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DOPAMINERGIC SYSTEM INVOLVEMENT IN THE MEMORY-MODULATING EFFECTS OF GLUCOSE

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Presented in Partial Fulfillment of the Requirements for the Degree of

Master of Arts

Specialization in Neuroscience

University of Ottawa

December 1998

ACKNOWLEDGMENTS

This research was supported by grants from the Natural Sciences and Engineering Research Council of Canada and from the University of Ottawa.

I would like to thank Dr. Claude Messier for his advice and supervision in the development, execution, and writing of this thesis.

I would also like to thank my family for their patience, understanding, and support throughout this project.

ABSTRACT

Darren G. Abrams

Dopaminergic System Involvement in the Memory-Modulating Effects of Glucose

We examined whether the dopaminergic system plays a role in the ability of glucose to improve memory. Specifically, we were interested in determining if glucose could reverse a memory-impairment produced by a dopaminergic D_2/D_3 receptor antagonist. The first three experiments used an operant training paradigm to test memory. Experiment 1 established the most effective memory-improving dose of glucose in our specific memory task. Experiment 2 was designed to establish which dose of eticlopride, a dopaminergic D_2/D_3 antagonist, was most effective at impairing memory in our specific task. Experiment 3 examined the effect of an injection of a 3 g/kg dose of glucose on the memory-impairing effect of a 0.1 mg/kg dose of eticlopride. Finally, Experiment 4 examined the effect of an injection of LY-171555, a dopaminergic D_2 agonist, on circulating blood glucose levels. The results of Experiment 1 demonstrated that a 3 and 4 g/kg dose of glucose significantly improved memory. Experiment 2 revealed that a 0.1 mg/kg dose of eticlopride significantly impaired memory. Experiment 3 demonstrated that glucose could not reverse the memory-impairment produced by eticlopride and Experiment 4 showed that LY - 171555 did not result in an increase in circulating blood glucose.

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GENERAL INTRODUCTION

How are Memories Modulated?

It has been recognized for a long time that memory can be modulated; either improved or impaired. The study of the brain mechanisms that underlie this modulation have been conducted within the theoretical framework of the memory consolidation hypothesis (Gold & McGaugh, 1975).

This theory holds that new memory traces are kept in a labile state susceptible to interference or facilitation. The trace is stored in a more permanent way later. This transition from a labile state to a more permanent state is called memory consolidation (Gold & McGaugh, 1975).

Three arguments are primarily responsible for the consolidation hypothesis: 1) studies that demonstrated post-training improvement of memory (Huston, Mondadori, & Waser, 1974), 2) studies that have demonstrated post-training impairment of memory (Kesner & Conner, 1972), and 3) studies that demonstrated a time gradient for post-training memory improvement and impairment (Huston, et. al., 1974; Kesner & Connor, 1972).

Experiments that have demonstrated post-training memory modulation through the use of reinforcers, either positive or negative, lend support to Gold and McGaugh's (1975) theory. The memory modulating effect of post-training reinforcers was demonstrated in an early study by Huston, et. al. (1974). This study showed that a food reward provided to animals immediately after training on a passive avoidance task improved performance, but had no effect when it was

provided before training. The effect was also diminished when the post-training reward was delayed. Messier & White (1984) extended these findings by demonstrating that drinking sucrose solutions immediately after training on a conditioned emotional response also improved retention.

White and Legree (1984) also demonstrated the non-contingent effect of negative post - training reinforcers on memory. In this study, they examined the effect of a post-training aversive electric shock on memory consolidation. Rats were trained in a conditional emotional response paradigm with two paired presentations of a tone and a mild shock. Immediately following training, the animals were removed from the training apparatus and given a single strong shock. Twenty-four hours later the rats were tested for the effect of the tone on drinking. The results demonstrated improved retention in the animals that received the immediate post-training non-contingent shock.

These results hint at one of the central questions associated with memory consolidation: Why are certain traces consolidated and others not. For example, we do not remember everything that happened to us in a day; only certain events are well remembered.

Earlier theorists have suggested that the selection of memories to be consolidated depends on the importance of those events for the organism. This view was taken as early as 1933 by Thorndike who, in his formulation of the Law of Effect, suggested that unconditioned stimuli can promote memory (Thorndike, 1933).

The idea that reinforcement could strengthen memories was

developed later by other theorists (Milner, 1970; Huston, Mueller, & Mondadori, 1977; Landauer, 1969; Routtenberg, 1975). Gold and McGaugh (1975) in their formulation of the consolidation process also postulated that memories are modulated by physiological changes in arousal or hormonal levels that are induced by a new learning situation. The search for these physiological changes involved examining the physiological consequences of either aversive reinforcement, such as electric shocks (McGaugh, 1989) or appetitive reinforcers such as food or sugar sweetened water (Messier & White, 1984). These studies led to the discovery that raising blood glucose levels modulated memory (Gold, 1986; Messier & White, 1987). These experiments showed that some aspects of the post-ingestive consequences of food intake appeared to promote memory for events surrounding a new learning situation. Because in the past, most studies investigating memory modulation had focused on pharmacological agents and synaptic mechanisms, the facilitative effect of glucose on memory was studied within this context. Several researchers examined the interactions of glucose and neurotransmitter function in the context of memory modulation (Durkin, Messier, de Boer, & Westerink, 1992; Messier, Durkin, Mrabet, & Destrade, 1990; Ragozzino, Wenk, & Gold, 1994; Stone, Walker, Gold, & Gold, 1991).

The next section will briefly discuss some results implicating the classic neurotransmitter systems, their interaction with memory function, and whether glucose appears to act on memory through these systems.

Classes of Neurotransmitters that Modulate Memory

Based on the hypothesis that it is the underlying neural and hormonal substrates that are responsible for the memory-modulating effects of appetitive

and aversive reinforcers, a number of studies have examined the neurochemical systems in the brain which can modulate memory. It has been demonstrated, through the use of neurotransmitter-related drugs, that the cholinergic, dopaminergic, GABAergic, glutaminergic, and opioidergic systems can all modulate memory, in either a positive or negative manner (Gold, 1994). Moreover, it has been shown that hormones, specifically epinephrine, can modulate memory as well (McGaugh, 1989). More recently, microdialysis has been employed to further substantiate the relationship between certain neurotransmitter systems and memory (Orsetti, Casamenti, & Pepeu, 1996; Ragozzino, Unick, & Gold, 1996). In the following section, a brief review of systemic injection studies, central injection studies, and microdialysis studies will be presented.

The GABAergic System and Memory

The GABAergic system has been studied quite thoroughly in terms of its relationship to memory consolidation. The results obtained from GABAergic studies demonstrate that GABAergic agonists impair memory and GABAergic antagonists improve memory (Castellano, Introini-Collison, Pavone, & McGaugh, 1989; Castellano & McGaugh, 1989). Castellano and McGaugh (1989) studied the effect of picrotoxin, a GABAergic antagonist, on memory consolidation in mice. The mice were trained on a step-through inhibitory avoidance task and immediately following the training session were injected with either saline or picrotoxin subcutaneously. The results demonstrated that step-through latencies of the mice that received picrotoxin were significantly higher than those of the saline controls. Thus, demonstrating that picrotoxin has a memory-improving effect. The memory-improving actions of GABAergic antagonists has been

demonstrated in other studies as well. In a study conducted by Castellano, et. al. (1989) the effects of two GABAergic antagonists, picrotoxin and bicuculline, and one GABAergic agonist, muscimol, on memory consolidation were studied using a one-trial inhibitory avoidance task. The results demonstrated that both of the antagonists enhanced, whereas muscimol impaired retention on a 24-hr test. Baclofen, a GABA - B receptor agonist has also been shown to impair working memory in a radial arm maze task (Stackman & Walsh, 1994). Hence, these studies not only support the results of the Castellano and McGaugh (1989) study but also demonstrate that GABAergic agonists have the reverse effects of the antagonists.

Diazepam, a prototypical benzodiazepine (BZD) which acts on the GABA/BZD receptor, has been shown to impair the retention of spatial information in a group of rats trained to locate a hidden platform (Brioni & Arolfo, 1991). Additionally, it was shown to produce significant dose - dependent memory impairments in rhesus monkeys trained on an operant task (Schulze, Slikker, & Paule, 1989). Therefore, it appears as though the GABA/BZD receptor is important for the retention of spatial information and operant responses.

Central injection studies examining the role of the GABAergic system in memory modulation have supported the findings of the systemic injection studies. For example, intraseptal injections of the GABA - A receptor agonist muscimol have been shown to impair working memory (Parent, Laurey, Wilkniss, & Gold, 1997) as have intrahippocampal injections of muscimol (Givens & Olton, 1990; Ohno, Yamamoto, & Watanabe, 1992). Moreover, a memory improving effect has been demonstrated by intrahippocampal injections of the GABA-A receptor

antagonist, picrotoxin (Izquierdo, da Cunha, Rosat, Jerusalinsky, Ferreira, & Medina, 1992).

