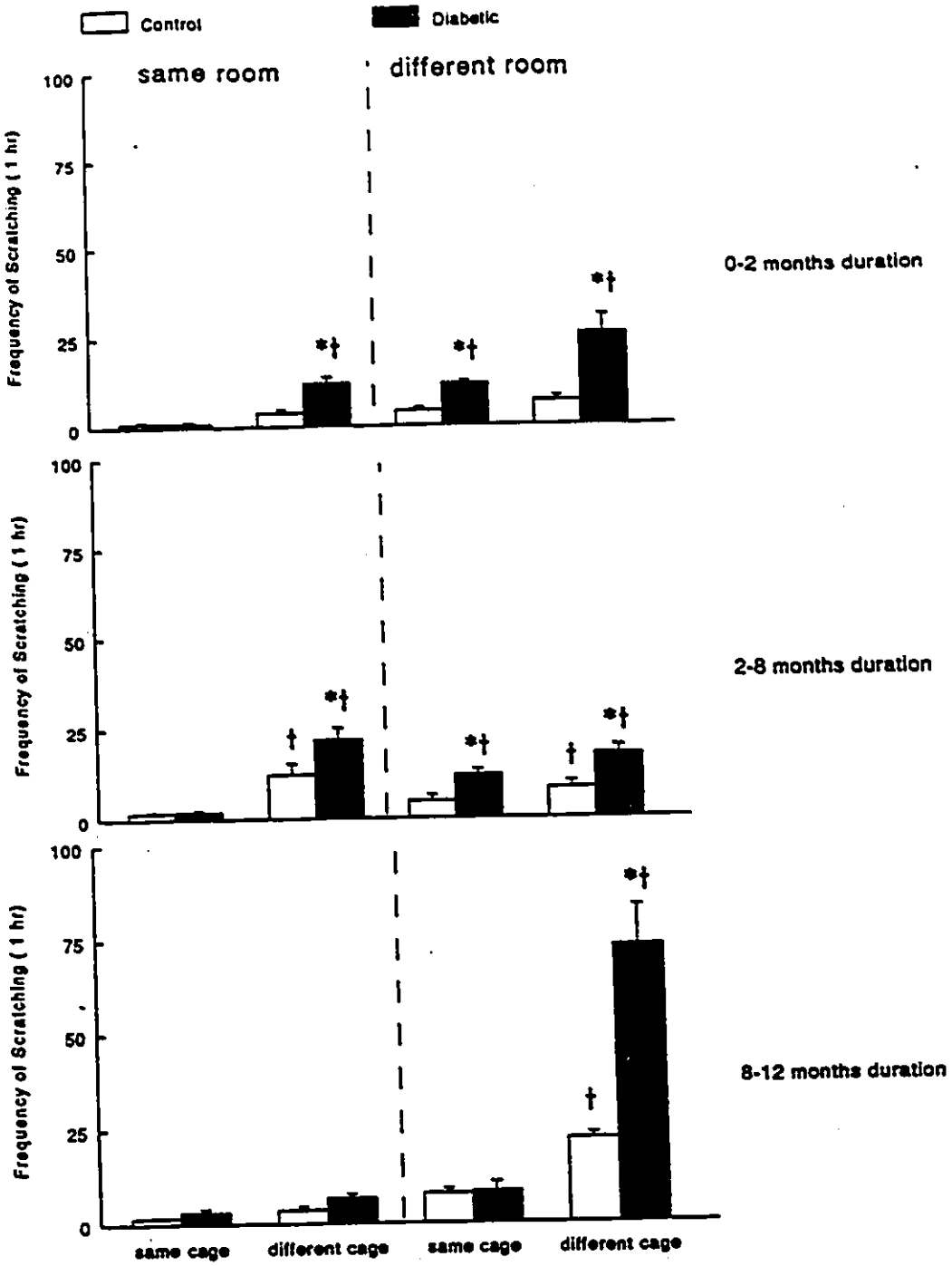

Fig. 6. The novelty-stress induced scratching behavior response of the diabetic rat. Each column represents the mean ± S.E.M. † Significantly different from respective baseline (same cage/same room) at $p<.05$. * Significantly different from respective control group value at $p<.05$.

TABLE 4

Effects of novelty-induced stress on the behavior of the spontaneously diabetic rat with 0-2 months duration of diabetes.

Group	Environment (cage/room)	Frequency of Behavior					
		HW	HS	BW	BS	EXP	REST
CTL	Same cage Same room	0.12 ±0.12	0.5 ±0.2	0.37 ±0.2	0.6 ±0.3	5.6 ±0.3	212 ±0.7
CTL	Diff cage Same room	4.0[a] ±0.4	1.6 ±0.5	4.1 ±0.7	2.1 ±0.6	51.2[a] ±2.8	159[a] ±1.5
CTL	Same cage Diff room	3.9[a] ±0.7	1.0 ±0.4	6.5[a] ±1.0	3.1 ±0.5	70.5[a] ±1.8	135[a] ±2.0
CTL	Diff cage Diff room	12.4[a] ±1.9	2.0 ±0.8	19[a] ±3.3	4.6[a] ±0.9	62.0[a] ±3.6	142[a] ±3.1
SDR	Same cage Same room	0.5 ±0.2	0.4 ±0.2	1.1 ±0.3	0.7 ±0.4	4.4 ±1.5	210 ±1.9
SDR	Diff cage Same room	7.6[a] ±1.1[b]	7.1[a] ±1.3[b]	17.7[a] ±1.3[b]	5.0[a] ±0.8[b]	47.1[a] ±4.6	141[a] ±4.1[b]
SDR	Same cage Diff room	9.2[a] ±0.9[b]	4.5[a] ±0.7	15.5[a] ±2.0[b]	7.4[a] ±0.4[b]	22.0[a] ±1.6[b]	169[a] ±1.4[b]
SDR	Diff cage Diff room	31.2[a] ±1.3[b]	10.0[a] ±3.2[b]	45.2[a] ±2.9[b]	15.5[a] ±2.2[b]	66.7[a] ±3.1	96.4[a] ±8.2[b]

Cells contain the mean ± S.E.M. [a] $p<.05$ from respective baseline condition (same cage/same room). [b] $p<.05$ from control condition at that novelty environment.

when compared to the baseline condition. In the SDR, the highest frequencies of behavior always occurred in the most novel of the environments (different cage/different room). In the control group, novelty was more effective at stimulating washing behaviors. The controls also displayed a trend towards exhibiting the highest frequencies of behavior in the most novel of the environments. A comparison of the SDR and control response indicated that the SDR displayed significantly higher frequencies of grooming behavior in the different cage/different room, different cage/same room and body washing and body scratching in the same cage/different room condition. Overall, the SDR responded to novelty-induced stress with an increase in grooming and exploratory behavior and in most conditions, this increase was significantly higher than the control response.

Experiment 3.2

The effect of novelty stress on the diabetic rat with 2-8 months duration of diabetes.

This experiment employed a total of two groups with an n=8 per group.

An analysis of the combined head and body washing scores and of the combined scratching scores resulted in a significant effect of group (SDR vs control) in both cases: washing $F(1,14)=126.5$, $p<.05$; scratching $F(1,14)=30.0$, $p<.05$. We also obtained was a significant effect for environment (novelty condition): washing $F(3,42)=95.7$, $p<.05$; scratching $F(3,42)=17.6$,

P<.05. a significant interaction was obtained for washing $F(3,42)=23.3$, $p<.05$ but not for scratching. As depicted in Fig. 5 (middle panel), the SDR displayed a significant increase in the frequency of washing when compared to the baseline environment (same cage/same room). The controls also manifested a significant rise in the frequency of behavior when compared to baseline. However, the extent of the stimulation in the SDR was significantly greater than that of the controls. In Fig. 6 (middle panel), the SDR displayed a significant increase in the frequency of scratching when compared to their respective baseline. The controls however, displayed a significant increase in behavior in two out of the three environments. Post hoc comparisons (Tukey) between the SDR and the controls revealed that the SDR manifested a significantly higher frequency of scratching behavior. Interestingly, scratching behavior in the same room/different cage environment was higher than that which occurred in the different cage/different room environment, the most novel of the presented environments.

Analyses of the individual behavioral elements resulted in a significant group effect (SDR vs control) for each of the elements: head washing $F(1,14)=14.7$, $p<.05$; body washing $F(1,14)=217.2$, $p<.05$; head scratching $F(1,14)=16.5$, $p<.05$; body scratching $F(1,14)=24.9$, $p<.05$; exploration $F(1,14)=58.1$, $p<.05$; resting $F(1,14)=6.1$, $p<.05$. A significant effect for novelty environment was also obtained for each of the elements: head washing $F(3,42)=32.3$, $p<.05$; body washing $F(3,42)=110.9$, $p<.05$;

head scratching $F(3,42)=15.3$, $p<.05$; body scratching $F(3,42)=14.8$, $p<.05$; exploration $F(3,42)=107.2$, $p<.05$; resting $F(3,42)=157.6$, $p<.05$. A significant interaction between group (SDR vs control) and environment (novelty condition) was obtained for each element: head washing $F(3,42)=5.2$, $p<.05$; body washing $F(3,42)=30.1$, $p<.05$; head scratching $F(3,42)=3.6$, $p<.05$; body scratching $F(3,42)=2.8$, $p<.05$; exploration $F(3,42)=17.5$, $p<.05$; resting $F(3,42)=11.5$, $p<.05$.

As outlined in Table 5, in the different cage/different room environment, both groups displayed a significant increase in behavior as compared to their respective baseline conditions. Also, in this environment, the SDR displayed a significantly higher frequency of grooming behavior than the controls, the exception being head scratching. A similar trend occurred in the same cage/different room environment. Notable differences between the groups also occurred in body washing, where the SDR manifested a significantly higher frequency of behavior than the controls in each of the three novel environments. Also of interest is the significantly lower levels of exploratory behavior demonstrated by the SDR in the different cage/different room and the same cage/different room environments.

Overall, as was the case in Experiment 3.1, the SDR with a 2-8 month duration of diabetes responded to novelty-induced stress with an increase in grooming behavior. The greatest difference in response occurred in the environment with the highest degree of novelty.

TABLE 5

Effects of novelty-induced stress on the behavior of the spontaneously diabetic rat with 2-8 months duration of diabetes.

Group	Environment (cage/room)	Frequency of Behavior					
		HW	HS	BW	BS	EXP	REST
CTL	Same cage Same room	0.87 ±0.35	0.6 ±0.2	1.4 ±0.6	1.1 ±0.5	25.0 ±4.9	169 ±2.6
CTL	Diff cage Same room	5.6[a] ±2.0	4.2[a] ±1.9	11.8[a] ±0.9	7.8[a] ±1.5	63.6[a] ±1.0	133[a] ±0.9
CTL	Same cage Diff room	1.5 ±0.5	1.2 ±0.4	8.6[a] ±1.0	3.6 ±1.5	118[a] ±5.8	99.8[a] ±2.0
CTL	Diff cage Diff room	8.1[a] ±1.4	2.8 ±0.6	19[a] ±1.5	5.4[a] ±1.3	137[a] ±6.5	58[a] ±5.6
SDR	Same cage Same room	0.75 ±0.3	0.6 ±0.3	2.1 ±0.8	1.4 ±0.5	15.2 ±2.1	200 ±2.6
SDR	Diff cage Same room	8.6[a] ±2.5	10.7[a] ±1.8[b]	24.6[a] ±2.9[b]	11.0[a] ±1.7	69.6[a] ±3.5	114[a] ±6.6[b]
SDR	Same cage Diff room	5.5[a] ±0.9[b]	1.5 ±0.5	26.7[a] ±3.4[b]	10.6[a] ±1.4[b]	65.5[a] ±6.2[b]	139[a] ±10[b]
SDR	Diff cage Diff room	18.2[a] ±1.4[b]	5.1[a] ±0.9	59.4[a] ±2.4[b]	12.2[a] ±1.5[b]	80.2[a] ±7.9[b]	52[a] ±8.9

Cells contain the mean ± S.E.M. [a] $p<.05$ from respective baseline condition (same cage/same room). [b] $p<.05$ from control condition at that novelty environment.

Experiment 3.3

The effects of novelty stress on the diabetic rat with 8-12 months duration of diabetes.

This experiment employed a total of 16 animals with an n=8 per group.

As in Experiments 3.1 and 3.2, an analysis of the combined washing and the combined scratching scores produced a significant group effect in both behaviors: washing $F(1,14)=40.9$, $p<.05$; scratching $F(1,14)=22.2$, $p<.05$. The analyses also revealed a significant environment effect: washing $F(3,42)=110.2$, $p<.05$; scratching $F(3,42)=63.1$, $p<.05$. A significant interaction of group x environment was also evident: washing $F(3,42)=12.6$, $p<.05$; scratching $F(3,42)=20.9$, $p<.05$.

As depicted in Fig. 5 (lowest panel), both groups displayed a significant augmentation in the frequency of washing, in all 3 novel environments as compared to their respective baseline environments. In two of the three novel environments (different cage/different room; different cage/same room), the SDR had a significantly higher frequency of washing than the controls. In both groups the highest frequency of washing occurred in the environment with the greatest degree of novelty (different cage/different room). In both groups only the environment with the greatest degree of novelty was effective in stimulating an increase in the frequency of scratching behavior (Fig. 6, lowest panel), as compared to their respective baseline environments. It

is important to note, that in the different cage/different room environment, the SDR scratched at a significantly higher frequency than the controls.

A summary of Table 6, indicates that the different cage/different room environment was most effective in stimulating behavior in both groups. In this environment, in terms of grooming, the SDR manifested a significantly higher frequency of behavior as compared to controls. Interestingly, the exploratory response of the SDR was significantly lower in all three novelty environments, as compared to controls. In general, it would appear that a qualitatively similar response by the SDR was obtained in all three experiments.

TABLE 6

Effects of novelty-induced stress on the behavior of the spontaneously diabetic rat with 8-12 months duration of diabetes.

Group	Environment (cage/room)	Frequency of Behavior					
		HW	HS	BW	BS	EXP	REST
CTL	Same cage Same room	1.4 ±0.9	0.5 ±0.2	4.0 ±0.5	1.0 ±0.3	15.7 ±1.1	190 ±2.5
CTL	Diff cage Same room	6.5[a] ±1.0	1.0 ±0.4	15[a] ±2.2	2.5[a] ±0.5	56.2[a] ±4.0	38.5[a] ±2.6
CTL	Same cage Diff room	5.2 ±0.6	2.6 ±0.3	19.6[a] ±2.1	5.1 ±1.2	47.2[a] ±7.0	36.5[a] ±5.2
CTL	Diff cage Diff room	16.6[a] ±1.5	15.8[a] ±2.0	26[a] ±1.5	6.1[a] ±1.5	133[a] ±4.2	51.4[a] ±1.7
SDR	Same cage Same room	1.4 ±0.5	1.8 ±1.0	9.6 ±1.7	1.1 ±0.6	18.8 ±1.9	179.7 ±2.4
SDR	Diff cage Same room	8.5[a] ±1.4	1.6 ±0.5	22.4[a] ±1.9[b]	4.8[a] ±0.7	32.7[a] ±4.3[b]	40[a] ±2.5
SDR	Same cage Diff room	9.2[a] ±0.9	3.5 ±1.3	23.2[a] ±2.7	4.7[a] ±1.4[b]	25.2[a] ±3.1[b]	44.4[a] ±4.2
SDR	Diff cage Diff room	31.9[a] ±2.8[b]	61.2[a] ±9.9[b]	48[a] ±3.4[b]	11.9[a] ±2.1[b]	114[a] ±5.2[b]	29.2[a] ±7.0

Cells contain the mean ± S.E.M. [a] $p<.05$ from respective baseline condition (same cage/same room). [b] $p<.05$ from control condition at that novelty environment.

Summary

This study examined the effect of duration of diabetes and novelty-induced stress on the behavioral responsiveness of the insulin treated male SDR. In essence, it was found that the SDR exhibited an enhanced grooming response to the stress incurred by varying degrees of novelty. Moreover, this enhanced behavioral reactivity is chronic and endures for over a year following the detection of diabetes. The behavioral enhancement also endures despite insulin replacement therapy.

In Experiment 3.1, there were no differences in baseline behavior, however, as the degree of novelty increased, the frequency of washing and scratching behaviors increased significantly in both groups. This graded behavioral response is characteristic of experiments which use increasing degrees of novelty as a stressor (Flaherty et al, 1985). The SDR always displayed a higher frequency of grooming behavior as compared to the controls.

In Experiment 3.2, a similar response pattern to that observed at 0-2 months occurred during the 2-8 month time frame. In terms of the magnitude of behavioral output, a slightly higher frequency of washing occurred at the intermediate levels of novelty, in the SDR, at 2-8 months as compared to 0-2 months. The washing response of both groups to the different cage/different room condition was similar in magnitude to that observed at 0-2 months. The scratching response of both groups was highest in the

same room/different cage condition. This response is puzzling because this environment was not considered to be the most novel. However, it is possible that there was less competition from other behaviors allowing scratching to surface. There is also the possibility that there were unidentified factors unique to this condition at this phase, which elicited the observed response. In general, there was a higher magnitude of scratching frequency at 2-8 months as compared to 0-2 months.

The washing response profile of the SDR at 8-12 months was for the most part, qualitatively similar to that of the 0-2 and 2-8 month time frames. Although, there was no statistically significant difference between the SDR and controls in the same cage/different room condition, the SDR did exhibit a higher frequency of washing. In general, at the 8-12 month time frame, the SDR manifested a higher frequency of washing, across the three novelty environments, as compared to the SDR response during the other two time frames. In concordance with this finding, Kametani (1988) demonstrated that grooming, in particular washing, in the aged rat (26-28 months old) was enhanced following novelty stress compared to rats 5-6 months of age.

In terms of scratching behavior at the intermediate levels of novelty, neither group responded to the extent observed at the 0-2 and 2-8 month time frames. There was a significant augmentation in the frequency of scratching at the most novel condition in both groups. As in the earlier time frames, the SDR

once again exhibited a significantly higher frequency of scratching at the most novel condition.

This study also examined the exploratory response of the SDR. In general, the control exploratory response could be described as 'bell shaped' for each environment across time frames (beginning from 0-2 to 8-12 months duration of diabetes). In the SDR, there was an increase in baseline exploratory behavior over time frames. Similar to the controls, a 'bell shaped' response over time frame for the intermediate levels of novelty was evident in the SDR. In the most novel condition, the SDR demonstrated an increase in exploratory activity over time frames.

