



National Library
of Canada

Bibliothèque nationale
du Canada

Canadian Theses Service

Service des thèses canadiennes

Ottawa, Canada
K1A 0N4

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

Characterization of Sigma and
Phencyclidine Binding Sites in Bovine
Adrenal Medulla

by

Cheryl Rogers

Thesis presented to the Faculty of
Graduate Studies and Research in
partial fulfillment of the requirements
for the degree of MSc.

Department of Anatomy
Faculty of Medicine
University of Ottawa
Ottawa, Ontario
Canada





National Library
of Canada

Bibliothèque nationale
du Canada

Canadian Theses Service Service des thèses canadiennes

Ottawa, Canada
K1A 0N4

The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-315-60053-5



UNIVERSITÉ D'OTTAWA
UNIVERSITY OF OTTAWA

Acknowledgements

I am deeply indebted to Dr. Simon Lemaire, department of Pharmacology, for providing the guidance, support and timeless discussions throughout the course of this work. The generous advice of Dr. Wm. Staines is sincerely appreciated.

For their friendship and competent assistance with laboratory techniques, I am grateful to Dr. M. Dumont, Chantal Lapierre, Danielle Cecyre and Barbro Tinner. I also wish to thank Brian Murphy and Pierre Drouin and for expert graphical and photographic assistance.

I also wish to thank Dr. L. Maler, Dr. J. Fryer, Dr. Wm. Staines and Dr. Hrdina for taking the time to review and evaluate this thesis.

Finally, I would like to extend thanks to the Department of Anatomy for granting the permission for this interdepartmental project.

List of Presentations and Publications

1. Rogers C., D. Cecyre, and S. Lemaire. (1988) "Characterization of the sigma/PCP receptor in membrane preparations of bovine adrenal medulla." Abstract. 72nd annual meeting of the Fed. Am. Soc. Exp. Biol. (FASEB), May 1-5, 1988. Las Vegas, Nevada.
2. Rogers C., D. Cecyre, and S. Lemaire. (1988) "Binding characteristics and the physiological role of the sigma/PCP receptor in bovine adrenal medulla membrane preparations." 31st annual meeting of the Can. Fed. Biol. Soc. (CFBS) June 15-19, 1988. Quebec City, Quebec.
3. Rogers C., D. Cecyre, and S. Lemaire. (1989) "Presence of sigma and Phencyclidine (PCP)-like receptors in membrane preparations of bovine adrenal medulla. *Biochemical Pharmacology*. 38(15): 2467-2472.
4. Murphy B. J., C. Rogers, R. K. Sunahara, S. Lemaire, and B. S. Tuana. (1989) "Identification and characterization of a high affinity binding site for calcium channel blockers in the bovine adrenal medulla." 32nd annual meeting of the Can. Fed. Bio. Soc. (CFBS) June 14-18, 1989. Calgary, Alberta.
5. Murphy B. J., C. A. Rogers, R. K. Sunahara, S. Lemaire and B. S. Tuana. (1989) "Identification, characterization and photoaffinity labeling of the dihydropyridine receptor associated with the L-type calcium channel from bovine adrenal medulla." *Molecular Pharmacology*. In press
6. Rogers C. A. and S. Lemaire (1989) "Characterization of [³H]3(+)-PPP and [³H]TCP binding sites in bovine adrenal medulla." Int. Narcotics Research Conference (INRC) Ste. Adele, Quebec. July 9-14, 1989.
7. Rogers C. A. and S. Lemaire. (1989) "Characterization of [³H]3(+)-PPP and [³H]TCP binding sites in bovine adrenal medulla." In, (eds. R. Quirion, K. Jhamandas, and C. Gianoulakis) *Progress in Clinical and Biological Research*. Vol. 38, In press.
8. Lemaire S., C. Rogers, D. Cecyre, C. Lapierre, P. Parent, B. Jeannotte, L. Sabourin, and M. Dumont. (1989) "Structural identification of bovine adrenomedullary histogranin, a novel peptide possessing PCP and/or sigma receptor binding and behavioural activities." Submitted.

9. Rogers C. and S. Lemaire. (1989) "Autoradiographic visualization and binding characteristics of histogranin, an endogenous PCP and/or sigma receptor ligand in bovine adrenal medulla." In preparation.

1. REVIEW OF THE LITERATURE	1
1.1. PHENCYCLIDINE.....	1
1.2. SIGMA AND PHENCYCLIDINE RECEPTORS.....	3
1.3. THE NMDA CONNECTION.....	9
1.4. ENDOGENOUS LIGANDS.....	12
1.5. PHYSIOLOGICAL EFFECTS	13
1.5.1 Central Nervous System.....	13
1.5.2. Endocrine System.....	15
1.6 THE BOVINE ADRENAL MEDULLA.....	17
1.6.1 Morphology	18
1.6.2 Adrenal Chromaffin Cells	19
1.6.3. Stimulus-Secretion Coupling.....	24
1.6.4. Adrenomedullary Opioid Receptors.....	29
1.6.5 Adrenal medullary Sigma and PCP receptors.....	31
1.6.6. Endogenous PCP and/or Sigma Ligands in the Adrenal Medulla.....	32
2. SPECIFIC AIMS	33
3. METHODS.....	34
3.1 The Radioligand Binding Assay.....	34
3.2 Membrane Preparation.....	40
3.3 Radioligand Binding.....	42
3.4 Preparation of [¹²⁵ I] histogranin.....	44
3.5 Autoradiography	45
4. RESULTS.....	48
4.1 [³ H](+)-SKF-10047 binding.....	48
4.2 [³ H]TCP and [³ H]3(+)-PPP binding in bovine adrenal medulla.....	58

4.3	Autoradiography of [^{125}I]histogranin Binding in Bovine	
	Adrenal Medulla.....	70
5.	DISCUSSION.....	73
6.	REFERENCES.....	84

LIST OF FIGURES

	Page
1. Structure of sigma and PCP radioligands	8
2. The binding model of the NMDA receptor	11
3. Excitation-secretion coupling	26
4. Preparation of adrenal medulla membranes	41
5. Methodology of radioligand binding	43
6. Autoradiography methods	47
7. Time and protein dependence of (+)[³ H]SKF-10047	50
8. Saturation of [³ H]SKF-10047 receptors	52
9. Saturation of [³ H]SKF-10047 sigma receptors	55
10. Saturation of [³ H]SKF-10047 PCP-like receptors	56
11. Competition studies against [³ H]SKF-10047	57
12. Association of [³ H]TCP and [³ H]3(+)-PPP binding	61
13. Saturation of [³ H]TCP binding	63
14. Saturation of [³ H]3(+)-PPP binding	64
15. Hill Plot of [³ H]TCP and [³ H]3(+)-PPP	66
16. Autoradiographic illustration of [¹²⁵ I]histogranin	71

List of Tables

	Page
1. Constituents of the chromaffin cell granule	23
2. List of materials and suppliers	39
3. Specificity of [³ H]SKF-10047 binding	59
4. Stereoselectivity of [³ H]SKF-10047 binding	60
5. [³ H]TCP and [³ H]3(+)-PPP inhibitory constants I	69
6. [³ H]TCP and [³ H]3(+)-PPP inhibitory constants II	70
7. [¹²⁵ I]histogranin displacement by sigma and PCP ligands	72

LIST OF ABBREVIATIONS USED

<u>Abreviation</u>	<u>Product</u>
BW 234U	rimcazole
DTG	ditolylguanidine
Hal	haloperidol
NMDA	N-methyl-D-aspartate
PCP	phencyclidine
3(+)PPP	3-(3-hydroxyphenyl)-N-(1-propyl)piperidine
SKF-10047	N-allylnormetazocine
TCP	1-thienylcyclohexylpiperidine

Abstract

The adrenal medullary sigma and phencyclidine (PCP) receptors have been characterized by radioligand binding studies in bovine adrenal medulla. Using the prototypic sigma receptor ligand [³H](+)-SKF-10047, two binding components were revealed, firstly, a high affinity sigma site and secondly, a low affinity "PCP-like" binding site. These sites have proven to be of interest in the central nervous system due to their association with psychotic behaviour. Sigma agonists have previously been reported to induce hyperexcitability in animals, whereas stimulation of the PCP receptor in humans produces a behaviour that resembles the disease state known as paranoid schizophrenia. Investigation of sigma and PCP ligands in brain has revealed their distinct binding sites, but has failed to explain their mechanism of action.

Investigation of the sigma and PCP-like binding sites in the adrenal medulla using the highly selective sigma ([³H]3(+)-PPP) and phencyclidine receptor ([³H]TCP) ligands has demonstrated that these two binding sites share many similar features. The radioligands [³H]3(+)-PPP and [³H]TCP saturate a similar maximal population of receptors ($B_{max} = 218-250$ fmol/mg protein) and both are most potently displaced by sigma ligands. This binding profile thus differs from that found in the central nervous system where [³H]TCP is most potently displaced by PCP-analogues. Although [³H]TCP and [³H]3(+)-PPP binding is potently displaced by sigma ligands, slight differences in stereoselectivity suggest that the binding of [³H]TCP and [³H]3(+)-PPP may be to multiple subtypes of sigma receptors.

Thus, the binding of [^3H]3(+)-PPP would correspond to a subtype designated σ_1 while [^3H]TCP binding would correspond to a subtype designated σ_2 .

Our laboratory has recently isolated a novel peptide from adrenal chromaffin cell granules that shares an 80% primary structural homology with a segment of histone H₄. The autoradiographical analysis of the binding of this peptide revealed its specificity for the adrenal medulla and its potent displacement by sigma and PCP ligands. Evidence accumulated suggests that the peptide, namely histogranin, may act as an endogenous ligand for one or both sigma subtypes defined in this study.

1. REVIEW OF THE LITERATURE

1.1. PHENCYCLIDINE

Phencyclidine (PCP) an arylcyclohexamine, was introduced over 20 years ago as a potent, fast-acting anaesthetic that did not cause respiratory or cardiovascular depression (Chen, Ensor et al. 1959). However, it has had limited use in clinical medicine due to the "emergence phenomena" experienced by patients coming out of anaesthesia (hallucinations, maniacal excitations, 'drunkenness', and unmanageable agitation), for recent review see (Clouet 1986). Research over the last decade has revealed that there has been a dramatic increase in the abuse of PCP as a recreational drug known as 'angel dust' by young people in the U.S.A. (Petersen and Stillman 1978). At subanaesthetic doses, phencyclidine, a structural analogue of the "dissociative" anaesthetic ketamine (Domino 1964) induces pronounced global disturbances of the sensory modalities and psychotomimetic symptoms in man that are unlike those induced by other psychotropic compounds such as lysergic acid diethylamine (LSD) (Domino 1964; Luby, Gottlieb et al. 1962; Meyer, Greifenstein et al. 1959; Morgenstern, Beech et al. 1962; Showalter and Thornton 1977). These unique psychotropic effects are similar in some respects to those seen with the mental disorder schizophrenia, which lead to the suggestion that PCP may provide an experimental model for schizophrenia (Allen and Young 1978; Luby and Cohen 1959). Until a few years ago, very little was known about the mechanism of action of PCP. Various groups had suggested that PCP was interacting with either muscarinic (Vincent, Cavey et al. 1979), nicotinic

(Albuquerque, Tsai et al. 1980), dopaminergic (Doherty, Simonovic et al. 1980; Johnson and Oeffinger 1981), serotonergic (Martin, Berman et al. 1979), GABAergic (Hsu, Smith et al. 1980), and/or opiate (Vincent, Cavey et al. 1979) systems. Similar diverse hypotheses had been proposed some years earlier to explain the effects of morphine and its congeners before the discovery of highly selective opiate receptors in rat brain (Pert and Snyder 1973; Simon, Hiller et al. 1973). In 1979, two independent groups reported the presence of specific [³H]PCP receptors in rat brain and suggested that they were the major site of action of PCP (Vincent, Cavey et al. 1979; Zukin and Zukin 1979). These results were challenged by Maayani and Weinstein (Maayani and Weinstein 1980) who reported that the binding data could be explained as a filtration artifact. Despite this, the existence of the receptor has been substantiated through the elimination of some of the high non-specific binding observed in the original assays by pretreatment of the filters with polyethylenimine. The relevance of the binding site labeled by [³H]PCP is supported by numerous studies comparing the structure-activity profiles for inhibition of the binding of [³H]-PCP to induction of stereotyped behaviour, (Contreras, Quirion et al. 1986), ataxia (Vignon, Vincent et al. 1982; Vincent, Cavey et al. 1979), or generalization to PCP stimulus in drug discrimination assays (Shannon 1981; Shannon 1982). The existence of the PCP receptor was further substantiated through autoradiography which allowed biochemical characterization as well as visualization and localization of these receptors in specific brain areas (Quirion, Hammer et al. 1981)

1.2. SIGMA AND PHENCYCLIDINE RECEPTORS

In humans and other mammalian species, the psychotomimetic syndrome caused by PCP resembles that elicited by synthetic opiates such as (+)SKF-10047 (N-allylnormetazocine) (Haertzen 1970; Keats and Telford 1964), implying mediation by a unique opiate receptor subtype designated as sigma. Sigma receptors were first defined based on the unique effects reportedly described as "mania" in the chronic spinal dog. These effects were induced by (+)SKF-10047, the prototypic sigma agonist from the benzomorphan structural class of opioids (Martin, Eades et al. 1976). Although psychotic effects are elicited by widely used opiate analgesics, such as pentazocine, certain pharmacological features of these effects now indicate that they are not mediated via a classically defined type of opiate receptor. Most strikingly, the psychotomimetic behavioural effects of sigma drugs in animals are not antagonized by opiate antagonists such as naloxone (Brady, Balster et al. 1982; Iwamoto 1981; Katz, Spealman et al. 1985; Vaupel 1983; Young and Khazan 1985). Moreover, the sigma-like behavioural effects manifest reversed stereoselectivity from classical opiate receptors being caused essentially by the (+)form of SKF-10047 (Brady, Balster et al. 1982; Iwamoto 1981; Katz, Spealman et al. 1985; Young and Khazan 1985). In addition, the sigma site can be differentiated clearly from all types of brain opiate receptors (μ , κ , δ) through autoradiography. The prototypic sigma agonist [^3H](+)SKF-10047 labels specific brain receptors that are

pharmacologically distinct from the opiate receptor site of [³H]etorphine (Tam 1983).

As some psychotomimetic opioids interact with receptor sites for the psychotomimetic drug phencyclidine, it has been suggested that sigma-like behavioural effects may involve specific phencyclidine sites rather than opiate receptors (Zukin and Zukin 1981). Studies examining the binding of SKF-10047 in the past, produced conflicting results. Until recently, studies on the drug specificity of (+) or (±)-[³H]SKF-10047 binding resembled that of PCP receptor binding sites indicating that (+)SKF-10047 and PCP were labeling the same site (Itzhak, Hiller et al. 1984; Johnson 1983; Mendelsohn, Kalra et al. 1985). However, both rat and guinea pig brain in vitro autoradiography and membrane binding studies revealed that (+)[³H]SKF-10047 labeled two distinct sites. The higher affinity site for (+)[³H]SKF-10047 which is highly stereoselective for the (+) isomer of SKF-10047, displayed the drug specificity and anatomical localization characteristic of putative sigma receptor sites as labeled by the prototypic sigma receptor ligand, (+)[³H]3-PPP (Largent, Gundlach et al. 1986). In contrast, the binding characteristics of the low affinity binding site for (+)-[³H]SKF-10047 was essentially the same as that of PCP receptors labeled with [³H]TCP (Vignon, Chicheportiche et al. 1983). Competition binding studies with [³H](+)SKF-10047 indicated that sigma or PCP receptors can be selectively blocked by the addition of the appropriate concentration of sigma or PCP receptor ligand without affecting [³H](+)SKF-10047 binding to the other receptor class (Largent,

Gundlach et al. 1986). The binding characteristics of the two sites resolved in this way were then determined and compared with each other. These studies revealed that the two sites have a similar drug specificity profile. Both sites bind PCP and some benzomorphans with submicromolar affinity, however, the PCP binding site has a higher affinity for PCP and its analogues than for (+)SKF-10047; conversely the sigma binding site binds (+)SKF-10047 with higher affinity than PCP and its congeners. The main pharmacological distinction between the two binding sites is that the sigma site is highly selective for haloperidol and other classes of neuroleptic drugs including phenothiazines, whereas PCP binding is not displaced by haloperidol (Largent, Gundlach et al. 1984; Largent, Gundlach et al. 1986; Mendelsohn, Kalra et al. 1985; Sircar, Nichtenhauser et al. 1986; Su 1982). It is important to note that the sigma receptor is not a dopamine receptor despite the fact that several D₂ receptor antagonists (such as haloperidol and phenothiazines) interact with this site. Support for this notion is derived from the following two lines of evidence. Firstly, the sigma site has reversed stereoselectivity for butaclamol enantiomers compared to the D₂ receptor. The affinity of (-)butaclamol is greater than that of (+)butaclamol for the sigma site, whereas the reverse is true at the D₂ receptor (Largent, Gundlach et al. 1986; Weber, Sonders et al. 1986). Secondly, the distribution of sigma sites as revealed by autoradiography is different from that of D₂ receptors (Largent, Gundlach et al. 1986). Identification of drugs lacking opiate structures, but demonstrating even higher affinity for sigma

receptors than many opiate-associated sigma drugs has emphasized the unique conformational requirements of sigma sites.

Binding Characteristics of Sigma and PCP Receptors

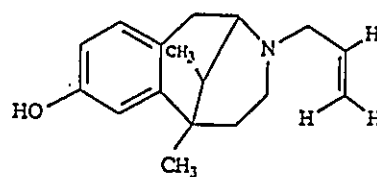
<i>Sigma</i>	<i>PCP</i>
1. High affinity for (+)SKF-10047	1. High affinity for PCP
2. Selective for haloperidol	2. Lacks affinity for low concentrations (10^{-8}) of haloperidol
3. Distinct from the D2 site by its stereoselectivity for (-)butaclamol	3. Associated with the NMDA receptor channel demonstrated by its selectivity to MK-801.
4. High degree of selectivity for (+)3-PPP.	4. High degree of selectivity for TCP
5. Psychotic effects unaffected by the opiate antagonist naloxone.	5. Psychotic effects unaffected by the opiate antagonist naloxone.

The considerable progress that has been made in the characterization of the two binding sites is mainly due to the development of selective radioligands for the sigma site, [^3H]3-(3-hydroxyphenyl)-N-(1-propyl)piperidine ([^3H](+)3-PPP), and for the PCP site [^3H]TCP (Fig. 1). Although it is still not clear whether PCP or sigma receptors mediate the psychotomimetic effects of (+)SKF-10047, potential antagonists for the sigma receptor have been synthesized. In preclinical trials, these drugs, BW 234U (Ferris, Tang et al. 1986), HR-375 (Su, Weissman et al. 1986), and BMY 14802 (Taylor and Dekleva 1987), were characterized as 'atypical' antipsychotics that do not exert their effects through dopaminergic antagonism. The compound BW 234U, commonly named rimcazole is particularly successful in treating cases of acute schizophrenia. Binding studies with rimcazole indicate that its affinity for the D₁ and D₂ subtypes of dopamine receptors is a thousand fold lower than that demonstrated at sigma sites (Ferris, Tang et al. 1986). BMY-14802, possesses a relatively low affinity for D₂ receptors in vitro and appears not to block dopamine receptors in vivo (Taylor and Dekleva 1987).