The Opioid System and Memory

The opioid system also plays a role in learning and memory. Opioid agonists and antagonists have both been used as a means to determine the role of the opioid system in memory consolidation. Like the GABAergic system, opioid agonists have been shown to impair memory (Gallagher & Knapp, 1978) whereas opioid antagonists improve memory (Aigner & Mishkin, 1988; Canali, Cook, & Miczek, 1990). In a study conducted by Canali and his colleagues (1990) the effects of two opioid antagonists, naloxone and naltrexone, on the spatial working memory of rats was examined. The results demonstrated that both drugs significantly improved working memory-based performance in the radial maze. The memory-improving effects of naloxone, which is a mu opioid receptor antagonist, have also been demonstrated in non-human primates. In a study by Aigner and Mishkin (1988) the effects of naloxone on visual recognition in monkeys was examined. The results demonstrated that naloxone produced a significant increase in the number of objects correctly recognized. The delta opioid receptor antagonist ICI 174,864, has also been shown to improve memory. In a study by Ilyutchenok and Dubrovina (1995), ICI 174,864 and naloxone were shown to attenuate amnesia and forgetting as indicated by prolongation of step-through latency. However, microinjections of dynorphin, an opioid peptide which acts on both the mu and kappa receptors, into the hippocampus has been shown to impair spatial learning in rats (McDaniel, Munday, & Tilson, 1990). Furthermore, dynorphin and the kappa opioid receptor agonist U69,593 have been shown to inhibit induction of long-term

potentiation in the dentate gyrus of the Guinea pig hippocampus (Terman, Wagner, & Chavkin, 1994).

The Glutaminergic System and Memory

The role of the glutaminergic system in learning and memory has also been studied quite extensively. It seems that the NMDA receptor has received most of the attention. This system appears to be similar to the cholinergic and dopaminergic systems, in that, glutaminergic agonists tend to improve memory (Flood, Baker, & Davis, 1990), whereas, glutaminergic antagonists tend to impair it (Ward, Mason, & Abraham, 1990). Flood and his colleagues (1990) studied the effect of intracerebroventricular injections of various classes of glutamate receptor agonists and antagonists. Mice were trained on a shock avoidance learning paradigm and then, immediately following training, injected centrally with either non-NMDA or NMDA agonists and antagonists. Retention was tested one week after training. The results revealed that the NMDA and non-NMDA agonists, kainic acid and quisqualic acid, enhanced retention in a dose-dependent manner, whereas, both the NMDA and non-NMDA antagonists produced dose-dependent impairments of retention. The glutamate receptor antagonists AP5 and CNQX when infused into the amygdala or hippocampus impair memory as well (Jerusalinsky, Ferreira, Walz, Da Silva, Bianchin, Ruschel, Zanatta, Medina, & Izquierdo, 1992). The NMDA antagonists CPP and MK-801 have also been shown to impair radial-arm maze performance in rats (Ward, et. al., 1990). Moreover, the NMDA antagonist NPC 12626 has been shown to produce impairments in spontaneous alternation performance of mice (Walker & Gold, 1992). Therefore, it appears as though the glutaminergic system, especially the NMDA receptor, plays a role in a variety of memory paradigms.

Epinephrine and Memory

A large amount of research suggests that epinephrine plays a key role in modulating memory consolidation. Epinephrine at low doses generally enhances retention, whereas at higher doses it tends to impair retention (Sternberg, Isaacs, Gold, & McGaugh, 1985). When epinephrine was administered shortly after training, at physiological significant circulating levels, memory tested at a later time was enhanced (Gold, 1991). Epinephrine has been shown to influence later retention in rats and mice trained on both aversively (Gold & Zornetzer, 1983) and appetitively motivated tasks (Sternberg, et. al., 1985). Peripherally administered epinephrine has also been shown to enhance the development of long-term potentiation in a dose-related manner (Gold, Delanoy, & Merrin, 1984). Studies of the release of peripheral epinephrine demonstrate that injections of the hormone enhance or impair memory in a dose- and time-related manner. They also suggest that post-training circulating levels of epinephrine predict later memory effects when the hormone is either released or injected exogenously. Finally, peripherally administered adrenerigic antagonists block the effects on memory of a wide variety of other drugs (Gold, 1991).

Because epinephrine does not enter the brain from blood in large amounts, it is postulated that epinephrine must act on a peripheral substrate to modulate memory. The substrate which has received the most attention is glucose, primarily because a major physiological action of epinephrine is to release glucose from the liver, which results in increased circulating glucose levels (Gold, 1994).

The Cholinergic System and Memory

The neurotransmitter system which has received the most attention in memory research has been the cholinergic system. Many experiments have looked at the memory modulating effects of cholinergic agonists and antagonists in both animals (Aigner & Mishkin, 1986; Levin & Rose, 1991; Riekkinen, Sirvio, Aaltonen, & Reikkinen, 1990) and humans (Sevush, Guterman, & Villalon, 1991).

Systemic Injection Studies

In an experiment conducted by Levin and Rose (1991), the relationship between the cholinergic system and memory was examined. In this experiment, rats were trained on a working memory task in an 8-arm radial maze. Following training, the rats received acute injections of the muscarinic or nicotinic agonists, pilocarpine and nicotine, alone or in combination with the muscarinic or nicotinic antagonists, scopolamine and mecamylamine. The results demonstrated that nicotine administration caused a significant improvement in the rats ability to remember where they had been, compared to the saline controls, and that pilocarpine caused a marginally significant memory improvement. However, the memory improvement caused by these two cholinergic agonists was reversed by both the nicotinic and muscarinic antagonists. In a similar study, the effect of physostigmine (a cholinesterase inhibitor) and scopolamine on visual recognition in rhesus monkeys was examined using a delayed non-matching-to-sample task. Physostigmine and scopolamine produced dose-related improvements and impairments, respectively, in the number of objects correctly remembered (Aigner & Mishkin, 1986).

Similar results have also been found in human studies. Oral physostigmine therapy has been shown to improve verbal learning in patients with dementia of the Alzheimer type (Sevush, et. al., 1991) and cholinergic antagonists have impaired memory in normal human subjects (Drachman & Sahakian, 1980). In a study conducted by Drachman and Leavitt (1974) it was shown that blockade of central muscarinic receptors, by scopolamine, induced memory deficits in young subjects qualitatively similar to those occurring naturally in aged subjects.

Central Injection Studies

Further evidence for the memory impairing effect of cholinergic antagonists, such as scopolamine and mecamylamine, has also been gathered by Riekkinen, et. al. (1990). In their experiment, rats received central injections of either scopolamine, mecamylamine, or a combination of the two, and were then trained on both spatial (Morris water-maze) and passive avoidance learning tasks. The results demonstrated that scopolamine, mecamylamine, and the combination of the two impaired acquisition of the water-maze task and retention of the passive-avoidance task.

Scopolamine, injected directly into the amygdala has also been shown to impair working and reference memory in a double Y-maze task in rats (Ingles, Beninger, Jhamandas, & Boegman, 1993). Moreover, two other anticholinergic drugs, atropine and pirenzepine, when injected introcerebroventricularly, significantly impaired acquisition of an 8-arm radial maze task (Sala, Braida, Calcaterra, Leon, Comotti, Gianola, & Gori, 1991). It has also been shown by Ohno, Yamamoto, and Watanabe (1994) that direct injections of pirenzepine, a

selective M1 muscarinic receptor antagonist, into the hippocampus impairs working memory performance. These researchers also demonstrated that the M_2 muscarinic receptor antagonist, methoctramine, impairs working memory when injected into the hippocampus. The ventral tegmental area (VTA) and the substantia nigra (SN) have also been examined as possible sites for cholinergic effects on memory. In a study by Levin, Briggs, Christopher, & Auman (1994) the effect of direct injections of the nicotinic antagonist, mecamylamine, into the VTA and the SN was examined. The results demonstrated that mecamylamine impaired memory when injected into both the VTA and SN.

Microdialysis Studies

The potential role of acetylcholine in memory formation has also obtained support through in vivo release experiments utilizing microdialysis technique. In a study by Orsetti, et. al. (1996) it was demonstrated that acetylcholine release in the hippocampus and cortex of rats was increased during the acquisition of an operant behavior. Acetylcholine release in the hippocampus has also been shown to increase during the acquisition of a spatial task (Ragozzino, et. al., 1997). Furthermore, Ikegami (1994) demonstrated that aged mice, who performed poorly on a spatial memory task, had significant decreased ACh content in the hippocampus and striatum when compared to young mice who performed well on the same task. Microdialysis studies have also demonstrated that memory-improving drugs, such as nimodipine, elevate hippocampal ACh levels as well (Levy, Kong, Stillman, Shukitt-Hale, Kadar, Rauch, & Lieberman, 1991).

These results demonstrate that the cholinergic system plays a very important role in memory modulation. Cholinergic agonists, both muscarinic and

nicotinic, exert a positive effect by enhancing memory, and cholinergic antagonists have a negative effect by impairing memory. Moreover, these positive and negative effects seem to be pervasive across tasks (i. e., working/spatial memory, recognition memory, and passive avoidance learning) as well as species (i. e., humans, primates, & rodents). Furthermore, acetylcholine appears to be released during acquisition of a memory task, which would indicate that cholinergic systems are important in memory formation.