In the rodent challenged with states of high arousal, grooming behavior is considered to be a pivotal response in returning the animal to homeostasis (Cohen & Price, 1979). From this perspective, grooming is considered as an important index of the animals response to stressful conditions (Kametani, 1988). It would seem that in this context, the grooming behavior displayed by the SDR is indicative of an heightened adaptive response to the stress of a novel environment during specific time frames both proximal and distal to the development of the overt diabetic condition. This finding is in general agreement with studies undertaken in the hyperglycemic chemically diabetic rat, which have demonstrated an altered behavioral response of the diabetic rodent to various stressors (for a review, Rowland & Bellush, 1989).

The results of this study are in agreement with Ahmad and Merali (1988), which demonstrated that the SDR displayed a heightened grooming response to the mild stress of a novel environment but, not to the more intense stress of multiple saline injections. This study is also in general agreement with the findings of Bellush et al, (1991) which demonstrated a disruptive effect of novelty on the behavioral response of the hyperglycemic STZ diabetic rat. There are however, some aspects of this study which are unique to the literature. First, this study examined the stress response of the SDR during the diabetic phase of these animals. Other studies have focussed largely on the prediabetic phase of the SDR (Carter et al, 1987; Nakhooda et al, 1981; Nakhooda et al, 1978). Second, this study examined the behavioral responsiveness of the SDR over an extended period of time. This approach allows for the establishment of a behavioral profile of the SDR and its response to novelty stress. Most studies in the literature investigating behavior in the diabetic rodent have not considered as a main factor, the duration of diabetes. The majority of studies investigating the behavioral and neurochemical response of the diabetic rat have employed rats with a duration of diabetes of 2 months or less. Considering diabetes is a progressive disease, it is important to chart the changes which may accompany the progression. Such an approach allows for a more dynamic view of the effects of stress on the diabetic condition.

It is also of interest to note, that the behavioral deficits endured despite insulin treatment. Lee et al, (1989) also found that footshock induced alterations in basal circulating levels of E and plasma levels of E, in the chemically diabetic rat, endured despite insulin treament. Ahmad and Merali (1988) also found that novelty stress induced grooming was enhanced in the SDR, treated with insulin. In the present study, direct physiological measures were not taken. Thus, it is difficult to ascertain if any alterations in glycemic level occurred during the stress challenge. However, urine glucose values in the SDR, immediately following the stressor test, were found to be in the normal range.

In summary, the literature regarding the response of the diabetic rat to stress, although small, is in general agreement. It is evident that the diabetic rat when challenged with a stressor, demonstrates an altered compensatory response. Overall, these studies are indicative of a behavioral sensitization of the diabetic rat to stress.

EXPERIMENT 4: The behavioral effects of chlordiazepoxide on the diabetic rat exposed to the open field and elevated plus maze.

Introduction

The findings of Experiment 3, essentially demonstrated that the SDR has a heightened reactivity to novelty stress. However, the contributive role of anxiety was not assessed in Experiment 3. The following experiment was designed to provide an acute investigation of the SDR (2-8 months duration of diabetes) response to the anxiogenic effects of the elevated plus maze and open field. Furthermore, the response of the SDR to chlordiazepoxide, a drug with anxiolytic properties, was also assessed.

The elevated plus maze was chosen because this method does not involve nociceptive stimulation, food deprivation or training as confounding variables. In addition, it allows for the reliable detection of the effects of anxiolytic drugs. The elevated plus maze has also been validated behaviorally as well as physiologically (Handley & Mithani, 1984; Pellow et al, 1985). In essence, the demonstration that rodents display a preference for the closed arms of this apparatus reflects an aversion towards the open arms caused by fear or anxiety induced by high and open spaces (Pellow & File, 1986). Furthermore, it has been reported

that with the administration of anxiolytic or anxiogenic drugs the responses or approaches towards the open arms can be altered (Pellow & File, 1986).

The open field is a widely used apparatus for the testing of exploratory, locomotor and anxiogenic responsiveness in rodents (Gentsch et al, 1987; Archer, 1973). Entry into the center area of the open field is considered anxiogenic to the rodent, due to the rat's inherent thigmotaxic nature. Finally, chlordiazepoxide is a well established drug believed to reduce anxiety through its effects on the CNS GABA/benzodiazepine receptor complex (Tallman et al, 1980).

In summary, anxiety was a term used to describe the rat's avoidance towards specific areas of the apparatii employed in this experiment. Thus, depending on the rat's level of anxiety, it will avoid the anxiogenic areas of the apparatii more or less frequently.

General Methods

Animals

All experiments were conducted on male Wistar rats from the following three groups: 1) Genetically distinct group (controls); 2) The genetic littermates of the SDR who have not developed diabetes (non-diabetics); 3) Spontaneously diabetic rats (SDR). Housing and environmental conditions were as outlined in Experiment 1 (Methods). Maintenance of the diabetic animals was as described in Experiment 1 (Methods).

Behavioral Tests

Rats were transferred to the observation room 2 hrs prior to the behavioral testing. The observation room was illuminated by a 60 watt red light bulb. The experimenter was separated from the subjects by a one-way mirror. The control, non-diabetic (NDR) and SDR were tested alternately, in a counterbalanced order.

Open Field: General activity and anxiety were measured in an open field chamber measuring 45 x 45 x 26 cm. The chamber was equipped with four sets of infrared cells, two per adjacent wall, located 2.5 cm above the floor into nine 15 x 15 cm virtual squares which were represented with red tape. The infrared cells relayed information to a single digital counter. It is the center square which is considered to be most anxiogenic.

Elevated Plus Maze: The maze was constructed according to the specifications published by Pellow and File (1986). The maze consisted of two opposing open arm planks and two closed arm planks which were surrounded by black plexiglas walls. The width of the arms was 10 cm and the length was 50 cm. The closed arms were surrounded by 38 cm high walls. The arms were elevated 50 cm above the ground with a wooden stand. It is the open arms which are considered to be most anxiogenic.

Drug Administration

Chlordiazepoxide-HCL (Librium, Hoffman-La Roche Ltd, Basle Switzerland) was dissolved in distilled water and injected intraperitoneally (i.p.) at the following doses (0.0, 2.0, 4.0, 6.0 mg/kg) 60 min prior to behavioral testing.

Procedure

Open Field: Animals received their injection of chlordiazepoxide 60 min prior to behavioral testing. Following this time period, the rat was placed in a randomly determined corner of the open field. The animal was subsequently monitored for 10 min and the number of beam interruptions and frequency of entries into the central square were assessed.

Elevated Plus Maze: The injection of chlordiazepoxide followed the same protocol as that used in the open field. The rat was subsequently placed in the center of the elevated plus maze facing a closed arm, and monitored for a duration of 10 min. The animals were assessed for the frequency of entries and the duration of time spent in the various arms.

Results

Open Field: As depicted by Fig. 7 (upper panel) chlordiazepoxide dose dependently increased locomotor activity (# of beam interruptions) in each group. A two-way ANOVA repeated over the factor dose revealed no statistically significant group differences, indicating the SDR exhibited similar levels of locomotor activity to both the controls and NDR. An analysis of the frequency of entries into the central area of the open field resulted in a dose dependent increase in the frequency of entries into the central area of the open field for all groups [control vs NDR vs SDR, $F(2,21)=57.1$, $p<.001$.; dose of chlordiazepoxide $F(3,63)=421.9$, $p<.001$] There was also a significant interaction of Group x Dose $F(3,63)=11.14$, $p<.001$. Multiple post hoc comparisons using Tukey demonstrated that the interaction was largely accounted for by the fact that the SDR had a significantly lower frequency of entries (Tukey, $p<.01$) into the central area than both the controls and NDR (see Fig.7, lower panel).

Elevated Plus Maze: A two-way ANOVA repeated over dose of chlordiazepoxide was used to analyze the time spent by each group in the various arms of the elevated plus maze. As depicted by Fig. 8 (lowest panel) chlordiazepoxide dose dependently increased the amount of time spent by each group on the open arms of the elevated plus maze [control vs NDR vs SDR $F(2,21)=10.9$, $p<.001$;

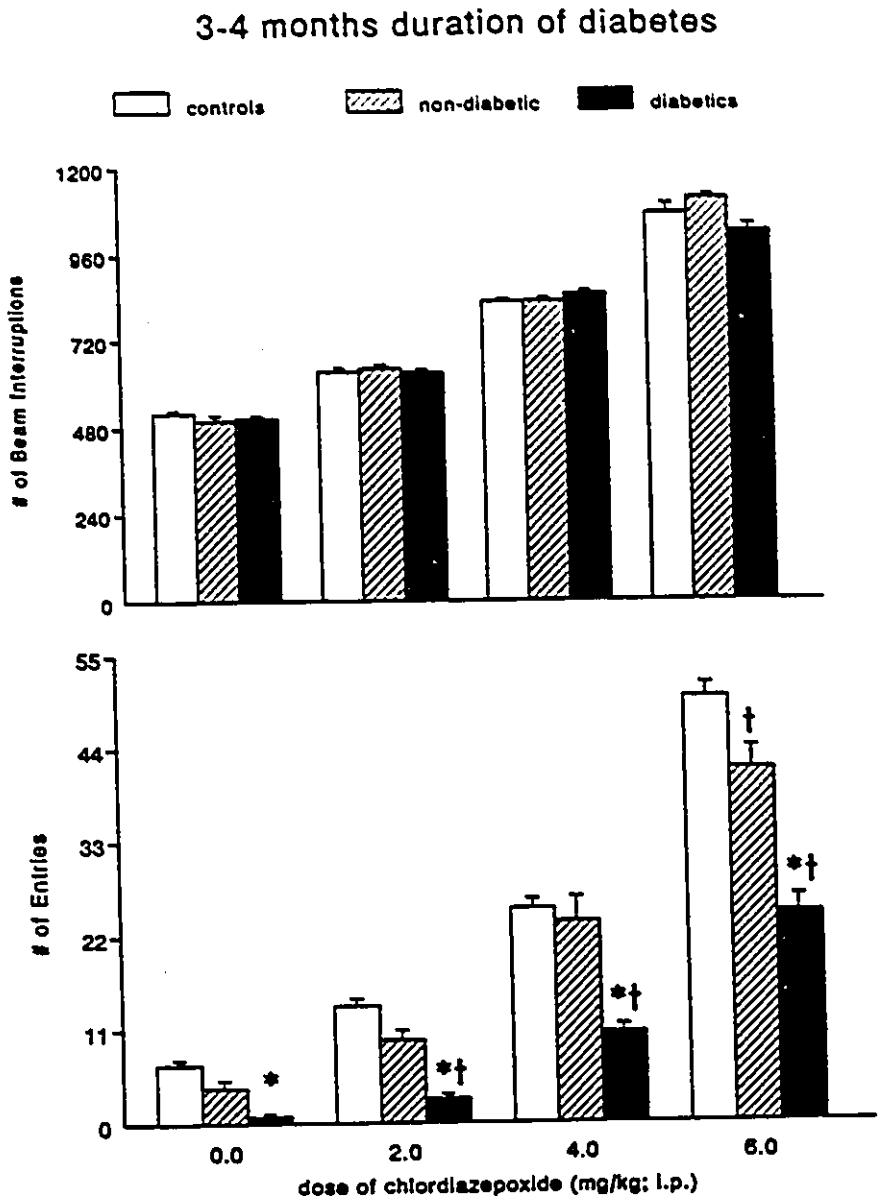

Fig. 7. The locomotor response (top) and frequency of entries into the center square (bottom) of the diabetic rats exposed to the open field and various doses of chlordiazepoxide for 10 min. Each column represents the mean ± S.E.M. † Significantly different from the control group value at $p<.05$. * Significantly different from the non-diabetic group value at $p<.05$.

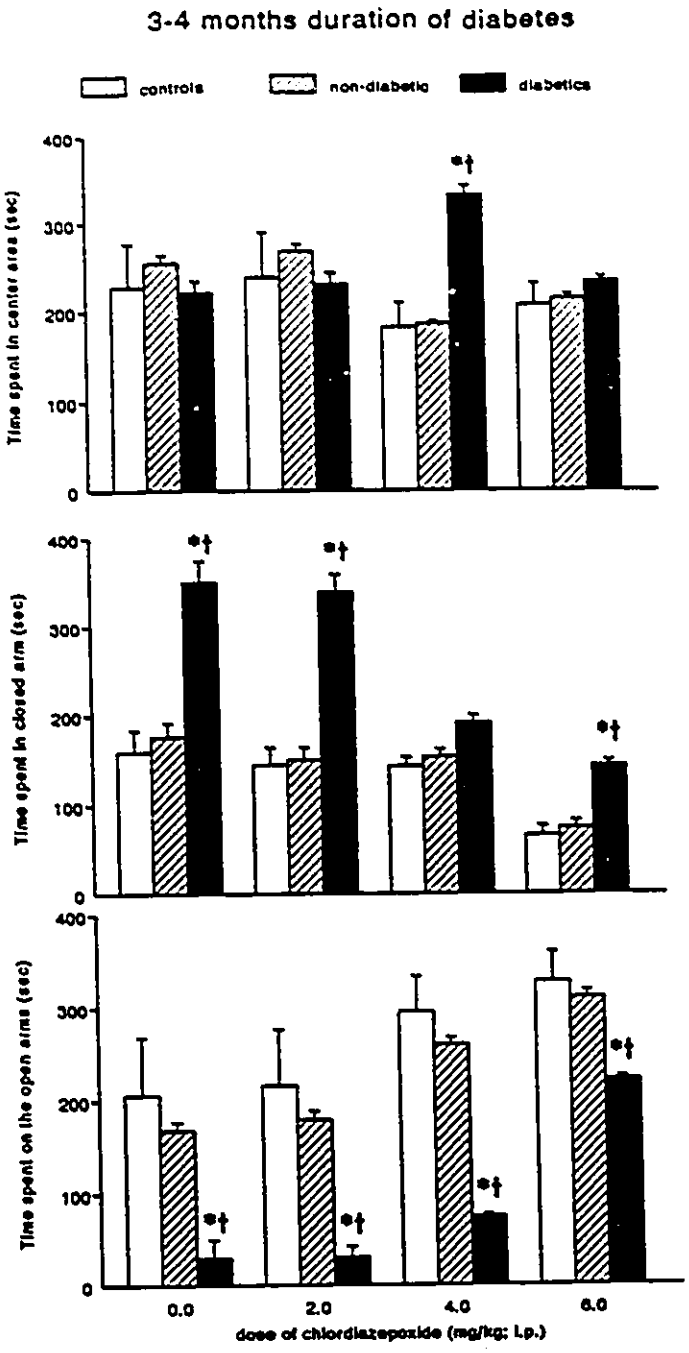

Fig. 8. The duration of time spent in the elevated plus maze by the diabetic rat following various doses of chlordiazepoxide. Each column represents the mean ± S.E.M. † Significantly different from the control group value at $p<.05$. * Significantly different from the non-diabetic group value at $p<.05$. The top panel shows the time spent in the center portion of the maze, the middle panel the time spent in the closed arms, and the bottom panel the time spent in the open arms.

dose of chlordiazepoxide $F(3,63)=87.8$, $p<.001$]. There was also a significant interaction of Group x Dose $F(3,63)=3.91$, $p<.01$. The interaction was largely the result of the significantly lower amount of time spent by the SDR (Tukey, $p<.01$) on the open arms, across all doses, compared to both the controls and NDR. A similar analysis indicated that the overall behavioral trend of the SDR as depicted by Fig. 8, was to spend a significantly greater amount of time on the closed arms of the maze at the 0.0-2.0 mg/kg doses (Fig. 8, middle panel) than the control and NDR groups. However, at the 4.0-6.0 mg/kg doses, the SDR spent increasing amounts of time on the center area and open arms of the maze (see Fig. 8, top panel) but, never to the extent of the control and NDR groups. An analysis of the frequency of entries by the SDR on the open arm of the maze, revealed a similar trend to that observed with regards to the duration of time spent on the open arm (see Fig. 9).

Summary

A comparison of the three groups in both apparatii showed that: 1) the insulin treated SDR had a clear aversion to the anxiogenic areas of both apparatii; 2) chlordiazepoxide was effective in attenuating this aversion in all groups, but its efffects were more potent in the diabetic group. Nevertheless, the effects of chlordiazepoxide on the response of the SDR were not as efficacious when compared to controls.

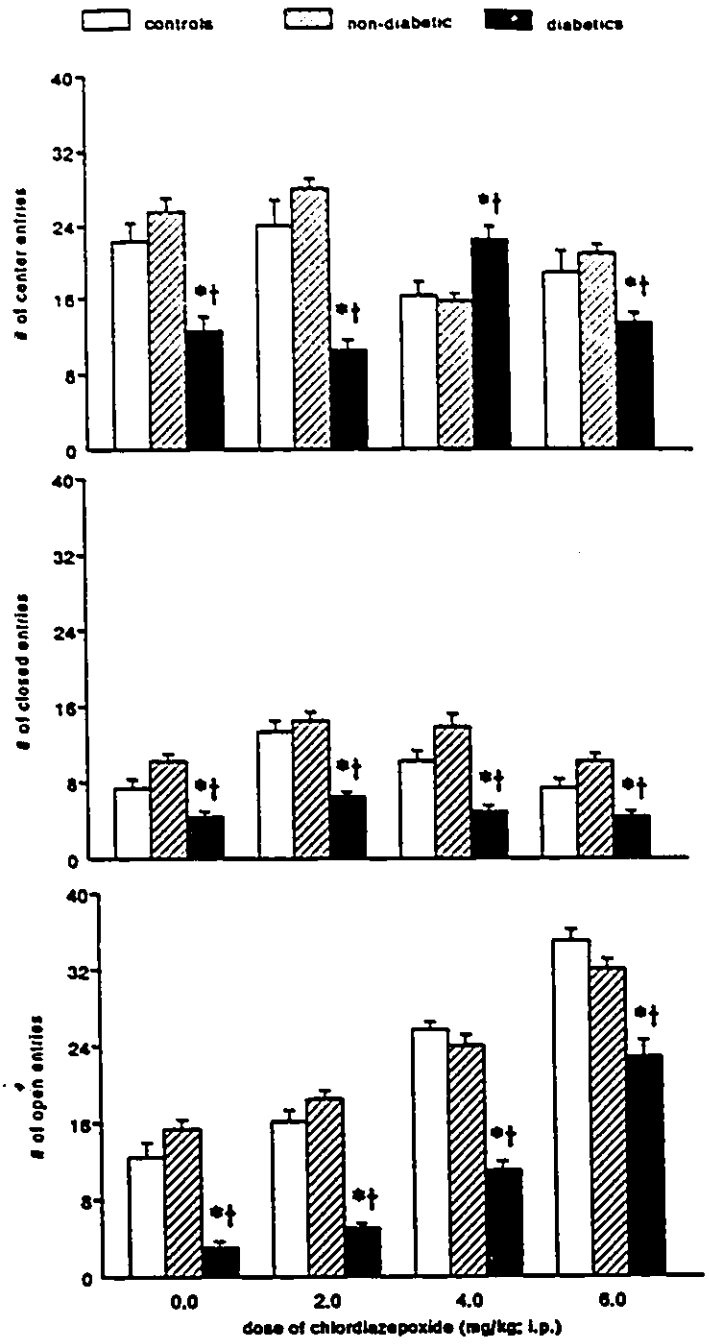

Fig. 9. The frequency of entries into the various sections of the plus maze by the diabetic rat following various doses of chlordiazepoxide. Each column represents the mean ± S.E.M. † Significantly different from the control group value at p<.05. * Significantly different from the non-diabetic group value at p<.05. The top panel shows the frequency of entries into the center portion of the maze, the middle panel the frequency of entries into the closed arms, and the bottom panel the frequency of entries into the open arms.