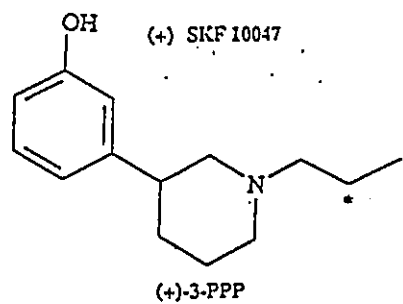
Figure 1

Structure of sigma and PCP ligands

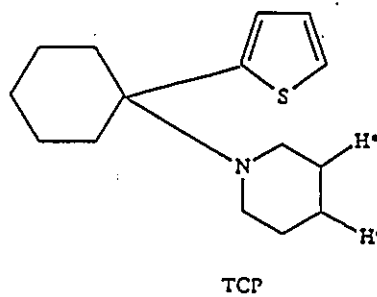
Sigma and PCP Receptors



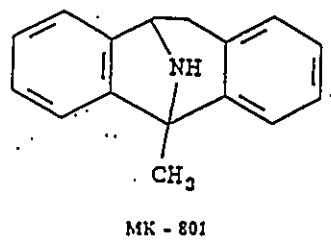
Sigma Receptor



PCP Receptor



PCP/NMDA Receptor



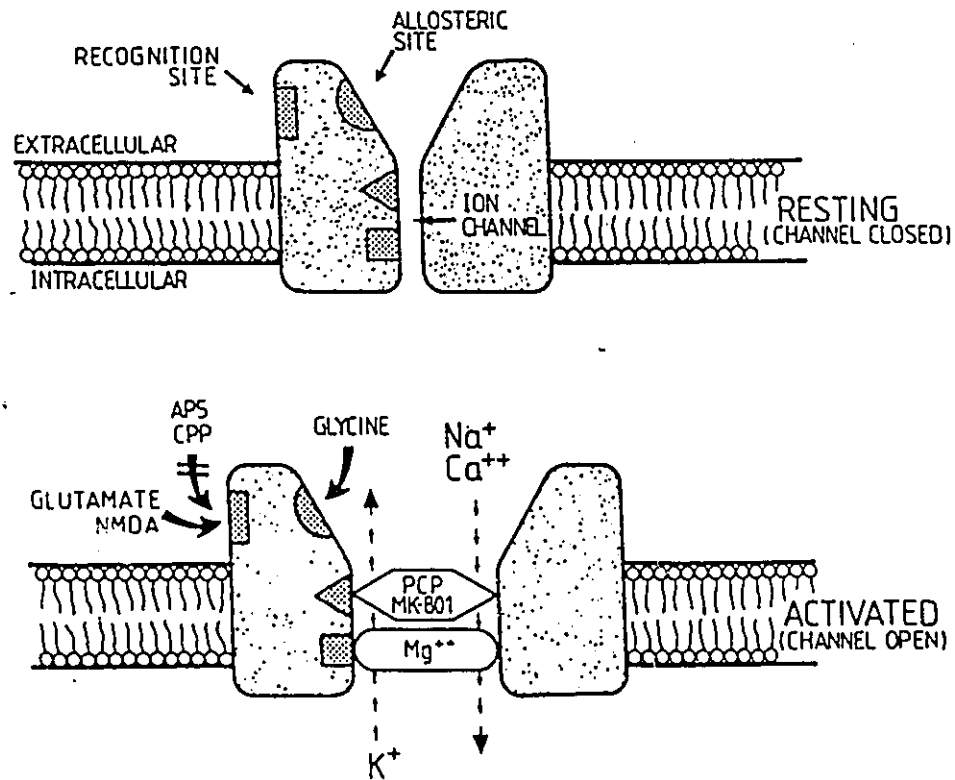
1.3. THE NMDA CONNECTION

Recent studies have demonstrated that PCP and ketamine selectively block the N-methyl-D-aspartate (NMDA) excitation of central mammalian neurons and exert little effect on excitation produced by quisqualate and kainate (Anis, Berry et al. 1983). These findings along with additional electrophysiological data (Berry, Dawkins et al. 1984; Johnson and Ascher 1987; MacDonald, Schneiderman et al. 1986) and neurochemical experiments (Fagg, Foster et al. 1986; Fagg and Matus 1984; Jones, Snell et al. 1987; Jones, Snell et al. 1989) provide evidence for the noncompetitive nature of the blocking of NMDA-induced potentials by PCP. In membranes from the rat brain, PCP appears to interact with at least two distinct binding sites. One of these, the phencyclidine-NMDA site (PCP₁) which may represent an interaction with the NMDA receptor complex (Fagg 1987; Maragos, Chu et al. 1986) can be differentiated pharmacologically and anatomically from a second site, the phencyclidine-sigma site (PCP₂), that is labelled by some sigma "opiates", 3-(3-hydroxyphenyl) N-n-propylpiperidine (3-PPP) and haloperidol (Downes, Lewis et al. 1986; Gundlach, Largent et al. 1986b; Vignon, Privat et al. 1986). Since PCP has been identified as a non-competitive antagonist of the N-methyl-D-aspartate (NMDA) class of excitatory amino acid receptors (Anis, Berry et al. 1983) a PCP/NMDA receptor model has emerged wherein PCP and PCP-like drugs such as MK-801 block the open NMDA receptor ion channel (Foster and Wong 1987), for which five binding sites for drugs and regulatory cations have been proposed (Reynolds and Miller 1988)

(Fig. 2). The binding of PCP and its congeners may be potentiated by the presence of glutamate (Loo, Braunwalder et al. 1987). Glutamate is thought to bind to the NMDA agonist binding site on the receptor complex which in turn causes the ion channel to open. The presence of NMDA ion channels in the open state allows the access of PCP to its receptor site within the channel pore. The effect of glutamate may also be potentiated by glycine (a positive allosteric modulator) and inhibited by NMDA antagonists such as AP5 or CPP. It has been suggested that the blockade of NMDA evoked excitation of central mammalian neurons is manifested by steric hindrance or "clogging" of the receptor ion channel by PCP (Honey, Miljkovic et al. 1985).

Figure 2

The NMDA receptor complex adapted from Foster and Fagg, 1987. The binding site for PCP and MK-801 is thought to exist within the channel pore. The binding efficacy of PCP receptor ligands is favoured when the NMDA ion channel is open, thus when NMDA agonist such as glutamate are present. Glycine acts at a modulatory site to potentiate the opening caused by the agonist binding of while AP5 and CPP-competitive antagonists block this effect.



1.4. ENDOGENOUS LIGANDS

Several laboratories have described the presence of endogenous compounds in brain extracts which displace selective radioligands from PCP or sigma receptors (Contreras, Dimaggio et al. 1987; Quirion, Dimaggio et al. 1984; Su, Weissman et al. 1986; Zukin, Zukin et al. 1987). These factors were roughly described as peptides of 2 to 3 kilodaltons, however their structure has not yet been identified. It has been suggested that one single prohormone may give rise to a family of PCP and sigma ligands which preferentially interact with subsets of receptors in a fashion similar to the prodynorphin or proenkephalin derived peptides (Quirion, Dimaggio et al. 1984). The high degree of cross-reactivity between endogenous sigma and PCP factors in binding assays is not surprising, since the prototypic receptor ligands PCP and (+)SKF-10047 react with both types of receptors. A biologically significant endogenous ligand should manifest not only the receptor binding properties but also the biological effects of sigma or PCP like drugs. A lack of a suitable bioassay for psychotomimetic symptoms renders the task of screening endogenous ligands a difficult one. Very recent results suggest that sigma ligands cause a large increase in the amplitude of the electrically stimulated twitch of isolated mouse and guinea pig vas deferens (Campbell, Bobker et al. 1987). Until a suitable model for psychosis is developed one can only postulate the possible implications of these endogenous sigma and PCP factors in the central nervous system.

1.5. PHYSIOLOGICAL EFFECTS

1.5.1 Central Nervous System

At the present time it is entirely unknown whether PCP or sigma receptors or both are responsible for mediating the schizophrenic-like psychotomimetic effects. There is evidence that the potency of benzomorphan opiates in several animal behavioural models correlates well with their potency in PCP binding assays (Mendelsohn, Kerchner et al. 1984; Quirion, Hammer et al. 1981; Quirion, Rice et al. 1981; Vignon, Privat et al. 1986; Vincent, Cavey et al. 1979; Zukin and Zukin 1979). These observations lead to the suggestion that the PCP site is responsible for mediating the effects of both benzomorphans and PCP. However, many of these studies were carried out before the highly specific sigma and PCP receptor ligands became available. It should be noted that presently there is no animal test that can predict schizophrenia-like psychotomimetic effects of sigma and PCP-like drugs in humans.

The majority of the data on sigma receptors concerns the binding characteristics, anatomical localization, and behavioural effects induced by putative sigma agonists in animals. However, more would be revealed about the function of the central sigma system if the behavioural effects resulting from activation of the endogenous sigma system were known. The complex behavioural syndrome of lateral head weaving, hyperlocomotion and ataxia, induced in the rat by the prototypic sigma receptor ligand (+)SKF-

10047 is thought to reflect activation of the sigma receptor (Shannon 1983). Previous studies implicated sigma ligands in the regulation of movement and posture. This hypothesized function of sigma ligands is based in part on the high density of sigma receptors in many areas of the brain that control movement (eg. cerebellum, red nucleus, substantia nigra pars compacta) and the motor effects elicited by injections of sigma ligands (Bowen, Walker et al. 1988). However, the behaviours of sniffing, rearing, backpedalling, circling, and weaving can be induced by both DTG (a selective sigma ligand) and MK-801 (a selective PCP receptor ligand), suggesting that both PCP and sigma receptors are involved in mediating stereotyped behaviour and ataxia in the rat.

The release of neurotransmitters from nerve endings may be modulated by stimulation of presynaptic sigma receptors. A study by Kinouchi et al. (1989) on the effects of the sigma ligand pentazocine, demonstrated that d-pentazocine increased baseline release of [³H]noradrenaline from guinea pig cortical slices in a concentration dependent manner. This action was not antagonized by naloxone and this enhancing effect was also observed with PCP (10⁻⁵M). Other opioids did not affect baseline release. This non-involvement of opiates was also confirmed by Werling et al. (1987) who reported that the baseline release of [³H]noradrenaline was not affected by DAGO, U-50488H, and DPDPE, three selective opioid ligands binding mu, kappa and delta receptors, respectively. Whether the effect is related to sigma receptors or not remains to be elucidated.

To date the best characterized mechanism of action of PCP involves the N-methyl-D-aspartate receptor. Several studies suggest that the effect of PCP may be to inhibit the ionic conductance mechanisms underlying activation of the NMDA receptors that lead to the release of neurotransmitters in rat brain (Jones, Snell et al. 1987). (+)SKF-10047 also demonstrated selective NMDA antagonism, however, the potent sigma compound 3(+)-PPP had no effect on NMDA activated neuronal systems. It has been observed that PCP inhibited the release of dopamine and ACh induced by excitatory amino acids in the striatum and nucleus accumbens (Snell and Johnson 1985; Snell and Johnson, 1986). Lesions of the dopaminergic innervation to the nucleus accumbens resulted in a loss of 62% of the binding sites for PCP suggesting that many of these sites are on dopaminergic nerve terminals in the nucleus accumbens, although studies by Hattori, (1982) have demonstrated the degeneration of dendritic spines on postsynaptic neurons after lesion of the afferents. Transsynaptic degeneration of the postsynaptic neuron indicates that it is inappropriate to conclude that loss of receptor binding after these types of lesions necessarily indicates that these receptors are located presynaptically.

1.5.2. Endocrine System

In addition to their psychotomimetic effects in the central nervous system, PCP and SKF-10047 have been reported to alter neuroendocrine function. Specifically, both compounds have been reported to stimulate hypothalamic-pituitary-adrenocortical

secretion (Boggan, Wallis et al. 1982; Pechnick, George et al. 1985a; Pechnick, George et al. 1985b), and suppress LH (Pechnick, George et al. 1985b), and PRL (Bayorh, Lozovsky et al. 1983; Lozovsky, Saller et al. 1983; Pechnick, George et al. 1985a; Saller, Zerbe et al. 1983) secretion in rats. (+)-[³H]SKF-10047 binding sites have been demonstrated in the anterior pituitary (Tam 1983), and PCP and SKF-10047 also bind to cultured pituitary cells and decrease LH release in vitro (Stojilkovic, Dufau et al. 1987). Since PCP and SKF-10047 bind with high affinity to both PCP and sigma receptors (Contreras, Quirion et al. 1986; Gundlach, Largent et al. 1986b; Largent, Gundlach et al. 1986; Tam 1985; Vignon, Privat et al. 1986), the identity of the receptor(s) mediating the endocrine effects of these compounds is unknown. Furthermore, it is unclear whether the effects of PCP and SKF-10047 are mediated in brain or through direct actions on the pituitary or target endocrine organs. A direct endocrine effect of these drugs is supported by the autoradiographic labelling of sigma receptors in membrane homogenates of rat pituitary, testis, and ovary (Wolfe, Culp et al. 1989). Other studies have also demonstrated the existence of sigma receptors in spleen homogenates as well as on peripheral blood lymphocytes (Su, London et al. 1988; Su, Schell et al. 1988). These results suggest that sigma receptors may also play a role in immune functions. If so, the potential therapeutic application of sigma agonist or antagonists in pathophysiological states involving the lymphoid sigma receptors should not be overlooked.

1.6 THE BOVINE ADRENAL MEDULLA

The adrenal medulla has been used extensively as a model to study the mechanism of stimulus-secretion coupling. In short, the bovine adrenal medulla has been used in whole gland perfusion studies to evaluate the effect of secretogues (Lemaire, Livett et al. 1981; Viveros and Wilson 1983), in isolated cell preparations to study basic release mechanisms (Douglas 1966; Kirshner and Viveros 1972; Livett, Day et al. 1982; Rahwan and Borowitz 1973) and in membrane preparations for radioligand binding studies on opiate (Castanas, Bourhim et al. 1985a; Castanas, Bourhim et al. 1985b; Castanas, Giraud et al. 1984; Dumont and Lemaire 1984); dopaminergic (Gonzalez, Artalejo et al. 1986), nicotinic (Wilson and Kirschner 1977), muscarinic (Kayaalap and Neff 1979), adrenergic (Greenberg and Zinder 1982; Helle and Serck-Hanssen 1975; Serck-Hanssen 1974; Wada, Sakuri et al. 1982), substance P (Boksa, Seidah et al. 1982), GABA (Kataoka, Gutman et al. 1984), vasopressin (Antoni 1984), and angiotensin II (Healy, Maciejewski et al. 1985) receptors. There are several advantages to using the bovine adrenal gland as a model for research; i) the tissue is readily available ii) the intact gland may be perfused with secretagogues via the adrenal vein to provide a secretory system that closely resembles physiological conditions iii) a relatively homogenous large population of adrenomedullary cells can be isolated by enzyme digestion of the dissected tissue iv) the cells are quite stable and remain functional in culture for long periods of time. Thus many chromaffin cell release properties and cellular processes have been thoroughly investigated

in vitro and are well documented. The adrenal medulla is derived from a neuronal origin, and like neuronal systems provides all components of both pre and postsynaptic activity; for example, electrical excitability, synthesis and release of neuropeptides and transmitters, storage and reuptake of biogenic amines (Marley and Livett 1985; Stjarne 1972; Von Euler 1972). Therefore, drug-receptor interactions here may prove to be similar to those in the central nervous system. In this study, the adrenal medulla was employed as a model to investigate the binding of sigma and PCP receptor ligands.

1.6.1 Morphology

The suprarenal or adrenal glands are roughly pyramidal, flattened organs, one at the cranial pole of each kidney. A sectioned bovine adrenal gland shows two regions; an outer cortex, the main part of the gland which when fresh appears reddish brown, and secondly, a thin inner medulla that appears light beige in colour. These regions are distinct structurally, developmentally, and functionally in both human and bovine. The cortex originates from epithelium (mesothelium) and the medulla from the neural crest (Coupland 1965). Each gland is surrounded by a connective tissue capsule which sends radial trabeculae, consisting principally of reticular fibers, into the cortex. Capillaries penetrate the gland along the delicate trabeculae. The cortex is divided into three layers. i) a thin, outer zone, the zona glomerulosa (15%) ii) a thick middle zone,

the zona fasciculata (78%) iii) a very thin inner zone, the zona reticularis, adjacent to the medulla (7%) (Bloom and Fawcett 1986).

The human adrenal gland receives a rich vascular supply from a network of arterioles and capillaries that originate from the following arteries, i) superior suprarenal arteries, (arising from the inferior phrenic artery ii) middle suprarenals (arising from the aorta) iii) inferior suprarenals (which branch from the renal artery)

The cortex of the adrenal gland is presumably not innervated, but preganglionic fibers originating from the cell bodies in the intermediolateral column of the spinal cord, pass through the sympathetic chain and via splanchnic nerves surround medullary chromaffin cells. These preganglionic neurons appear to be surrounded by fibers containing acetylcholine, and neuropeptides including enkephalin, serotonin, substance P and somatostatin (Holets and Elde 1982). These peptides may modulate the cholinergic action of stimulation of adrenal chromaffin cells. The adrenal medulla also receives adrenergic innervations from sympathetic postganglionic neurons. It is thought that these may regulate regional blood flow (Unsicker, Habura-Fluh et al. 1978).

1.6.2 Adrenal Chromaffin Cells

Adrenal chromaffin cells possess an irregular shape and have an approximate diameter of 20 μm . They contain either adrenaline (A cells) or noradrenaline (NA cells) or both in membrane bound

vesicles located in the cytoplasm. Adrenal chromaffin cells can be considered as typical endocrine paraneurons which secrete both peptides and biogenic amines. Fujita (Fujita, Kobayashi et al. 1974), introduced the term "paraneuron" to describe cells which are not regarded as neurons but are related to neurons on the basis of their structure, function and metabolism. The paraneurons fulfill the following criteria: i. Production of substances identical or related to neurotransmitters or neurohormones; ii. Presence of neurosecretory-like and /or synaptic vesicle-like granules; iii. Release of granule contents into into the extracellular space in response to adequate stimuli acting upon a cell membrane receptor (mostly nicotinic for the chromaffin cells)

Some members of this paracrine family include gastroenteric endocrine cells, mast cells, melanocytes, pancreatic islet cells, pinealocytes, adenohipophysial cells, parafollicular and Merkel cells (Fujita, Kobayashi et al. 1974). As neurons and paraneurons share much in common in terms of structure and function, the latter may serve as a preliminary test system for certain questions of nerve function.

The major cell types of the adrenal medulla are outlined below.

1. Adrenaline containing (A). Adrenaline containing cells contain large granular vesicles of 180 nm in diameter, of low electron density. The presence of the enzyme phenylethanolamine-N-methyl-transferase (PNMT) allows the conversion of noradrenaline to adrenaline in these cells.