The Dopaminergic System and Memory

Another neurotransmitter system which has received attention in terms of its role in learning and memory is the dopaminergic system. Dopaminergic agonists of both the D_1 and D_2 receptor subtype have been shown to improve memory consolidation, whereas, D_1 and D_2 antagonists have been shown to impair it (McGaugh, 1989; Castellano, Cestari, Cabib, & Puglisi-Allegra, 1991).

Systemic injection studies

In a study conducted by Castellano, et. al. (1991) the question of whether post-training administration of dopaminergic agonists and antagonists would affect memory consolidation was examined. Mice were trained on a step-through inhibitory avoidance task, which was then followed by an injection of either the D_1 or D_2 agonists SKF 38393 and LY 171555, or the D_1 or D_2 antagonists SCH 23390 and (-)sulpiride. Twenty-four hours later, the mice were tested. The results demonstrated that post-training administration of the selective D_1 and D_2 agonists dose-dependently improved retention of the inhibitory avoidance response, while the selective D_1 and D_2 antagonists produced an impairment of

retention. The D_2 antagonist (-)sulpiride has also been shown to impair the retention of conditioned avoidance behavior when injected centrally into the ventral striatum of the rat (Wadenberg, Ericson, Magnusson, Ahlenius, 1990).

Other dopaminergic antagonists have also been shown to produce memory impairments. For example, Eticlopride, a dopamine D_2 and D_3 receptor antagonist, has been shown to dose-dependently impair the performance of rats on a conditioned avoidance task. Moreover, when combined with the D_1 antagonist SCH23390 performance was impaired in an additive fashion (White & Rebec, 1994).

Conversely dopaminergic agonists have also been shown to produce memory facilitation in other studies. Packard and White (1989) examined the role of dopamine receptor subtypes in the acquisition of two memory tasks in the 8-arm radial maze (win-shift & win-stay). Rats received post-training injections of an indirect dopamine receptor agonist, d-amphetamine, a selective D_2 receptor agonist, LY 171555, and a selective D_1 receptor agonist, SKF 38393. The results demonstrated that both d-amphetamine and LY 171555 significantly improved performance on both of the radial arm maze tasks, however, SKF 383393 had no effect. The authors concluded that the D_2 receptor appears to mediate the memory-improving properties of dopamine agonists, specifically on working memory tasks.

Minaprine, an atypical antidepressant drug, which reportedly facilitates dopaminergic activity (Biziere, Kan, Goniot, Garattini, & Wermuth, 1983) has also been shown to enhance memory storage in mice. Puglisi-Allegra, Cabib, Cestari, and Castellano (1994) found that post-training administration of

minaprine dose-dependently improved retention of an inhibitory avoidance response. Moreover, this improvement was reversed by pretreatment with either of the selective D_1 or D_2 receptor antagonists SCH 23390 and (-)sulpiride. Hence, indicating that both D_1 and D_2 receptor subtypes are similarly involved in the effects of minaprine on memory consolidation.

Central Injection Studies

Direct injections of specific dopaminergic agonists and antagonists into different areas of the brain have also been conducted. In a study by White and Viaud (1991) the effect of direct injections of the dopaminergic D_2 receptor agonist, LY171555, and the indirect catecholamine agonist, d-amphetamine, into the posteroventral (PV) and ventrolateral (VL) areas of the caudate nucleus, on a conditioned emotional response (CER) was examined. The results of the study demonstrated that post-training injection of the two drugs into the VL improved retention of a CER with an olfactory CS, but had no effect on a CER with a visual CS. However, when the same two drugs were injected into the PV, the reverse occurred.

Packard and White (1991) have also demonstrated that d-amphetamine, an indirect dopamine agonist, LY-171555, a D_2 agonist, and SKF-38393, a D_1 agonist, when injected directly into the caudate nucleus improved acquisition of a win-stay task but had no effect on a win-shift task. However, when these three drugs were injected into the hippocampus retention of a win-shift task was improved but there was no effect on the win-stay task.

The dopaminergic D_1 receptors in the prefrontal cortex (PFC) have also been implicated in memory formation. Sawaguchi and Goldman-Rakic (1994) demonstrated that direct injections of the dopamine D_1 antagonists SCH23390 and SCH39166, as well as the indirect dopaminergic antagonist, haloperidol, into the PFC impaired the performance of rhesus monkeys on a oculomotor delayed-response task.

Therefore, it can be stated that the dopaminergic system seems to be an important neurotransmitter system in memory consolidation. Also, in a similar fashion as with the cholinergic system, dopaminergic agonists exert a positive effect by facilitating memory consolidation and dopaminergic antagonists have a negative effect by impairing consolidation. It should be noted that the dopaminergic system, does not solely consist of the D_1 and D_2 receptor subtypes, but that there are several other receptor subtypes which range from D_3 to D_5. These other receptor subtypes were not covered in this section because they have not been studied extensively in relation to memory consolidation processes.

Glucose and Memory

Based on the findings of the epinephrine studies, it became important to determine whether glucose itself could produce memory improvement (Gold, 1986). Hence, a number of studies were conducted, using several tasks, to investigate the potential memory-modulating effects of glucose (Gold, 1986; Messier & Destrade, 1988; Messier & White, 1987). Messier and White (1987) examined the effect of post-training injections of several doses of glucose on the retention of a conditioned emotional response in rats. The results of the study demonstrated that a 2 g/kg dose of glucose significantly improved retention

compared to control conditions. Gold (1986) also demonstrated the memory-improving action of glucose. Rats were trained in a one-trial inhibitory avoidance task and then, immediately after the training footshock, received an injection of glucose (1-500 mg/kg). When the animals were tested for retention 24 hrs later, the animals that received 10 or 100 mg/kg doses of glucose showed enhanced retention. Messier and Destrade (1988) looked at the effect of glucose on memory in an appetitively motivated task. The mice were trained to bar-press for food reinforcers in an operant box and then were systemically injected with glucose (2, 3, 4, & 5 g/kg). When the mice were tested for retention of the bar-pressing behavior, 24 hrs after training, the group that received 3 g/kg performed significantly better than the controls.

Packard and White (1990) also looked at the effect of post-training injections of glucose on appetitive tasks. Two appetitive tasks in an 8-arm radial maze were studied: (1) win-stay and (2) win-shift. The results demonstrated that a glucose dose of 2 g/kg significantly improved memory for the win-stay task, whereas, glucose doses of both 2 g/kg and 100 mg/kg improved memory for the win-shift task. Therefore, the above studies lead to the conclusion that glucose improves memory in both aversively motivated and appetitively motivated tasks. It is also possible to conclude that, generally, there are two optimal doses of glucose that produce a memory-improving effect, one at around 100 mg/kg and the other around 2-3 g/kg.

A major question in the search for glucose's role in learning and memory is how glucose produces its memory-improving effect. Research examining this question has pointed to two different possible physiological mechanisms of action. One mechanism of action originating outside the brain in the peripheral nervous

system, whereas, the other involves direct interaction of glucose with brain function (White, 1991). This hypothesis was first proposed on the basis of the results demonstrating that there were two optimal memory-improving doses of glucose.

Research examining glucose's physiological mechanism of action outside the brain has indicated that glucose may act on the liver, resulting in a peripheral neural signal being sent to the central nervous system, thus influencing the physiological processes underlying memory formation. This hypothesis is based on two sets of experimental data. The first set of data demonstrated that when rats received coeliac ganglion lesions, which block most of the efferents coming from the liver, large doses of glucose were unable to improve memory in these animals (White,1991). The second set of data underlying the suggestion that glucose may act peripherally comes from experiments which have studied fructose, a sugar that does not cross the blood-brain barrier and does not produce a significant rise in blood glucose (Rodriguez, Horne, Mondragon, & Phelps, 1994). The results from their study indicated that both glucose and fructose affected memory in the same way. Moreover, fructose was shown to improve memory at the same doses as glucose in a learning task. Messier and White (1987) also showed that the same doses of fructose and glucose affected memory in the same way. Therefore, these experiments suggest that glucose and other related substances can act on peripheral mechanisms which in turn affect memory processing.

Possible brain mechanisms underlying the effect of glucose on memory have been suggested primarily from data documenting interactions between glucose and neurotransmitter functions (Gold, 1992; Sara, 1988). Several pharmacological experiments have indicated a possible connection between

glucose and the cholinergic system (Kopf & Baratti, 1994; Messier, Durkin, Mrabet & Destrade, 1990). According to Messier et al. (1990), glucose may improve memory by increasing the availability of acetylCoenzymeA (one of the acetylcholine precursor), which facilitates acetylcholine re-synthesis, at times when there is a high neuronal demand for acetylcholine. Further support for the connection between glucose and the cholinergic system comes from studies which have looked at the effect of glucose on scopolamine-induced amnesia (Stone, Croul, & Gold,1988; Stone, Rudd, & Gold, 1992; Stone, Walser, Gold, & Gold, 1991). In these studies, a post-training injection of scopolamine, a competitive cholinergic muscarinic antagonist, along with a post-training injection of glucose, reversed scopolamine-induced amnesia. It was argued, therefore, that glucose interacted with the cholinergic system to reverse the memory-impairing effect of scopolamine. A recent study conducted by Ragozzino, Unick, and Gold (1996), provided further evidence for a connection between glucose and the cholinergic system. These researchers examined whether enhancement of spontaneous alternation performance by systemic glucose treatment is related to an increase in hippocampal ACh output. Rats received intraperitoneal injections of saline or glucose and, 24-min later, were tested for spontaneous alternation behavior combined with microdialysis collection. The results indicated that glucose at 250 mg/kg, but not 100 or 1000 mg/kg, improved memory and ACh output compared to the saline controls. More interestingly, however, none of the glucose doses affected hippocampal ACh output when the rats remained in the holding chamber. The results indicate that glucose may enhance memory by increasing the release of ACh and that this only occurs during a period when the animals are challenged with a memory task.