It is important to note that the behavioral deficits in the response of the SDR do not seem to be the result of a locomotor deficit. This is evidenced by the fact that the magnitude of the locomotor response of the SDR to chlordiazepoxide in the open field was virtually identical to that of the control groups. However, it differed in that the SDR failed to visit the central area of the open field as frequently. Thus, although the SDR sustained a level of activity similar to that of the control groups, it was achieved by activity relegated to the edges of the open field.

The overall trend of the SDR in the elevated plus maze was to display a significantly lower frequency of entries and shorter duration of time on the open arms (the most anxiogenic section of the maze) of the elevated plus maze. It is of interest to note, that the time spent by the SDR on the center section of the maze at 4.0 mg was quite similar in magnitude to the control groups response on the open arms at 6.0 mg. It is possible perhaps, that the milder degree of anxiogenesis produced by the center section of the elevated plus maze combined with the anxiolytic effects of chlordiazepoxide at 4.0 mg, produced a maximal exploratory response by the SDR.

The frequency of entries into the various sections of the elevated plus maze were generally lower in the SDR. This patrolling pattern is indicative of a decreased exploratory response by the SDR. This reduced exploratory response could be due to novelty. If so, results in this experiment in combination

with those of Experiment 4.1 are indicative of a decreased propensity of the SDR to explore when placed in novel conditions.

Converse to the results of this experiment, Hilakivi-Clarke et al, (1990), found that the STZ diabetic mouse (19 days duration of diabetes) untreated with insulin, demonstrated a higher total activity in the elevated plus maze when compared to controls. In light of the SDR's response to the elevated plus maze it is difficult to explain the discrepancy in results. The source(s) of the difference may of course be due to differences in treatment, species, procedure and/or duration of diabetes.

The results of the present experiment, indicate that the SDR demonstrate a greater degree of reactivity to the anxiogenic areas of both the open field and elevated plus maze. Furthermore, the altered anxiolytic effects of chlordiazepoxide may suggest a possible physiological substrate(s) for the observed deficits.

EXPERIMENT 5: The spontaneous behavioral profile of the diabetic rat: effects of duration of diabetes.

Introduction

There exists few studies which have investigated the spontaneous behavioral profile (i.e. behavioral investigations devoid of pharmacological intervention and novelty) of the diabetic rat. Marshall (1978) demonstrated that the non-insulin treated Alloxan-diabetic rat (2-3 weeks duration of diabetes) manifested a lower level of spontaneous locomotor activity. In support of Marshall (1978), Chu et al, (1986) also reported that the non-insulin treated STZ-diabetic rat (2 weeks diabetic) exhibited a lower level of spontaneous motor activity when compared to controls. In both of these studies, the behavioral assessments were undertaken for an acute period of 90 min and during the "lights on" period of the daily light/dark cycle, a time period when the rat has a typically lower level of spontaneous behavior.

In a more comprehensive analysis, Shimomura et al, (1988) observed, continuously over 14 days, decreased spontaneous ambulatory activity during the dark phase of the daily cycle in the non-insulin treated STZ diabetic rat (duration of diabetes 0-2 weeks). Moreover, as the ambulatory activity decreased, there was a concomitant increase in blood glucose. In contrast to the

findings of Marshall (1978) and Chu et al, (1986), no significant alterations in ambulatory activity were observed during the diurnal (lights on) portion of the cycle.

Merali et al, (1988) reported that the insulin treated SDR (2-4 months duration of diabetes) exhibited a significantly attenuated spontaneous nocturnal locomotor and rearing response. The SDR was particularly insensitive to the transitional photoperiodic cues. Similar to Shimomura et al, (1988), Merali et al, (1988) found no significant differences in spontaneous behavior between the diabetic and controls during the diurnal portion of the cycle.

In the present context, the experiments presented to this point were carried out during the diurnal portion of the light cycle (9 AM to 6 PM) and concordant with Merali et al, (1988), there were no significant differences in the baseline spontaneous behavioral activity of the SDR when compared to the control groups. However, since the drug, novelty-stress, and plus maze stimulated behavioral responses differed significantly from the control response, it became of interest to examine whether behavioral differences were apparent during a cyclically higher level of spontaneous activity, such as the nocturnal phase of the daily light cycle. Furthermore, previous investigations had utilized animals with a specific durational time frame of diabetes. Therefore, this study incorporated experiments which used animals with acute (0-2 months), intermediate (4-8 months) and long-term (8-12 months) duration of diabetes.

General Methods

Animals

This experiment involved the use of two groups (n=8 each); a diabetic and a control group. The care of the SDR and the housing conditions for the animals were as described in Experiment 1 (Methods).

Procedure

Animals were acclimatized to the monitoring chambers for a period of 1 week prior to testing and allowed free access to food and water throughout the monitoring period. Behavioral monitoring took place over a period from 1:00 PM to 10:00 AM; however it was concentrated during the dark phase of the light cycle (7:00 PM to 7:00 AM). The animals were assessed on the following behavioral parameters: locomotion, rearing frequency and rearing duration, as described earlier in Experiment 1 (Methods). In this experiment however, the data were collected in 60 min bins, throughout the 20 hr monitoring period.

Results

Experiment 5.1

The spontaneous behavioral profile of the diabetic rat with 0-2 months duration of diabetes.

A two-way ANOVA repeated over the factor time was used to

assess the spontaneous behavior of the two groups for each behavioral parameter. The analyses revealed no significant group differences (SDR vs Control) for locomotion $F(1,15)=2.33$, p=N.S., rearing duration $F(1,15)=0.66$, p=N.S., or rearing frequency $F(1,15)=4.2$, $p<.055$. There was however, a significant effect for time at each behavioral parameter: locomotion, $F(20,280)=31.0$, $p<.0001$; rearing duration, $F(20,280)=20.1$, $p<.0001$; rearing frequency, $F(20,280)=24.0$, $p<.0001$. A significant interaction (Group x Time) was obtained for locomotion $F(20,280)=2.45$, $p<.001$ and rearing frequency $F(20,280)=5.2$, $p<.0001$ but, not for rearing duration, $F(2,280)=2.1$, p=N.S.

As illustrated by Fig. 10 (top panel), both groups maintained similar levels of locomotor activity during the diurnal period. Upon the offset of the lights (i.e. nocturnal period), both groups exhibited a sharp rise in locomotion. In the SDR, this was followed by a sharp drop and gradual decline in activity. The controls also exhibited a decline in activity. This decline however, was followed by a gradual rise in locomotion which peaked at 2:00 am. A sharp rise in locomotion was exhibited by both groups several hours prior to the onset of light and diurnal period. During the diurnal period, both groups manifested comparable levels of locomotion.

Post hoc analyses (Tukey) of the interaction in the case of locomotion revealed that it was largely accounted for by the sharper rise and decline in locomotion by the SDR between

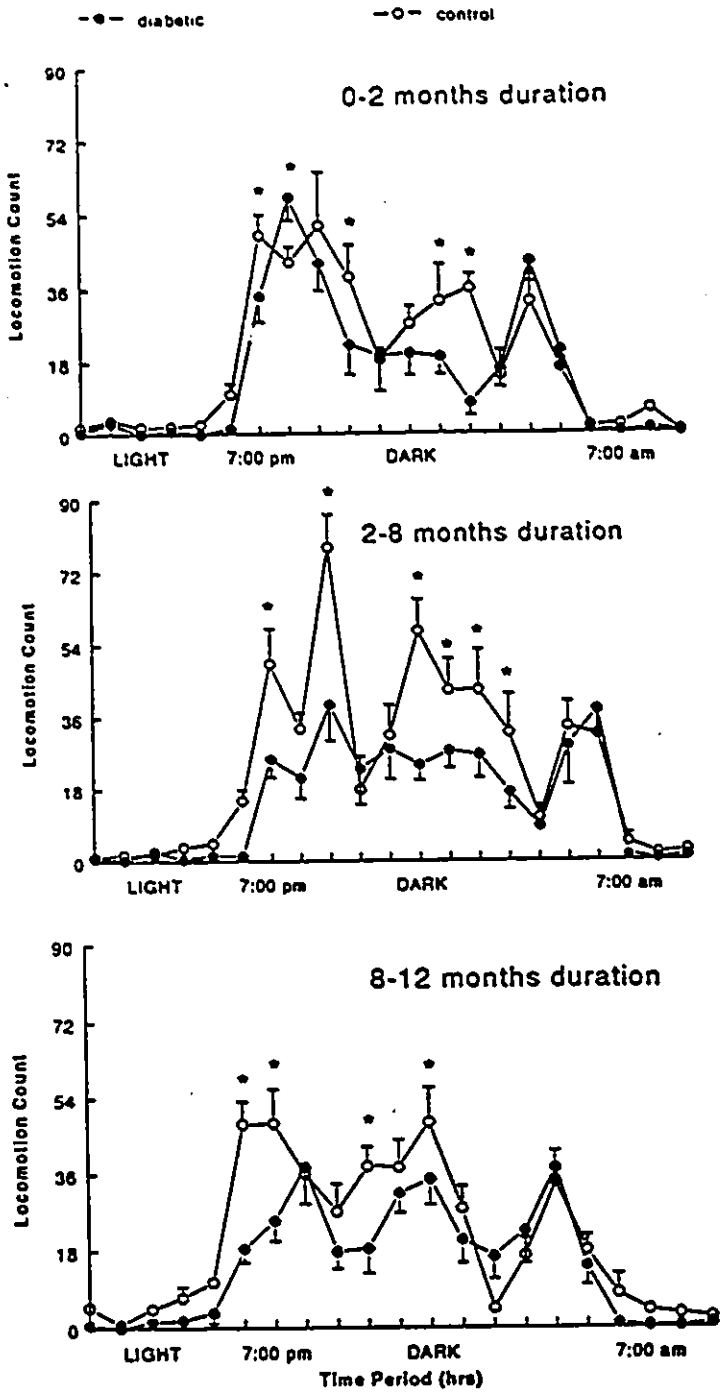

Fig. 10. The spontaneous diurnal and nocturnal locomotor response of the diabetic rat. Each point represents the mean ± S.E.M.
* Significantly different at $p<.05$.

6-10 PM. Also, there was a significantly lower level of locomotion by the SDR between 11 PM and 2 AM.

As illustrated by Fig. 11 (top panel), a qualitatively similar response was obtained for rearing duration. In the case of rearing frequency (see Fig. 12, top panel), both groups exhibited a sharp rise in behavior proximal to the offset of the light. In the control group the increase in behavior was sharper than that of the SDR and occurred right at the offset of the lights. In the SDR, the rise was more gradual and followed by a generally lower period of activity than that exhibited by the controls. The controls appeared to be exhibiting a more bimodal response pattern, whereas the increases in rearing frequency in the SDR appeared to be largely stimulated by the offset and onset of light. This is exemplified by the sudden rise in behavior of the SDR at 6 AM. Overall, the spontaneous behavioral response profile of the SDR is altered when compared to the control profile. Specifically, there are distinct periods during the nocturnal phase where the SDR had generally lower levels of activity than the controls.

Experiment 5.2

The spontaneous behavioral profile of the diabetic rat with 2-8 months duration of diabetes.

Statistical analysis was as described under Experiment 5.1. A significant group effect (SDR vs Control) was obtained for each behavioral parameter: locomotion, $F(1,15)=8.4$, $p<.01$; rearing

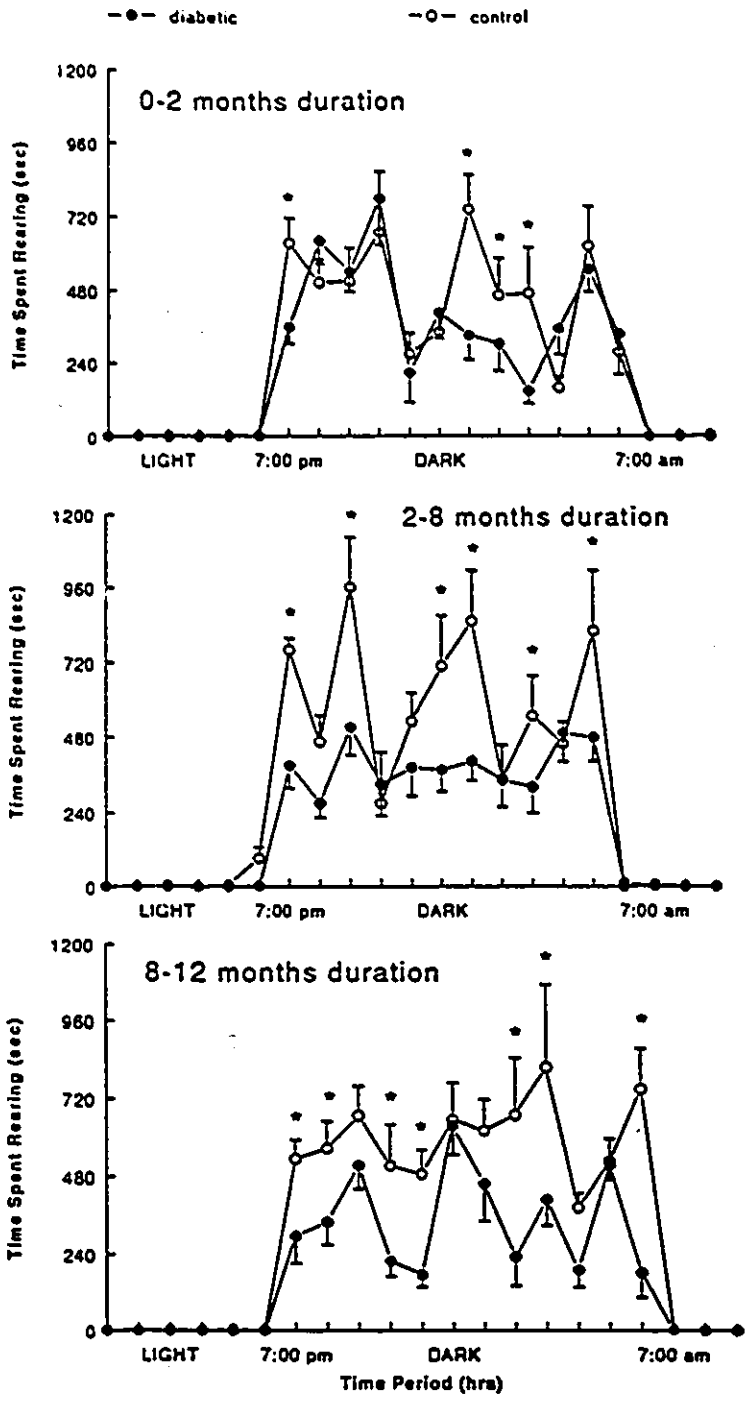

Fig. 11. The spontaneous diurnal and nocturnal rearing duration response of the diabetic rat. Each point represents the mean ± S.E.M. * Significantly different at $p<.05$.

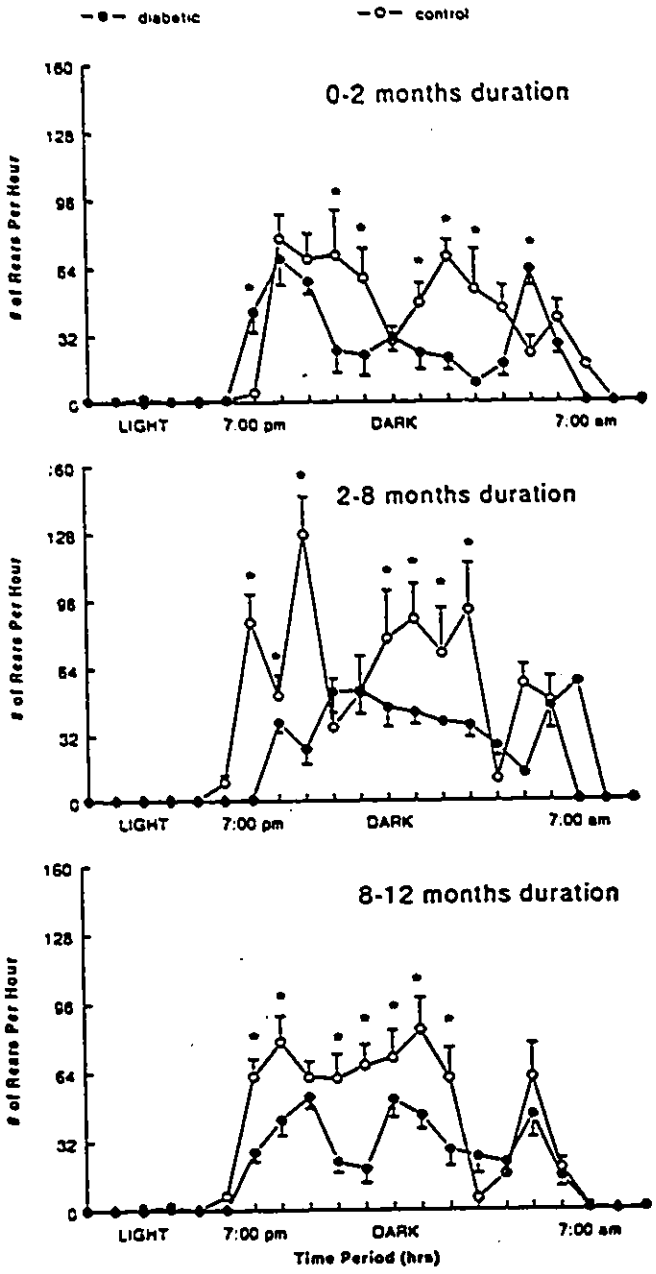

Fig. 12. The spontaneous diurnal and nocturnal rearing frequency response of the diabetic rat. Each point represents the mean ± S.E.M. * Significantly different at $p<.05$.

duration, $F(1,15)=6.3$, $p<.05$; and rearing frequency, $F(1,15)=4.5$, $p<.05$. A significant effect for time was also obtained for each behavioral parameter: locomotion, $F(20,280)=23.4$, $p<.0001$; rearing duration, $F(20,280)=26.9$, $p<.0001$; and rearing frequency, $F(20,280)=24.0$, $p<.0001$. In each case, a significant interaction (Group x Time) was obtained: locomotion, $F(20,280)=2.7$, $p<.001$; rearing duration, $F(20,280)=2.7$, $p<.0001$; and rearing frequency, $F(20,280)=4.4$, $p<.0001$.