2. Noradrenaline containing (NA). These cells typically contain vesicles of 80 nm in diameter, of high electron density.

3. Small granule containing cell (SGC). These cells differ from the NA and A containing cells by the following: i) a smaller cell body ii) higher nucleus to cytoplasm ratio iii) smaller granules iv) sparse innervation by cholinergic neurons.

The SGC cells form processes which are found both in the adrenal cortex and medulla contacting blood vessels including sinusoidal capillaries, steroid producing cells of the reticularis and fasciculata zones, and processes that are seen to belong to medullary nerve cells (Unsicker, Habura-Fluh et al. 1978).

Three different catecholamines are found in the adrenal medulla; two of these (epinephrine and norepinephrine) occur in high concentration (Aldrich 1901; McGoodall 1951) the third, dopamine is present only in small amounts, and possibly exists just as a precursor of norepinephrine (Shepard and West 1953). It is now well established that catecholamines in this gland are stored in a specialized subcellular particle, the chromaffin granule (for review see Kirshner 1969 and Smith 1968). The first clear indication that the hormones of the adrenal medulla are stored in some kind of subcellular particle was obtained in 1953 by Blaschko and Welch and by Hillarp and Nilson in 1953. The first electron micrographs of the adrenal medulla were published by Lever in 1955. Osmiophilic membrane-limited granules in the cell that were smaller than mitochondria were suggested to be the hormone granules and hence

the term chromaffin granule was introduced for the characteristic particles of the chromaffin cell. Later studies revealed the costorage and corelease of neuropeptides within chromaffin cells of the adrenal medulla (Evans, Erdelyi et al. 1983; Livett, Day et al. 1982; Livett and Dean 1980; Livett, Dean et al. 1981; Lundberg, Hamberger et al. 1979; Schultzberg, Hokfelt et al. 1978), (see Table 1 "Constituents of Chromaffin Cell granules")

Table 1**Constituents of the Chromaffin Cell Granule**

Catecholamines
 Nucleotides
 ascorbic acid
 cytochrome b561
 dopamine β hydroxylase

Peptides

Chromogranins (A,B,C)
 Preproenkephalin A
 Met-enkephalin
 Leu-enkephalin
 BAM-12-P, 20-P,22-P
 Peptide I
 Peptide E
 Amidorphin
 Metorphamide
 Preproenkephalin B
 Dynorphin A (related peptides)
 Proopiomelanocortin
 Bombesin
 GRP
 Neuromedin C
 Neuropeptide Y
 Neurotensin
 Substance P
 Somatostatin
 Vasoactive Intestinal Peptide
 Aldosterone Secretion Inhibitory Factor
 Calcitonin Gene Related Peptide
 Histogranin*

References:

(Marley and Livett 1985) , *(Lemaire,1989)

1.6.3. Stimulus-Secretion Coupling

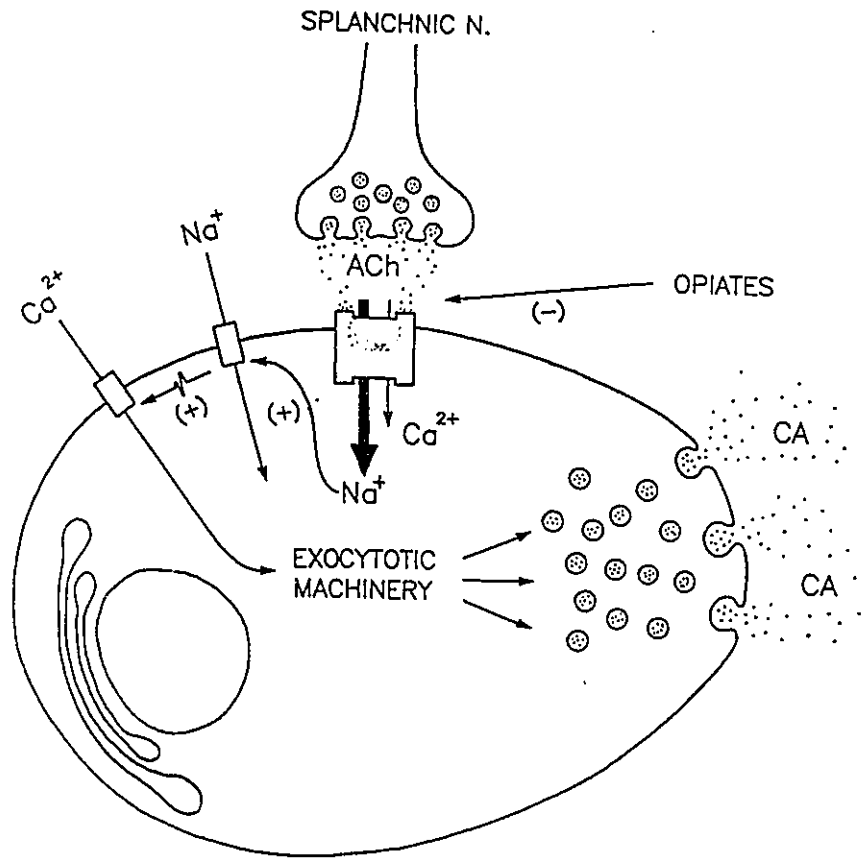
The main focus of research on chromaffin cells has revolved around their secretory mechanisms. The release of catecholamines from the adrenal medulla is triggered by a process termed stimulus-secretion coupling, coined by Douglas and Rubin, 1965 (Douglas and Poisner 1965; Douglas and Rubin 1961). The ACh receptor is responsible for the initiation of events related to stimulus secretion coupling. The dependence of the secretory function of chromaffin cells on time, ACh concentration, potassium depolarization, calcium concentration, nicotinic and muscarinic receptor stimulation has been thoroughly investigated. The cholinergic receptor of the bovine adrenal medulla is thought to be predominantly nicotinic based upon: i) the ability of nicotine to stimulate secretion ii) the lack of substantial secretion induced by muscarinic agonists iii) the blockade of secretion by hexamethonium.

Stimulation of the splanchnic nerve resulting in the release of acetylcholine (ACh) which binds to and activates the ACh nicotinic receptor complex allows the entry of Na^+ and to a lesser extent Ca^{++} into the chromaffin cell. (Douglas 1968; Douglas and Rubin 1961; Douglas and Rubin 1968; Kirshner 1969). This ACh activation results in sufficient depolarization of the chromaffin cell membrane to allow the opening of voltage-sensitive Na^+ and Ca^{++} channels. This combination of events allows the entry of Ca^{++} from the extracellular fluid and the resulting rise in intracellular Ca^{++} is the primary trigger

for exocytosis of chromaffin granules containing catecholamines (Fig.3). The precise machinery, however, that transduces the cytosolic free Ca^{++} signal into the mechanics of granule fusion with the cell membrane is unknown. Calcium binding proteins (eg. calmodulin) Ca^{++} -dependent kinases and cytoskeletal elements appear to be involved (Strittmatter 1988).

Figure 3

The receptors and known intracellular events of excitation secretion coupling in the adrenal chromaffin cell.



High affinity muscarinic receptors are also present on chromaffin cells (Kayaalp and Neff 1979). Muscarinic agonists have been shown to stimulate the release of catecholamines in cat (Douglas and Poisner 1965; Feldberg, Mintz et al. 1934), rat (Wakade 1981), and guinea pig (Role, Leeman et al. 1981), but inhibit secretion in bovine chromaffin cells (Fischer, Holz et al. 1981). In the case of bovine chromaffin cells, stimulation of muscarinic receptors leads to three intracellular events; i) an increase in intracellular cGMP levels (Lemaire, Derome et al. 1981; Schneider, Hollis et al. 1979) ii) the stimulation of inositol phosphate turnover (Azila and Hawthorne 1982; Fischer, Holz et al. 1981) iii) increased intracellular calcium concentrations (Baker, Cheek et al. 1985; Kao 1985). The question as to whether the stimulation of muscarinic receptors in bovine adrenal chromaffin cells leads to stimulation, inhibition or no effect on secretory processes remains to be determined (Baker, Cheek et al. 1985; Derome, Tseng et al. 1981; Fischer, Holz et al. 1981).

The release of catecholamines (CA) induced by ACh may be modulated by neuropeptides released from nerve terminals, interneurons or adrenal chromaffin cells. These peptides acting on specific receptors located on nerve terminals and/or adrenal chromaffin cells may block the release of ACh and therefore decrease splanchnic nerve action on the adrenal chromaffin cells. Neuropeptides acting directly on adrenal chromaffin cells have also been demonstrated to stimulate or inhibit the release of catecholamines. Indeed, it is possible to delineate two groups of

peptides that modulate adrenal catecholamine secretion. Firstly, peptides with positive effects on catecholamine secretion at nanomolar concentrations, such as, bradykinin, angiotensin II (Feldberg and Lewis 1964), CRF (Udelsman, Harwood et al. 1987), and gastrin releasing peptide (Okubo, Kaku et al. 1985) can be differentiated from a second group of peptides. This second group includes vasoactive intestinal peptide, somatostatin, substance P (Livett, Kozousik et al. 1979), vasopressin (Porter, Wiley et al. 1988), oxytocin (Porter, Wiley et al. 1988), and certain opiate peptides (discussed in the following section) that inhibit catecholamine secretion at micromolar concentrations.

1.6.4. Adrenomedullary Opioid Receptors

The adrenal medulla may be a suitable model system to study neuronal opioid receptors because of the relative homogeneity of its constituent cells. A large number of opioid peptides have been identified in the mammalian adrenal medulla, (for review see Marley and Livett 1985). Immunohistochemical studies have demonstrated that these opioid peptides are present in both the adrenal chromaffin cells and nerve terminals within the adrenal medulla (Lemaire, Chouinard et al. 1986; Lemaire, Dumont et al. 1983; Livett, Day et al. 1982; Schultzberg, Hokfelt et al. 1978). Membranes from the bovine adrenal medulla or isolated bovine adrenal chromaffin cells possess saturable, high affinity, stereospecific opioid binding sites (Boublik, Clements et al. 1983; Chavkin, Cox et al. 1979; Costa, Guidotti et al. 1981; Kumakura, Karoum et al. 1980; Lemaire, Livett et al. 1981; Saini and Guidotti 1982). More recently, ligand binding studies have provided evidence for a multiplicity of opioid receptors within the adrenal medulla (Castanas, Bourhim et al. 1985a; Castanas, Bourhim et al. 1985b; Dumont and Lemaire 1984; Kamikubo, Murase et al. 1986). Secretion of adrenal opioid peptides has been demonstrated both in vivo, by stimulation of the splanchnic nerve (Chaminade, Foutz et al. 1984; Hexum, Hanbauer et al. 1980) and in vitro from isolated bovine chromaffin cells in cultures stimulated with nicotine or high potassium ion concentrations (Livett, Day et al. 1982; Rossier, Dean et al. 1981). The released endogenous opiates may act locally on receptor sites on adrenal chromaffin cells to modulate the release of catecholamines by feedback inhibition or may have effects on

distant organs. Hughes, (Hughes 1981) reviewed the many peripheral sites where opioid receptors have also been identified and where peripheral opiates may play important functions. These tissues include kidney, pancreas, stomach, jejunum, ileum, colon, vas deferens, sympathetic ganglia, and adrenal cortex. The presence of endogenous opiate peptides has also been reported in human placenta (Lemaire, Valette et al. 1983) and rat lung. (Day, Lemaire et al. 1985).

Despite extensive investigation, the function assigned to adrenal opiate receptors remains largely speculative. Opioid receptors in the adrenal medulla were originally thought to play modulatory roles in catecholamine secretion from chromaffin cells (Kumakura, Karoum et al. 1980). Opioid agonists such as morphine and etorphine produce concentration dependent inhibition of endogenous CA secretion induced by nicotine. Similar effects were produced by opioid peptides endogenous to the adrenal including Met- and Leu- enkephalin, Met enkephalin-Arg-Phe, BAM-22-P, dynorphins, rimorphin and metorphamide (Dean, Lemaire et al. 1982). However these peptides were very weak, some requiring 10-1000 μ M to produce effects. Lemaire and his coworkers, (Lemaire, Lemaire et al. 1980) provided the reasoning and experimental results to prove that the inhibition of CA secretion by opiates did not occur in the low nanomolar concentration range that would allow the postulation that high affinity opiate receptors were involved. Furthermore, they noted that these effects were mimicked, not reversed by the opiate antagonists naloxone and naltrexone

(Lemaire, Lemaire et al. 1980). Therefore it is thought that the inhibition of secretion produced by opiate receptor ligands is via a non-specific interaction with the ACh nicotinic receptor complex, or via a non-opiate modulatory site associated with the receptor (Lemaire, Livett et al. 1981). Since the opiate inhibition is not reversed by naloxone it is also possible that this effect is produced through the non-opiate sigma binding site.

1.6.5 Adrenal medullary Sigma and PCP receptors

Of all the opiates tested, (\pm)SKF-10047 was one of the most potent inhibitors of catecholamine release evoked by nicotinic agonists in the adrenal medulla (Dumont and Lemaire 1984). Phencyclidine, has also been reported to inhibit the ACh induced secretion of CA from isolated chromaffin cells and perfused adrenal glands (Malave, Borowitz et al. 1983; Purifoy and Holz 1984). The mechanism of PCP induced inhibition of ACh responses is thought to be the result of the interaction of PCP with the ACh receptor complex. In binding assays, (\pm)SKF-10047 was found to bind to a high affinity site in the presence of a high concentration of DADLE- which selectively blocks all subtypes (μ , κ , δ) of opiate receptors (Dumont and Lemaire 1984). This residual high affinity site was then postulated to be the sigma receptor.

1.6.6. Endogenous PCP and/or Sigma Ligands in the Adrenal Medulla

The presence of endogenous ligands for sigma and PCP receptors in the periphery has not been documented, although sigma and PCP receptors are reportedly present (Malave, Borowitz et al. 1983; Purifoy and Holz 1984; Rogers, Cecyre et al. 1989; Su, London et al. 1988; Wolfe, Culp et al. 1989). The recent isolation of an endogenous peptide from the bovine adrenal gland that can displace the binding of PCP and sigma ligands has also been of interest in our laboratory (Lemaire, 1989). The purified peptide known as "histogranin", was named after its structural similarity with that of a fragment of histone H4 and its origin in bovine adrenal chromaffin granules (Lemaire, 1989). Preliminary results have provided insight into the size of the precursor as well as the characteristics of histogranin release and its ability to modulate the secretion of CA from the bovine adrenal medulla induced by nicotinic agonists. Most convincingly, histogranin produces PCP-like stereotyped behaviour, ataxia, and locomotion in the rat after intracerebroventricular injection (Lemaire, 1989). The complete characterization of sigma and PCP binding sites in the bovine adrenal medulla will provide a model to evaluate the validity of histogranin as an endogenous ligand at these sites.

2. SPECIFIC AIMS

The objectives of this research are outlined below.

1. To fully characterize the binding of the prototypic sigma drug, (+)SKF-10047 in the adrenal medulla.
2. To establish the existence of and characterize the binding sites for PCP -like drugs in this tissue.
3. To characterize the binding of the highly selective sigma ligand [³H](+)-3-PPP and the PCP ligand [³H]TCP in bovine adrenal medulla.
4. To establish the similarities and/or dissimilarities that these may have in comparison with CNS binding sites using the available specific congeners of PCP and sigma ligands.
5. To monitor the specific binding of histogranin by autoradiography in tissue sections of the bovine adrenal glands.

3. METHODS

3.1 The Radioligand Binding Assay

Probably, the most important technical advance in the study of receptors was the development of the direct ligand binding assay. The ability of the receptor binding assay to directly quantitate the affinity of a ligand for the receptor provides a powerful screening method for drug selection as well as an ideal tool used in structure activity relationships. This assay also permits the characterization of multiple receptor sites for a given receptor ligand. It has generally been found that the potency of a drug measured by radioligand binding in vitro predicts the potency of that drug in biological systems. The technique itself therefore, offers a primary screening method for drugs which is quantitative and rapid. The measurement of ligand binding to receptors has become very routine with the commercial availability of many radiolabeled drugs for numerous receptor systems. In most tissues, the concentrations of receptors are very low, ie. in the range of femtomoles to picomoles per mg original weight of tissue and therefore, a ligand must be developed which possesses three basic properties:

1. The ligand must have high affinity for the receptor.
2. The ligand must exhibit high selectivity for the receptor.
3. The ligand must be readily radiolabeled to high specific activity so that low concentrations can be accurately measured.

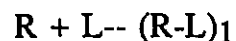
The binding of a ligand to a membrane or soluble preparation does not constitute definition of a receptor. In order to define a binding site as a receptor, certain important criteria must be met. The binding site must: i) be saturable ii) the binding of the ligand must be reversible iii) the receptor must exhibit the appropriate distribution, iv) binding must demonstrate the proper rank order of potencies and stereospecificity characteristic of the receptor and v) be heat labile, (that is under thermal denaturing conditions the binding site should no longer exhibit the proper binding characteristics). For the study of receptor systems the property of rank order is probably the most critical. If the potencies of a group of drugs are known for a particular biological function and there is good correlation with the potencies for competition of the binding site, then it is highly likely that the receptor has been labeled. Similarly, if one enantiomer is known to be potent on a system and the other is not this stereoselectivity must also be apparent in the radioligand binding assay. Thus receptors are not defined based upon the type of [^3H]-ligand that attaches, but by the order of potency by which the radiolabel is displaced by ligands specific for the site.

The binding data reveals two features of the ligand-receptor interaction. Firstly, the affinity (K_D) of the ligand for the receptor and secondly the maximal receptor population (B_{max}) that can be bound by the ligand. These values are determined by the completion of a saturation binding experiment, wherein, an increasing concentration of [^3H] ligand is incubated in the presence of a fixed

concentration of tissue. The results of this experimental array are plotted as *Bound Ligand* on the ordinate and *Concentration of Free Ligand* plotted on the abscissa. The curve obtained from this plot is a rectangular hyperbola. Transformation of this saturation data in the Scatchard Plot allows the estimation of the affinity of the ligand for the receptor (K_D) and the density of the receptor in the tissue (B_{max}). The Scatchard plot consists of *Bound/Free* on the ordinate vs *Bound* on the abscissa. The negative-inverse of the slope of this linearized saturation data reveals the apparent K_D while the x-intercept represents the apparent B_{max} . In complex systems, where one ligand has affinity for more than one receptor or subtype of receptor, the Scatchard plot will be curvilinear. Plotting the saturation data according to the Hill equation, will allow the determination of whether the ligand is binding to one or multiple sites (Hill,1910). The Hill plot was derived to quantitate enzyme-substrate interactions from classic mass-action, rectangular hyperbolic behaviour. One of the earliest applications of the Hill equation was the determination of the oxygen saturation of hemoglobin, where it is known that binding of the first O_2 molecule facilitates binding of the second O_2 molecule and so forth. Instead of obeying the classic mass action law,

$$B = \frac{B_{max} [\text{ligand}]}{K_D + [\text{ligand}]}$$

such systems could be adapted to a sequential binding model,



describing the net ligand binding isotherm (n=theoretical number of ligand binding sites per receptor molecule)

The resulting Hill equation,

$$B = \frac{B_{\max} [\text{ligand}]^n}{K_{D1} + [\text{ligand}]^n}$$

may be transformed into a logarithmic form for convenience in plotting.

$$\log \frac{B}{(B_{\max} - B)} = n \log[\text{ligand}] - \log K_{D1}$$

Saturation data of multireceptor binding, must be analyzed by nonlinear regression using various computer programs designed for this purpose, eg. Ligand (Munson and Robard, 1980) or BDATA,(EMF Softwares, Knoxville, TN USA).