Although the cholinergic system appears to be the means through which glucose acts to improve memory, other neural systems may also be involved in learning and memory, as described above. In fact, a number of studies have looked at whether glucose could reverse memory impairments produced by either agonists or antagonists which act on these other systems (Lennartz & Gold, 1995; Ragozzino & Gold, 1991; Ragozzino, Parker, & Gold,1992).

Glucose has been shown to attenuate morphine-induced impairments in spontaneous alternation and inhibitory avoidance, through both peripheral (Ragozzino, et. al., 1992; Stone, et. al., 1991) and central injections (Ragozzino & Gold, 1994). However, Lennartz and Gold (1995) were unable to reverse memory-impairments produced by MK-801, an NMDA antagonist, with glucose. Therefore, it appears as though glucose may interact with some systems and not with others.

Rationale of Experiments

One system that has received little or no attention in terms of its relationship with glucose, has been the dopaminergic system. Moreover, it has been suggested that the dopaminergic system may interact with the cholinergic system in the regulation of memory storage (Gasbarri, Introini-Collison, Packard, Pacitti, & McGaugh, 1993; Levin & Rose, 1991). In a study by Gasbarri, et. al. (1993), the interaction of cholinergic-dopaminergic systems in the regulation of memory storage in aversively motivated tasks was examined. Mice were trained in an inhibitory-avoidance task and in a Y-maze discrimination task. Following training, mice received injections of either cholinergic or dopaminergic agonists and/or antagonists. Retention was tested 48 hrs later. The

results demonstrated that in the inhibitory-avoidance task, quinpirole (a dopaminergic D_2 agonist) blocked the memory-impairing effects of the muscarinic cholinergic antagonist atropine, and that sulpiride, a dopaminergic D_2 antagonist, significantly attenuated the memory enhancing effects of the muscarinic cholinergic agonist oxotremorine. Whereas, in the Y-maze task atropine blocked the memory enhancing effects of quinpirole, and oxotremorine attenuated the memory impairing effect of sulpiride. These results suggest an interaction of the dopaminergic and cholinergic systems, whereby, one system mediates the action of the other depending on the memory task.

The dopaminergic D_1 receptor has also been shown to interact with the cholinergic system. Levin and Rose (1991) looked at the effect of the D_1 receptor agonist SCH 38393 on the memory-impairing effect of scopolamine, a muscarinic antagonist. The results showed that the D_1 agonist significantly alleviated a scopolamine-induced memory deficit. Similarly other studies have shown

interactions between the dopaminergic and cholinergic systems in relation to memory modulation. For instance, the memory-impairing effects of scopolamine have been shown to be reversed by the dopamine receptor blocker haloperidol (Levin, McGurk, Rose, & Butcher, 1990), whereas, the nicotinic cholinergic agonist nicotine has been shown to reverse the memory-impairing effect of SKF38393, a D_1 agonist, in a choice accuracy test in the radial-arm maze (Levin & Eisner, 1994). However, other results have shown that the memory-improving effects of nicotine can be reversed by the dopaminergic D_1 and D_2 receptor antagonists, SCH 23390 and (-)sulpiride (Nitta, Katono, Itoh, Hasegawa, & Nabeshima, 1994). In fact, biochemical studies have shown that dopamine agonists can increase acetylcholine release in the cortex and hippocampus (Acquas, Day, & Fibiger, 1994).

Overall, these results, although somewhat contradictory, demonstrate a definite interaction between the dopaminergic and cholinergic systems. Therefore, the dopaminergic system, because of its interaction with the cholinergic system, appears to be a very interesting system to investigate in relation to glucose's effects on memory. Hence, in the present study we decided to look at the interaction between glucose and the effects of eticlopride, a dopamine D_2/D_3 receptor antagonist, and quinpirole, a dopamine D_2 receptor agonist, on memory.

Experiment 1

In the present experiment, we examined the effect of a variety of doses of glucose (0.05, 0.1, 0.5, 1, 2, 3, & 4 g/kg) on the bar-pressing behavior of mice in an operant box using an operant training paradigm. The purpose of Experiment 1 was to determine which dose (s) of glucose improved the animals retention of the bar-pressing response. The experiments conducted by Gold (1986), Messier and White (1987), and Messier and Destrade (1988) revealed that more than one dose of glucose was effective at producing a memory improvement. This difference in dose effectiveness could be related to the type of memory task being used, therefore, it was important to establish the most effective dose in our memory task.

MATERIALS AND METHODS

SUBJECTS

The subjects in the present experiment were male BALB/cByJ mice (refer to Fig. 1 for group sizes). The mice weighted between 26-38 g at the start of the experiment. Throughout the experiment, the mice were maintained on a 12/12 h dark/light cycle in a temperature controlled room (20°C).

MATERIALS

The mice were tested in an operant cage (12.5 x 13.5 x 18.5 cm) made of black Plexiglas on three sides and translucent Plexiglas on the front with a grid floor (Van den Bergen, Spratt, & Messier, 1997). A metal bar (3 x 6 cm) and a food cup extended from one wall and were separated by a 5-cm long partition so that, after a bar press, the mouse was required to go around the partition in order to retrieve the food. A pair of photocells were placed in front of the food-cup and the metal bar was connected to a control box. Therefore, a computer was able to monitor and record each bar-press and each occurrence of a broken food-cup photocell beam. If the animal did not take the food pellet after pressing the bar, the computer did not record it as a reinforced bar press and the pellet was automatically discarded.

The operant cage controls were programmed for continous reinforcement (CRF 1). The reinforcers were small pieces of pasta (7 mg). All post-training injections were administered subcutaneously on the animal's back about 1 cm from the base of the tail. The saline injections consisted of 0.5 ml of a sterile

normal solution of sodium chloride. The glucose injections consisted of a sterile 50% (w/v) glucose solution. Injections were made in the animals holding room.

PROCEDURE

Animals from group cages were put into individual cages with food and water freely available for approximately one week prior to the start of the experiment. At the end of this week, all food was removed from the cages and mice were put on a 4-day food restricted schedule designed to bring them to 90-95% of their ad libitum weights by the fifth day. The animals were given a few pasta reinforcers in their cages every day so that they were accustomed to the reinforcers on the training day.

On the fifth day of the experiment, each animal was individually placed in the operant cage and given 7 reinforced training trials. A reinforced trial was defined as a bar-press followed by the consumption of a reinforcer within approximately 30 seconds. Seven trials were used to produce partial learning so that the memory-improving effect of glucose could be observed.

Following the training trials, the animal was immediately removed from the operant cage and injected with saline, or glucose (0.05, 0.1, 0.5, 1, 2, 3, & 4 g/kg, s. c.), or no treatment. After the injection, each animal was returned to its cage. Five hours later, each animal received a food ration adjusted so that its weight the next day would be about 90-95% of its ad libitum weight.

Twenty-four hours after the end of training, each animal was placed back into the operant cage and given a 20-min retention test under a continous reinforcement (CRF 1) schedule. The number of reinforced responses made

during the 20-min period was used as a measure of retention of the bar-pressing training.

RESULTS

The dose-response curve data for glucose obtained during the retention test is summarized in Figure 1. The data was analyzed using a one-way analysis of variance followed by a priori comparisons between certain groups within the experiment. The results of the glucose-dose response curve demonstrated that glucose caused a dose-related increase in bar-pressing behavior. The results revealed an overall significant difference among groups ($F(13, 312) = 1.767$, $p < 0.05$). More specifically, the group of mice that received the 4 g/kg glucose injection emitted significantly more reinforced responses during the retention test ($p<0.05$) than the group that received no treatment and the group that received saline. This was also true for the group of mice that received the 3 g/kg glucose injection.