As illustrated by Fig. 10 (middle panel), both groups demonstrated fluctuations in locomotor activity over time. Both groups also evidenced sharp rises in activity during the time frame proximal to the offset of light. The magnitude of the locomotor response of the SDR during the first four hours following the offset of light, was significantly attenuated in the SDR. Furthermore, the controls maintained higher levels of locomotion between 12 pm to 3 am. Both groups demonstrated comparable increases in behavior two hours prior to the onset of light. The response of the SDR paralleled that of the controls but in an attenuated profile.

Figure 11 (middle panel), illustrates the time spent rearing by both groups. The response profiles of both groups were qualitatively similar to the locomotor response, the important exception being the significantly attenuated response of the SDR just prior to the onset of light.

Figure 12 (middle panel), depicts the frequency of rearing by both groups. Again, the rearing profile is quite similar to

the locomotor profile. In this case however, it appears that the SDR profile is phase shifted to the right. Characteristic of the SDR locomotion and rearing duration profiles, the rearing frequency profile of the SDR lacks the episodic peaks and valleys evident in the controls.

Overall, it is evident that the spontaneous behavioral profile of the SDR, 2-8 months diabetic, is altered when compared to the control profile. The SDR displayed an attenuated response to the photoperiodic cues, particularly the offset of light and in the case of rearing frequency, the offset and onset of light.

Experiment 5.3

The spontaneous behavioral profile of the diabetic rat with 8-12 months duration of diabetes.

The statistical analysis was as described in Experiment 5.1. A significant group effect (SDR vs Control) was obtained for each behavioral parameter: locomotion $F(1,15)=16.9$, $p<.01$; rearing duration $F(1,15)=7.7$, $p<.01$; and rearing frequency $F(1,15)=14.6$, $p<.01$. A significant effect for time was also obtained for each behavioral parameter: locomotion, $F(20,280)=23.2$, $p<.0001$; rearing duration, $F(20,280)=24.6$, $p<.0001$; and rearing frequency, $F(20,280)=29.7$, $p<.0001$. In each case, there was a significant interaction of group x time: locomotion, $F(20,280)=2.9$, $p<.001$; rearing duration $F(20,280)=2.5$, $p<.001$; and rearing frequency, $F(20,280)=4.0$, $p<.0001$.

Figure 10 (bottom panel), depicts the spontaneous locomotor response of the SDR and controls. During the diurnal period, there were no significant group differences. However, during the time periods just prior to and immediately following the offset of the lights there was a sharp rise in locomotion by the controls. The SDR also demonstrated an increase in locomotion, but the rise was more gradual and peaked at 8 PM, 1 hour later than the control peak response. Between 10 PM and 1 AM, the controls maintained a higher level of locomotion than the SDR. It is interesting that the SDR response although attenuated, paralleled the control response profile. Both groups exhibited a sharp augmentation in locomotion at 4 AM, prior to the onset of light. Following the onset of light (diurnal period), both groups manifested comparable levels of diminished locomotion.

As illustrated by Fig. 11 (bottom panel), the controls in general, spent more time rearing than the SDR. The SDR response profile for the most part was parallel to that of the controls, but at a significantly attenuated level. Also, peaks and valleys in the SDR profile were more severe.

Figure 12 (bottom panel), depicts the rearing frequency response of the SDR and controls. Throughout the majority of the dark (nocturnal) period, the controls manifested a significantly higher frequency of rearing than that of the SDR. It is interesting that the rearing frequency response profile of the SDR is qualitatively similar to the SDR locomotor response profile. As was the case in locomotion, the rise in rearing

frequency by the SDR was more gradual than the control response, following the offset of light. Both groups manifested an augmentation in the frequency of behavior in the hour (6 AM) just prior to the onset of light. During the diurnal phase, both groups exhibited similar levels in the frequency of rearing.

In summary, the spontaneous behavioral response of the SDR is different from the control profile in several respects. In general, the SDR manifested a lower level of spontaneous activity than the controls. Also, the SDR response to the onset of dark was often more sluggish than the control response. The SDR response to the onset of light was near normal (i.e. sharp rise), but generally lower in amplitude than the control response. Overall, the SDR 8-12 months diabetic has inherent to it an altered spontaneous behavioral profile as compared to the controls.

Waveform Analysis

Behavioral components associated with circadian rhythm can be described according to the following parameters; period, phase and amplitude (Goodless-Sanchez et al, 1991). Period refers to length of time over which the waveform occurs (i.e. min, hrs etc). Phase refers to a particular position in the cycle. This is usually measured from a reference point such as the onset or offset of light. Amplitude describes the magnitude of the rhythm, such as the frequency of a behavior. A qualitative comparison of SDR locomotor behavior waveforms across time frames revealed that

the SDR 0-2 months diabetic, exhibited a generally higher amplitude of behavior at the offset and onset of lights, as compared to the response at 2-8 and 8-12 months duration of diabetes (see Fig. 10). The SDR 2-8 and 8-12 month waveforms were essentially similar in amplitude. However, there was a phase shift to the right in the peak response proximal to the offset and onset of light, in the 2-8 month SDR locomotor waveform. The 0-2 and 8-12 month SDR locomotor waveforms appear to be in phase, however, the peak response proximal to the offset of light is higher in amplitude at 0-2 months. The control locomotor response when compared across time frames, revealed that at 2-8 months the peak response following the offset of lights was higher in amplitude and shifted to the right when compared to the 0-2 and 8-12 months waveforms. The peak response proximal to the onset of lights, at 2-8 months was also shifted to the right, when compared to the 0-2 and 8-12 month responses. When examining the 0-2 and 8-12 month waveforms, the only period where the curves were in phase was during the time period proximal to the onset of light. Morphologically the 0-2 and 8-12 month control locomotor waveforms had different peak periods.

A similar comparison of spontaneous rearing duration (see Fig. 11) revealed that the control waveforms at 0-2 and 2-8 months are morphologically similar in terms of "peak and valley" periods. The 2-8 month waveform was higher in amplitude at the peak periods proximal to the offset and onset of light than the 0-2 month response. This was particularly evident at 9 PM. A

comparison of the SDR 0-2 and 2-8 month waveforms revealed that the peak period following the offset of the lights was higher in amplitude and longer in periodicity at 0-2 months.

A comparison of the control 2-8 and 8-12 month waveforms for rearing duration revealed that there was a higher amplitude (between 6 to 9 PM) during the peak period proximal to the offset of the lights at 2-8 months. Also, there was a shift to the left in the peak response proximal to the onset of lights at 2-8 months as compared to 8-12 months. A similar comparison of the SDR response, indicated that the 8-12 month waveform had sharper "peaks and valleys" when compared to the 2-8 month SDR rearing duration waveform. The amplitudes of the peak responses which occurred at the offset and onset of the lights were essentially similar at both time frames.

A comparison of the control rearing duration waveforms at 0-2 and 8-12 months revealed that the 0-2 month peak response at the offset of the lights was shifted to the right when compared to the 8-12 month response. In contrast, there was a shift to the right in the 8-12 month peak response proximal to the onset of the lights. Overall, "peak and valley" periods of the waveforms were different morphologically. In the SDR, there was a higher amplitude and longer periodicity of the 0-2 month peak response proximal to the offset of the lights. The peak periods at the onset of the lights were in phase. As was the case with the control waveforms, the SDR waveforms were morphologically different.

A qualitative comparison of the spontaneous rearing frequency (see Fig. 12) responses at 0-2 and 2-8 months duration of diabetes revealed that the 2-8 month peak period proximal to the offset of the lights, occurred earlier than the 0-2 month response. There was also a longer periodicity of the 2-8 month peak response proximal to the onset of the lights. The SDR 0-2 month peak response proximal to the offset of lights was both higher in amplitude and longer in periodicity than at 2-8 months. In general, there was a lower amplitude in the 0-2 month waveform as compared to the 2-8 month response. The 2-8 month peak response at the onset of the lights was shifted to the right but, similar in amplitude when compared to the 0-2 month response.

A comparison of the 2-8 and 8-12 month control rearing frequency response revealed that the peak response proximal to the offset of the lights was higher in amplitude than the 8-12 response. The peak response in the hours proximal to the onset of the lights, were in phase but, there was a longer periodicity (1 hr) at the 2-8 month time frame. In the SDR, the peak 8-12 month response at the offset of the lights was more gradual and phase shifted when compared to the 2-8 month response. The peak 2-8 month response at the onset of lights was shifted to the right and longer in duration when compared to the 8-12 month response.

Finally, a comparison of the control rearing frequency waveforms at 0-2 and 8-12 months revealed that the 8-12 month peak period proximal to the offset of lights had an earlier onset but was similar in amplitude to the 0-2 month response waveform.

The 0-2 month peak period proximal to the onset of lights was shifted to the right and also lower in amplitude than the 8-12 month response. In general, the waveforms were dissimilar, with the 0-2 month waveform being more bimodal in shape. The SDR 8-12 month peak period at the offset of lights was shifted to the right, more gradual and lower in amplitude than the 0-2 month SDR response. The peak response at the onset of lights was similar in phase but, the 0-2 response was higher in amplitude.

Overall, there were waveform alterations evident within each group, from time frame to time frame. Moreover, there were alterations in phase and periodicity within each group from time frame to time frame. Despite the changes in the SDR waveform across time frames, the SDR response remained consistently altered in comparison to the control waveforms.

Summary

This study examined the spontaneous behavioral profile of the insulin treated male SDR at acute (0-2 months), intermediate (2-8 months), and long-term (8-12 months) time frames relative to the onset of the overt diabetic syndrome. In essence, it was found that the SDR demonstrated an altered spontaneous nocturnal locomotor and rearing behavioral profile as compared to matched controls. Specifically, the SDR exhibited attenuated levels of spontaneous nocturnal activity and depending on the time frame and behavior examined, a diminished response to photoperiodic

cues such as, the offset and onset of light. Furthermore, the altered behavioral profile of the SDR is chronic, as it endured to different extents from a period up to twelve months following the onset of the diabetic syndrome. The altered profile of the SDR also endured despite insulin treatment.

The results of the present study are in agreement with those of Merali et al, (1988). However, in the present context, it was demonstrated that the findings of Merali et al, (1988), can be extended to time periods both proximal (0-2 months) and distal (8-12 months) to the onset of the overt diabetic syndrome.

Shimomura et al, (1988) investigated the spontaneous behavioral response of the non-insulin treated STZ diabetic rat. However, in the Shimomura study, there were some distinct methodological differences from the present study. First, their animals were not treated with insulin. Second, their measurements were undertaken during a specific duration of diabetes (26 days). Also, their measure of activity was more general, as they employed a tilting floor, which upon movement activated a counter. Despite these differences, it is interesting that in general, their findings corroborate those presented here. In essence, Shimomura et al, (1988), found an attenuated nocturnal ambluatory reponse in the diabetics as compared to controls.

Shimomura et al, (1988) also reported that the post-mortem striatal turnover rate of DA (as measured by the DA to DOPAC ratio) was significantly lower in the diabetic rats. They also reported a negative correlation between striatal DA turnover rate

and blood glucose levels. In essence, as blood glucose levels rose, there was a concomitant decrease in DA turnover in the diabetic rats. These researchers speculated that the alterations in striatal DA turnover may be influencing the decrease in nocturnal ambulatory behavior in the diabetic rats. A similar inverse relationship was also reported between ambulatory activity and blood glucose levels in the diabetics.

In the Shimomura study, it is evident that blood glucose level is an important factor in the diabetic neurochemical and behavioral response. In the present study, the diabetic animals were kept normoglycemic with protamine zinc insulin (P.Z.I., 100 I.U. concentration) which was administered at 4:00 PM daily. The action of P.Z.I. is known to last 12-16 hrs in humans. Glycemic (glucosuria) levels were only measured immediately prior to and post experimental session. These levels were found to be normoglycemic in both the SDR and controls. Urine glucose levels were not taken during the experimental period in order to avoid confounds (i.e. stress from handling) in behavioral measurement. However, in a preliminary study (Ahmad, personal observations), urine glucose measures were taken at several nocturnal time points, from both groups and found to be slightly elevated as compared to diurnal levels, but still in the normoglycemic range. The finding that the attenuated nocturnal response of the diabetic rat occurs regardless of insulin therapy, would seem to indicate the possibility that other nonglycemic related factors may be implicated.

Given the multitude of hormonal and neurochemical alterations which occur nocturnally and with diabetes, one can only speculate on the altered mechanisms at play in the SDR. Given the findings of reduced DA turnover by Shimomura et al, (1988) coupled with other studies indicating alterations in the diabetic CNS DA system(s) (Lozovsky et al, 1981; Trulson & Himmel, 1983; Saller, 1984; Kwok et al, 1986; Merali et al, 1988; Bellush & Reid, 1991) the DA system(s) may be one possible source of the observed behavioral deficits in the SDR. Indeed, the CNS mesolimbic and striatal DA systems have been implicated in the mediation of nocturnal locomotor behavior (Bruinink et al, 1983; Cools, 1986).

A number of factors however, need to be addressed before conclusions can be made regarding the source(s) of the altered spontaneous behavior in the SDR. A neurochemical assessment of both the insulin treated and insulin deprived SDR must be achieved to identify if CNS alterations are evident. Second, a more pharmacodynamic measurement (i.e. *in vivo* microdialysis, *in vivo* voltammetry) of neurochemistry and behavior must be undertaken as post-mortem measures are static and may not reveal fluctuations in these factors over the circadian cycle.

EXPERIMENT 6: Post-mortem neurochemical assessment of brain catecholamine and metabolite levels in the diabetic rat.

Introduction

As reviewed in the General Introduction, there are numerous studies in the literature reporting alterations in the status of CNS biogenic amines in the pancreotoxin-based diabetic rat (for review, Rowland & Bellush, 1989; McCall, 1991). With regards to the SDR, Kwok and Juorio (1985) investigated the status of CNS DA, 5-HT, and their metabolites. It was found that in the acutely diabetic (11-23 days duration of diabetes) SDR, untreated with insulin, there was a significant reduction in the concentration of DOPAC in the striatum. The concentrations of DOPAC, HVA, and 5-HIAA were also significantly reduced in the olfactory tubercle of these animals. No significant alterations in the levels of DA and 5-HT were reported. In deference to this study, it would appear that the SDR has associated with it specific alterations in CNS neurotransmission. Further, the authors concluded that there was a marked reduction in striatal and mesolimbic DA and mesolimbic 5-HT metabolism. They suggested that the reduction in metabolism could be a consequence of a reduction in the formation of DA and 5-HT. Their findings in conjunction with the behavioral alterations observed in Experiments 1-5 may be indicative of

alterations in the CNS neurotransmission of the SDR. Thus, it was of interest to assess the neurochemical status of the SDR.

Atlthough revealing, the study by Kwok and Juorio (1985), investigated the acute untreated diabetic condition in the SDR. In the present study, we investigated the response of the SDR with a 2-8 month duration of diabetes. In addition, we assessed the CNS neurochemical status of the SDR following acute insulin withdrawal and a pharmacological challenge with amfonelic acid.

Experiment 6.1

The neurochemical status of the insulin treated diabetic rat.

Animals

This experiment consisted of three groups: an SDR group, a Non-diabetic group (NDR) and a control group (n=10, each). Animals housing and environmental conditions were as described as Experiment 1 (Methods). Maintenance of the diabetic animals was as described in Experiment 1 (Methods).

Procedure

All rats were food deprived for 12 hr prior to sacrifice. Immediately after decapitation, the brain was rapidly removed and dissected into the following regions: cortex, striatum, hypothalamus, hippocampus, pons-medulla, midbrain, and olfactory bulbs (Glowinski and Iversen, 1966). Each region was quickly

weighed and deposited in 1.0 ml of 0.1 M $HClO_4$ containing 40 μM sodium meta-bisulfite, sonicated and centrifuged for 3 minutes. The supernatant was then stored at -70°C for neurochemical analysis. Immediately following decapitation, trunk blood samples were collected in heparinized tubes which were promptly centrifuged for 10 min, and analyzed for glucose content using a Beckman Glucose Analyzer II.

Neurochemical Analysis

Levels of dopamine (DA), norepinephrine (NE), epinephrine (EPI), homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxytyramine (3-MT), and 3-methoxy-4-hydroxyphenyl-glycol (MHPG) were separated and quantitated simultaneously using high performance liquid chromatography with electrochemical detection (HPLC-ED) (for a representative chromatogram of a standard run, see Appendix I, top panel). The method involved the use of a Waters M-6000A pump, WISP or Rheodyne manual injector, guard column, radial compression column (5 μm, C_{18} reverse phase, 8 mm x 10 cm), a three cell coulometric-amperometric electrochemical detector (ESA model 5100A) and an Hewlett Packard integrator. The mobile phase used for the separation was a modification of that used by Chuieh et al (1983). Each liter consisted of the following: 1.3 g of heptane sulfonic acid, 0.1 g of dissodium EDTA, 4.5 ml triethylamine, 60 ml acetonitrile, pH was adjusted to 2.5 with phosphoric acid. The conditioning cell was positioned first in the series of the three

electrodes and used to oxidize at +400 mV. The next cell was set at -100 mV and the final high-sensitivity cell was set at -380 mV (gain 8000). The last analytical cell was hooked up to an integrator and calibrated with an external standard.

Results

A statistical comparison of blood glucose content between the three groups revealed no significant group differences (see Table 7). A separate statistical analysis (one-way ANOVA) of each region and its brain catecholamine and metabolite content in the insulin treated SDR and control groups, revealed no significant differences (data not shown). It can therefore be concluded in the context of this study, that brain catecholamine and metabolite levels remain largely unchanged in the diabetic rats maintained on insulin replacement therapy.

Experiment 6.2

The effects of cessation of insulin treatment on the neurochemical status of the diabetic rat.

Animals

Since the above experiment revealed no significant differences between the NDR and the controls, this experiment focused on two groups (n=10 each): 1) the SDR, and 2) the NDR group.

TABLE 7

Plasma glucose levels following insulin treatment and cessation.

Group	Glucose (mg/dl)
Genetically distinct controls	109 ± 16
Non-diabetic controls	106 ± 6
SDR insulin treated	123 ± 5
SDR insulin deprived	357 ± 24*

Each value represents the mean ± S.E.M.
* Significantly different from all groups (Tukey, $p<.05$).