Establishing rank order of potency of compounds active at the binding site involves the completion of competition displacement curves. These experiments are carried out in tubes containing a fixed concentration of membrane protein, along with a fixed concentration of radiolabel in the presence of increasing

concentrations of the unlabelled drug. The displacement data is plotted as *Percent Bound Radiolabel vs Concentration of Unlabelled Ligand*. Competition data is analyzed by nonlinear regression using computer programs such as CDATE (EMF SOFTWARES, Knoxville, TN., USA) or Ligand (Munson, and Robard, 1980). IC₅₀ values obtained by non-linear analysis are converted to K_i values using the following equation of Cheung and Prusoff, 1959.

$$K_i = \frac{IC_{50}}{1 + [L]/K_d}$$

Table 2**Compounds Used in Binding and Autoradiography**

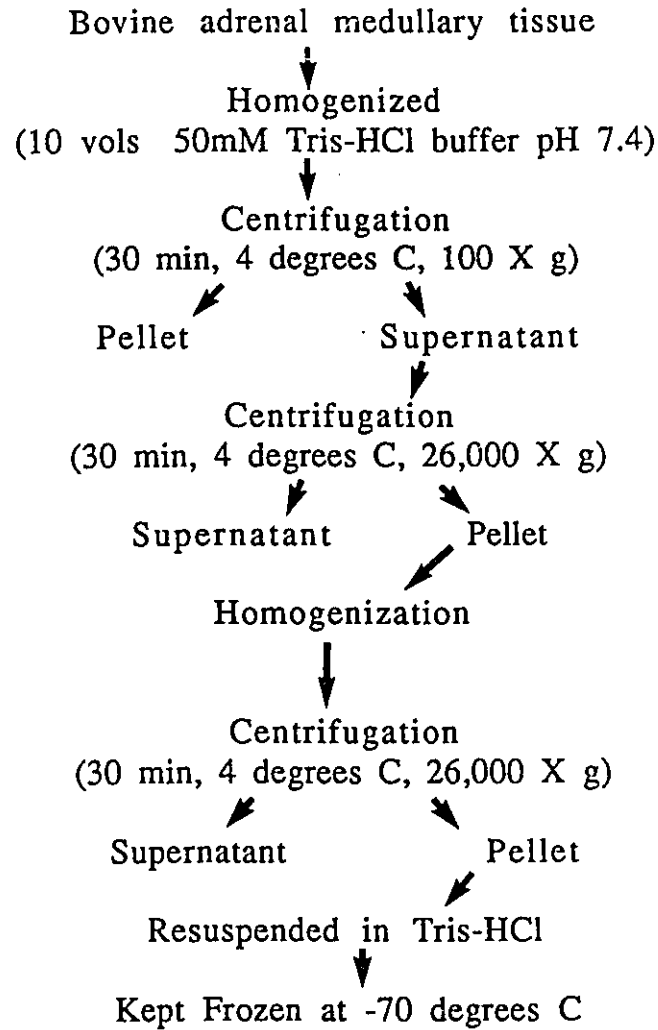
<u>Materials</u>	<u>Supplier</u>
[³ H]SKF-10047	New England Nuclear, Boston, Mass. USA
[³ H]TCP	New England Nuclear, Boston, Mass. USA
[³ H]3(+)-PPP	New England Nuclear, Boston, Mass. USA
[³ H]MK-801	New England Nuclear, Boston, Mass. USA
[¹²⁵ I] histogranin	Our Laboratory
acetylcholine	Sigma Chemical Corp., St. Louis, MO
AP5,AP7	Tocris Neuramin, Essex, UK
atropine	Sigma Chemical Corp., St. Louis, MO
BMY-14802	Bristol Myers Corp.
alpha-bungarotoxin	Sigma Chemical Corp., St. Louis, MO
(+),(-)butaclamol	Research Biochemicals, Wayland, MA
carbamylocholine	Sigma Chemical Corp., St. Louis, MO
chlorpromazine	Kindly donated by Dr. P. Hrdina, Ottawa
decamethonium	Sigma Chemical Corp., St. Louis, MO
diltiazem	Aldrich Chemicals, Milwaukee, WI
ditolylguanidine	Dr. E. K. Weber, Portland, OR. USA
haloperidol	McNeil Pharmaceuticals, Don Mills, Ont.
hexamethonium	Sigma Chemical Corp., St. Louis, MO
MK-801	Tocris Neuramin, Essex, UK
muscarine	Sigma Chemical Corp., St. Louis, MO. USA
nicotine	Sigma Chemical Corp., St. Louis, MO. USA
nifedipine	Dr. A. Scrabine, Miles Lab., IN. USA
phencyclidine	Natl. Inst. Drug Abuse, Bethesda, MD.
mOH-PCP	Dr. H. Avdovich, Ottawa, Ontario
3(+)-PPP	Nat., Inst. Drug Abuse, Bethesda, MD.
rimcazole	Dr. R. Ferris, Burroughs Wellcome Lab.
(+),(-)SKF-10047	Dr. R. L. Hanks, NIDA, Baltimore, MD
spiperone	Dr. H. Niznik, Toronto, Ontario
TCP	Dr. H. Avdovich, Ottawa, Ontario
verapamil	Knoll Pharmaceuticals

3.2 Membrane Preparation

Bovine adrenal medulla were obtained from a local slaughterhouse (St. Albert, Ontario) within five minutes after death and immediately transported to the laboratory on ice. The boundary between the adrenal medulla and cortex may be quite irregular and thus during dissection of the medulla, a distinct ring of medulla is left behind in order to avoid contamination by non-medullary tissues. The dissected adrenal medullary tissue was then minced with razor blades and processed (2X20s) in a Waring blender with 10 vol. of ice-cold 50 mM Tris-HCl, pH 7.4 (Buffer A). The tissue suspension was then homogenized with a glass-teflon homogenizer, and particulate matter was removed by centrifugation at 1000 X g for 30 min at 4 degrees C in a Sorvall SS34 rotor. The pellets were discarded and the resulting supernatant fraction was centrifuged at 26,000 X g for 30 min at 4 degrees C. The resulting membrane pellets were resuspended in Buffer A, incubated for 20 minutes at 37 degrees C and recentrifuged at 26,000 X g for 30 min. During this last step, the incubation at 37 degrees C facilitates the dissociation of endogenous ligands which may be present in the membrane homogenate. The final membrane pellets were resuspended in buffer A at a concentration of 2 mg/ml (Lowry determination) and frozen at -70 degrees C, (see fig 4).

Figure 4

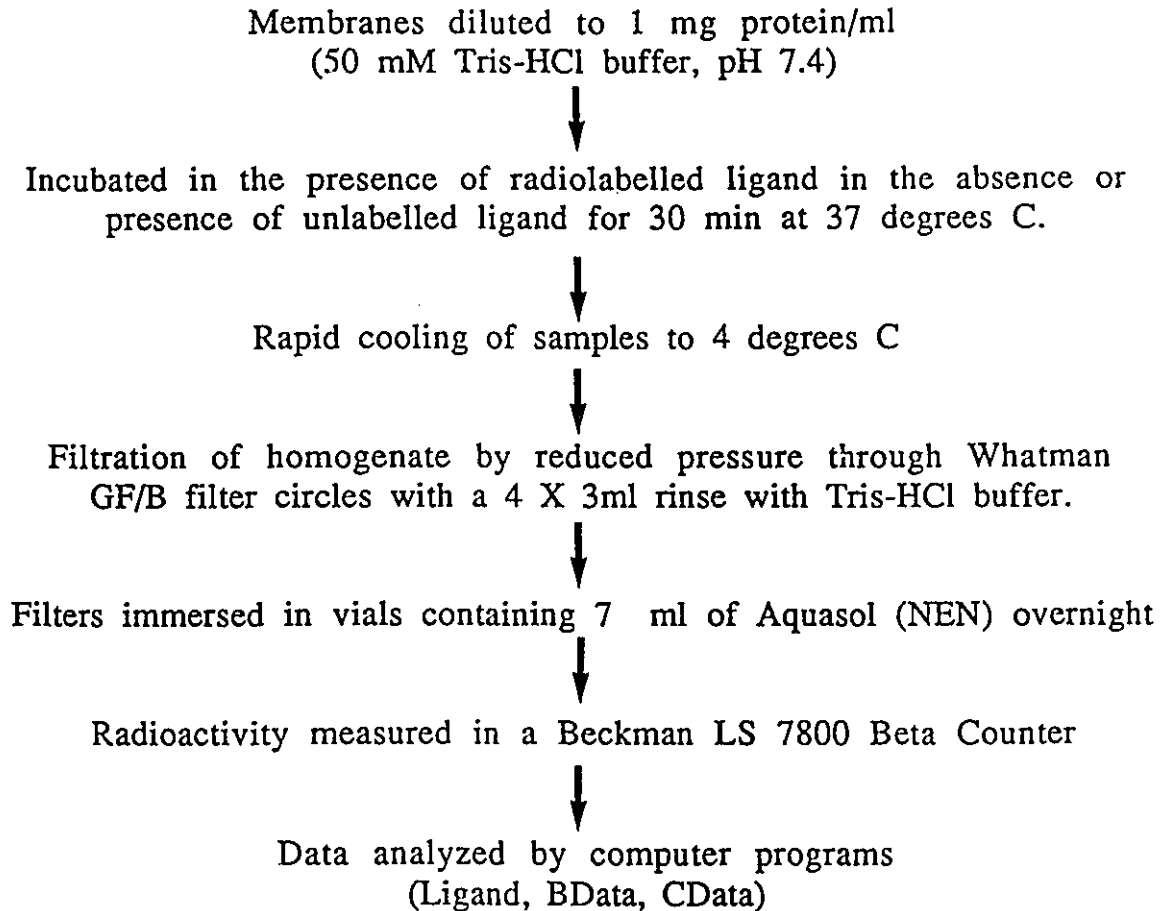
Preparation of Bovine adrenomedullary membranes



3.3 Radioligand Binding

The thawed bovine adrenal membrane homogenate was diluted to a final concentration of 0.5 mg protein/ml. Typical binding assays were performed at 37 degrees C for 30 min with 2-ml aliquots of the membrane preparation in the presence of labelled and unlabeled drugs at the indicated concentrations. Non-specific binding was measured in the presence of 10^{-6} M of the unlabelled ligand and this value was subtracted from the total binding for quantification of the specific binding. Incubations were terminated by placing the samples on ice for 15 min followed by filtration under reduced pressure through GF/B Whatman filters pretreated with 0.05% polyethylenimine. Filters were washed with four 3-ml aliquots of ice cold Buffer A and placed in liquid scintillation vials along with 10 ml Aquasol (NEN). Radioactivity was measured in a Beckman LS 7800 liquid scintillation counter at 45% efficiency. Binding data were analyzed by the iterative curve fitting program, BDATA (EMF Softwares, Knoxville, TN, USA) and the nonlinear least-square computer-fitting program CDATA (EMF software Inc.) All binding experiments were repeated two or three times in duplicate and the values represent the mean (+/-) the SD.

Figure 5

Radioligand binding technique

3.4 Preparation of [¹²⁵I] histogranin

Histogranin, a peptide of 15 amino acids, was synthesized by the solid phase procedure as described by Lemaire et al, 1989. The synthetic peptide was purified by gel filtration (Sephadex G-10) and preparative high performance liquid chromatography on neucleosil C18. The purity of the peptide was assessed by amino acid analysis, thin layer chromatography and analytical high performance liquid chromatography on μ -Bondapak C18, SP-5PW and Zorbax ODS columns. The peptide was iodinated with Na¹²⁵I in the presence of chloramine T as described by (Hunter and Greenwood 1962). The iodinated peptide was separated from free iodine by passage through a cartridge of Sep Pak and the monoiodinated form of the peptide was isolated by high performance liquid chromatography on μ -Bondapak C18.

3.5 Autoradiography

Bovine adrenal glands obtained fresh from a local slaughterhouse, were retrogradely perfused via the suprarenal vein with cold saline and placed on ice during transport to the laboratory. Tissue was frozen in isopentane that was cooled with liquid N₂. Frozen adrenal tissue was mounted onto cryostat chucks within cryostat embedding medium, (Tissue Tek, Miles, IN. USA) and sectioned at 10 microns at -17 degrees C using a Bright cryostat. Sections were thaw-mounted onto HCl-cleaned, chrome-alum/gelatin subbed microscopic slides (Canlab) and stored in a dessicator for an hour at -20 degrees C.

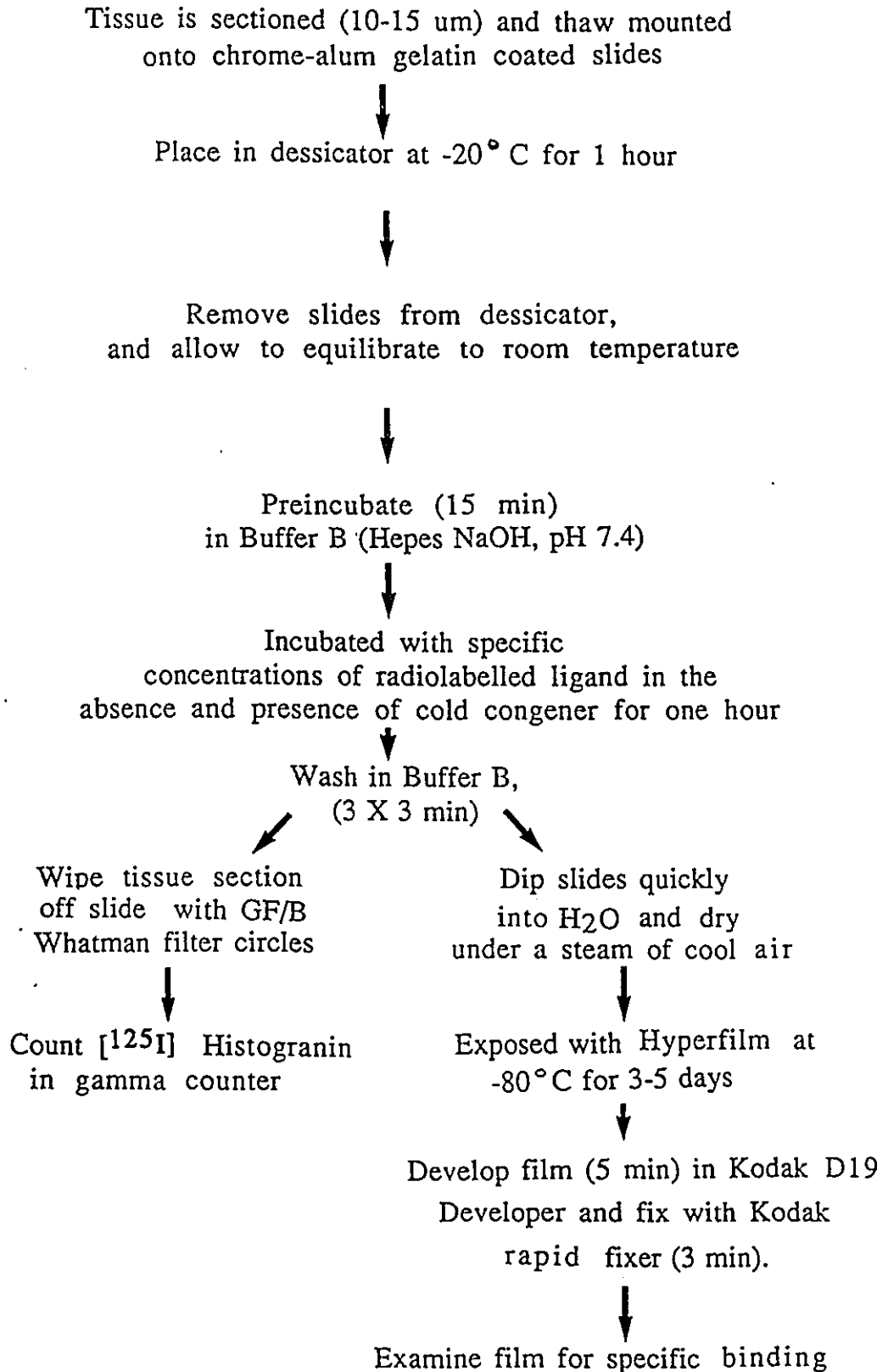
Slides containing tissue sections were preincubated for 15 min at room temperature in Buffer B, (10 mM Hepes NaOH pH 7.4, with the addition of 130 mM NaCl, 0.47 mM KCl, 0.5 mM MgCl₂·6H₂O, 0.1% BSA and the peptidase inhibitor Bacitracin). The buffer was removed and the slides were then incubated in Buffer B containing 60 nM [¹²⁵I] histogranin for one hour according to the following procedure. Slides were placed in flat plexiglass humidity trays. After one hour, slides were washed (3 X 3 min) in fresh ice cold Buffer B. Tissue sections for competition studies were wiped from the slide using GF/B Whatman filter circles. The filter circles were placed in polypropylene tubes and counted in a Beckman gamma counter. In every experiment, nonspecific binding values were determined by including 1 μM concentrations of unlabelled histogranin in the

incubation solution. Specific binding was calculated as the difference between total and nonspecific binding.

Slides processed for visualization were dipped twice rapidly in distilled water and dried under a stream of cold dry air. Dried radiolabelled slides were apposed to Hyperfilm (Amersham) and stored at -80 degrees C for 5 days. After exposure the autoradiograms were developed in Kodak D-19 developer, fixed in Kodak Rapidfixer, and examined for localized binding of [^{125}I] histogranin (see fig. 6).

Figure 6

Flow chart of autoradiographic technique as described in materials and methods.