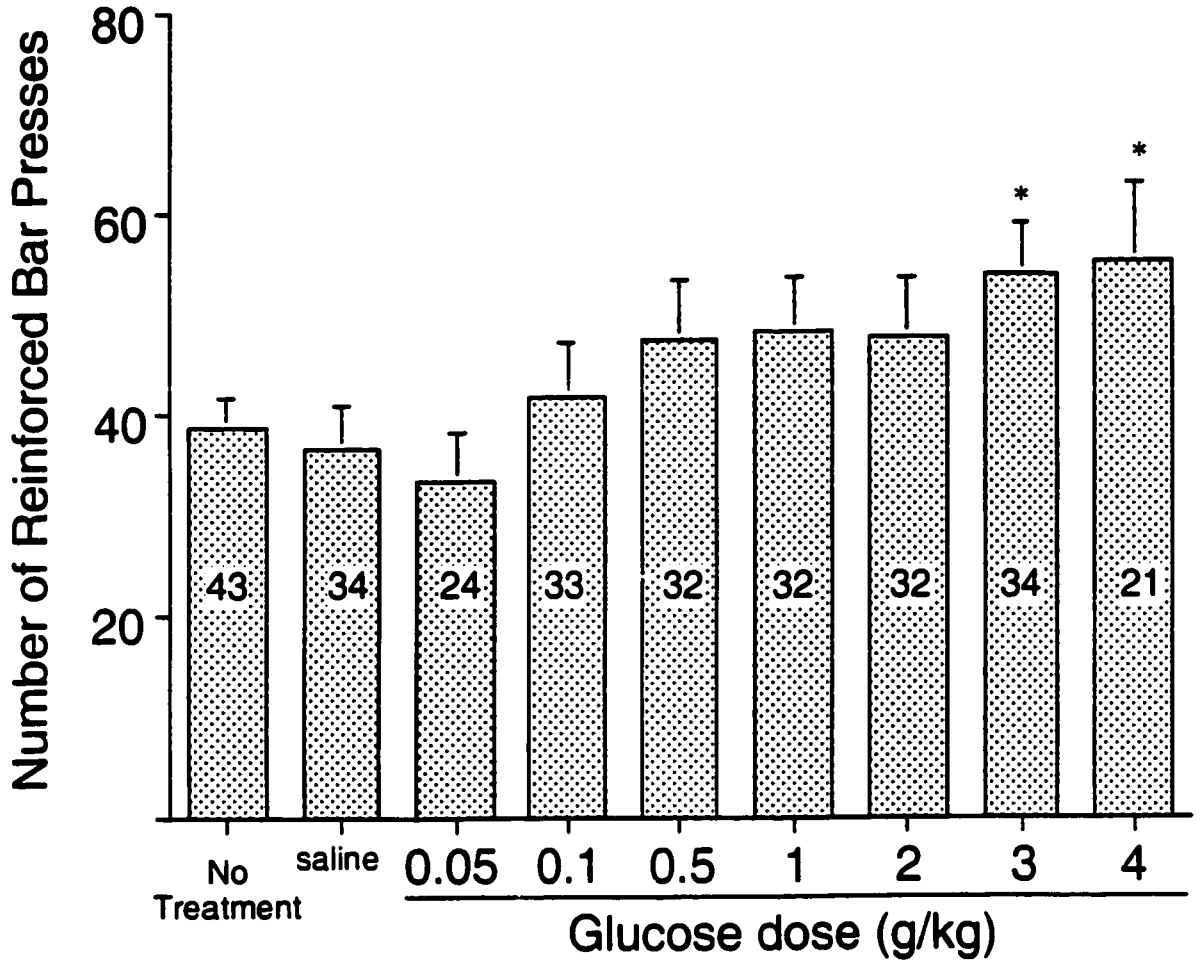

Figure 1: Number of reinforced responses (mean ± S.E.M.) made during the 20-min retention test, 24 h later by animals that received either saline, glucose or no treatment at the end of the training session.

DISCUSSION

The results of the present experiment showed that post-training injection of glucose immediately following an appetitively-motivated operant training produced increased operant responding the next day. This increase was interpreted as a demonstration that a 3 and 4 g/kg dose of glucose can retroactively and non-contingently improve memory.

The results of the present study are consistent with and support the findings of Messier and Destrade's (1988) glucose-dose response curve study. However, the results do tend to differ from other glucose-dose response curves (Messier & White, 1987; Gold, 1986; Rodriguez, Van Ausdle, Dhanens, & Mondragon, 1993). For example, Gold (1986) demonstrated that a 100 mg/kg dose of glucose was most effective at improving the memory of rats in a one-trial step through inhibitory avoidance task, and Messier and White (1987) demonstrated that a 2 g/kg dose of glucose improved retention of a conditioned emotional response. However, although the Messier and White (1987) study found that a 2 g/kg dose of glucose, as opposed to a 3 or 4 g/kg dose, was most effective at producing memory improvement, it is the large difference between the 100 mg/kg dose, reported in the Gold (1986) study, and the 3 and 4 g/kg dose found in the present study that is of most concern.

One possible explanation for the different optimal doses of glucose could be related to the strain and species of animals being used in the studies. For example, it has been shown that in some strains of mice (BALB/cAnNCrlBR) glucose does not produce memory-improvement at any dose (Messier, 1998).

Moreover, Gold's (1986) study was conducted with Sprague-Dawley rats, Messier and White's (1987) study was conducted with Long-Evans rats, and the present study was conducted with Balb/cbyJ mice.

Another possible explanation for the observed differences between the studies could be due to the type of learning task. In the Gold (1986) study, as well as the Messier and White (1987) study, aversively motivated tasks were used, whereas, in the present study an appetitively motivated task was used. To date there have not been any studies reporting the memory-improving effect of the 100 mg/kg dose of glucose with an appetitive task.

Although, these explanations are plausible they are somewhat less likely considering the relatively similar optimal doses of glucose found in the present study with mice (3-4 g/kg), in an appetitively motivated task, and those found by Messier and White (1987) with rats (2 g/kg), in an aversively motivated task. However, in the absence of the appropriate experimental data, such explanations cannot be ruled out.

A more likely explanation for the large difference between the two optimal doses of glucose is that they correspond to the activation of two distinct physiological mechanisms that both produce memory improvement. One of these mechanisms could be activated centrally, whereas, the other could be in the peripheral nervous system. Experiments have shown that fructose, a sugar that does not cross the blood - brain barrier and does not produce a significant rise in blood glucose, improved memory at the same doses as glucose in two learning tasks (Rodriguez, et. al.,1994). Moreover, it has been shown that glucose and fructose both improved memory at 2 and 3 g/kg but that fructose did not

improve memory at 100 mg/kg dose (White, 1991). These findings strongly suggest that the lower optimal dose of glucose may act centrally, whereas, the higher optimal dose may act peripherally.

The main purpose, however, of Experiment 1 was to establish the most effective dose of glucose in the present study. Therefore, since it was demonstrated that 3 and 4 g/kg of glucose improved retention of the operant training we went on to do the next series of studies with a dopaminergic drug.

Experiment 2

Dopaminergic antagonists which act on the D_1 and D_2 receptors have been studied extensively with respect to memory consolidation (Castellano, et. al., 1991; Packard & White, 1989). It has been widely demonstrated that dopaminergic D_2 antagonists, when injected both centrally or peripherally, produce memory impairment on a number of tasks (Castellano, et. al., 1991; Wadenberg, et. al., 1990; White & Rebec, 1994).

Therefore, in the present study we attempted to examine the effect of eticlopride, a dopaminergic D_2/D_3 receptor antagonist, on retention of a bar pressing response. White and Rebec (1994) showed that eticlopride impaired performance on a lever release task at a dose of 0.01 and 0.05 mg/kg. Based on their data, we once again ran a dose-response curve in order to determine the optimal memory-impairing dose of eticlopride for our specific memory task.

MATERIALS AND METHODS

SUBJECTS

The subjects in the present experiment were male BALB/cByJ mice (refer to Fig. 2 for group sizes). The mice weighted between 26-38 g at the start of the experiment. Throughout the experiment the mice were maintained on a 12/12 h dark/light cycle in a temperature controlled room (20°C).

MATERIALS

The materials and methods used in the present study were identical to those used in Experiment 1. Eticlopride was dissolved in 0.5 ml of a sterile normal solution of sodium chloride.

PROCEDURE

The procedure for testing the animals was identical to Experiment 1 except that the mice were given 15 reinforced training trials as opposed to 7. Fifteen trials were used because if you give less training trials, like 7 for example, there will not be any spontaneous improvement. However, when animals are given 15 training trials or more they show a spontaneous improvement the next day and if the memory for that training is not completely stored it can be disrupted by post-training treatment. Once the 15 training trials were completed, the animals were removed from the operant cage and administered saline, or eticlopride (0.01.

0.05, & 0.1 mg/kg; s. c.), or no treatment. Finally, one group of mice received a 5-hr delayed injection of 0.1 mg/kg of eticlopride.

RESULTS

The dose-response data obtained for eticlopride during the retention test is summarized in Figure 2. The data was analyzed using a one-way analysis of variance followed by a priori comparisons between certain groups within the experiment. The results of the eticlopride dose-response curve demonstrated that there was no overall difference between the groups ($F(5, 52) = 1.524$, $p > 0.05$). However, the results of the a priori comparisons revealed that the 0.1 mg/kg dose of eticlopride significantly impaired memory ($p<0.05$) compared to the no treatment and saline control groups. It is worth noting that the delayed injection of 0.1 mg/kg of eticlopride had no effect ($p>0.05$), demonstrating that the impairment observed after the immediate eticlopride injection was not due to non-specific effects. Rather, it suggests that the immediate injection of eticlopride interacted with memory processes that occur shortly after training to impair memory.