Procedure

The SDR was deprived of insulin replacement for 4 days prior to sacrifice. Neurochemical and blood glucose analysis was achieved through the method outlined in Experiment 6.1.

Results

The SDR under insulin therapy displayed near normal blood glucose levels. The cessation of insulin therapy however, resulted in a marked elevation of blood glucose content as compared to the Non-diabetic and the genetically distinct control group (see Table 7).

As depicted in Table 8, insulin deprivation in the SDR resulted in discrete region specific changes in brain catecholamine metabolism. Statistical analysis (one-way ANOVA) of regional brain catecholamine and metabolite content revealed that the diabetic rats withdrawn from insulin for 4 days, displayed the following neurochemical changes: significantly increased NE at the hypothalamus $F(1,18)=11.53$, $p<.001$ and the cortex $F(1,18)=8.45$, $p<.01$; increased DA levels at the hippocampus $F(1,16)=6.03$, $p<.05$; increased HVA at the striatum $F(1,18)=8.67$.

TABLE 8

Effect of cessation of insulin treatment on the regional catecholamine and metabolite levels in the Non-diabetic (NDR) and Spontaneously Diabetic Rat (SDR)

Tissue	Group	NE	EPI	DA	DOPAC	HVA
HYPOTHALAMUS	NDR	1745 ±55	123 ±6	248 ±20	82 ±5	354 ±20
	SDR	2110* ±91	120 ±5	269 ±20	86 ±7	333 ±16
HIPPOCAMPUS	NDR	441 ±45	ND	24 ±2	42 ±3	90 ±3
	SDR	334 ±37	ND	37* ±5	44 ±5	74 ±6
STRIATUM	NDR	161 ±15	92 ±7	5607 ±338	1260 ±62	662 ±29
	SDR	170 ±6	103 ±8	5465 ±214	1430 ±130	878* ±67
MIDBRAIN	NDR	416 ±21	19 ±5	119 ±9	46 ±4	106 ±9
	SDR	437 ±17	22 ±9	125 ±9	37 ±3	119 ±13
OLFACTORY BULB	NDR	282 ±25	28 ±3	70 ±4	38 ±2	164 ±14
	SDR	312 17±	23 ±2	68 ±6	31 ±2	171 ±11
PONS-MEDULLA	NDR	608 ±48	33 ±4	49 ±6	27 ±2	100 ±17
	SDR	503 ±20	31 ±2	44 ±5	27 ±3	98 ±4
CORTEX	NDR	196 ±7	37 ±4	28 ±6	16 ±2	32 ±4
	SDR	241* ±14	31 ±3	53 ±15	19 ±4	44 ±3

The values are means ± S.E.M. (ng/g). * significantly different from Non-diabetic group (Tukey, $p<.05$). ND denotes not detected.

Experiment 6.3

The effects of amfonelic acid on the neurochemical status of the diabetic rat.

Given the behavioral results observed following d-amphetamine and amfonelic acid in Experiments 1 and 2, it was of interest to examine the response of the SDR to a pharmacological challenge.

Animals

In this exploratory experiment, we investigated the neurochemical response of the SDR (5-6 months duration of diabetes) and NDR (n=6 each) to amfonelic acid (1.0 mg/kg;i.p.). Housing and environmental conditions were as described in Experiment 1.

Procedure

The SDR was maintained as described in Experiment 1. All rats were food deprived for 12-15 hr prior to sacrifice. Amfonelic acid was administered in a sequential manner, at 30 min intervals, prior to sacrifice. Neurochemical analysis was as described in Experiment 6.1.

Results

In consideration of the behavioral differences obtained with amfonelic acid we examined the neurochemical response of the SDR and NDR to 1.0 mg/kg of amfonelic acid. A statistical comparison

of trunk blood glucose content between the two groups revealed no significant differences $F(1,10)=0.9$, N.S. Analysis of regional brain catecholamine and metabolite content using a one-way ANOVA revealed the following differences in the SDR as compared to the NDR (see Table 9): a significant reduction in DA in the striatum $F(1,10)=4,5$, $p<.05$; hypothalamus $F(1,10)=8.0$, $p<.05$; olfactory bulbs $F(1,10)=6.9$, $p<.05$; midbrain $F(1,10)5.4$, $p<.05$. A significant increase in striatal NE content was observed in the SDR as compared to the NDR $F(1,10)=4.8$, $p<.05$. The DA metabolite levels were not significantly different between the groups tested. In the SDR, there was a trend towards lower levels of the metabolites, DOPAC, HVA and 3-MT in the striatum.

Summary

The results from Experiment 6.1 demonstrated that when the SDR is maintained in a normoglycemic state, its levels of biogenic amines and metabolites are comparable to those of their non-diabetic littermates and genetically distinct controls. In general, this finding is in agreement with neurochemical studies in the STZ and alloxan diabetic rat treated with insulin. In this regard, it has been demonstrated that alterations in the levels of biogenic amines and metabolites in the CNS of the non-insulin treated chemically diabetic rats can be 'normalized' with insulin treatment (Trulson & Himmel, 1983; Kazmi & Baquer, 1985; Kwok & Juorio, 1986). An exception to these findings

TABLE 9

The effects of 1.0 mg/kg of amfonelic acid on the regional levels of dopamine in the Non-diabetic (NDR) and Spontaneously Diabetic Rat (SDR).

GROUP	HYPOTHALAMUS	STRIATUM	OLFACTORY BULB	MIDBRAIN
NDR	318.6 ±41	5915 ±748	74.8 ±13	147 ±13
SDR	185.8* ±22	4108* ±408	34.9* ±7	87* ±11

The values are means ± S.E.M. ng/g wet weight. * significantly different from Non-diabetic group (Tukey, $p<.05$).

was reported by Bellush and Reid (1991) and Oliver et al, (1989). Bellush and Reid (1991) found that the DA turnover (as indicated by DOPAC/DA ratio) was reduced in the insulin treated STZ rat in several brain regions. Oliver et al, (1989) found an increase in DA turnover in the ventromedial nucleus of the hypothalamus of the insulin treated STZ diabetic rat. In these studies alterations in CNS DA neurotransmission endured despite insulin treatment. In the present study, no significant alterations were found in DA neurotransmission. The discrepant findings between this study and that of Bellush and Reid (1991) and Oliver et al, (1989) may be due to the inherent differences between the animals, regimen and techniques employed.

In Experiment 6.2, it was found that the cessation of insulin treatment for 4 consecutive days resulted in region specific increases in the levels of: NE in the hypothalamus and cortex; DA in the hippocampus and HVA in the striatum. In this context, Bellush and Reid (1991) and Oliver et al, (1986) reported that the acute withdrawal (48-72 hrs duration) of insulin in the STZ diabetic rat resulted in altered monoamine turnover. Specifically, following insulin withdrawal, Bellush and Reid (1991) reported a significant reduction in 5-HT turnover in the frontal cortex and Oliver et al, reported a decrease in DA turnover in the medial preoptic nucleus of the hypothalamus. Although their results were not similar to those reported here, in general, it appears that insulin cessation can affect CNS monoamine neurotransmission. The source of the differences

between the aforementioned studies and the present, may be multifold. In this study, rats with a longer duration of diabetes were employed and thus, insulin treatment occurred over a longer duration of time. The effects of chronic insulin replacement on CNS neurotransmission cannot be ruled out. The existance of insulin receptors in the CNS (Unger et al, 1991) and the fact that peripherally administered insulin can affect CNS neurotransmission (Lozovsky et al, 1985; McCaleb & Myers, 1979) may have influenced the present results in an as yet undetermined mechanism(s). Differences may have also been due to genetically versus chemically induced diabetes. Overall, there appear to be no specifically consistent trends in the few studies available investigating insulin withdrawal and brain monoamine status.

In Experiment 6.1 the basal levels of biogenic amines were found to be normal in the SDR. Experiment 6.2 demonstrated that insulin cessation resulted in an altered neurochemical response in the SDR. In Experiment 6.3 it became of interest to examine the neurochemical response of the SDR following the pharmacological challenge of a single dose of amfonelic acid. It was found that there was a greater depletion in the post-mortem levels of DA in the striatum, hypothalamus, olfactory bulb, and midbrain of the SDR. Also, a significant increase in striatal NE content of the SDR was obtained. In the striatum there was a tendency towards increased levels of DOPAC in the SDR as compared to the NDR. There were however, no statistically significant differences in metabolite levels between the two groups. It is

important to note that the SDR were receiving insulin to the day they were sacrificed. Insulin is reported to accelerate the turnover of brain monoamines in normal and diabetic rats (Kwok and Juorio, 1988; Kwok and Juorio, 1987; Sauter et al, 1983). The most dramatic impact of insulin on CNS monoamines typically occurs in the hypoglycemic rat (Lozovsky et al, 1985). The SDR used in this study were maintained in a normoglycemic state. An alternative explanation to the observed alterations is that the diabetic rat may not be able to sustain high levels of DA release (Merali et al, 1988; Rowland & Bellush, 1989). It is possible that the reduced levels of DA may be the result of a reduced biosynthetic capacity in the SDR. Thus, the lower levels of DA following amfonelic acid administration may in part account for the reduced availability of DA and the behavioral response of the SDR to amfonelic acid, observed in Experiment 2. The alterations observed in striatal NE may suggest that the deficit extends to this receptor system as well. Indeed there have been reports of alterations in NE levels in the diabetic rat (Rowland & Bellush, 1989; Trulson & Himmel, 1985). The precise mechanisms and underlying substrates need further investigation.

In summary, it appears that under basal conditions the SDR treated with insulin is able to maintain normal levels of biogenic amines. However, when insulin treatment is terminated, region specific alterations in CNS biogenic amines are observed. Moreover, the pharmacological challenge of amfonelic acid resulted in a significantly greater depletion of DA in the SDR.

Although the results of Experiment 6 do suggest alterations in CNS neurotransmission of the SDR under certain conditions, total regional levels of transmitters and/or metabolites may not always reflect altered dynamics of transmitter availability at the synaptic level. Nevertheless, this approach represents an important first step in understanding altered neurotransmission in the SDR. A comprehensive assessment of intrasynaptic transmitter release requires the use of more dynamic techniques such as *in vivo* microdialysis.

EXPERIMENT 7: The concomitant characterization of dopamine release in the ventral striatum and spontaneous nocturnal locomotion using *in vivo* microdialysis.

Introduction

Given the post-mortem neurochemical alterations found in the diabetic rat (either pancreotoxin based or genetic), it is surprising that neither direct in vivo measurements nor the effects of central drug administration have been explored. In this regard, direct access to the CNS (avoiding the blood brain barrier) may allow for a more comprehensive comparison of specific CNS structures, neurochemistry and behavior in the diabetic rat.

To date the majority of studies on the CNS of the diabetic rat have used post-mortem measures of neurochemical levels and metabolism. Although, this approach has provided important information on the neurochemical profile of the diabetic rat, it has inherent limitations. First and foremost, it is difficult to interpret changes in tissue neurotransmitter levels and how they related to synaptic availability. Second, behavioral and biochemical functions are dynamic and multiphasic, while conversely, post-mortem measures are static. Thus, post-mortem measures may not reflect dynamic alterations in brain neurochemistry. Given this limitation, potentially important contributions of specific neuronal systems to the behavioral

functioning of the diabetic rat may not be detected.

In the last decade, the *in vivo* microdialysis technique has been introduced widely and used to circumvent the aforementioned limitations and add to the findings of post-mortem investigations (Ungerstedt, 1991). Microdialysis is in principle, a unique technique which enables the collection of virtually any substance from remote brain regions, with a limited amount of tissue trauma (Beneviste & Hanson, 1991). The basic principle underlying microdialysis, involves the positioning of a dialysis membrane, that allows free diffusion of water and small solutes between the solution of interest (brain interstitial space) and a solution lacking the substances concerned (Ringers solution). Continuous perfusion via a pump, through an inlet tube, creates a concentration gradient which causes a diffusion of substances from the interstitial space into the dialysis membrane. The continuous flow through the dialysis membrane carries the substances from the sampled site, via an outlet tube, to a collection vial, where the constituent elements of the dialysate can be analyzed by HPLC-ED (see Appendix I). The use of microdialysis is still a relatively new technique and is constantly undergoing reassessment (Beneviste & Hanson, 1991). However, it has been shown to yield reliable and consistent results when used within its limitations (Ungerstedt, 1991). The use of microdialysis in the investigation of the CNS of the SDR, could contribute to the clarification of some as yet, unanswered questions.

Given the attenuated behavioral response of the SDR to the DA agonists d-amphetamine and amfonelic acid, and also to spontaneous nocturnal locomotion, it was of interest to examine whether this was a consequence of diminished release or synaptic availability of DA. Thus, in the present experiment, the diurnal and nocturnal interstitial DA levels were measured using microdialysis, for 13 consecutive hours, from an initial target, the nucleus accumbens. The majority of the sampling occurred during the nocturnal phase. Concomitantly, spontaneous locomotor activity was also assessed in order to determine the behavioral status of the SDR during the sampling of the dialysate.

The ventral striatum was chosen as the sampling site as it is a structure known to be rich in DA terminals and is implicated in the mediation of locomotor behavior (Pijnenburg et al, 1973; Rajne & Ungerstedt, 1977; Mogenson et al, 1980; Robinson & Camp, 1990). The ventral striatum is also known to be connected to a variety of limbic structures believed to be involved in the initiation of movements in response to motivational and emotional stimuli (Heimer et al, 1982). Finally, the ventral striatum is also an important site of action for DA stimulant drugs such as d-amphetamine (Robinson & Camp, 1990).

Methods and Materials

Animals

This experiment involved two groups, n=6 each: 1) SDR weighing 400-450 g, with a 4-5 months duration of diabetes;

2) genetically distinct Wistar control rats weighing 400-450 g, matched in age to the SDR group. The animals were maintained under a reverse 12 hr red light (9:00 am to 9:00 pm), 12 hr white light cycle. Housing conditions were otherwise as described in Experiment 1, (Methods).

Maintenance of the Diabetic Animals

In this experiment, the SDR were implanted subcutaneously (upper abdominal region) with insulin pellets (courtesy of Dr. P.Y. Wang, University of Toronto). The pellets are made by high pressure compression of a powder admixture of insulin and re-crystallized palmitic acid. The pellet dimensions are 7 mm in length and 2 mm in diameter. The action of the implant occurs through slow surface erosion *in vivo*, which causes the gradual release of the entrapped insulin. The implant is designed to release a set basal dose (2.0 I.U.) of insulin continuously each hour of the day. The action of the insulin pellet can be detected in less than 1 hr following implantation. The duration of the implant has been charted to last approximately 45 days. The advantage of the insulin pellet is that it reduces the handling and injection stress associated with daily systemic insulin administration. More importantly, insulin is maintained at a constant and sustained level in the body with minimal fluctuations which can occur with the injectable insulin. Monitoring of urine glucose and body weight was as described under Experiment 1, (Methods).

Surgery

Each animal was anesthetized with sodium pentobarbitol (Somnatol©, 60 mg/kg), placed in a stereotaxic device, and implanted unilaterally with a custom stainless steel guide cannula (Kinetrode, Canada) at the following co-ordinates: anterior-posterior, 1.8 mm; lateral 1.3 mm; dorsal-ventral, 5.5 mm relative to bregma with a flat skull (Paxinos & Watson, 1982). The cannula was held in place with jewellers screws and dental acrylic. The patency of the cannula was maintained with a stainless steel stylet. All animals were allowed to recover for 5 days prior to experimentation.

Probe Construction

The probe design selected was vertical and concentric in shape with a total length of 24.5 mm (see Appendix II). Probe construction and assembly was custom designed by Z.M. and subsequently constructed and modified by Q.A. The probe inlet tubing consisted of PE 20 tubing and the outlet was made of fused silica (0.25 mm I.D. x 0.76 mm O.D.). The cannula housing for the probe was a 25 g stainless steel tube crimped to a 20 g stainless steel outer sleeve. The inner stainless steel tubing had a total length of 24.5 mm, the outer sleeve was cut to a length of 13 mm and crimped 1 mm from the top of the inner tubing. The silica outflow was fed through the PE 20 via a tiny hole made 3 cm from the top of the PE 20. The silica tube was fed through the PE 20

until it protruded from the opposite end. The juncture through which the silica was fed was sealed with 2-ton epoxy and then reinforced with a web of 5 min epoxy. Twenty-four hours later, the silica/PE 20 assembly was fed through and attached to the stainless steel cannula. The junction between the cannula and the PE 20 was sealed with 2-ton epoxy. Twenty-four hours later, the exposed silica tip protruding from the base of the cannula, was cut to a length of 2.5 mm. Under a stereoscopic microscope, the dialysis tip was placed over the silica tip, until the silica tip was flush with a 2-ton epoxy plug which sealed the open end of the dialysis membrane. The membrane extended into the cannula, and where the two components met, 2-ton epoxy was applied to form an airtight junction. The active region (i.e. area of exchange) of the dialysis probe was 2.5 mm in length.

Membrane Characteristics

The dialysis membrane was made of a copolymer, polyacrylnitrile sodium methallyl sulfonate. The membrane had an outer diameter of 270 μ. The membrane is semipermeable and allows the passage of molecules smaller than 15,000 daltons.

Probe Sterilization

Before use, the probe was prepared using the following procedure:

1) The probe was flushed with filtered double deionized water at a rate of 2 μl/min.

2) The dialysis tip was then placed in a filtered solution of 70% ethyl alcohol for 20 min. This was to remove the isopropyl myristate contained in the tip.

3) The tips of the probes were then placed in filtered double deionized water and flushed overnight at a rate of 0.5 µl/min.

4) The next day, the double deionized water was removed and replaced with modified Ringers solution (NaCl, 7.88g; KCl, 223.8 mg; $CaCl_2$ $2H_2O$, 176.4 mg; MgCl, 203.3 mg; ascorbate, 17.6 mg, dissolved in 276 mg/l of monobasic phosphate and adjusted to a pH of 7.4 with 5M NaOH) (Moghaddam et al, 1989).

5) Subsequently, the tips of the probes were placed in Ringers solution which was pumped through at a rate of 1.5 µl/min for 30 min.

6) *In vitro* tests at 20° C revealed that on average, a probe with a 2.5 mm active membrane had a relative recovery rate of approximately 15% of the bathing solution.