4. RESULTS

4.1 [3H](+)SKF-10047 binding

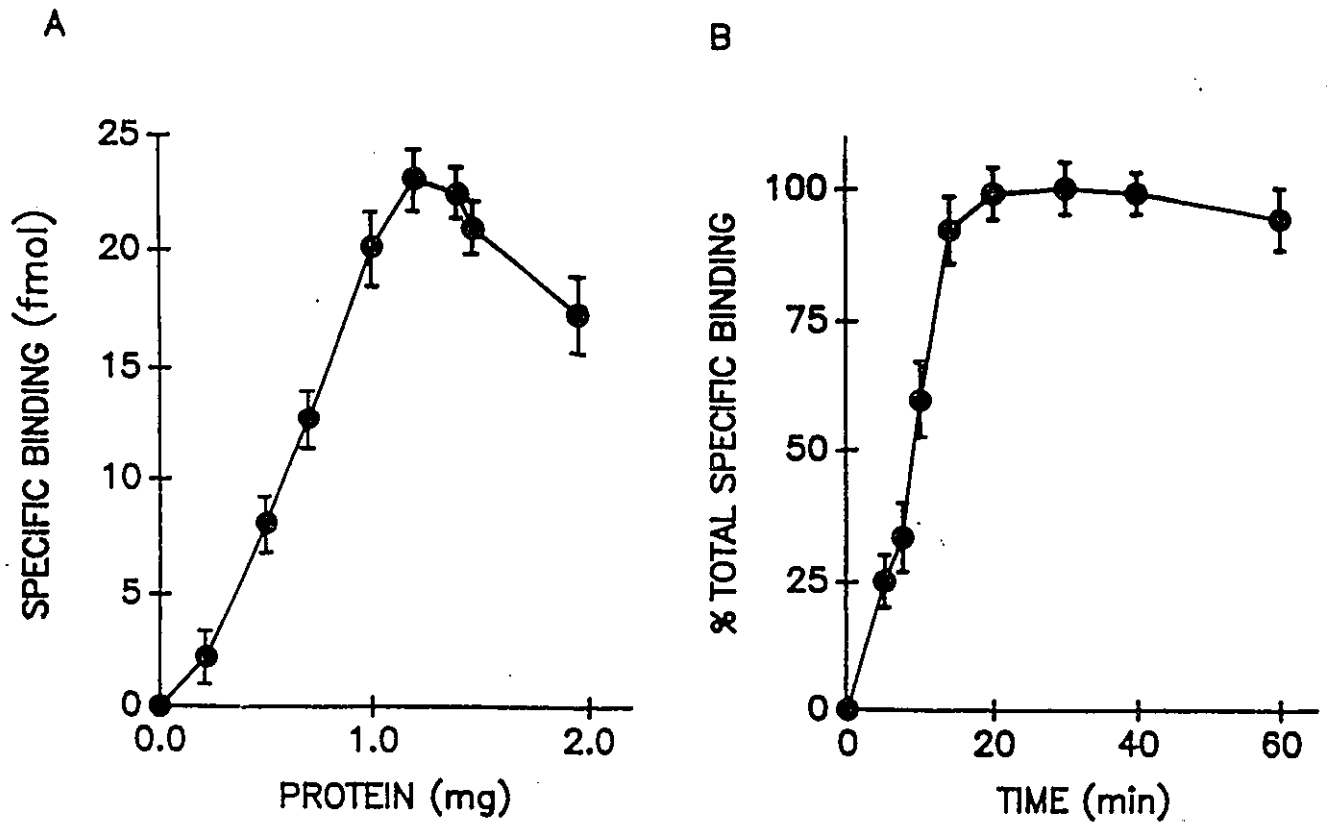
Binding dependence on time and protein concentration.

The binding of [³H]-(+)-SKF-10047 was investigated as a function of time in order to determine when equilibrium was achieved (Fig 7B). Equilibrium was reached rapidly at 37 degrees C, 75% of the specific binding being obtained at 10 min with no statistically significant increases after 20 min. The amount bound was stable up to 60 min. Specific binding of radioligand was also measured as a function of protein concentration. Tissue linearity is important in binding studies to demonstrate the absence of artifacts, such as receptor-ligand degradation during the incubation. Ideally, binding experiments should be carried out with minimal tissue concentrations in order to avoid artifacts caused by non-specific binding. Specific binding of [³H](+)SKF-10047 increased linearly as a function of protein concentration up to 0.6 mg/ml (Fig. 7A). At higher concentrations, specific binding decreased, which reflected the maximum protein limit of GF/B Whatman filter circles. A minor degree of downward curvature in the graphs plotting binding against added tissue is unlikely to lead to incorrect receptor identification, it will certainly yield incorrect or ambiguous values for such parameters as density of binding sites. Therefore, subsequent binding assays were performed in the linear portion of the tissue dependence curve at 0.5 mg protein/ml for 30 min at 37 degrees C. Temperatures ranging from 0 to 37 degrees C will strongly affect the

rates of association and dissociation of binding reactions and may affect equilibrium dissociation constants. Although, this type of information is useful in the consideration of energy barriers and receptor mechanisms, it is rarely critical in receptor identification. An exception is that preincubation of tissue much above 40 degrees C should reduce specific receptor binding. At higher temperatures the tertiary structure of the receptor protein becomes disrupted leading finally to the heat denaturation of the receptor. Maintained or increased binding at higher temperatures may reflect covalent binding of the radioligand or a breakdown product rather than the actual reversible receptor binding. Denaturation of bovine adrenal medulla by preheating membranes to 60 degrees C and then cooling to 37 degrees C before the binding assay reduced over 95% of the specific [³H](+)-SKF-10047 binding.

Figure 7

Dependence of [^3H](+)-SKF-10047 binding on time and protein concentration. [^3H](+)-SKF-10047 was incubated with bovine adrenal medullary membranes for increasing time periods or with increasing concentrations of protein at 37° C. The binding proved maximal after a 30 min incubation, and was also shown to increase linearly with protein concentration until values of 0.6mg/ml were reached.

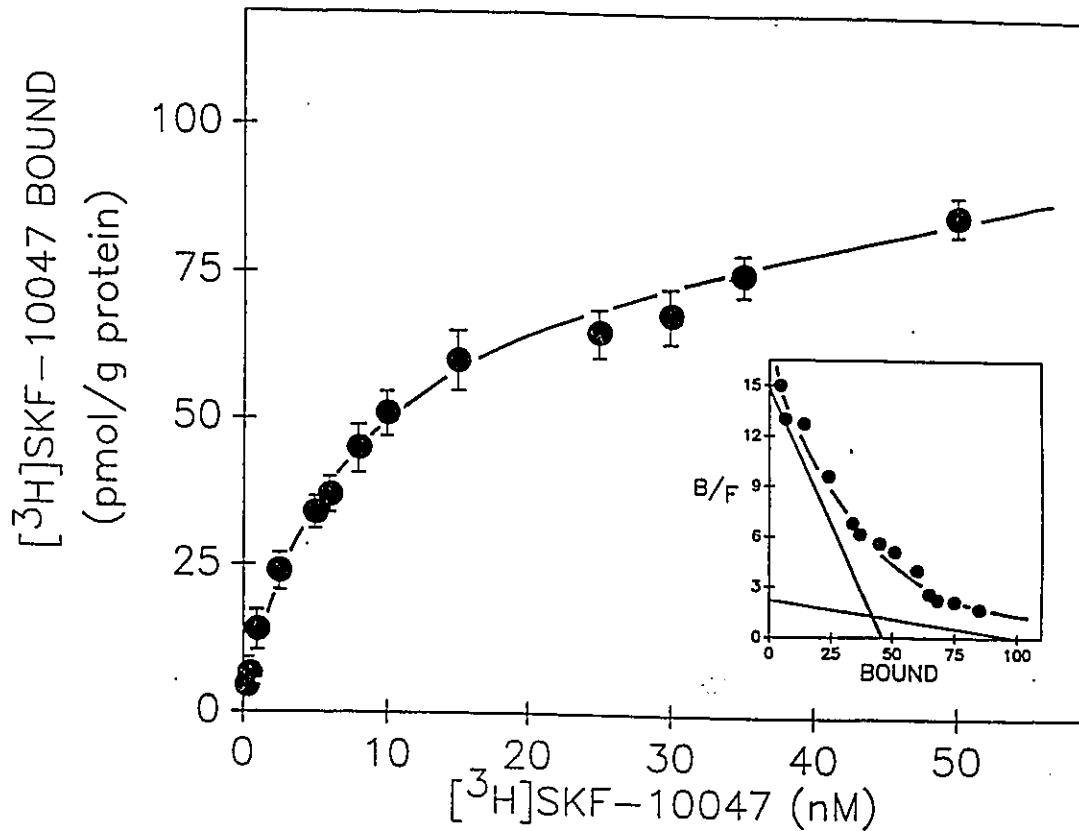


Saturable binding of [³H](+)-SKF-10047 to adrenomedullary membranes.

The saturation of [³H](+)-SKF-10047 binding sites with increasing concentrations of the radiolabel (1-50) nM is shown in Fig. 8. Transformation of these data in the form of a Scatchard Plot reveals a non-linear curve, implying that [³H]SKF-10047 binds to multiple sites in bovine adrenal medullary membrane preparations (Fig 8,inset). Analysis of these data using the binding program (BDATA) revealed that [³H](+)-SKF-10047 bound to a high affinity site with a $K_D = 3 (\pm) 0.5$ nM and $B_{max} = 45 (\pm) 7$ fmol/mg protein, as well as a low affinity site with a $K_D = 55 (\pm) 6$ nM and a $B_{max} = 93 (\pm) 9$ fmol/mg protein.

Figure 8

Saturation and Scatchard plot (inset) of [^3H](+)-SKF-10047 binding sites in bovine adrenal medulla. Incubation of bovine adrenal medulla with increasing concentrations of [^3H](+)-SKF-10047 was performed at 37 degrees C for 30 min. Analysis of the binding by BDATA resulted in a two-site best fit model. The high affinity site was shown to possess a $K_D = 3$ nM, and $B_{\text{max}} = 45$ fmol/mg protein. The low affinity site displayed a $K_D = 50$ nM and $B_{\text{max}} = 100$ fmol/mg protein. (n= 2 experiments completed in duplicate)



Saturation binding studies in presence of specific receptor blockers

Saturable and reversible binding of [³H](+)-SKF-10047 was performed at 37 degrees C for 30 min. Analysis of saturation data in the presence of 0.2 μM TCP, (to block the PCP receptor), utilizing the iterative curve-fitting program, revealed a high affinity component of specific [³H](+)-SKF-10047 binding with an apparent dissociation constant (K_D) of 8.3 (±) 0.2 nM and a B_{max} of 67 (±) 7 pmol/g protein (Fig. 9). In the presence of 1 μM haloperidol (to mask the sigma receptor), a lower affinity site was observed with an apparent K_D of 32.7 (±) 2 nM and a B_{max} of 83 (±) 6 pmol/g protein (Fig. 10).

Competition Assays

Drug competition binding studies also indicated a discrimination of at least two sites labelled by [³H](+)-SKF-10047 in bovine adrenomedullary membranes (Fig. 11). At a 5 nM concentration of the radiolabelled ligand, competition binding studies with selective sigma ligands, namely haloperidol (Largent, Gundlach et al. 1986), produced biphasic inhibition curves. The addition of low concentrations of haloperidol (10⁻⁹-10⁻⁷ M) displaced 72% of the total [³H](+)-SKF-10047 bound while the remaining 28% of the bound radiolabel required higher concentrations for complete displacement (Fig. 11). The K_i values for the high and low affinity binding components were 3.14 and 2475 nM respectively (Table 3). In

contrast, the addition of increasing concentrations of the PCP receptor ligand TCP revealed a 23% displacement of [³H](+)-SKF-10047 at low concentrations, while the remaining 77% of the radiolabel was displaced at concentrations of 10⁻⁷ to 10⁻⁵ M. TCP displayed K_i values of 0.88 and 1011 nM for its high and low affinity binding components respectively. PCP showed marked selectivity for the PCP binding site with a K_i value of 1.3 nM as compared with 3778 nM for the sigma receptor. The benzomorphans, pentazocine and cyclazocine, also produced biphasic inhibition patterns (Table 3). The high affinity component of pentazocine (K_i= 5.5 nM) corresponded to the sigma site (76% displacement of total binding), whereas the high affinity component of cyclazocine (K_i= 2.75 nM) corresponded to the PCP site (18% displacement of total binding).

Figure 9

Saturation and Scatchard plot (inset) of the sigma component of [^3H](+)-SKF-10047 binding. Saturation of binding sites for [^3H](+)-SKF-10047 completed in the presence of 0.2 μM TCP allowed the examination of the sigma binding site. This high affinity binding site for [^3H](+)-SKF-10047 displayed a $K_D=8.3$ nM and a $B_{\text{max}}=67$ fmol/mg protein after equilibrium was reached at 37 C. The binding of the tritiated ligand was carried out as described in Materials and Methods. The binding data were analyzed by the BDATA softwares program and resulted in a one-site best-fit model. Results are the mean \pm the S.D of two sets of experiments completed in duplicate ($n=4$).

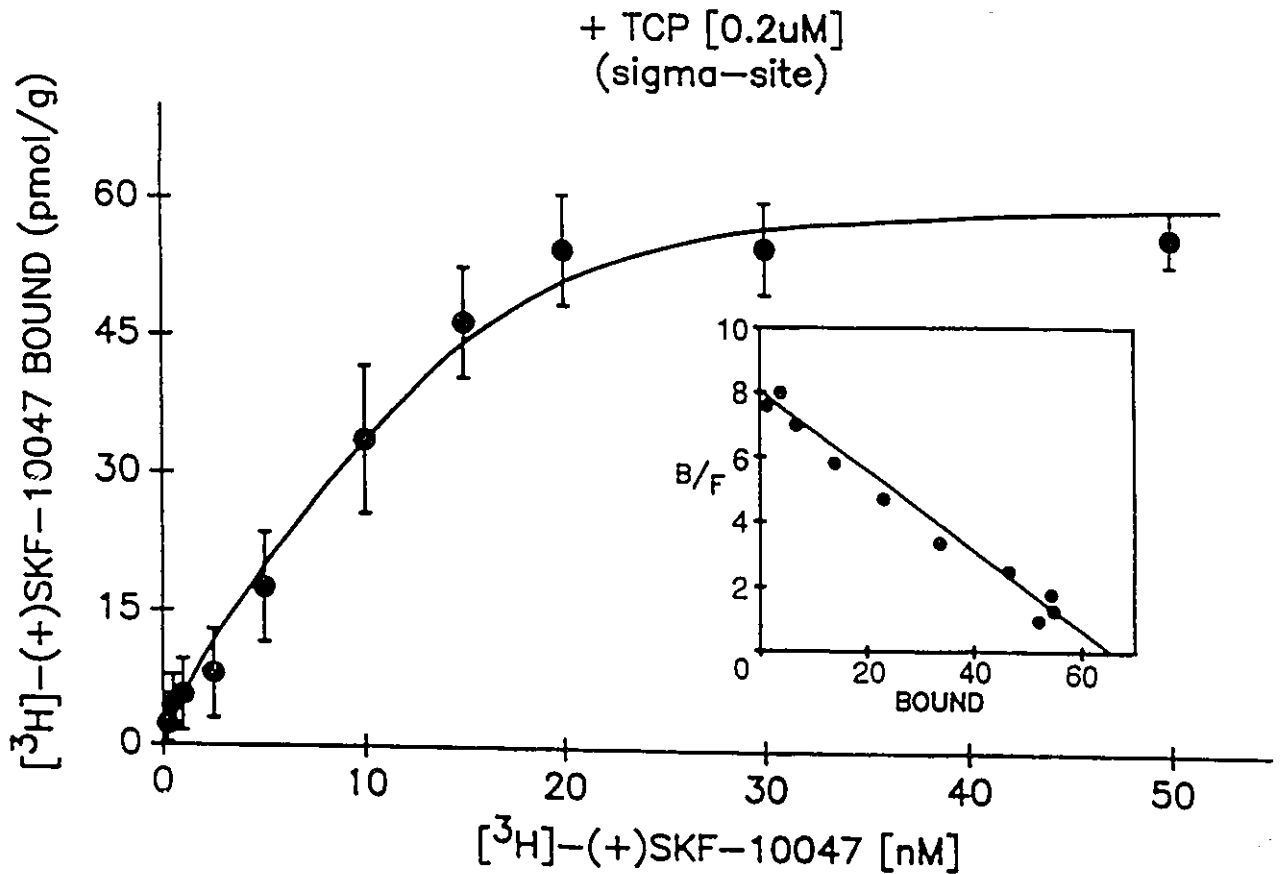


Figure 10

Saturation and Scatchard plot (inset) of the PCP-like site of [^3H]SKF-10047 binding. Increasing concentrations of [^3H](+)-SKF-10047 incubated in the presence of bovine adrenal membranes and 1 μM haloperidol revealed a secondary component of [^3H](+)-SKF-10047 binding. This low affinity site, displayed a $K_D = 32.7 \text{ nM}$ and $B_{\text{max}} = 83 \text{ fmol/mg protein}$. Results are the mean \pm S.D. or two separate experiments ($n=4$ samples per concentration).

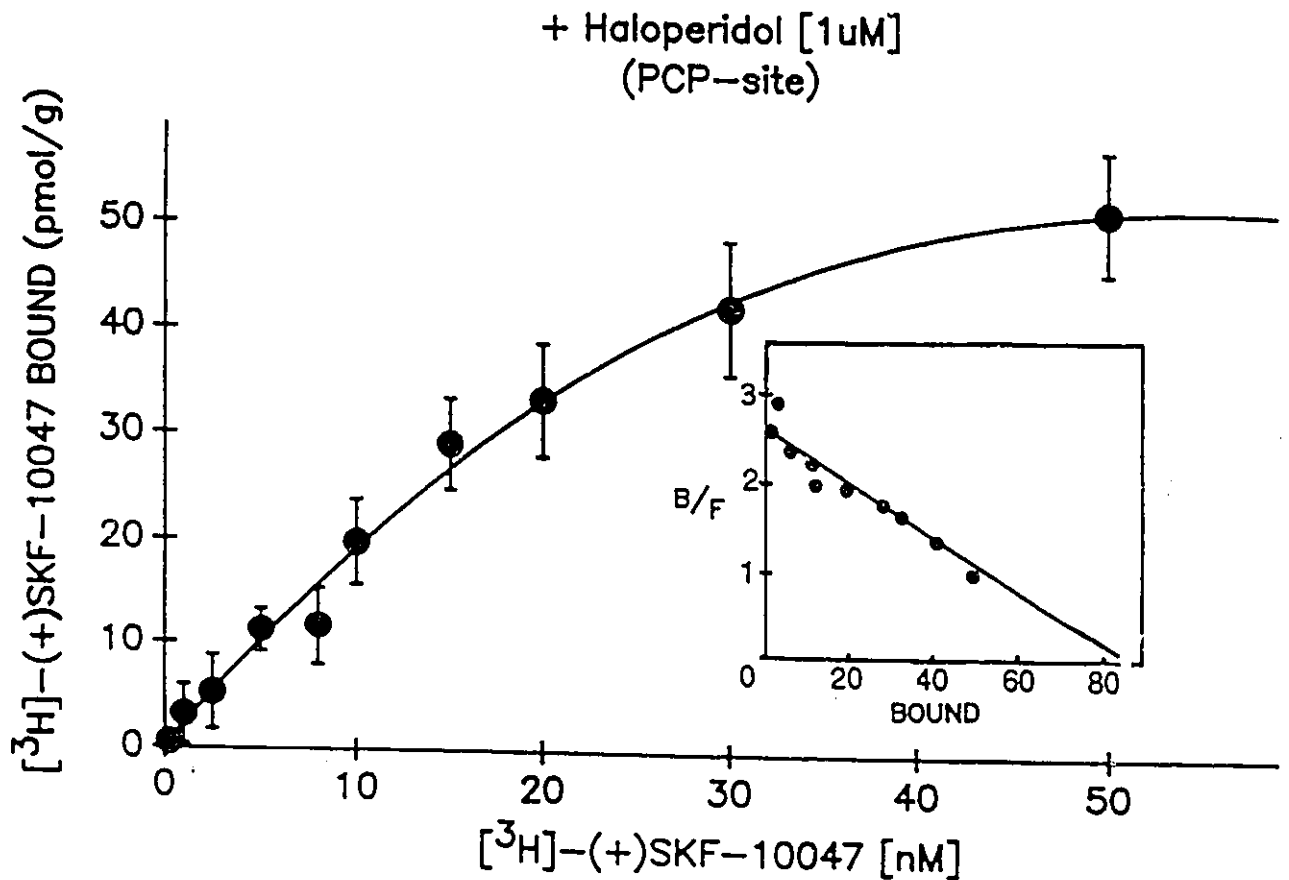
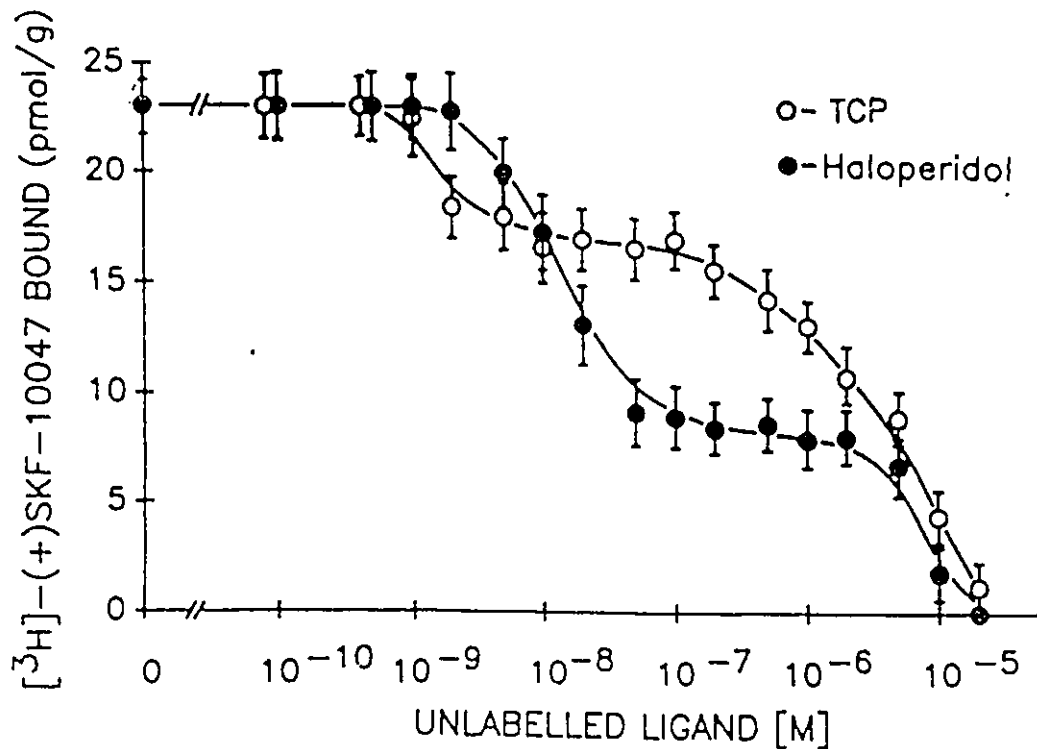