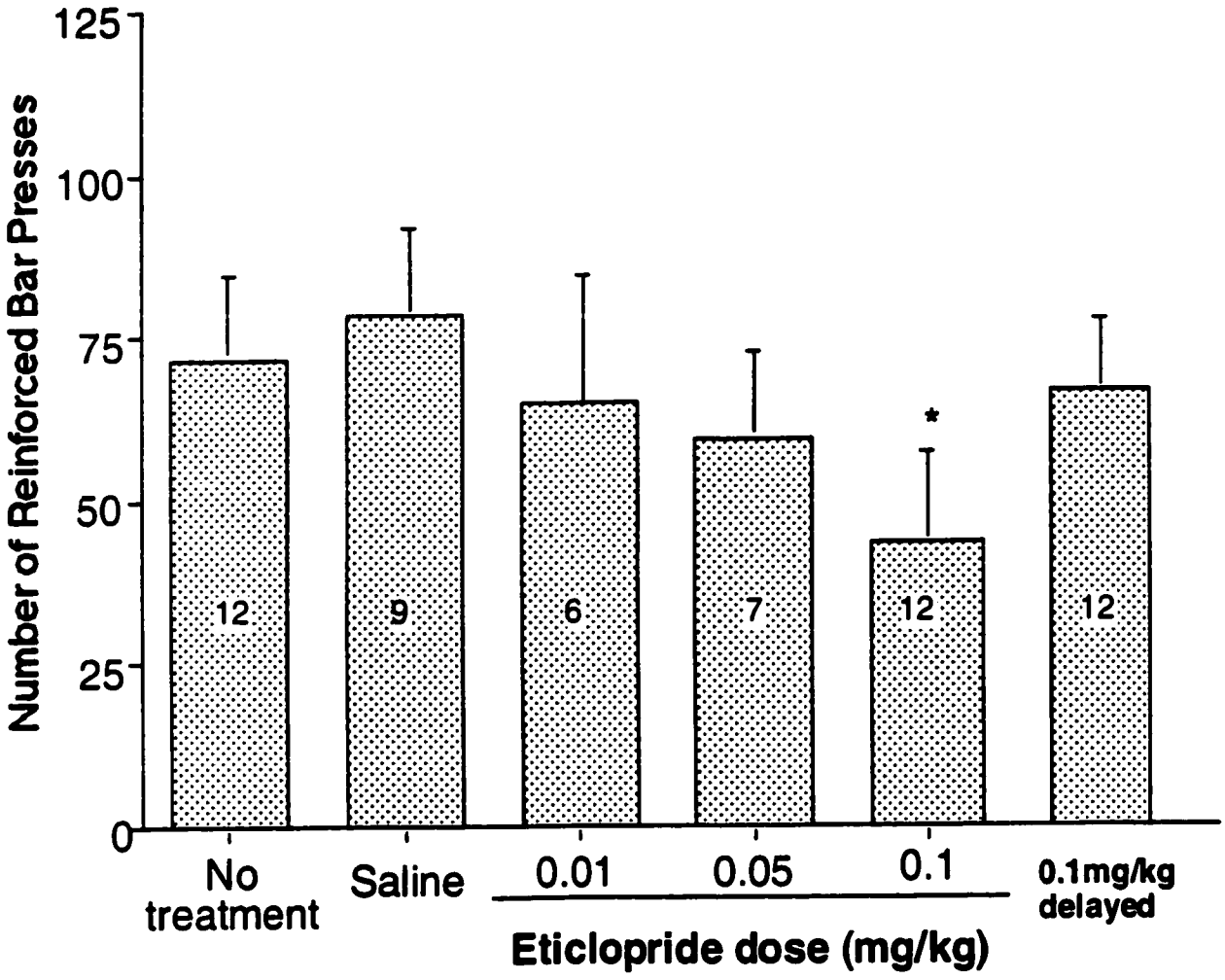

Figure 2: Number of reinforced responses (mean ± S.E.M.) made during the 20-min retention test, 24 h later by animals that received either saline, eticlopride or no treatment at the end of the training session.

DISCUSSION

The results of the present experiment showed that post-training injection of eticlopride, a dopaminergic D2/D3 receptor antagonist, immediately following an appetitively-motivated operant training, decreased operant responding the next day. This decrease was interpreted as a demonstration that a 0.1 mg/kg dose of eticlopride can retroactively and non-contingently impair memory.

The present results tend to support the findings of other studies that have examined the effect of dopaminergic antagonists on memory, in that, we have shown that a D2/D3 antagonist impairs memory (Castellano, et. al., 1991; Sawaguchi & Goldman-Rakic, 1994; Wadenberg, et. al., 1990;White & Rebec, 1994). Moreover, the present results demonstrate that eticlopride, specifically, can impair memory of an operant response. To our knowledge, this is the first demonstration of this drugs effect on an appetitively motivated memory task. White and Rebec (1994) showed that eticlopride could decrease performance on a lever-release task, which was aversively motivated, and therefore, our results extend these findings.

One small difference between the White and Rebec (1994) study and the present study is the optimal dose of eticlopride. In the White and Rebec (1994) study the optimal dose was 0.01 and 0.05 mg/kg, whereas, in our study we found that the optimal dose (from the doses we tested) was 0.1 mg/kg. However, we did not have a large effect. Both 0.01 and 0.05 mg/kg resulted in a decrease in operant responding in the present study, however, the decrease was not significant. Once again, as in Experiment 1, some possible explanations for this

difference in optimal dose could be the nature of the memory task (aversive vs. appetitive training) or the strain and species of the animals, we used mice, whereas, White and Rebec (1994) used rats. Also, a reason why we did not have a large effect could be because we used to many training trials. It may be possible that the animals learned the task to well, therefore, the doses of the present study were not high enough to perturb their memory.

The present results also demonstrate that dopaminergic antagonists which act on the D_3 receptor, or at least partially, modulate memory in the same direction as pure D_1 and D_2 receptor antagonists, in that, they all tend to impair memory. However, because eticlopride acts on both the D_2 and D_3 receptor, it will be necessary in the future to investigate the relationship between pure dopaminergic D_3 receptor antagonists and memory consolidation. Moreover, it would also be interesting to look at the effect of D_4 antagonists, as well as agonists, on retention. It would also be interesting to look at higher doses of the drug and if its potency differs in different species.

The primary reason for conducting Experiment 2, however, was to establish 1) the memory-impairing effect of eticlopride in our specific task, and 2) if eticlopride did impair memory, what was the optimal dose. Therefore, in Experiment 3, we examined the interaction between glucose and eticlopride.

Experiment 3

The purpose of the present study was to determine whether a relationship exists between the dopaminergic system and the memory-improving effects of glucose. More specifically, we were interested in determining whether an injection of glucose could reverse the memory-impairing effects of eticlopride established in Experiment 2. If glucose could reverse a memory impairment produced by eticlopride, then this could indicate that glucose interacts with the dopaminergic system in some fashion to produce memory improvement. Also, it could lend support to the idea that part or all of the memory-improving action of peripherally injected dopamine drugs may be mediated by glucose (Messier & Gagnon, 1996).

The present study was based on findings that indicate a relationship between the cholinergic system and the dopaminergic system in relation to memory modulation (Gasbarri, et. al.,1993; Levin, et. al., 1990; Levin & Rose, 1991), findings that show that the cholinergic system is involved in the memory modulating effects of glucose (Messier, et. al., 1990; Stone, et. al., 1988; Stone, et. al., 1991), and findings that showed that certain dopaminergic agonists increase blood glucose levels (Saller & Kreamer, 1991) (see general introduction). Essentially, we postulated that since cholinergic agonists can reverse the memory-impairing effect of dopaminergic antagonists (Levin, et. al., 1990) it would be possible for glucose, a substance which has been shown to increase acetylcholine levels in the brain (Raggozzino, et. al., 1996), to reverse a memory-impairment produced by a dopaminergic antagonist.

MATERIALS AND METHODS

SUBJECTS

The subjects in the present experiment were male BALB/cByJ mice (refer to Fig. 3 for group sizes). The mice weighed between 26-38 g at the start of the experiment. Throughout the experiment the mice were maintained on a 12/12 h dark/light cycle in a temperature controlled room (20°C).

MATERIALS

The materials and methods used in the present study were identical to those used in Experiment 1 and 2.

PROCEDURE

The procedure for testing the animals was identical to Experiment 2. However, once the 15 training trials were completed the animals were removed from the operant cage and received injection of saline, eticlopride (0.1 mg/kg) and saline, subcutaneous injections of glucose (3 g/kg) and saline, or eticlopride (0.1 mg/kg) and glucose (3 g/kg), or no treatment. All injections were delivered subcutaneously.

RESULTS

The data obtained in Experiment 3 was analyzed using a one-way analysis of variance followed by a priori group comparisons. The data is summarized in Figure 3. The results demonstrated no overall differences between groups ($F(4, 100) = 1.919$, $p > 0.05$). However, a priori comparisons showed that the combined injection of eticlopride and saline significantly impaired memory compared to the no treatment control group ($p < 0.05$). Also, the combined injection of eticlopride (0.1 mg/kg) and glucose (3g/kg) significantly impaired memory compared to the no treatment group ($p < 0.05$). This last result is of most interest because it demonstrates that glucose could not reverse the memory-impairing effect of eticlopride.