Probe Insertion

Prior to implantation, the animals (with swivels attached) were acclimatized for 2 days to the monitoring apparatus. Following the acclimatization period, the probe was fed through a swivel assembly (Instech) with the inflow attached to a 1 ml glass syringe containing Ringers solution, which was housed in a digitally calibrated Harvard infusion pump. The outflow silica tube was fed into a microvial which was secured to the swivel assembly. Under light halothane anesthesia, the stainless steel

stylet was removed from the headcap guide cannula assembly and replaced with the dialysis probe. The probe was carefully lowered into the brain aimed at the ventral striatum, 8.5 mm below the skull surface. The probe was secured to the head cap assembly with a retaining screw and the animal was then placed into a custom made monitoring chamber which was designed to hold the probe and swivel at the top of the cage. The testing chamber was well ventilated and constructed of clear Plexiglas. The floor of the chamber was covered with wood chips. The animal was given free access to food and water. The design of the chamber allowed for the undisturbed movement and monitoring of the animal and collection of the dialysate.

The probe was implanted at around 5:00 PM. The probe was perfused at a rate of 0.5 µl/min for 15 hrs prior to the actual experiment. This procedure was undertaken for the following reasons: 1) to allow for the acute trauma from implantation to subside, 2) to maintain the patency of the probe whilst in the brain, and 3)to allow the animal to acclimate to the testing chamber (Benveniste & Hansen, 1991).

Collection and Analysis of the Dialysate

At 7:00 AM, the flow rate was increased to 1.5 µl/min, yielding 30 µl of dialysate every 20 min. The first two samples were discarded. The next samples were used to establish a baseline. Two 20 min samples were collected each hour (the first and last 20 min of each test hr). These samples were immediately

analyzed ex vivo for DA content with the HPLC (for representative chromatograms of control and SDR responses, see Appendix I). Neurochemical analysis was as described in Experiment 6 (Methods) with the exception that integration and analysis of the chromatogram was achieved with a computerized system and software (EZCHROM ©). Collection and analysis of dialysate proceeded in this manner until 1 hr following the onset of lights (i.e. return to the diurnal phase). Simultaneous monitoring of locomotor behavior was achieved by computer (Hyperion) and custom made software which was interfaced with the monitoring chamber. The distance traversed (cm) was computed when the animal moved between quadrants established by infrared beams. The beams projected through the base of the monitoring chamber from an outer rectangular chamber.

Histology

At the conclusion of the experiment, the animals were sacrificed with an overdose of sodium pentobarbitol. Their brains were perfused with 10% formalin, frozen and sliced (30 μm thick) in a cryostat microtome (Hacker International). The brain slices containing the tracks of the microdialysis probe were fixed on slides and then stained with thionine for subsequent macroscopic verification.

Statistics

The data were analyzed using a two-way ANOVA with repeated measures. Multiple post hoc comparisons were achieved using Tukey tests. Correlative analysis was calculated using Pearson r test.

Results

Histological Verification

Macroscopic examination of the histological sections indicated that the tips of the microdialysis probes were found to range coronally and ventrally between the ventral portion of the caudate nucleus and the nucleus accumbens (see Fig. 13). Five of the control animals had their dialysis probe tips in the nucleus accumbens, however because the area of exchange was 2.5 mm in length, at least 2 of the probes were sampling solely from the accumbens and 2 from a combined accumbens-anterior caudate area. One control animal was sampling solely from the anterior caudate. A total of 3 diabetic rats had their probes in the nucleus accumbens (see Fig. 13, middle panel). At least 4 diabetic animals were primarily sampling from the accumbens. The remaining 2 diabetic animals had probes in the caudate nucleus. The general neuroanatomical area sampled is referred to as the ventral striatum (Heimer, 1978). The data from both the anterior caudate and nucleus accumbens animals were pooled within each group for statistical analysis.

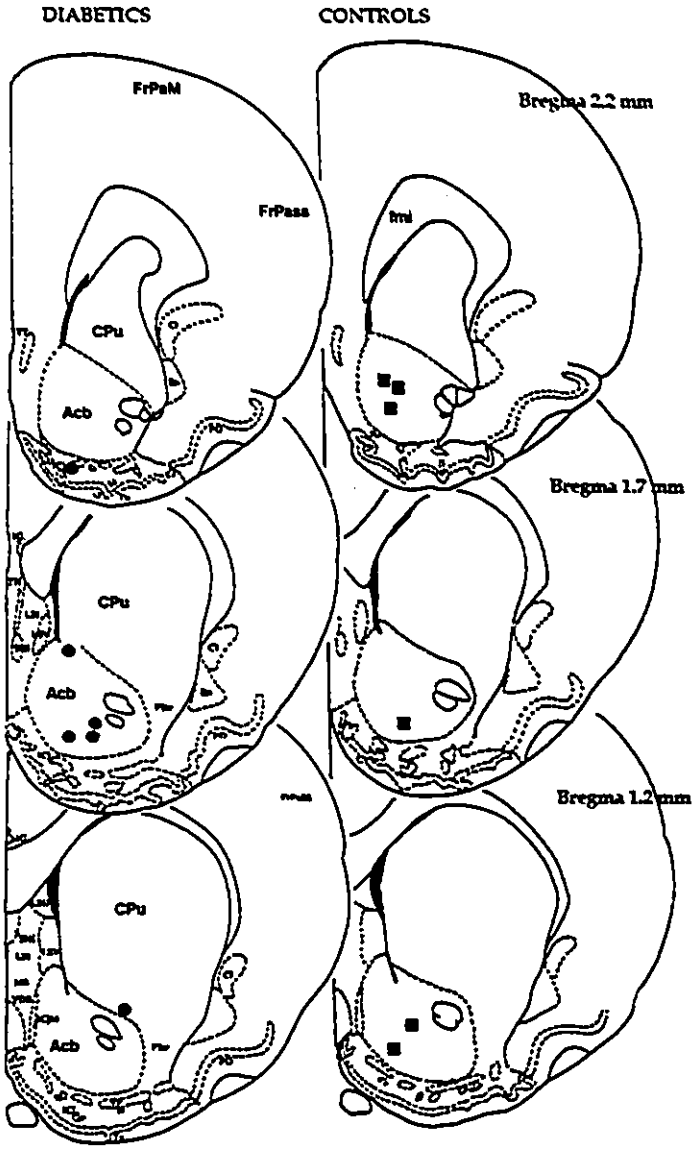

Fig. 13. The location of the microdialysis probe tips.
■ Represents the location of the tips in the control animals.
● Represents the location of the tips in the diabetic animals.
Acb is the abbreviation for the nucleus accumbens.
CPu is the abbreviation for the caudate putamen.

Nocturnal Locomotor Activity

The data were analyzed in 1 hr bins. A two-way ANOVA repeated over time revealed a significant group effect (SDR vs control) $F(1,11)=34.5$, $p<.001$. A significant effect of time was also obtained $F(13,130)=2.1$, $p<.05$. No significant interaction (Group x Time) effect was obtained $F(13,130)=1.6$, $p<.08$. Figure 14 (upper panel), depicts the spontaneous nocturnal distance traversed (cm) of the controls and SDR. The SDR response profile is relatively flat. While, the control profile is significantly elevated at almost all time periods. Moreover, the control profile consisted of the characteristic "peaks and valleys" found in a previous study (Merali & Ahmad, 1988) and in Experiment 5 of this thesis.

Interstitial Dopamine Levels

The two 20 min samples were combined to yield hourly values to coincide with the locomotor measurement. A two-way ANOVA repeated over time revealed no significant group differences $F(1,11)=0.007$, p=N.S. between the SDR vs controls in the levels of interstitial DA. There was also no significant effect of time $F(13,130)=0.98$, p=N.S. There was however, a significant interaction $F(13,130)=4.47$, $p<.0001$. Figure 14 (lower panel), depicts the DA response of both groups over time. It can be seen that the mean control DA levels are initially lower than the SDR; however, at the sixth hour of monitoring, the mean control DA levels rose above the SDR levels. For the most part, SDR response

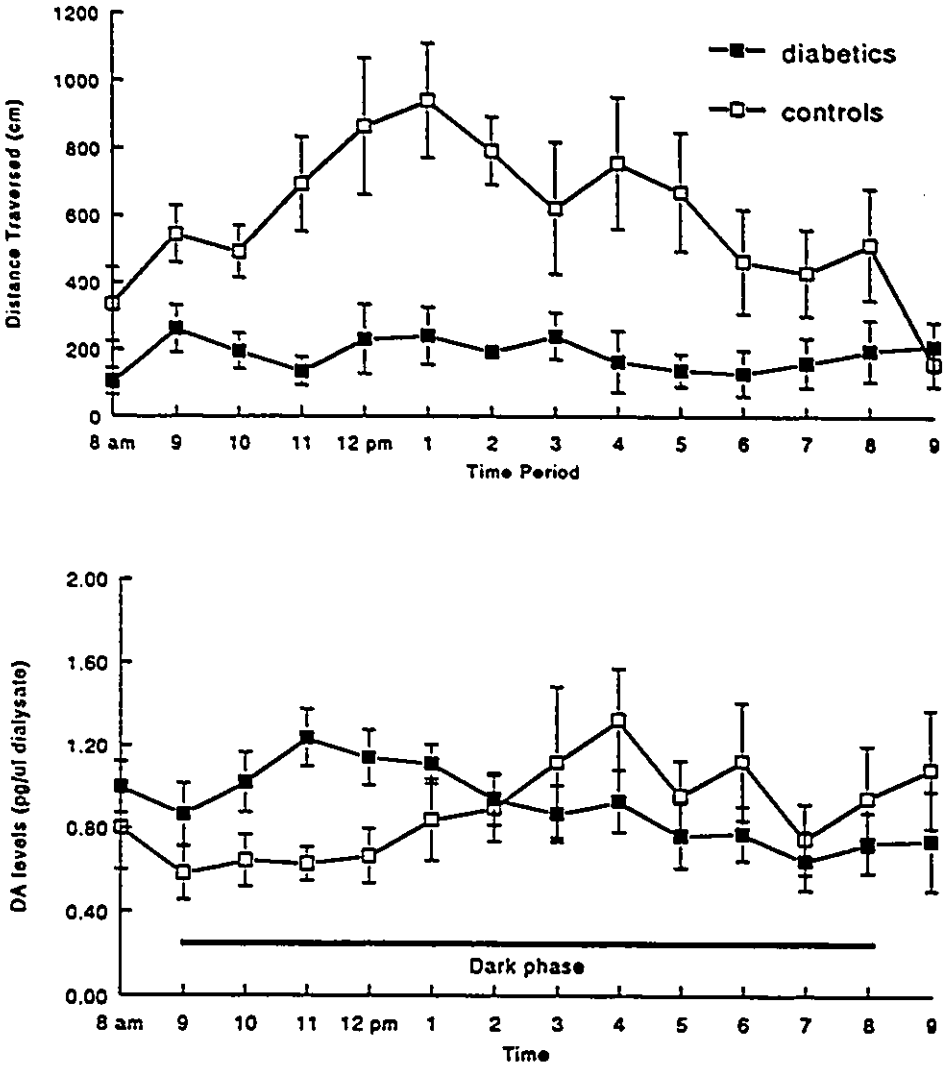

Fig. 14. The top panel illustrates the spontaneous nocturnal locomotor response of the diabetic rat during simultaneous microdialysis sampling. Each point represents the mean ± S.E.M. from 6 animals.The lower panel illustrates the nocturnal interstitial dopamine levels as measured by microdialysis. Each point represents the mean ± S.E.M. from 6 animals.

appears to be relatively stable. Although the controls demonstrated a rise in mean interstitial DA levels during the latter portion of the monitoring period, the differences were not significantly different.

Individual variations in DA level and locomotor responsivity were compared through correlational analyses, at all time intervals for both groups. No significant correlation were found.

Summary

The results presented in this experiment were an attempt to establish whether the blunted locomotor response of the SDR was associated with diminished interstitial DA release from the ventral striatal area in the insulin treated SDR, 4-5 months diabetic.

In essence, it was found that the SDR exhibited a significantly attenuated nocturnal locomotor response when compared to the controls. This finding is in agreement with Merali et al, (1988), Shimomura et al, (1988) and Experiment 5 of this thesis. It is important to note, that because the SDR received insulin via subcutaneous pellets, the possible fluctuations in the availability of insulin, which might occur when insulin is administered via daily injection, were circumvented.

The nocturnal interstitial levels of DA were not statistically different from the control levels. The controls

exhibited a gradual rise in DA level particularly during the latter portion of the dark phase. Conversely, the SDR exhibited a slight but gradual decline in the level of DA during the same period. The results of this experiment suggest that the attenuated nocturnal locomotor response of the SDR is not accompanied by diminshed interstitial levels of DA in the ventral striatal region.

The results obtained may be due to a number of factors. First, the common assumption that interstitial levels of neurotransmitters monitored by microdialysis accurately reflect the release at the synaptic level has not been validated. For instance, if the alterations in the release are associated with concomitant dynamic changes in the reuptake and/or metabolism of DA, the overflow into the extracellular compartment being sampled may not accurately reflect alterations in synaptic release. Second, although not supported by our data, it is possible that the attenuated nocturnal locomotor response of the SDR may be due altered neurotransmission at the post-synaptic and/or post-receptor level(s). Also, it may be that the source of the deficit in the SDR is located at other neuroanatomical structures. At this point the source of the attenuated response of the SDR remains unidentified.

The lack of correlation between the locomotor response and the level of DA in both the control and SDR groups was surprising, particularly with regards to the heightened nocturnal locomotor response in the controls. Regarding this point, Paulson

and Robinson (1992) found no significant alterations in the day/night levels of DA (as measured by microdialysis) from the ventral striatal region of non-diabetic rats, despite a marked increase in nocturnal locomotor activity. These researchers suggested that DA release from the ventral striatal region plays only a minor role in regulating large changes in diurnal and nocturnal motor activity.

It would appear that the reputed relationship between release of DA and enhanced locomotor activity is not a simple uniform phenomenon (Kuczenski & Segal, 1989). Several studies using either push-pull perfusion techniques or microdialysis have shown a dissociation between d-amphetamine enhanced locomotion and extracellular striatal DA release (Kuczenski & Segal, 1989; Kuczenski & Segal, 1988; Segal & Kuczenski, 1987b). These researchers cite a number of factors which may explain the lack of correlation between DA release and behavior. First, behaviors such as locomotion are complex and multifactorial and therefore, it is difficult to obtain a correlation between release of DA for example, and a global behavior such as locomotion. It has been suggested that a more effective approach may be to assess release of neurotransmitter to a more specific element of behavior (Kuczenski & Segal, 1989).

In a different vein, it is possible that other neurotransmitter systems may be involved in the mediation of spontaneous nocturnal locomotor activity. Indeed, it is known that adrenergic, NE and 5-HT neurotransmitter systems also

undergo nocturnal alterations (Wirz-Justice, 1987). Certainly 5-HT and NE have been implicated in the modulation of locomotor activity mediated by the DA system (Beninger, 1983; Kuczenski & Segal, 1989; Layer et al, 1992). Other neuroanatomical sites may be more pivotal in the mediation of spontaneous locomotor activity. Cools (1986) has suggested a more pronounced role for DA located in the olfactory tubercles in the mediation of nocturnal locomotor activity.

Experiment 7, demonstrated an absence of a correlative relationship between interstitial DA release and changes in the spontaneous nocturnal locomotor behavior. This finding suggests the lack of a simple quantitative association between DA and spontaneous nocturnal locomotion, as dialysed from the ventral striatal region. Other factors may have also contributed to the lack of correlation. These factors include, a small group number (n=6 per group) and within group variability.

GENERAL DISCUSSION

The SDR is a genetically determined, clinically relevant, animal model of Insulin Dependent Diabetes (IDDM). Although, in existence since 1974, few if any studies have characterized the behavioral and neurochemical profile of the SDR.

The present studies investigated the behavioral response of the SDR following exposure to DA agonists, normal dark/light cycles as well as novelty-stress and anxiogenic tests. In addition, the post-mortem neurochemical status of the biogenic amines in the CNS of the SDR was investigated. Finally, the nocturnal release of interstitial DA from the ventral striatal region was assessed in the SDR, using *in vivo* microdialysis. An important facet of this research was the assessment of the behavior of the SDR, during the progression of the diabetic condition. Thus, at various time frames (0-2, 2-8, and 8-12 months duration of diabetes) the SDR response to d-amphetamine, novelty-stress, and spontaneous behavior was assessed. The purpose of the latter was to chart the possible sequelae of changes which might have occurred as a result of duration of diabetes. Taken together, the results of this research revealed some interesting findings and trends as well as future research possibilities.

Locomotor and Rearing Behavior

Overall, the SDR manifested an attenuated locomotor and rearing response to d-amphetamine, amfonelic acid and the nocturnal phase of the light/dark cycle than the control animals. Moreover, in both Experiments 1 and 5, the attenuated behavioral responsiveness of the SDR endured to different extents, across all three time frames, thus indicating that the differential responsivity between the SDR and controls was robust and long lasting. These findings are unique in that they are the first to demonstrate long-term alterations in the behavioral response of the SDR. Further, our data demonstrated that other behaviors such as rearing were also diminished in the diabetic rat. It is of import, that the attenuated behavioral response of the SDR occurred despite the fact that the rats were maintained on insulin replacement therapy.

The findings of the diminished response of the SDR to d-amphetamine, presented in Experiment 1, are concordant with those of Marshall et al, (1976 & 1978) who reported that female rats with 5-6 weeks of alloxan induced hyperglycemia manifested a diminished behavioral response to peripherally administered d-amphetamine. Specifically, Marshall reported a significant reduction in locomotor, stereotyped, and anorectic responses to a wide range of doses of d-amphetamine. Rowland et al, (1985) also reported that rats made hyperglycemic with STZ manifested a significant decrease in d-amphetamine-induced stereotyped

behavior. In both of the aforementioned studies, an amelioration of the observed deficits occurred after the acute administration of insulin.

The fact that insulin reversed the observed deficits in d-amphetamine responding in pancreotoxin-based diabetes is in contrast to the results presented here. In the present context, the administration of insulin to the SDR was undertaken with the aim of correcting the metabolic and physiological alterations which occur as a result of severe hyperglycemia circumventing the possible adverse effects of uncontrolled diabetes on drug metabolism and uptake. The finding that insulin can reverse the deficits in responding to d-amphetamine in pancreotoxin based diabetes and not in the SDR is puzzling. The source of the differences in this regard may come from different models of diabetes (genetic vs chemical), duration, and/or severity of the diabetic condition.

Marshall et al, (1976) reported that reduced tissue access by d-amphetamine to the CNS, as measured by the [^{3}H] d-amphetamine, was not a factor in their hyperglycemic animals. However, Curzon and Fernando (1977) reported significant decreases in d-amphetamine uptake in the brains of STZ diabetic rats. In contrast to Curzon and Fernando (1977), and in agreement with Marshall et al, (1976), Rowland et al, (1985) did not find significant differences in [^{3}H] d-amphetamine accumulation in the brains of their STZ diabetic rats. In the present study, this issue was not addressed and therefore alterations in the

pharmacokinetics of d-amphetamine in the SDR cannot be ruled out. However, an important difference here is that the SDR were treated with insulin, which is known to reverse the hyperglycemia related abnormalities in pharmacokinetics (Ackerman, 1976). There is no doubt it would be interesting in future studies to assess the uptake of pharmacological substances such as, d-amphetamine in the brains of the SDR.