Figure 11

Competition against 5.0 nM [^3H](+)-SKF-10047 binding using specific ligands for sigma and PCP binding sites, haloperidol and TCP respectively. The competition curves were generated by using increasing concentrations of the unlabelled ligand in the presence of a fixed concentration of tissue and [^3H](+)-SKF-10047. Both ligands reduced 100% of the [^3H](+)-SKF-10047 binding in a biphasic manner. Analysis of IC_{50} values were completed with the computer program CDATA. Results are expressed as the mean \pm the S.D. of two sets of duplicated experiments (n=4 samples per concentration).



Specificity of [³H](+)-SKF-10047 binding sites.

(+)-SKF-10047 was more than 120 and 18.4 times as potent as the levorotatory isomer at competing with [³H](+)-SKF-10047 for its PCP and sigma receptors respectively (K_i of 570 and 160 nM as compared with >69,000 and 2,900 nM for the (-)-form respectively (Table 3). The racemate (±)-SKF-10047 was 4 times less potent than the dextroisomer at interacting with both receptors. In addition, the selective opioid receptor ligands DAGO (μ), DSLET (δ), and U-69593 (κ), were ineffective in displacing the binding of [³H](+)-SKF-10047 (IC₅₀ >10,000 nM; Table 4).

4.2 [³H]TCP and [³H]3(+)-PPP binding in bovine adrenal medulla

Time Dependence of [³H]TCP and [³H]3(+)-PPP Binding

The specific binding of [³H]TCP and [³H]3(+)-PPP was examined in membranes prepared from bovine adrenal medulla. Fig. 12 (A,B) shows the time dependence of [³H]TCP and [³H]3(+)-PPP binding respectively. The binding of both ligands was rapid, reaching equilibrium after 30 min and remaining stable for up to one hour at 37 degrees C. In all subsequent assays the binding of [³H]TCP and [³H]3(+)-PPP was measured after a 30 min incubation at 37 degrees C.

Table 3 Relative potency of various compounds in displacing the binding of [³H]-(+)-SKF-10047 to sigma and PCP receptors

Compound	Sigma K_i (nM)	PCP K_i (nM)
Haloperidol	3.14 ± 0.44	2475 ± 312
Pentazocine	5.5 ± 2.1	1449 ± 220
TCP	1011 ± 190	0.88 ± 0.28
PCP	3778 ± 600	1.3 ± 0.4
Cyclazocine	677 ± 80	2.75 ± 1.5

Values represent the means ± SD of three sets of duplicated experiments. [³H]-(+)-SKF-10047 binding was performed with a 5 nM concentration of the radiolabelled ligand as described in Materials and Methods. Competitive displacers were used at sixteen concentrations between 10⁻¹¹ and 10⁻⁵ M. Biphasic competition curves were obtained, and the σ and PCP binding components were analysed with the non-linear least-square computer-fitting program CDATA. The standard deviation is derived from individual curves analysed separately.

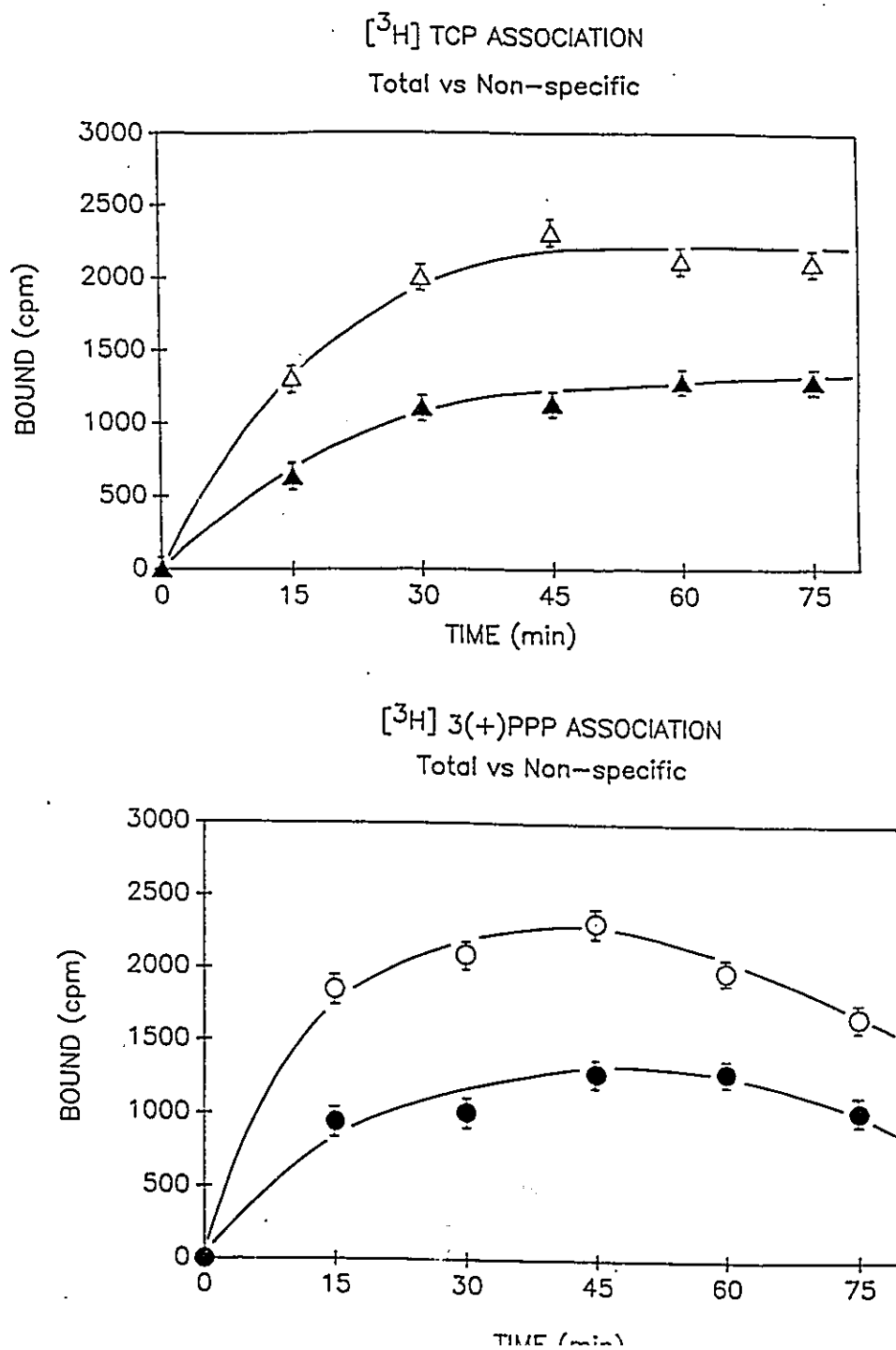
Table 4 Stereospecificity and selectivity of the binding of [³H]-(+)-SKF-10047 to membrane preparations of bovine adrenal medulla: Competition binding studies

Compound	PCP/ σ sites	σ site	PCP site
	IC ₅₀ (nM)	(+0.2 μ M TCP) K _i (nM)	(+1 μ M haloperidol) K _i (nM)
(+)-SKF-10047	128 \pm 26	160 \pm 38	570 \pm 62
(-)-SKF-10047	2,700 \pm 109	2,900	> 69,000
(\pm)-SKF-10047	482 \pm 65		
DAGO	> 10,000		
DSLET	> 10,000		
U-69593	> 10,000		

Competition binding studies were performed in the presence of 0.2 μ M TCP or 1 μ M haloperidol to block PCP or σ sites or without discrimination of the two binding sites (PCP/ σ sites). Values are expressed as the means \pm SD of three sets of duplicated experiments. Binding experiments were performed as described in Materials and Methods.

Figure 12

The dependence of [^3H]TCP and [^3H]3(+)-PPP binding on time was analyzed. Incubation of 3 nM radiolabel in the presence and absence of (10^{-6} M) unlabelled ligand for increasing periods of time revealed the results shown. Both ligands associated rapidly and remained stable for up to 40 min at 37 degrees C. The maximal binding activity was observed at 30 min and remained stable for up to 45 min at 37 degrees C.



Saturation Experiments

Analysis of [^3H]TCP saturation isotherms revealed a one-site best fit model with a K_D of 54 ± 4 nM and a B_{max} of 250 ± 21 pmol/g protein (Fig.13). The calculated Hill coefficient for [^3H]TCP binding was 0.92 (Fig. 15). Analysis of [^3H]3(+)-PPP binding also revealed a linear Scatchard Plot (Fig. 14 (inset)) with a $K_D = 30 \pm 2.5$ nM and a B_{max} of 250 ± 26 pmol/g protein. The Hill coefficient of 0.99 confirmed the one-site binding model of [^3H]3(+)-PPP in the adrenal medulla (Fig. 15).

Competition Experiments

Competition binding studies were carried out using 2 nM [^3H]TCP and 2nM [^3H]3(+)-PPP (Table 5). The sigma specific ligands haloperidol, DTG, rimcazone, BMY-14802, 3(+)-PPP, and (-)-butaclamol were the most potent competitors of both [^3H]TCP and [^3H]3(+)-PPP binding, with K_i values ranging from 0.13 to 0.28 μM against (+)[^3H]3(+)-PPP and 0.019 to 4.4 μM against [^3H]TCP (Table 5). Surprisingly, the specific brain PCP receptor ligands TCP, PCP and m-OH-PCP, were less effective than sigma drugs in displacing the binding of [^3H]TCP, although m-OH-PCP was more potent in displacing [^3H]TCP than [^3H]3(+)-PPP. In contrast to [^3H]TCP, [^3H](+)-3PPP displayed a greater degree of stereoselective preference for the dextrorotatory isomer of SKF-10047 and the levorotatory form of butaclamol.

Figure 13

Saturation of [^3H]TCP binding was investigated in bovine adrenal medullary membranes for 30 min at 37 degrees C. [^3H]TCP was found to bind to one site when analyzed by BDATA. This site resembled that of the low affinity [^3H](+)SKF-10047 (PCP-like) binding component (see Fig. 10). Analysis of the binding of [^3H]TCP (inset) in the Scatchard plot revealed a $K_D=54$ nM and a $B_{\text{max}}=250$ fmol/mg protein. The results represent the mean \pm S.D. of two sets of duplicated experiments. ($n=4$ samples per concentration)

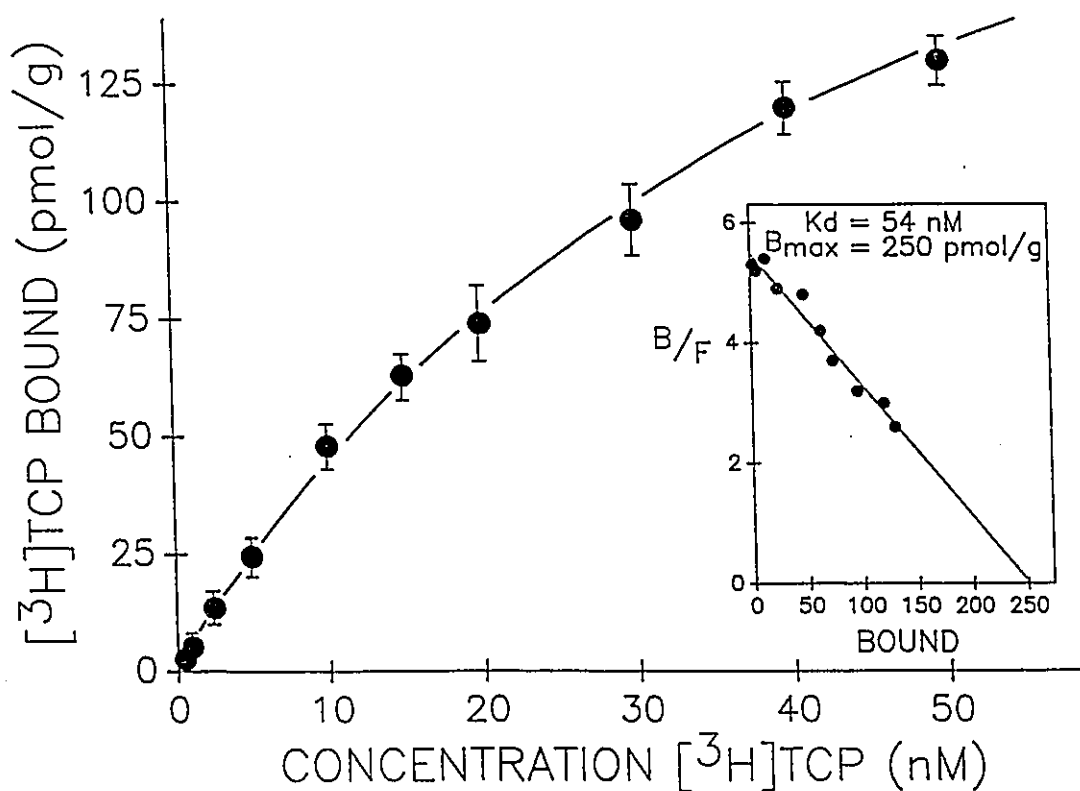
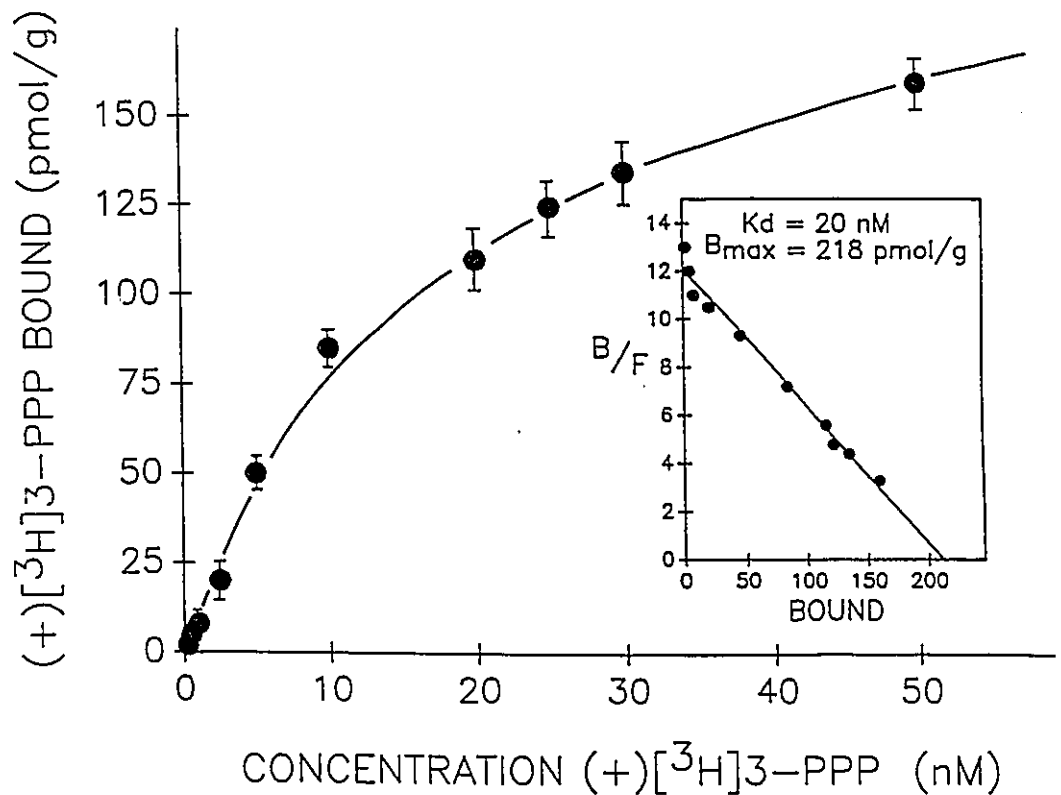


Figure 14

Saturation of the binding site for [^3H]3(+)-PPP in bovine adrenal medullary membranes. Increasing concentrations of [^3H]3(+)-PPP incubated with membranes of adrenal medulla for 30 min at 37°C revealed a one-site binding model when analyzed by BDATA. Scatchard plot analysis revealed a $K_D = 30$ nM and a $B_{\text{max}} = 218$ fmol/mg protein (inset). These results represent the mean \pm S.D. of two sets of duplicated experiments. (n=4 samples per concentration)



The effectiveness of dopaminergic, nicotinic, muscarinic, and calcium channel ligands were analyzed in competition studies against [^3H]3(+)-PPP and [^3H]TCP, (see Table 6). The dopaminergic antipsychotic drug, chlorpromazine has considerable affinity for each of [^3H]3(+)-PPP and [^3H]TCP binding sites. This affinity however, does not reflect typical dopaminergic binding since the more potent dopaminergic receptor ligands spiperone and (+)-butaclamol were less effective in reducing the radioligand binding. The calcium channel ligand verapamil proved to be quite potent in competition for [^3H]3(+)-PPP and [^3H]TCP binding sites. Although the cholinergic drugs were not as potent as sigma ligands, nicotinic compounds proved to be more potent than muscarinic compounds in displacing both radiolabels from their respective binding sites. Interestingly, decamethonium (known to block neuromuscular depolarization) but not hexamethonium (an agent capable of blocking ganglionic depolarization) was able to displace [^3H]3(+)-PPP and [^3H]TCP binding with a potency that parallels that of PCP.