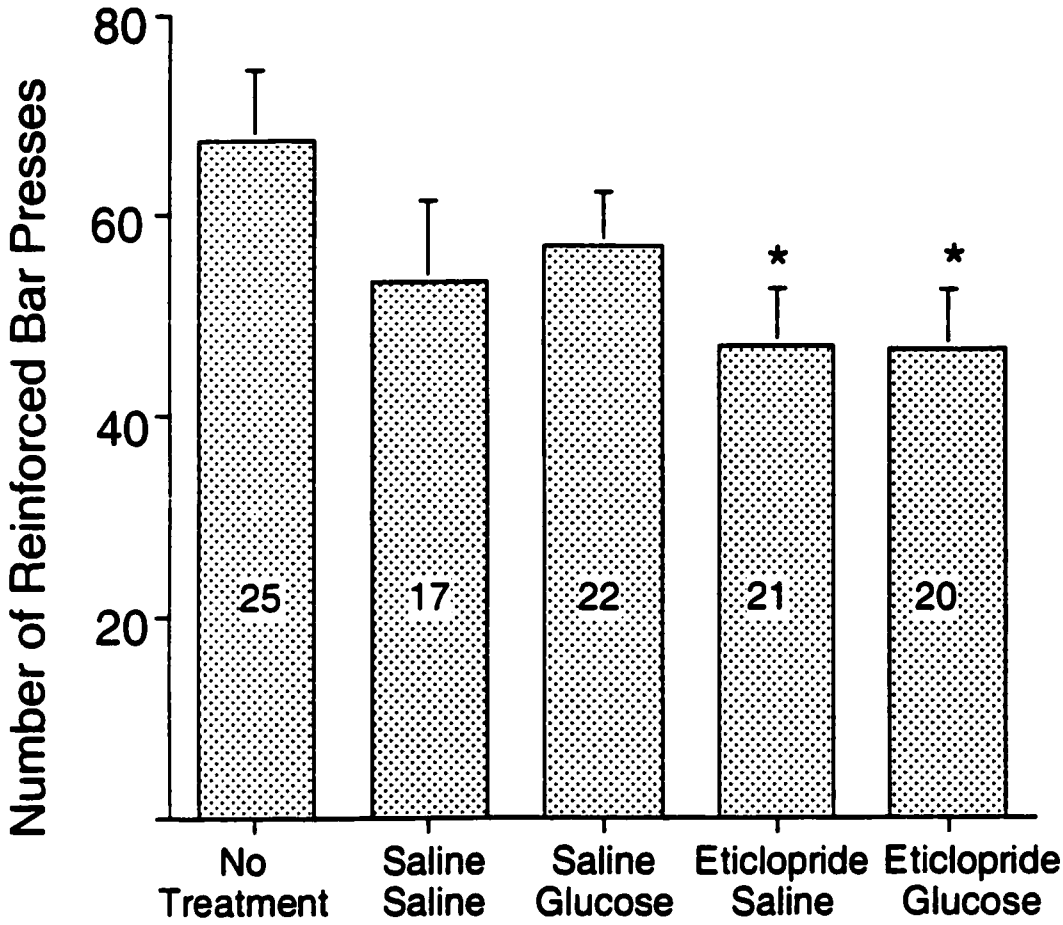

Figure 3: Number of reinforced responses (mean ± S.E.M.) made during the 20-min retention test, 24 h later by animals that received either two saline injections, a combination of saline and glucose (3 g/kg), of eticlopride (1 mg/kg) and saline, of eticlopride (1 mg/kg) and glucose (3 g/kg) or no treatment.

DISCUSSION

The present results demonstrated that a 3 g/kg dose of glucose could not reverse the memory-impairment produced by a 0.1 mg/kg dose of eticlopride. These results appear to indicate that, although injections of glucose have been linked to increased circulating levels of acetylcholine (Raggozzino, et. al., 1996), glucose itself does not interact with the dopaminergic system in relation to memory modulation in this strain of mice with this task in our laboratory. The present findings also suggest that an increase in blood glucose is not the biochemical route through which dopamine agonists improve memory. However, because the two eticlopride groups were not different from the saline control group, the finding that they are not different from the no treatment group does not allow us to conclude that eticlopride perturbed memory in this experiment. It should be noted, however, that there was no significant difference between the no treatment control and the saline control group, but that there was a significant difference between the eticlo/saline, eticlo/glucose, and the saline control group.

It is possible, however, that we did not use the correct dose of glucose to reverse the memory-impairment produced by eticlopride. It may be that a higher dose of glucose is necessary to counteract the effect of eticlopride. A future study should be conducted to examine the effect of a variety of doses of glucose, both higher and lower, on the memory impairment produced by eticlopride. This explanation is unlikely, however, because we showed in Experiment 1 that the 3 g/kg glucose dose produced memory improvement, which suggests that the failure of the 3 g/kg glucose dose to reverse the memory impairment produced by the eticlopride was not due to the lack of effect of the 3 g/kg glucose dose. Rather, the present results indicate that glucose does not interact with dopamine

systems to improve memory. These results also suggest that the dopaminergic system is not a possible route through which glucose improves memory.

However, in order to fully examine the relationship between glucose and the dopaminergic system it will be necessary to conduct experiments that look at the effects of glucose on memory-impairments produced by D_1 and D_2 antagonists. It will also be important to conduct biochemical studies which look at the release of dopamine after glucose injections and the effect of dopaminergic drugs on blood glucose levels, in the present strain of mice and possibly during a memory test.

Experiment 4

The purpose of the present experiment was to examine the effect of LY-171555, a dopaminergic D_2 agonist, on circulating blood glucose levels in BALB/cByJ mice. Previous research has shown: 1) when glucose is injected into an animal, dopamine firing rates in the substantia nigra are decreased (Saller & Chiodo, 1980), and 2) injections of dopamine D_2 agonists increase circulating blood glucose levels (Saller & Kreamer, 1991). These findings seem to indicate a relationship between glucose and the dopamine system. However, because results from Experiment 3 demonstrated that glucose failed to interact with the dopaminergic system in relation to memory modulation, it was important to determine whether a D_2 agonist would increase circulating blood glucose levels in the present strain of mice.

MATERIALS AND METHODS

SUBJECTS

The subjects in the present experiment were male BALB/cByJ mice (refer to Fig. 4 for group sizes). The mice weighed between 26-38 g at the start of the experiment. Throughout the experiment the mice were maintained on a 12/12 h dark/light cycle in a temperature controlled room (20°C).

MATERIALS

The LY-171555 was dissolved in 0.5 ml of a sterile normal solution of sodium chloride.

PROCEDURE

In order to replicate the results of Saller & Kreamer (1991), we used a procedure similar to theirs. Mice were deprived overnight and then given an injection of LY-171555 (0.25, 1, 2.5 mg/kg; s.c.) or saline. Twenty minutes following the injections whole blood glucose was measured using a portable glucometer (Elite, Miles Canada). The meter measures whole blood glucose in samples of 5 μl using electrochemical test strips containing glucose oxidase and potassium ferrocyanide (Fe(III)). The range of linear measurements is 2.2 to 27.8 mmol/1. Once blood is drawn by capillary action into the test strip, the reaction is allowed to take place for 55 s; then an electric potential is applied to a carbon electrode for 5 s after which the current measured is converted into glucose concentration (Messier & Kent, 1995).

RESULTS

The blood glucose concentrations obtained 20 minutes after the injections of LY-171555 and saline are summarized in Figure 4. The data was analyzed using a one-way analysis of variance followed by a priori comparisons between groups. The results demonstrated that there was no overall difference between the groups ($F(3, 12) = 1.596$, $p > 0.05$).