The demonstration that the attenuated response of the SDR to d-amphetamine was chronically enduring is unique. Previous studies have been largely concerned with a specific time point in the duration of the diabetic condition. Studies by Lakovic et al, (1990) and Broderick and Jacoby (1988) demonstrated changes in neurochemical response of the diabetic rat over time. These studies emphasize the importance of measuring the behavioral response of the diabetic rat as the diabetic conditions endures.

Experiment 2 was undertaken to establish whether the altered response to d-amphetamine was unique to this drug. The results presented in Experiment 2, regarding the behavioral response of the SDR (5-6 months diabetic) following several doses of amfonelic acid (a non-amphetamine stimulant), demonstrated that the attenuated locomotor and rearing response of the SDR was not an idiosyncratic response to d-amphetamine. Although amfonelic acid is reputed to enhance DA synaptic availability by a different mode of action than d-amphetamine, it produces a behavioral profile similar to that of d-amphetamine (Shore, 1976; Schechter, 1987). In the present study, it was evident that the

behavioral response of the SDR to amfonelic acid was qualitatively quite similar to that of d-amphetamine.

Given that the drug-stimulated behavioral response of the SDR differed significantly, whereas the day-time baseline behaviors did not, it became of interest to examine the locomotor and rearing response of the SDR under conditions of naturally higher levels of activity. In Experiment 5, the spontaneous behavioral response of the SDR was assessed both during the nocturnal and diurnal phases of the daily 12 hr light cycle. It is well established that the rat, an inherently nocturnal animal, exhibits naturally higher levels of locomotor activity during the dark or nocturnal phase of the daily light/dark cycle. Thus, an examination of the locomotor and rearing profile of the SDR would not have been complete without an assessment during the nocturnal period. Overall, the data revealed that during the diurnal phase, when levels of activity are low, the SDR was able to maintain similar levels of locomotor and rearing behavior to those of the controls. During the nocturnal phase however, the SDR demonstrated an attenuated locomotor and rearing response which endured to different extents throughout the three time frames examined. Depending on the time frame and behavior examined, the SDR demonstrated a shifted or reduced locomotor and rearing response to photoperiodic cues such as the onset and offset of lights.

The findings of Experiment 5 corroborate those of Merali et al, (1988) and Shimomura et al, (1988). Merali et al, (1988)

demonstrated that insulin treated SDR (2-4 months duration of diabetes) exhibited an attenuated nocturnal rearing and locomotor response. Also, in agreement with the results of Experiment 5, the SDR showed an attenuated behavioral response to photoperiodic cues. Shimomura et al, (1988) demonstrated that the non-insulin treated STZ diabetic rat (26 days duration of diabetes) exhibited attenuated levels of nocturnal ambulatory activity over a period of 14 days. The findings of Experiment 5 are unique in that they are the first to demonstrate a chronic deficit in spontaneous nocturnal activity of the SDR. Further, this deficit is not corrected with insulin replacement.

An attempt was also made to identify the source(s) of the pharmacological and non-pharmacologically induced locomotor and rearing deficits in the SDR. There is convergence of data in the literature which postulates a role for central DA in the altered behavioral response of the diabetic rat.

It has been well established that global manipulations of the CNS DA system(s) alter locomotor behavior in the rat (Beninger, 1983; Fishman et al, 1983). Moreover, manipulations of the mesolimbic DA system (which projects from the ventral tegmental area to the ventral striatum) have also revealed that this system may play a pivotal role in the mediation of locomotor activity (Pijnenburg et al, 1973; Cools, 1983; Robinson & Camp, 1990).

In the present context, there exists a body of research which has demonstrated reduced CNS DA turnover in the diabetic

rat as reflected by the levels and metabolism of this neurotransmitter. This altered neurochemical responsiveness has been detected in the mesolimbic and nigrostriatal DA systems of the STZ, alloxan, and genetically diabetic rat (Lozovsky et al, 1981; Trulson & Himmel, 1983; Saller, 1984; Serri et al, 1985; Kwok et al, 1986; Merali et al, 1988; Shimomura et al, 1988; Bellush & Reid, 1991). It has been well established that diabetic rats (either pancreotoxin or genetically based) have an altered behavioral response to DA agonists such as amphetamine (Marshall, 1978; Rowland et al, 1985; Chu et al, 1986; Merali et al, 1988), amfonelic acid (Ahmad & Merali, 1989), apomorphine (Rowland et al, 1985; Merali et al, 1988; Walls et al, 1984), and SKF 38393 (Merali & Ahmad, 1989). This altered behavioral response also extends to DA antagonists such as haloperidol (Ahmad & Merali, 1988; Walls et al, 1984) and SCH 23390 (Lim et al, 1992; Merali & Ahmad, 1989) induced catalepsy. There is also an altered reponsiveness of the diabetic rat to non-pharmacological stimulated behaviors, believed to be in part mediated by the CNS DA system(s). Some examples include novelty-stress induced grooming (Ahmad & Merali, 1988) and spontaneous nocturnal activity (Merali et al, 1988).

Taken together, there appears to be alteration(s) in the CNS DA system(s) of the diabetic rat. Furthermore, these alterations may have behavioral consequences. The source(s) of this deficit(s) remains largely enigmatic. One possible hypothesis contends that there is a deficit in the biosysnthetic capacity of

the CNS DA system(s). This is evidenced by the demonstration that STZ diabetic rat, untreated with insulin, has a reduced tyrosine concentration (Bellush & Rowland, 1986). This reduction in synthesis could in turn affect the availability of DA (Trulson & Himmel, 1983; Saller, 1984; Kwok & Juorio, 1986). This deficit in release may in part explain the altered behavioral response of the diabetic rat to amphetamine and amfonelic acid. Both drugs are known to produce their behavioral effects via the release of DA (Merali et al, 1988). This hypothesis may also explain the increase in striatal DA receptor number which may be a compensatory response to decreased DA release (Lozovsky et al, 1981; Trulson & Himmel, 1983). Clearly, an investigation of CNS neurotransmission in the SDR was necessary to assess the aforementioned hypothesis. Furthermore, pharamacodynamic measures need be taken from the CNS of diabetic rats in general, in order to further substantiate the aforementioned hypothesis. These concerns were addressed by Experiments 6 and 7.

The neurochemical status of the biogenic amines in the CNS of the SDR is a relatively obscure area of research. Kwok et al, (1986), demonstrated that the SDR (with 11-23 days duration of diabetes) showed a significant reduction in striatal and mesolimbic DA and 5-HT metabolism. In the present study, the results of experiment 6.1 demonstrated that the insulin treated SDR (4-5 months diabetic) had similar levels of CNS biogenic amines to both genetically distinct and non-diabetic littermate control groups. This finding is in contrast to a study by Bellush

and Reid (1990) which found that insulin replacement did not normalize a significant reduction in hypothalamic DA turnover in the STZ diabetic rat.

In the present study, only when insulin was withdrawn for four consecutive days, were alterations in neurotransmitter levels observed. Specifically, it was found that there was a significant elevation in the level of NE in the cortex and hypothalamus and DA in the hippocampus. The level of the DA metabolite HVA was found to be significantly elevated in the striatum. Overall, the levels of DA in the striatum, an area implicated in the regulation of locomotion, was not significantly altered. This finding is in agreement with those of Bitar et al, (1986) and Kolta et al, (1986) who used insulin untreated STZ and alloxan diabetic rats, but in contrast to the findings of Trulson and Himmel (1983), Saller (1984), Shimomura et al, (1988) and Bellush and Reid (1990). At this point, it is difficult to explain the discrepant findings regarding DA level in the CNS of the diabetic rat. In the case of the chemically diabetic rats, some of the discrepant findings may be associated with variations in the type and dosages of the pancreotoxin used, the regimen of insulin replacement (the consequent metabolic decompensation), and the duration of the lesion.

In the present context, there is evidence that insulin treatment possibly contributed to the "normal" neurochemical status of the SDR. Kwok and Juorio (1986) have suggested that insulin treatment counteracted diabetes-induced changes in

striatal DA metabolism by correcting alterations in the availability of amino acid precursors and in tyrosine hydroxylase activity. Although these measures were not undertaken in the present context, it is possible that insulin treatment in the SDR had a similar effect on DA precursor availability.

In Experiments 1 and 2, behavioral alterations were observed in the SDR following d-amphetamine and amfonelic acid challenges. With this in mind, it became of interest to examine the neurochemical response of the SDR also following a pharmacological challenge. Therefore, amfonelic acid (1.0 mg/kg) was administered to the insulin treated SDR (with a 5-6 month duration of diabetes) and the post-mortem levels of biogenic amines from specific structures in the CNS were measured. It was found that there was a significantly greater depletion of DA levels in the striatum, hypothalamus, midbrain and olfactory bulbs of the SDR. There were no significant alterations in the levels of DA metabolites, but there was a significant reduction in the striatal level of NE in this group.

The mechanism by which amfonelic acid exerts its behavioral activation is believed to result from the release of DA from a more permanent inactive presynaptic pool (Miller & Shore, 1982). This reputed mechanism of action is evidenced by the fact that pretreatment with alpha-methylparatyrosine does not block the central effects of amfonelic acid whilst reserpine pretreatment does (Miller & Shore, 1982). Arbuthnott et al, (1990), suggest that alterations in the levels of this permanent inactive pool

can affect the availability of DA from more readily releasable presynaptic pools. The exploratory nature of Experiment 2 can only allow for speculative conclusions. Nevertheless, one possible source of the behavioral deficits observed in the SDR to d-amphetamine and amfonelic acid may be due to a dysfunction in the synaptic availability of DA following pharmacological challenge. If so, this reputed reduction in the availability of DA should be reflected by the diminished amounts of DA released.

In an effort to assess this hypothesis, in Experiment 7 we measured the interstitial levels of nocturnal DA via microdialysis from the ventral striatum of the SDR (with a 4-5 month duration of diabetes). Concomitant spontaneous locomotor activity was also measured. Experiment 7 is unique in that it is the first study to assess the dynamic status of neurochemical release in the diabetic rat using in vivo microdialysis.

The attenuated spontaneous nocturnal locomotor activity exhibited by the SDR in Experiment 7 was consistent with the findings of Experiment 5 and those of previous studies (Merali et al, 1988; Shimomura et al, 1988). The finding that there were no significant group differences in the levels of interstitial DA released from the ventral striatal region is in the least heuristic. Clearly, in the context of Experiment 7, an altered DA release profile in the SDR cannot account for the attenuated nocturnal locomotor response observed. It is possible that relegating the assessment to one specific time frame in the duration of the diabetic condition may have limited the

opportunity to observe neurochemical differences. In this context, Broderick and Jacoby (1988), found that extracellular levels of striatal 5-HT (as measured by *in vivo* voltammetry) were significantly increased in non-insulin treated STZ diabetic rats with a 3 day duration of diabetes. 5-HT levels returned to normal however, after 3-7 weeks duration of diabetes. Perhaps an assessment of the SDR at a time frame more proximal to the onset of the overt condition may have yielded different results? More importantly, the integrity of the site measured may be at question, given the lack of correlation observed between nocturnal locomotion and ventral striatal DA release, in both groups. Indeed, Paulson and Robinson (1992) reported that DA release (as measured by microdialysis) from the ventral striatum plays only a minor role in regulating transitions in the levels of day/night locomotor activity. This would suggest the importance of investigating other neuroanatomical sites. Certainly more research is required in the area of direct CNS measurement from the diabetic brain.

The reported alterations in DA turnover in the diabetic rat may also be indicative of impairments at different levels of the neurotransmission process. Neurotransmitter turnover is the result of synthesis, release, and uptake. It is quite plausible that alterations in any one or all of these processes may be occurring in the diabetic rat.

In this regard, there is evidence of postsynaptic "up regulation" of DA receptor sites in the striata of rats following

pancreotoxin-induced hyperglycemia (Lozovsky et al, 1981; Trulson & Himmel, 1983; Serri et al, 1985). Many consider changes in receptor number to be a compensatory response to presynaptic alterations. Indeed, as suggested by Rowland and Bellush (1989), the reported increase in the number of DA receptors is similar to that observed following extensive denervation of the striatum in the non-hyperglycemic rat. However, these findings have as their main basis uncontrolled severe hyperglycemia. The SDR must be treated with insulin within 24 hours of the onset of diabetes, otherwise subject mortality may be as high as 70%. Given this, the SDR is not as able to withstand the chronic hyperglycemia often associated with the pancreotoxin based experiments. Therefore, it is difficult to explain our results based upon those obtained from the hyperglycemic rat.

In this context, Rowland and Bellush, (1989), have levied some cogent concerns regarding the "positive" receptor binding studies. Namely, the severe physiological and behavioral abnormalities which typify DA denervation are not present in the the SDR or the STZ hyperglycemic rat. A case in point is the attenuated behavioral response of the diabetic rat to the direct DA agonist apomorphine and antagonist haloperidol (Rowland et al, 1985; Ahmad & Merali, 1988). Typically, non-diabetic rats with DA "denervation supersensitivity" exhibit a heightened behavioral response to direct DA agonists. Such a behavioral response is not evident in either chemically or genetically diabetic rats.

Thus, the source of the deficits may be more compatible with

a postreceptor defect. There is evidence for alterations in postreceptor function in the hyperglycemic rat and the SDR. Specifically, Gawler et al, (1987) found that STZ induced diabetes led to the loss of the expression of the G-protein Gi in the liver. This protein is known to mediate the inhibitory effects on adenylate cyclase activity. As suggested by Gawler et al, (1987), loss of the expression of this pivotal regulatory protein in Type-1 diabetes may lead to further dysfunctions. Moreover, these researchers also found that this deficit could be reversed with the acute administration of insulin. Thus, this deficit appears to be mediated by severe hyperglycemia, a condition kept under control in our animals. More important to the results in the present context was their finding that insulin treatment did not seem to reverse an overall reduction in the basal activity of adenylate cyclase. This perhaps indicates a possible dysfunction which is hyperglycemia independent but diabetes related.

In a similar vein, Palmer et al, (1983), found a reduction in the sensitivity of adenylate cyclase to NE but not DA mediated activation in the cerebrum and to NE and DA in the retina, in 8 week STZ rats. A consequence of adenylate cyclase abnormalities should evidence itself in responses directly related to the function of this enzyme.

Indeed, in the SDR (4-8 months diabetic), we reported an enhanced ability of the potent adenylate cyclase inhibitor SCH 23390 to block novelty-induced grooming behavior in the SDR

(Merali & Ahmad, 1987). Furthermore, we found that the SDR (4-8 months duration of diabetes) demonstrated an increased sensitivity to D-1 receptor stimulation with the specific agonist SKF 38393 (Merali & Ahmad, 1987). It is known that the D-1 subtype is defined as that receptor at which DA stimulates adenylate cyclase activity (White et al, 1988). These findings suggest a dysfunction of adenylate cyclase activity in the diabetic rat. Overall, this dysfunction may account in part for some of the behavioral and neurochemical alterations observed in the diabetic rat. Certainly, investigations into the postreceptor mechanisms in the diabetic rat should be an area of future concentration.

An alternative explanation for the observed deficits in the behavioral response of the SDR may come from alterations in other neurotransmitter systems. One prime candidate is 5-HT. There are numerous studies indicating that central 5-HT functioning is altered in both the chemically treated and genetically diabetic rat (for a review see Rowland & Bellush, 1989). Studies investigating the synthesis of 5-HT in the CNS of diabetic rats have revealed a reduced synthesis rate (Kwok & Juorio, 1985; Kwok & Juorio, 1986; Trulson et al, 1986; Bellush & Reid, 1990). Relevant to the present context, Kwok and Juorio (1985) demonstrated reduced 5-HT metabolism in the olfactory tubercle (an area not investigated in the present work) of the insulin treated SDR (11-23 days diabetic). It is also interesting to note that STZ diabetic animals (typically

untreated with insulin) have an subsensitive behavioral response to drugs known to affect the CNS 5-HT system (Trulson & MacKenzie, 1981; MacKenzie & Trulson, 1978).

As discussed earlier, Broderick and Jacoby (1988), using *in vivo* voltammetry, found a significant increase in extracellular 5-HT in the striatum of acute but not chronically hyperglycemic STZ diabetic rats, sedated with chloral hydrate. Thus, there is some evidence that CNS neurotransmission is adversely affected in the diabetic rat.

In relation to the present research findings, 5-HT is known to have a modulating effect on DA induced locomotor activity (Kuczenski & Segal, 1989; Layer et al, 1992). Furthermore, it has recently been demonstrated that the striatal DA and 5-HT systems may be functionally interdependent (Kuczenski & Segal, 1989). Also, in terms of nocturnal activity, lesions of the 5-HT fibers, (using 5,7-DHT) ascending to the suprachiasmatic nucleus of the hypothalamus from the medial forebrain bundle, are known to increase the duration, advance the onset, and delay the offset of nocturnal activity rhythms (Smale et al, 1990). There is no doubt that behavioral and neurochemical investigations of the CNS 5-HT system in the SDR would be an area of future interest.

In conclusion, the SDR, when treated with insulin, and unchallenged by: withdrawal of insulin treatment, pharmacological stimulation of the DA system(s), or environmental stimulation, is able to maintain relatively stable baseline levels of brain catecholamines and behavior. It is only

under challenging conditions that the SDR manifests a chronic abnormal behavioral and neurochemical response as compared to genetically distinct controls and genetically related non-diabetic littermates.

Novelty Stress and Anxiety Response

The findings presented in Experiment 3 are in broad agreement with previous research investigating stress and diabetes in experimental models using rodents. In general, it appears evident that the diabetic animal has inherent to it, an altered behavioral and biochemical response to stress. Unique to this Experiment is the finding that the heightened novelty-stress response of the SDR is chronic extending from a period of up to 12 months duration of diabetes. This altered response occurs despite insulin treatment. The results of Experiment 3 support the findings of Ahmad and Merali (1988), which demonstrated that the insulin treated SDR (5-6 months duration of diabetes) has a heightened grooming response to novelty-stress, when compared to non-diabetic littermates. The results of this study are also in agreement with previous research using acute, pancreotoxin based diabetes. In general, these studies have demonstrated a behavioral sensitization to stressors such as footshock (Bellush & Rowland, 1989; Lee et al. 1989; Leedom et al. 1987), restraint, novelty (Bellush et al. 1991), cold and hypoxia (Bellush & Henley, 1990; Forman et al, 1988).