Compounds such as L-Glu (100 μM), Gly (30 μM), Na^+ and Mg^{++} were ineffective in enhancing the binding of [^3H]TCP or [^3H]3(+)-PPP to bovine adrenal medulla membrane preparations.

Figure 15

Hill plot of [^3H]TCP and [^3H]3(+)-PPP binding. The data was analyzed according to the Hill equation described in the Materials and Methods. Both ligands exhibited Hill coefficients close to unity signifying that the binding of each radiolabel was to a homogenous population of receptors.

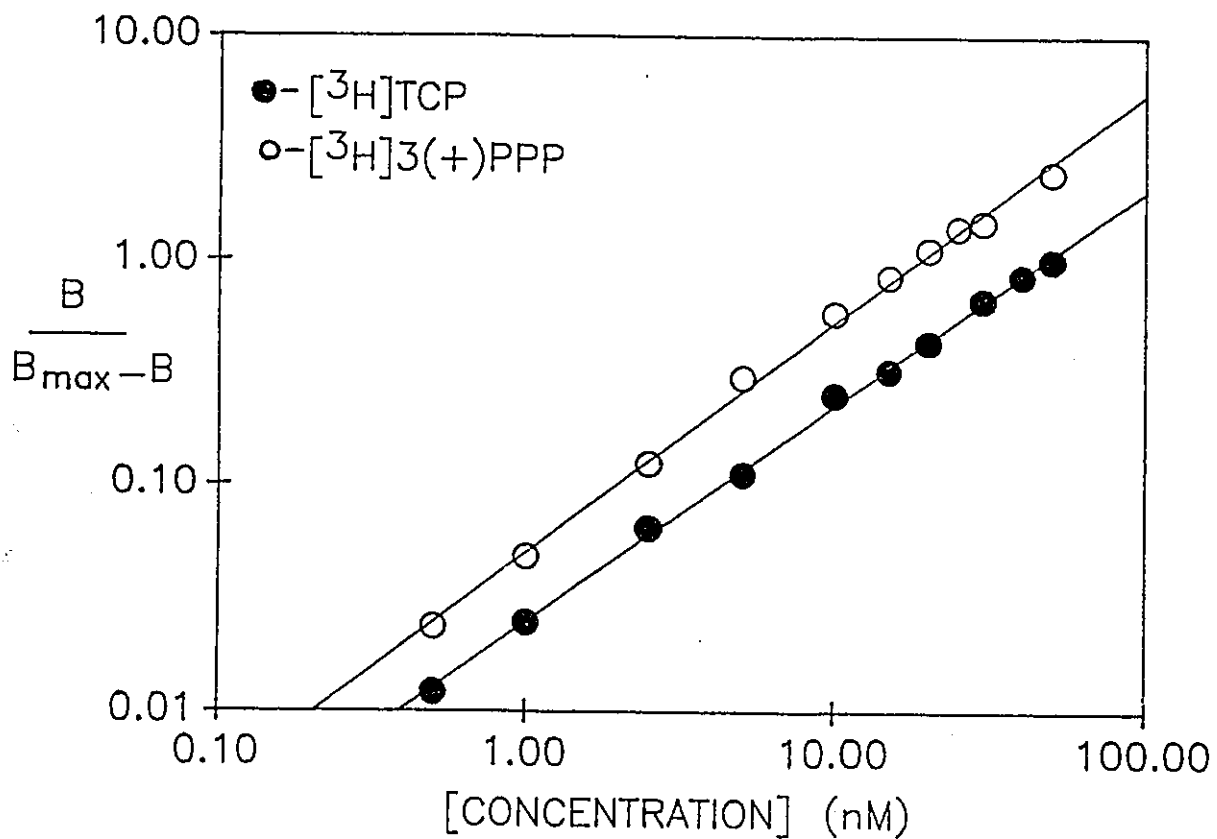


Figure 16

Correlation of K_i values for inhibition of $[^3\text{H}]\text{TCP}$ and $[^3\text{H}]\text{3(+)}\text{PPP}$ binding. Competition experiments were carried out according to that described in Materials and Methods. The inhibition constants correlate with an r^2 value of 0.54.

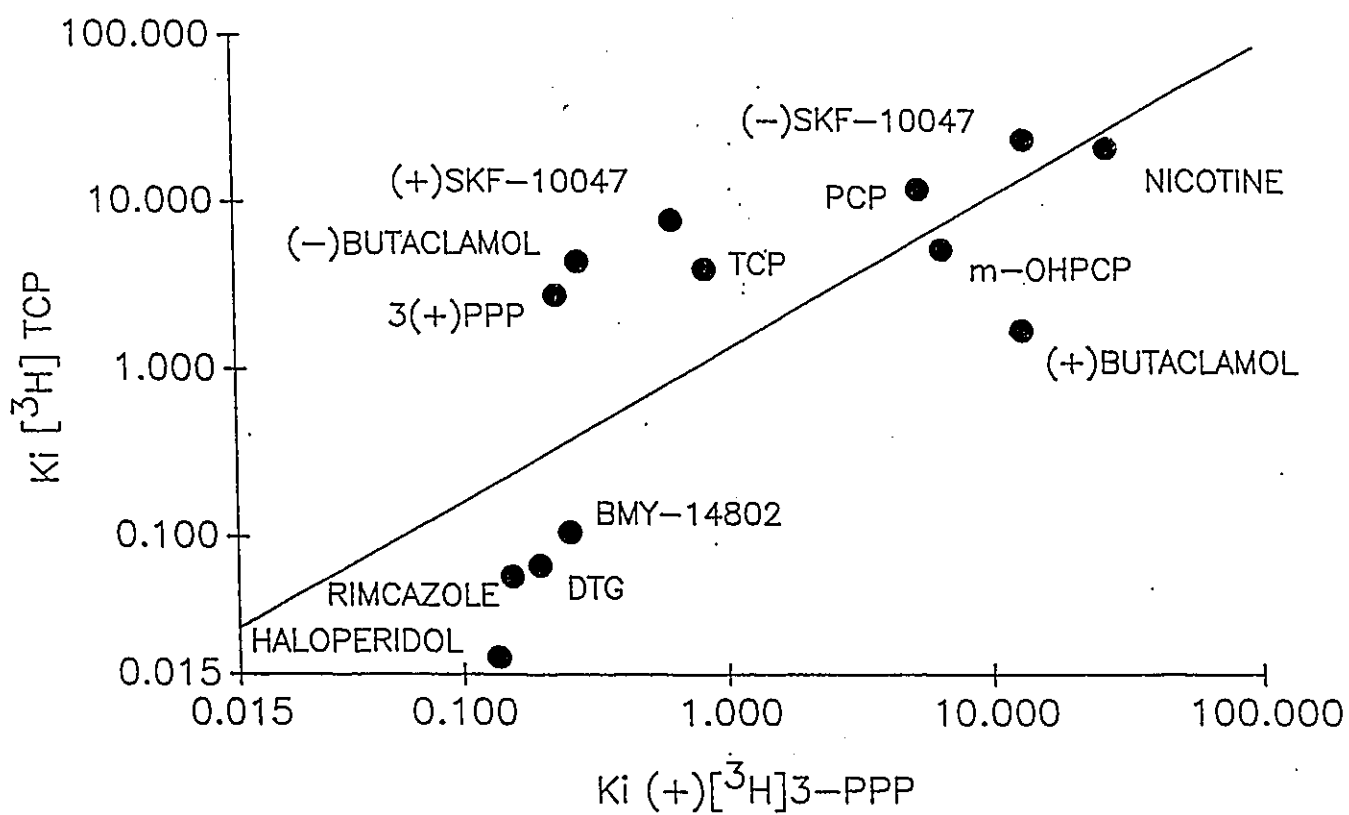


TABLE 5

Sigma Compounds	[³ H]TCP	[³ H] (+)3-PPP
	K _i	K _i
Haloperidol	0.19 (±) 0.06	0.13 (±) .03
DTG	0.06 (±) 0.01	0.15 (±) .02
BMY-14802	0.11 (±) 0.032	0.25 (±) .04
Rimcazole	0.07 (±) 0.035	0.20 (±) .02
3(+)-PPP	2.5 (±) 0.05	0.26 (±) .08
(-)-Butaclamol	4.4 (±) 0.29	0.28 (±) .02
(+)-SKF-10047	7.8 (±) 0.6	0.64 (±) .08
Phencylidine-like		
TCP	3.96 (±) 0.45	0.85 (±) .07
PCP	12 (±) 1.1	5.58 (±) .78
PCP-3OH	5.2 (±) 0.61	6.7 (±) .71
MK-801	>100	>100

Values represent the mean of six samples. [³H]TCP and [³H]3(+)-PPP were incubated at 2 nM concentrations as described in Materials and Methods. Competitive displacers of both sigma and PCP binding sites were used at concentrations between 10⁻⁹ M and 10⁻⁴ M. Inhibition curves obtained were analyzed by the non-linear curve fitting program CDATE.

TABLE 6

	[³ H]TCP	[³ H] (+)3-PPP
	K _i	K _i
DOPAMINERGIC		
Spiperone	10	10
(+)Butaclamol	1.7	13.3
Chlorpromazine	0.293	0.423
Ca ⁺⁺ CHANNEL		
Nifedipine	>100	100
Verapamil	1.5	1
Diltiazem	11	21
OPIATES		
(-)SKF-10047	24	14
Dynorphin A (1-13)	5	6
CHOLINERGICS		
ACh	>100	>100
Atropine	>100	>100
Muscarine	>100	>100
d-tubocurarine	>100	>100
alpha-Bungarotoxin	>100	>100
Carbamylcholine	60	22
Hexamethonium	>100	>100
Decamethonium	6	22
Nicotine	28	21
NMDA		
AP-5	>100	>100
AP-7	>100	>100

Values represent the mean of six samples. [³H]TCP and [³H]3(+)-PPP binding competitions proceeded as described in Materials and Methods. Competitive displacers of non-PCP and non-sigma related receptors were used at concentrations between 10⁻⁹ M and 10⁻⁴ M.

4.3 Autoradiography of [¹²⁵I]Histogranin Binding in Bovine Adrenal Medulla

Tissue sections of bovine adrenal glands were incubated in the presence of [¹²⁵I]histogranin at room temperature for 30 min. The specific binding (Fig. 17A) was calculated as the difference between total bound and that bound in the presence of unlabeled histogranin (2×10^{-5} M), (Fig. 17B). The radiolabelled peptide bound specifically to the adrenal medulla (Fig 17A). The non-specific binding was determined by incubation of the radiolabel in the presence of 20 μ M unlabelled histogranin (Fig.17B). The inhibition of [¹²⁵I] histogranin binding by various PCP and sigma ligands (1 μ M) was also determined (Table 7). The PCP/NMDA receptor ligand MK-801 and the dopaminergic antagonist spiperone were ineffective in displacing more than 10% of the iodinated ligand. The binding of [¹²⁵I]-histogranin was haloperidol sensitive, and sigma receptor ligands were slightly more potent than PCP receptor ligands. Therefore, histogranin may bind to both PCP and sigma-like adrenomedullary receptors, as characterized in this study.

Figure 17

Autoradiographic visualization of [125 I] histogranin in bovine adrenal medulla sections. Tissue sections were processed as described in Materials and Methods. A. Represents total binding of [125 I] histogranin B. Represents the binding of [125 I] histogranin in the presence of 20 μ M unlabelled histogranin. C= cortex, M= medulla

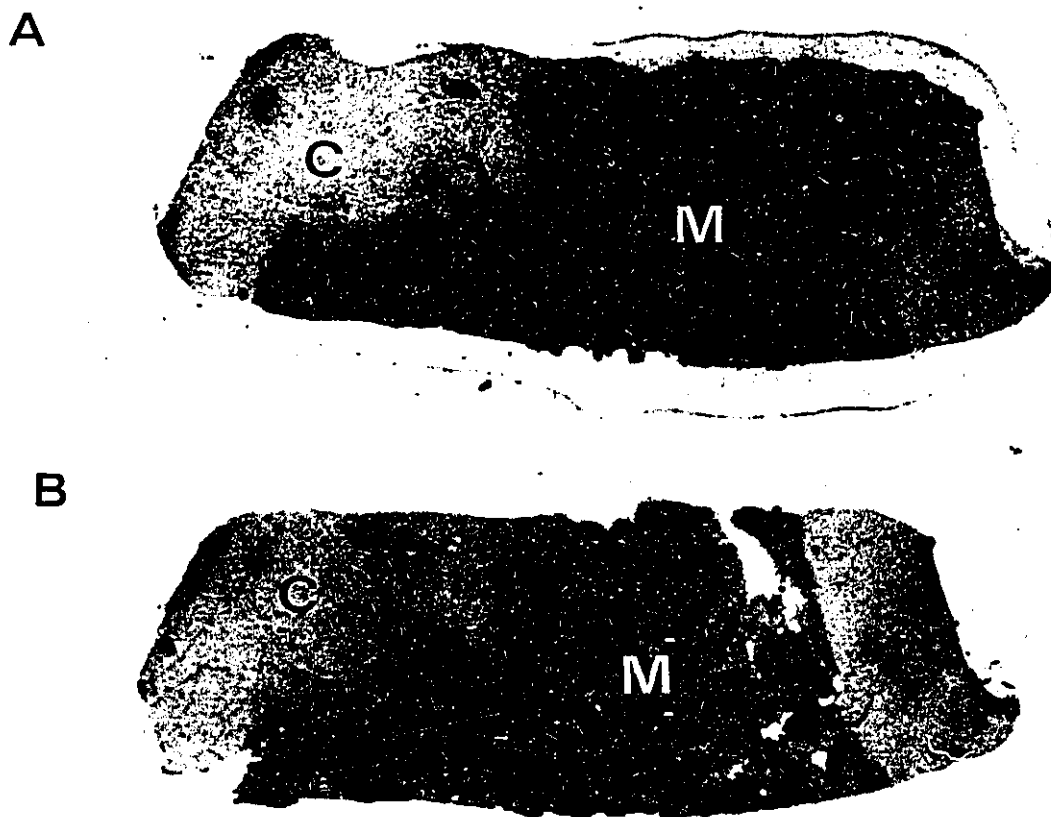


Table 7

The percent inhibition of [125 I] histogranin binding by various PCP and sigma compounds (1 μ M) in tissue sections of bovine adrenal medulla.

Compound	Percent Inhibition
(+)SKF-10047	46
(-)SKF-10047	41
TCP	45
Haloperidol	80
3(+)PPP	25
MK-801	10
Spiperone	6

5. DISCUSSION

The adrenal medulla is known to contain a large variety of opioid peptides and opioid receptors (Castanas, Bourhim et al. 1985a; Castanas, Bourhim et al. 1985b; Castanas, Giraud et al. 1984; Chavkin, Cox et al. 1979; Dumont and Lemaire 1984). The presence of the non-opioid sigma receptor in the adrenal medulla was first suggested by the observation of a receptor site for [^3H](\pm)SKF-10047 that was not displaced by a high concentration (5 μM) of (D-Ala $_2$,D-Leu-Enk,"DADLE") (Dumont and Lemaire 1984). At this concentration, DADLE is known to mask all opioid receptor sites (Attali, Gouarderes et al. 1982). While [^3H]($-$)SKF-10047 may bind opioid receptors (Castanas, Bourhim et al. 1985a; Castanas, Giraud et al. 1984), the present data provide direct biochemical evidence for the existence of selective receptors for [^3H]($+$)SKF-10047 in bovine adrenal medulla. [^3H]($+$)SKF-10047 is known to bind two specific sites in brain, sigma and PCP receptors (Largent, Gundlach et al. 1986; Sonders, Keana et al. 1988). This study has confirmed the existence of binding sites for sigma and PCP-like compounds in bovine adrenal medulla. Using radioligand binding we have reported the existence of two haloperidol-sensitive binding sites for the prototypic sigma receptor compound (+)SKF-10047. The two distinct components of [^3H]($+$)SKF-10047 binding were investigated independently by blocking either the high affinity site with [10^{-6}] M haloperidol or the low affinity site with [2×10^{-7}] M TCP. The binding of [^3H]($+$)SKF-10047 in each

condition was proven to be specific and saturable, binding to one sigma-like site (Fig. 9) and one PCP-like site (Fig. 10).

Competition experiments under conditions of blockade revealed the distinctive nature of these two sites. The sigma component was highly sensitive to haloperidol and pentazocine, whereas the PCP component was more sensitive to TCP and cyclazocine (Table 3). Cyclazocine, has been reported to bind to three different receptor sites in the rat brain (Zukin and Zukin 1981), and thus the specificity of this ligand for PCP receptors is questioned. Binding studies in rat brain suggest that cyclazocine binds to sigma receptors with high affinity (Largent, Wikstrom et al. 1987) although (Zukin and Zukin 1981) have reported its ability to potently displace [^3H]PCP. In contrast to stereoselectivity reported in rat brain (Largent, Gundlach et al. 1984), the PCP component of [^3H](+)-SKF-10047 binding in bovine adrenal medulla exhibited a greater degree of stereoselectivity for the (+)enantiomer of SKF-10047. The selective μ , κ , and δ -opiate receptor ligands DAGO, DSLET, and U-69593, were ineffective in reducing the specific binding of [^3H](+)-SKF-10047, providing evidence to indicate that the receptor sites for the (+)form of SKF-10047 are not opiate receptors.

To further investigate the nature of sigma and PCP-like receptors in the bovine adrenal medulla, selective radiolabelled ligands for PCP and sigma receptors were obtained. Evaluation of the binding of tritiated thienylcyclohexylpiperidine, ([^3H]TCP, known as a highly potent selective PCP receptor ligand in brain tissue) [Vignon,1980], and tritiated 3-(3-hydroxyphenyl)-N-(1-

propyl)piperidine ($[^3\text{H}]3(+)\text{PPP}$, a highly selective sigma ligand) (Largent, Gundlach et al. 1986), provided a means for characterizing these two binding sites separately. Thus the problem of cross-reactivity between sigma and PCP binding components encountered in the analysis of $[^3\text{H}](+)\text{SKF-10047}$ binding was overcome. The two specific ligands bound to adrenal membranes in a selective specific manner, saturating a similar population of binding sites (218 vs 250 pmol/g protein). The binding of $[^3\text{H}]\text{TCP}$ in the bovine adrenal medulla (K_D 54 nM, $B_{\text{max}} = 250$ pmol/g protein) was similar to that binding reported in brain tissue. The typical K_D values for $[^3\text{H}]\text{TCP}$ in the brain range between 5 and 65 nM, with B_{max} values between 163-1367 pmol/g protein (Largent, Gundlach et al. 1986; Rothman, Bykov et al. 1988; Wolfe, Culp et al. 1989). Similarly, $[^3\text{H}](+)3\text{-PPP}$ binding in bovine adrenal medulla ($K_D=30$ nM, $B_{\text{max}}= 218$ pmol/g protein) proved to be similar to binding studies in brain, which report affinities in the narrow range of (30 to 56 nM), although the bovine adrenal medulla demonstrated a lower B_{max} (218 vs 500 pmol/g protein) (Largent, Gundlach et al. 1984).