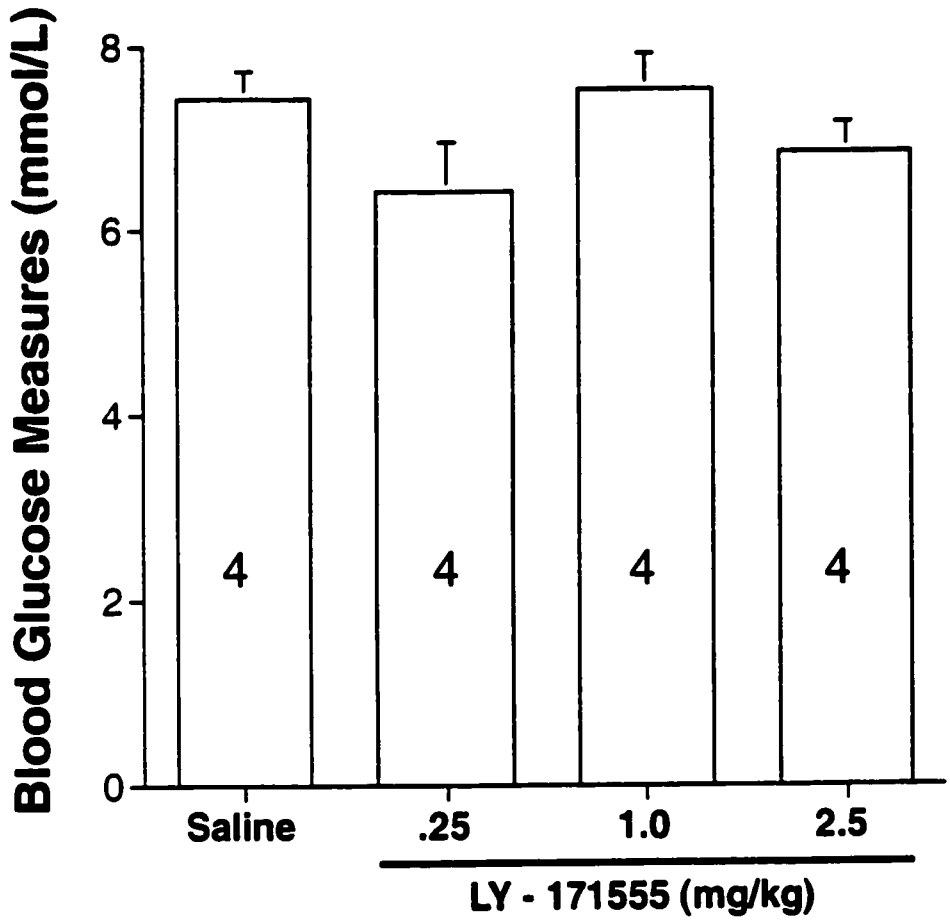

Figure 4: Blood glucose content in mmole/l (mean ± S.E.M.) measured in mice 20 minutes after receiving either a saline injection or three doses of LY-171555.

DISCUSSION

The present results demonstrated that the subcutaneous injection of LY - 171555 at all doses tested (0.25, 1, or 2.5 mg/kg) failed to increase blood glucose levels. These results appear to further indicate that glucose does not interact with the dopaminergic system (s), specifically, in the present strain of mice in this experiment, thus, further substantiating the results of Experiment 3.

These results also contradict the findings of Saller and Kreamer (1991) which demonstrated that D_2 agonists increase blood glucose levels in rats. Therefore, it would appear that the interaction between glucose and the dopaminergic system may be dependent upon the genetic composition of the animal.

Because we were unable to replicate the findings of Saller and Kreamer (1991) it would also seem plausible to argue that the doses of glucose and eticlopride used in Experiment 3 were not the variables responsible for the inability of glucose to reverse the memory impairment produced by eticlopride. The present results seem to indicate that the specific memory task used in Experiment 3 was also not an explanation for the results.

GENERAL DISCUSSION

The present studies were designed to investigate whether the dopaminergic system is involved in the memory modulating effects of glucose. More specifically, we were interested in determining whether a memory impairment produced by a post-training injection of a dopaminergic D_2/D_3 antagonist could be reversed by a post-training injection of glucose.

The questions which led to the design of these studies were based on previous research examining the role of the dopaminergic system in memory modulation. It has been shown that dopaminergic agonists of both the D_1 and D_2 receptor subtype improve retention for a variety of memory tasks (Packard & White, 1989; Packard & White, 1991; White & Viaud, 1991), whereas, dopaminergic D_1 and D_2 antagonists tend to impair memory (Castellano, et. al., 1991; Wadenberg, et. al., 1990). A number of studies have also suggested that the cholinergic and dopaminergic systems may interact in the regulation of memory storage (Gasbarri, et. al., 1993; Levin & Eisner, 1994; Levin & Rose, 1991). As it is thought that glucose improves memory by increasing the synthesis and release of acetylcholine (Messier, et. al., 1990; Ragozzino, et. al., 1996), it seemed possible that dopamine function may also be altered. This hypothesis was supported by research which has shown that glucose injections change the firing rates of dopaminergic neurons in the substantia nigra (Saller & Chiodo, 1980) and injections of D_2 agonists increase in circulating glucose levels (Saller & Kreamer, 1991).

In order to address these questions four experiments were conducted. Experiment 1 established that a 3 and 4 g/kg dose of glucose improved retention

of a bar press behavior in an operant training paradigm. Experiment 2 demonstrated that a 0.1 mg/kg dose of eticlopride, a dopaminergic D_2/D_3 receptor antagonist, retroactively and non-contingently impaired memory in the same operant training paradigm. Following Experiment 1 and 2, Experiment 3 demonstrated that a 3g/kg dose of glucose could not reverse the memory impairment produced by the 0.1 mg/kg dose of eticlopride. Based on the results of Experiment 3, we conducted Experiment 4, which demonstrated that an injection of LY-171555, a dopaminergic D_2 receptor agonist, did not raise blood glucose concentrations.

Taken together these results suggest that the dopaminergic system does not play a role in the memory modulating effects of glucose at least in the present strain of mice, with the present task, in the present experiment. Moreover, these results tend to indicate that dopaminergic drug effects on memory are not linked to circulating blood glucose levels. They also indicate that the dopaminergic system does not interact with glucose in the same way as the cholinergic system. However, in order to conclusively state that glucose injections do not change dopamine function, future research could use microdialysis to examine the effect of glucose injections on the release of dopamine in the brain, specifically, during a memory task.

The present results are also interesting because previous studies which have looked at the ability of glucose to reverse memory-impairments produced by agonists or antagonists of other neurotransmitter systems have had mixed results (Lennartz & Gold, 1995; Ragozzino & Gold, 1991; Ragozzino, Parker, & Gold,1992; Stone, Rudd, & Gold, 1992).

For the most part, glucose has been shown to reverse memory-impairments produced by drugs which act on the other neural systems. For example, in a study by Ragozzino et al. (1992) glucose was shown to attenuate morphine-induced impairments in spontaneous alternation and inhibitory avoidance. Glucose has also been shown to reverse scopolamine induced amnesia (Stone, et. al., 1992), indicating that glucose affects the role of these neural systems in memory consolidation.

Prior to the present study, the only system that had been investigated (and which did not interact with glucose) was the glutaminergic system, specifically the NMDA receptor. Lennartz and Gold (1995) were unable to reverse memory-impairments produced by MK-801, an NMDA antagonist, with glucose. Therefore, our results tend to further suggest that glucose may not be the universal underlying variable responsible for the memory-modulating effects of agonists and antagonists of different neural systems.

It is surprising, however, that glucose did not reverse the memory impairment produced by eticlopride. According to the research demonstrating that glucose increases the synthesis and release of acetylcholine (Messier, et. al., 1990) especially during a challenging memory task (Ragozzino, et. al., 1996), and the research showing that cholinergic agonists can reverse memory impairments produced by dopaminergic antagonists (Gasbarri, et. al., 1993), it would have been expected that glucose would reverse such impairments as well. Moreover, data indicating that glucose increases dopamine firing in the substantia nigra (Saller & Chiodo, 1980) would further lead to this hypothesis, indicating that glucose could reverse the memory impairment produced by a dopaminergic antagonist without involving the cholinergic system.

One hypothesis to explain the present results is that the 3 g/kg glucose injection did not result in a large enough release of acetylcholine as did the cholinergic agonist in the Gasbarri, et. al. (1993) study. It is also possible that the operant training paradigm does not challenge the animal in such a way that glucose would result in a large enough release of acetylcholine. Ragozzino, et. al. (1996) found that glucose increased levels of acetylcholine in the brain only when the animal was challenged with a memory task. It is therefore possible that different memory tasks challenge animals in different ways resulting in varying degrees of acetylcholine release.

However, as suggested above, according to the findings of Saller & Chiodo (1980), glucose could have still reversed the memory impairment without the involvement of acetylcholine. An explanation for why this did not occur is most likely due to the strain of the mice used in the present series of experiments. Experiment 4 demonstrated that glucose levels did not increase when the BALB/cByJ mice were injected with LY171555, a dopaminergic D_2 agonist. Therefore, although we did not directly test whether glucose increases dopamine firing in this strain of mice, it would follow that glucose most likely did not result in an increase of dopamine levels in the brain required to block the effects of eticlopride.

Overall, however, the results found in the present set of experiments add to the literature in a few ways. (1) they further support the findings that a 3g/kg glucose injection can improve memory consolidation, (2) dopaminergic antagonists, particularly of the D_2 receptor, can impair memory consolidation, (3) glucose can not reverse a memory impairment produced by a dopaminergic

D_2/D_3 antagonist. Further research should be conducted with different memory tasks, different dopaminergic antagonists and agonists, and different strains of mice to more clearly define the glucose/dopamine relationship.

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APPENDIX 1.

Experiment 1

	df	MS	df	MS		
Effect	Effect	Effect	Error	Error	F	p-level
1	9	1779.15	285	825.083	2.15633	.025164

Experiment 2:

	df	MS	df	MS		
Effect	Effect	Effect	Error	Error	F	p-level
1	5	1536.17	52	1008.31	1.52350	.198642

Experiment 3:

STATISTICA summary of all effects; design:

GENERAL 1-TREATMENT

MANOVA

	df	MS	df	MS		
Effect	Effect	Effect	Error	Error	F	p-level
1	4	2428.10	106	921.044	2.63625	.038030

Experiment 4:

One Factor ANOVA X 1 : Group Y 1 : Blood Glucose at 20min

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups	3	3.137	1.046	1.596
Within groups	12	7.862	.655	p = .242
Total	15	10.999		

Model II estimate of between component variance = .098