Due to the extreme sensitivity of the SDR to handling and surgical manipulation, Experiment 3 did not include measures assessing the biochemical correlates of stress. However, alterations in various biochemical measures have been reported in the alloxan and/or STZ rat. Specifically, following stress, acutely diabetic rats, often untreated with insulin, manifested significantly elevated levels of plasma corticosterone (Tornello et al. 1981; Leedom et al. 1987; Bellush et al. 1991) EPI, NE (Lee et al. 1989) and elevated levels of NE in urine (Bellush & Henley, 1990). Similar to our findings, the altered behavioral response by the diabetic rats in the Lee et al. (1989) study, endured despite insulin replacement therapy.

In the context of the present work, there is substantial literature which demonstrates that exposure to novel environments (i.e. environments different from the animals home cage) results in an activation of the pituitary-adrenal system (Jolles et al. 1979). Simply moving rats and their housing cages from one room to another can lead to an increase in corticosterone, prolactin, and thyroid stimulating hormone (Flaherty et al. 1986). Furthermore, the greater the degree of novelty, the greater the increase in plasma corticosterone (Flaherty et al. 1986; Hennessy & Levine, 1978). It is plausible that the SDR was responding biochemically in a manner similar to the pancreotoxin treated animals, namely through an altered hypothalamopituitary-adrenal and/or sympathoadrenal system(s) (Bellush & Reid, 1991). Indeed, such a biochemical reaction could result in an altered behavioral

response (Hennessy & Levine, 1978).

In the present study we employed as our main behavioral measure novelty induced grooming. Under conditions of high arousal, grooming behavior has been described as a displacement activity which has the purpose of enabling the rat to return to homeostasis (Kametani, 1988). Moreover, grooming is a well defined behavioral act consisting of discrete and sequential actions (Jolles et al. 1979; Colbern et al. 1979). Thus, grooming is often employed as a behavioral measure of adaptation to a stressor (Colbern et al. 1979). Although the precise substrates underlying stress induced grooming remain to be delineated, several peripheral and central factors have been implicated in stress induced grooming. Amongst the central factors, it has been well established that pro-opiomelanocortin peptide fragments (i.e. adrenocorticotropic hormone, ACTH), opioid, and dopaminergic systems are involved in excessive grooming (Green et al. 1979, Wiegant et al. 1977; Molloy & Waddington, 1985). In the periphery, hormones of the hypothalamopituitary-adrenal and sympathoadrenal systems appear to be involved in the triggering of stress induced grooming. Given that some of these systems are compromised during diabetes (for a review refer to McCall, 1992; Rowland & Bellush, 1989), it is possible that diabetes related biochemical alterations are responsible for novelty stress-induced grooming in the SDR, and that effect is chronic.

In a different vein, it is known that grooming appears to be the only behavior reliably induced by selective activation of the

DA D-1 receptor subtype (Murray and Waddington, 1989). In a study by Merali and Ahmad (1987) it was found that the SDR exhibited a heightened sensitivity to grooming induced by the D-1 receptor agonist SKF 38393. It was also found that the SDR exhibited an increased sensitivity to the blockade of novelty stress induced grooming with the D-1 receptor antagonist SCH 23390. It is possible, therefore that the heightened grooming response to novelty induced stress may be related to functional alterations at the D-1 receptor. Recently, Lim et al. (1992) found that the affinities of striatal D-1 receptors were significantly increased in the STZ diabetic rat; however there were no alterations in the densities of the receptors.

It is also of interest to note that the D-1 receptor subtype is defined as that receptor at which DA stimulates adenylate cyclase activity (White et al. 1988). The activity of adenylate cyclase has been found to be altered in diabetic rats. As referred to earlier, both Palmer et al. (1983) and Gawler et al, (1987) found altered adenylate cyclase activity in the STZ and alloxan diabetic rats. In terms of novelty stress, it was found that noradrenergic stimulation of adenylate cyclase maintains the integrity of the neophobic response in non-diabetic rats (Steketee et al. 1992). Thus, it is possible that postreceptor mechanisms may play a role in the altered behavioral response of the diabetic rat to novelty stress.

It is also possible that alterations in other neurotransmitter systems are adversely affecting the novelty

stress response of the SDR. The NE system(s) has long been shown to be affected by stress (Weiss et al, 1970; Weiss et al, 1981; Johnson et al, 1992). Trulson and Himmel (1985) found a decreased turnover rate of forebrain NE in the severely hyperglycemic STZ diabetic rat (4-6 weeks diabetic). Concordant with Trulson and Himmel (1985), Bitar et al, (1986) demonstrated a reduced concentration in several brain regions of the STZ diabetic rat (untreated with insulin) with 10, 30, and 90 days duration of diabetes. Massol et al, (1989), found that non-insulin treated STZ diabetic rats (4 weeks duration of diabetes) manifested an attenuated behavioral response (helpless behavior) to several antidepressants. Moreover, they suggested that alterations in the β-adrenoreceptor system may be implicated. In this regard, Bitar and DeSouza (1990), demonstrated an upregulation of β-1 adrenoreceptors in the hypothalamic-pituitary-testicular axis of non-insulin treated STZ diabetic rats (with 30 days duration of diabetes).

Taken together, the alterations in CNS NE functioning in the chemically diabetic rat suggest that similar investigations be undertaken in the SDR. In the future, it would be of interest to examine the NE response of the SDR to novelty stress using pharmacological agents known to interact with this system(s).

In Experiment 4, we investigated the behavioral response of the insulin treated SDR (with 4-8 months duration of diabetes) to the open field and elevated plus maze following the administration of the anxiolytic chlordiazepoxide. In summary, it

was found that the SDR exhibited a robust and significantly greater aversiveness to the center area of the open field and also to the open arms of the elevated plus maze. Both areas are known to be anxiogenic to the rat. When comparing the dose-response of chlordiazepoxide within each group, it is clear that the anxiolytic effects of the drug were more potent in the SDR. It is important to note that the source of this effect may be the result of an enhanced anxiety response by the SDR as compared to the control groups. This enhanced anxiety response is reflected by lower levels of baseline behavior. However, a between groups comparison reveals that overall, chlordiazepoxide is not as efficacious in increasing the response of the SDR to the anxiogenic areas of the apparatii, as compared to the control groups. This is the first such report for the SDR. Moreover, the altered behavioral response of the SDR to chlordiazepoxide may have a neurochemical substrate(s).

Specifically, chlordiazepoxide is a benzodiazepine, a class of tranquilizers known to have anti-convulsant, sedative, and anxiolytic properties (Greenblatt & Shader, 1974). Further, the effects of the benzodiazepines are known to be a result of activation at receptor sites on the gamma-aminobutyric acid (GABA-A) receptor complex. Martin et al, (1988) demonstrated that the non-insulin treated STZ diabetic rat manifested a marked decrease in cortical GABA-B receptor density, at 15 and 30 days duration of diabetes. There were no observed changes in GABA-A density. The status of the GABA system(s) and its relationship to

the altered response of the SDR remain to be elucidated.

As discussed in the previous section, the central 5-HT system in the diabetic rat is dysfunctional. It is possible that altered CNS 5-HT functioning may have contributed to the behavioral deficits displayed by the SDR in the open field and elevated plus maze. Indeed, alterations in GABA-B receptor function could be related to changes in 5-HT neurotransmission. More research is needed to assess the role of CNS neurochemical systems on neophobic and exploratory behavior in the diabetic rat.

In summary, the SDR manifests a chronic, maladaptive behavioral response to novelty stress. This response is not corrected by insulin treatment. The SDR also manifested a robust aversiveness to the anxiogenic areas of the elevated plus maze and open field as indicated by an altered response to the anxiolytic effects of chlordiazepoxide. This response also endured despite insulin treatment. The physiological substrates of this altered behavioral response by the SDR remain to be elucidated.

CONCLUSIONS

The following conclusions can be derived from the presented data:

1) The insulin treated SDR has an attenuated locomotor and rearing response to systemically administered d-amphetamine. This altered behavioral response is chronic as it was evident 0-2, 2-8, and 8-12 months following the onset of diabetes.

2) The insulin treated SDR (5-6 months diabetic) has an attenuated locomotor and rearing response to systemically administered amfonelic acid.

3) The insulin treated SDR has an attenuated spontaneous nocturnal locomotor and rearing response, particularly, during the transitional periods of the daily light/dark cycle. This altered behavioral response was evident 0-2, 2-8 and 8-12 months following the onset of diabetes.

4) The above conclusion (points 1-3) may be indicative of an alteration of CNS neurotransmission in the SDR. This applies particularly the DA system(s) which has been reported to play a pivotal role in the mediation of locomotion.

5) A post-mortem analysis of region specific CNS basal levels of biogenic amines and their metabolites in the insulin treated SDR revealed no significant alterations. These data indicate that the SDR is able to maintain relatively normal levels of CNS biogenic amines when metabolically compensated with insulin.

6) Cessation of insulin treatment for four consecutive days resulted in significant elevations of NE in the cortex and hypothalamus, DA in the hippocampus, and HVA in the striatum. These data indicate that hyperglycemia may be one important factor in altered CNS neurotransmission in the SDR.

7) The systemic administration of amfonelic acid (1.0 mg/kg) resulted in a significantly greater reduction in the post-mortem levels of DA in the striatum, hypothalamus, midbrain, and olfactory bulbs as well as striatal NE in the SDR as compared to control rats. These data may be indicative of an alteration in the storage and availability of DA at the presynaptic level in the SDR.

8) In an attempt to assess one possible substrate for the alterations described in points 1-4 and 7, nocturnal levels of CNS interstitial DA from the ventral striatal region of the SDR were measured using *in vivo* microdialysis. Although no significant differences were found between SDR and control interstitial levels of DA, the SDR did exhibit significantly

lower levels of nocturnal locomotor activity. The absence of a correlation between interstitial DA levels and nocturnal locomotion in both groups may indicate the following: i) that alterations may be occurring at other levels of the DA neurotransmission process; ii) the importance of assessing directly other neuroanatomical sites and neurotransmitter systems in the diabetic rat and iii) the complexity of neurochemical and behavioral interactions.

9) The insulin treated SDR has an increased behavioral sensitivity (as measured by grooming) to novelty-stress. This increased sensitivity is chronic as it was found at 0-2, 2-8 and 8-12 months following the onset of diabetes. These data indicate an enhanced sensitivity of the SDR to stress. These data may also be indicative of a maladaptive physiological stress response in the SDR.

10) The insulin treated SDR (4-8 months diabetic) has a significantly greater aversion to the anxiogenic areas of the elevated plus maze and open field.

11) Taken together, the findings described in points 9 and 10 may be indicative of a general deficit in the exploratory response in the SDR. The physiological substrates of this deficit remain to be elucidated.

12) Overall, the data from this thesis suggest that some of the behavioral, autonomic, and endocrine derangements associated with diabetes may be accounted for in part by alterations in brain monoamine functioning. These results may have clinical implications in the realm of neuro-endocrinological and/or behavioral complications associated with insulin dependent diabetes mellitus.

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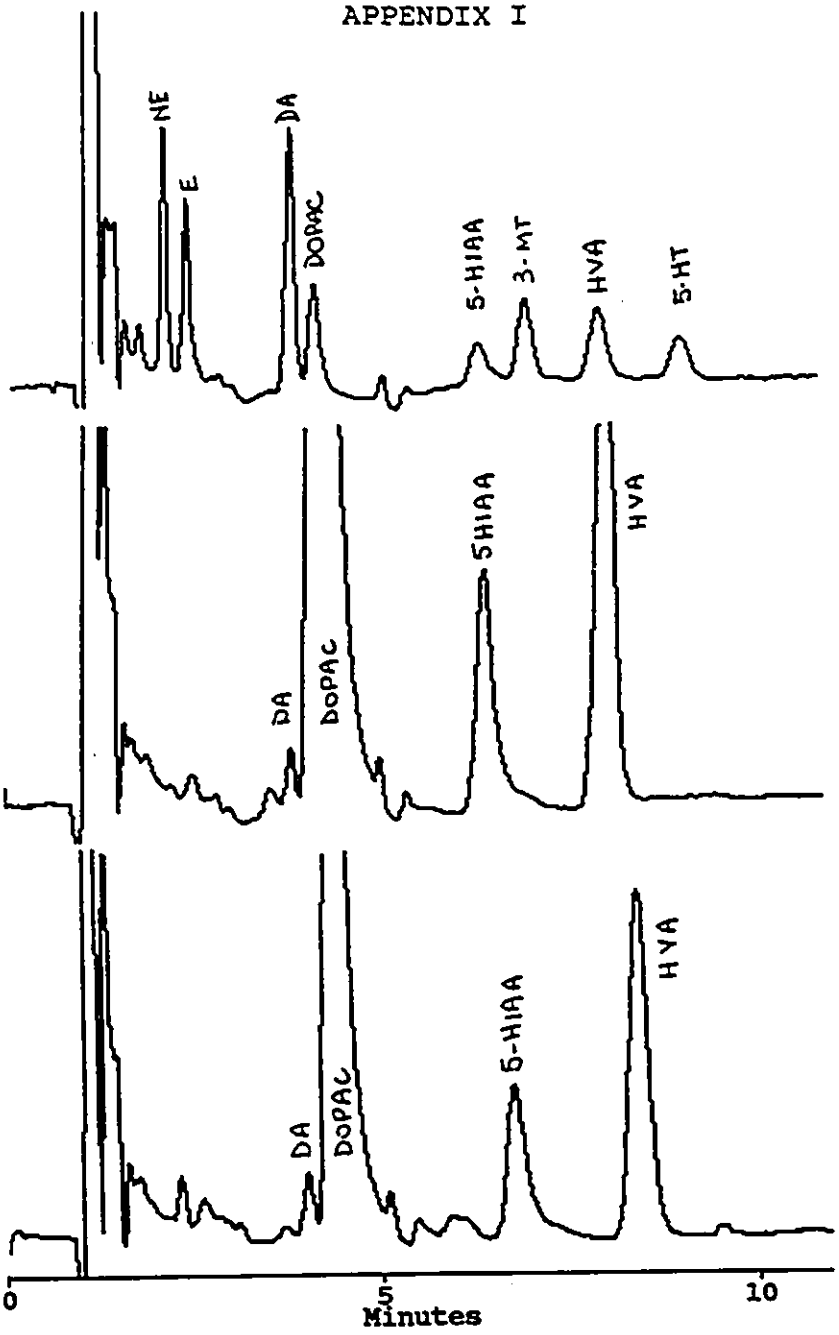
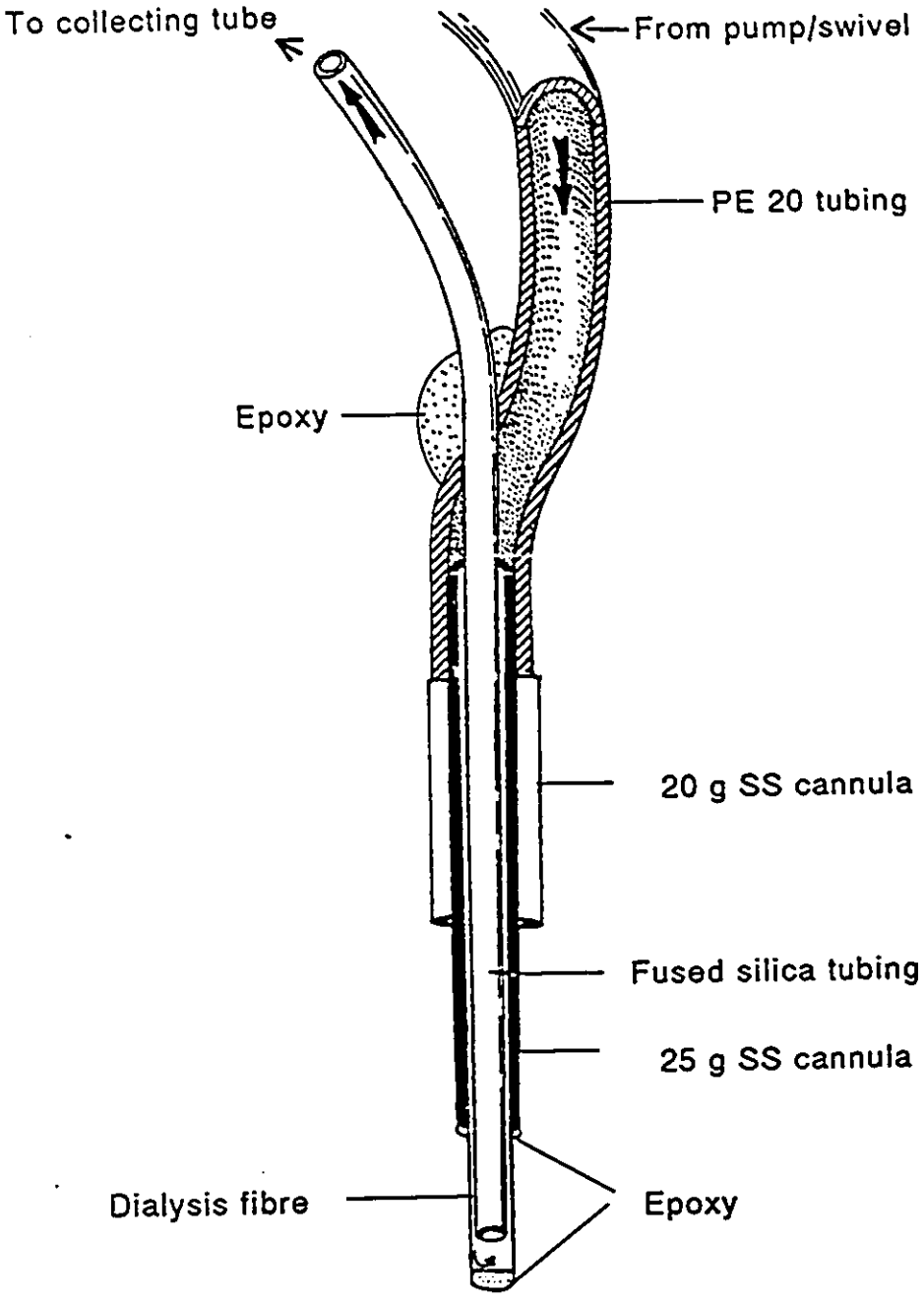
APPENDIX I



Chromatograms as obtained from the HPLC system. All chromotagrams were at an attenuation of 250 over a period of 15 min. The upper panel is a chromatogram of a standard solution (injection volume 20μl). The middle panel is a chromatogram of dialysate from the nucleus accumbens of a diabetic animal (30μl injection volume). The lowest panel is a chromatogram of dialysate from the nucleus accumbens of a control animal (30μl injection volume).

APPENDIX II



Schematic illustration of a concentric style dialysis probe as constructed in our laboratory. SS represents stainless steel.