The binding of $[^3\text{H}]\text{TCP}$ and $[^3\text{H}]3(+)\text{PPP}$ was measured in the presence of a wide range of compounds in order to establish rank order of potency (Tables 5,6). Surprisingly, the sigma ligands were more potent than PCP-analogues in displacing $[^3\text{H}]\text{TCP}$ from its binding site in bovine adrenal medullary membrane preparations. The rank order of compounds displacing $[^3\text{H}]\text{TCP}$ was also very similar to the rank order displacing $[^3\text{H}]3(+)\text{PPP}$ (Table 5)

Despite these similarities, subtle differences in the stereoselective nature of the binding were apparent. The (-)-isomer of butaclamol was 20 fold more potent than (+)butaclamol in displacing [^3H]3(+)-PPP from its binding site, whereas (+)butaclamol is the potent enantiomer in displacing [^3H]TCP. Gundlach, and Largent (1986), reported that in rat brain (-)butaclamol was 47 times more potent than (+)butaclamol in the inhibition of [^3H]3(+)-PPP, and Tam, (1983) described this reversed stereoselectivity of butaclamol for sigma and dopamine sites in brain. The (+)-isomer was found to be more potent at the dopamine site and the (-)-isomer, more potent at the sigma site. However, [^3H]TCP binding in the adrenal medulla does not resemble that of typical dopamine (D_2) receptor binding. The highly selective D_2 antagonist spiperone was not potent in competition experiments. The bovine adrenal medulla sigma sites exhibit a greater degree of stereoselectivity for the enantiomers of SKF-10047. [^3H]3(+)-PPP exhibited a greater degree of stereoselectivity (20 fold) over [^3H]TCP (3 fold) for the dextro-isomer of SKF-10047. In brain, (+)SKF-10047 is four fold more potent than (-)SKF-10047 at inhibiting the binding of [^3H]3(+)-PPP (Largent, Gundlach et al. 1986).

Interestingly, the calcium channel antagonist verapamil was moderately potent in displacing both [^3H]3(+)-PPP and [^3H]TCP in bovine adrenal medulla membrane preparations. In brain, skeletal muscle, smooth muscle, and heart, the binding of [^3H]phencyclidine was shown to be displaced by certain calcium antagonists (Bolger, Rafferty et al. 1985; El-Fakahany, Eldefrawi et al. 1984; Eldefrawi, El-

Fakahany et al. 1982; Quirion and Pert 1982). Studies with perfused adrenal glands, (Malave, Borowitz et al. 1983), reported that PCP resembled a Ca^{++} antagonist, inhibiting the Ba^{++} induced secretion of adrenal catecholamines. Further studies involving the actions of these ligands on ion channels may lead to clues to the nature of this interaction.

[^{125}I]histogranin bound specifically to the adrenal medulla (Fig. 17A). The displacement profile was characteristic of that seen with typical sigma ligands in this tissue. [^{125}I] histogranin binding was haloperidol sensitive, and displaced most potently by sigma ligands (Table 7). Most convincingly, the highly specific D_2 antagonist spiperone and the selective PCP_1 receptor ligand MK-801 failed to displace more than 10% of the [^{125}I] histogranin binding providing evidence for the peptide's selectivity. These preliminary results leave question as to whether histogranin represents an endogenous ligand for sigma or PCP receptors in brain. In rodents, PCP induces a dose-dependent hyperexcitability, stereotyped behaviours, motor incoordination and ataxia (Castellani and Adams 1981; Nabeshima, Ishikawa et al. 1987; Nabeshima, Kamei et al. 1988; Nabeshima, Sivam et al. 1982; Sturgeon, Fessler et al. 1979). Recently, Lemaire and collaborators (unpublished observation) have found that i.c.v. injections of histogranin can induce "PCP-like" behaviour in rats for longer duration than that produced by the same concentration of PCP. These data indicate that adrenal histogranin and its specific receptor sites may be present in the brain where they may subserve important functional roles in behaviour.

Comparison of the inhibitory constants of compounds displacing [^3H]3(+)-PPP and [^3H]TCP binding is plotted in Fig. 16. The similarity between the displacement of the PCP-receptor ligand [^3H]TCP and the sigma receptor ligand [^3H]3(+)-PPP is correlated with an r^2 value of 0.54. This significant correlation supports the possibility that these two radiolabelled compounds bind to a common receptor. The binding site profile for both radioligands resembles the sigma receptor reported in brain tissue, although the [^3H]TCP binding site does not possess the same degree of stereoselectivity for the enantiomers of SKF-10047 and exhibits reversed stereoselectivity for the enantiomers of butaclamol.

The [^3H]TCP binding in bovine adrenal medulla differs considerably from the PCP receptor characterized in brain. In rat brain, [^3H]TCP binds to two sites, one being associated with the NMDA receptor (PCP_1) and a second low affinity site (PCP_2) thought to be related to sigma receptors (Largent, Gundlach et al. 1986). The main discrepancies revolve around the lack of NMDA-like associated [^3H]TCP binding in bovine adrenal medullary membranes. Firstly, MK-801 does not bind specifically to bovine adrenal medullary membranes, whereas in brain, MK-801 inhibited 90% of the [^3H]TCP binding with an IC_{50} of 9 nM (Johnson, Saccaan et al. 1988; Loo, Braunwalder et al. 1987). Secondly, potent NMDA antagonists have been shown to cause a 40% inhibition of [^3H]TCP binding in rat brain although no inhibition of [^3H]TCP binding is noted in the adrenal medulla. Several investigators have also demonstrated enhancement of [^3H]TCP binding in the presence of exogenously added L-

glutamate, glycine and Mg^{++} . In the adrenal medulla however, [3H]TCP binding is unaffected by these modulators of the NMDA receptor. This low affinity PCP₂ binding site in brain is not related to the NMDA receptor. Many investigators have reported that this low affinity site is the sigma receptor, whereas others have suggested that the PCP₂ site is involved in the inhibition of [3H]norepinephrine and [3H]dopamine uptake (Vignon, Pinet et al. 1988). This later hypothesis is supported by the evidence that PCP inhibited [3H]dopamine uptake into striatal synaptosomes while MK-801 was 250 fold less potent (Johnson, Sacaan et al. 1988).

Due to the problem of cross-binding activity between high and low affinity [3H]TCP receptors in brain, little data has been published concerning the pharmacology of the PCP₂ site. Investigation of PCP and sigma receptors in the periphery by (Wolfe, Culp et al. 1989) suggest that there are no peripheral PCP receptors present in rat adrenal medulla. However, this binding study was conducted on membranes prepared from rat whole adrenal glands without separating the adrenal cortex from medulla. Due to the dilution of adrenal medullary membranes in the total gland homogenate, any specific binding of [3H]TCP to the medulla would be impossible to distinguish from background values produced by non-specific [3H]TCP binding to cortical tissue. Wolfe's finding also conflict with other investigators who have reported the significance of PCP in the adrenal gland. PCP has been reported to bind to cultured adrenal chromaffin cells (Wada, Arita et al. 1988), and to inhibit

catecholamine release from whole glands and cultured chromaffin cells (Malave, Borowitz et al. 1983; Purifoy and Holz 1984)

The nature of the described [^3H]TCP binding remains speculative, but not unique to the bovine adrenal medulla. The presence of low density, low affinity, haloperidol-sensitive [^3H]TCP binding has been reported in studies carried out on NCB-20 neurotumor cells in culture (Largent, Gundlach et al. 1986). This limited amount of [^3H]TCP binding was very sensitive to haloperidol ($\text{IC}_{50} = 20 \text{ nM}$) and thus, was apparently thought to represent binding of [^3H]TCP to the same pharmacological site as [^3H](+)SKF-10047 and [^3H](+)3-PPP (Largent, Gundlach et al. 1986)

In spite of all the data available in this study, the binding of [^3H]TCP does not resemble that of any known receptor. The "PCP-like" binding site more closely resembles the sigma receptor than the PCP receptor reported in brain. The displacement of the PCP component of [^3H](+)SKF-10047 binding in the adrenal by cyclazocine, (a sigma as well as PCP ligand), and the potent displacement of [^3H]TCP by sigma ligands suggests that the "PCP-like" binding may represent a subtype of the sigma receptor rather than a "PCP" receptor. The possibility of subtypes of the sigma receptor would provide a more suitable explanation of the data.

The theory of the existence of neuronal sigma receptor subtypes put forth by (Bowen, Hellewell et al. 1989) is gaining increased acceptance from investigators studying rat brain (Int. Narcotics Res. Conference, 1989). However, this model consists of a

high affinity haloperidol-sensitive site and a low affinity haloperidol-insensitive site. Since both the sigma and PCP-like components of [³H](+)-SKF-10047 binding, and the ligands [³H]3(+)-PPP and [³H]TCP are displaced by haloperidol in the bovine adrenal medulla; the sigma receptor haloperidol-insensitive subtype described in brain may not be applied here.

Due to the pharmacological differences observed between the brain sigma and PCP receptors and their counterparts in the adrenal medulla it may be more appropriate to consider the adrenal sigma and PCP-like receptors (Rogers, Cecyre et al. 1989) as sigma subtypes defined below.

Sigma 1:

1. Sensitive to haloperidol and prototypic sigma ligands.
2. Stereoselective for the (-)isomer of butaclamol.
3. Stereoselective for the (+)isomer of SKF-10047

Sigma 2:

1. Sensitive to haloperidol, prototypic sigma ligands and some prototypic PCP ligands such as TCP and PCP.
2. Stereoselective for the (+) isomer of butaclamol
3. Stereoselective for the (+)isomer of SKF-10047

A site on the ACh nicotinic receptor complex may be the low affinity site at which PCP could bind since our results demonstrate that nicotine but not muscarine is able to displace both the binding of [³H](+)-3-PPP and [³H]TCP from adrenal membrane receptors (Table 6). The two benzomorphans, cyclazocine and (+)SKF-10047 have been shown to inhibit [³H]PCP binding to the nicotinic receptor ion channel (Eldefrawi, Miller et al. 1982; Eldefrawi, El-Fakahany et al. 1982). A link between sigma binding sites and the ACh receptor complex would partially explain the inhibitory effect of PCP on adrenal catecholamine release.

The function of central nervous system sigma receptors is not clear nor has the physiological significance of sigma receptors in the periphery been revealed. However, it has been shown that sigma receptor-selective drugs cause an increase in the electrically stimulated twitch response of mouse *vas deferentia* (Campbell, Bobker et al. 1987). In addition, it has been shown that sigma receptor-selective drugs cause an increase in the electrically stimulated release of norepinephrine from the mouse *vas deferens*, raising the possibility that sigma receptors may be involved in stimulating catecholamine release (Campbell, Bobker et al. 1987). Investigation of the effects of sigma agonists on the secretion of catecholamines from the bovine adrenal medulla would clarify this function. Sigma receptors have also been implicated in the control of motor behaviour (Bowen, Walker et al. 1988), and the modulation of phosphoinositide turnover (Bowen, Kirschner et al. 1988). The

significance of these functional systems will await further investigation.

The presence of sigma receptor binding sites, in the absence of NMDA-associated (PCP₁) receptors in bovine adrenal medulla provides a useful cell model system for the study of sigma receptors in a manner that is not possible in brain tissue. Explanation of the similarities of [³H]3(+)-PPP and [³H]TCP receptor binding in the adrenal medulla would seem to favour the hypothesis of a multisite model for adrenal medulla sigma receptors. The question as to whether stimulation of these specific receptors is linked to a physiological function in this tissue remains to be answered and will necessitate further investigation.

Albuquerque, E. X., M.-C. Tsai, R. S. Aronstam, A. T. Eldefrawi and M. E. Eldefrawi. (1980). "Sites of action of phencyclidine." *Mol. Pharmacol.* **18**: 167-178.

Aldrich, T. B. (1901). "A preliminary report on the active principle of the suprarenal gland." *Am. J. Physiol.* **5**: 457-461.

Allen, R. M. and S. J. Young. (1978). "Phencyclidine-induced psychosis." *Am. J. Psych.* **135**: 1081-1084.

Anis, N. A., S. C. Berry, B. N. R and D. Lodge. (1983). "The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by N-methyl-aspartate." *Br. J. Pharmacol.* **79**: 565-575.

Antoni, F. A. (1984). "Characterization of high affinity binding sites for vasopressin in bovine adrenal medulla." *Neuropeptides.* **4**: 413-420.

Attali, B., C. Gouarderes, H. Mazarguil, Y. Audigier and J. Cros. (1982). "Differential interaction of opiates to multiple "kappa" binding sites in the guinea-pig lumbo-sacral cord." *Life Sci.* **31**: 1371-1375.

Azila, N. and J. N. Hawthorne. (1982). "Subcellular localization of phospholipid changes in response to muscarine stimulation of perfused bovine adrenal medulla." *Biochem. J.* **204**: 291-299.

Baker, E. M., T. R. Cheek and R. D. Burgoyne. (1985). "Cyclic AMP inhibits secretion from bovine adrenal chromaffin cells evoked by carbamylcholine but not high k^+ ." *Biochimica et Biophysica Acta.* **846**: 388-393.

Bayorh, M. A., D. Lozovsky, K. C. Rice, T. R. Burke Jr. and I. J. Kopin. (1983). "Cardiovascular and plasma prolactin responses to stereoisomers of phencyclidine." *Pharmacol. Biochem. Behav.* **19**: 365.

Berry, S. C., S. L. Dawkins and D. Lodge. (1984). "Comparison of sigma and kappa opiate receptor ligands as excitatory amino acid antagonists." *Br. J. Pharmacol.* **83**: 179-185.

- Blaschko, H. and A. D. Welch. (1953). "Localization of adrenaline in cytoplasmic particles of the bovine adrenal medulla." *Arch. Exptl. Pathol. Pharmacol.* 17: 22-36.
- Bloom, W. and D. W. Fawcett. (1986). "Adrenal glands and Paraganglia, In "A Textbook of Histology, 11th edition" W.B. Saunders Co., Philadelphia, PA." : 516-533.
- Boggan, W. O., C. J. Wallis and M. H. Owens. (1982). "Effect of phencyclidine on plasma corticosterone in mice." *Life Sci.* 30: 1581-1586.
- Boksa, P., N. G. Seidah, M. Chretien and B. G. Livett. (1982). "Effects of pro-opiomelanocortin-derived peptides in the human adrenal medulla." *Neurosci. Lett.* 28: 199-202.
- Bolger, G. T., M. F. Rafferty and P. Skolnick. (1985). "Phencyclidine increases the affinity of dihydropyridine calcium channel antagonist binding in rat brain." *Naunyn-Schmiedeberg's Arch Pharmacol.* 330: 227-234.
- Boublik, J. H., J. A. Clements, A. C. Herington and J. W. Funder. (1983). "Opiate binding sites in bovine adrenal medulla." *J. Receptor Res.* 3: 463-479.
- Bowen, W. D., S. B. Hellewell and K. A. McGarry. (1989). "Evidence for a multi-site model of the rat brain sigma receptor." *Eur. J. Pharmacol.* 163: 309-318.
- Bowen, W. D., B. N. Kirschner, A. H. Newman and K. C. Rice. (1988). "Sigma receptors negatively modulate agonist-stimulated phosphoinositide metabolism in rat brain." *Eur. J. Pharmacol.* 149: 399-400.
- Bowen, W. D., J. M. Walker, A. G. Yashar, R. R. Matsumoto, F. O. Walker and J. F. Lorden. (1988). "Altered haloperidol-sensitive sigma receptors in the genetically dystonic (dt) rat." *Eur. J. Pharmacol.* 147: 153-154.
- Brady, K. T., R. L. Balster and E. L. May. (1982). "Stereoisomers of N-allylnormetazocine: phencyclidine behavioral effects in squirrel monkeys and rats." *Science.* 215: 178-180.

Campbell, B. G., D. H. Bobker, F. M. Leslie, I. M. Mefford and E. Weber. (1987). "Both the sigma receptor-specific ligand (+)3-PPP and the PCP receptor-specific ligand TCP act in the mouse vas deferens via augmentation of electrically evoked norepinephrine release." *Eur. J. Pharmacol.* **138**: 447-449.

Castanas, E., B. Bourhim, P. Giraud, F. Boudouresque, P. Cantau and C. Oliver. (1985a). "Interaction of opiates with opioid binding sites in the bovine adrenal medulla: I Interaction with delta and mu sites." *J. Neurochem.* **45**: 673-687.

Castanas, E., N. Bourhim, P. Giraud, F. Boudouresque, P. Cantau and C. Oliver. (1985b). "Interaction of opiates with opioid binding sites in the bovine adrenal medulla: II Interaction with kappa sites." *J. Neurochem.* **45**: 688-699.

Castanas, E., P. Giraud, Y. Audigier, R. Drissi, F. Boudouresque, B. Conte-Devolx and C. Oliver. (1984). "Adrenal medullary opiate receptors. Pharmacological characterization in bovine adrenal medulla and a human pheochromocytoma." *Mol. Pharmacol.* **25**: 38-45.

Castellani, S. and P. M. Adams. (1981). "Acute and chronic phencyclidine effects on locomotor activity, stereotypy and ataxia in rats." *Eur. J. Pharmacol.* **73**: 143-154.

Chaminade, M., A. S. Foutz and J. Rossier. (1984). "Co-release of enkephalins and precursors with catecholamines from the perfused cat adrenal gland in situ." *J. Physiol.* **353**: 157-169.

Chavkin, C., B. M. Cox and A. Goldstein. (1979). "Stereospecific opiate binding sites in the bovine adrenal medulla." *Mol. Pharmacol.* **15**: 751-753.

Chen, G., C. R. Ensor, D. Rlussel and B. Bohmer. (1959). "The pharmacology of 1-(1-phenylcyclohexyl)piperidine-HCl." *J. Pharmacol. Exp. Ther.* **127**: 241-246.

Clouet, D. H. (1986). "Phencyclidine, an update." *NIDA Research Monograph Ser.* **64**: 77-84.

Contreras, P. C., D. A. Dimaggio and T. L. O'Donohue. (1987). "An endogenous ligand for the sigma opioid binding site." *Synapse.* **1**: 57-61.

- Contreras, P. C., R. Quirion and T. L. O'Donohue. (1986). "Autoradiographic distribution of phencyclidine receptors in the rat brain using [3H]TCP." *Neurosci. Lett.* **67**: 101-106.
- Costa, E., A. Guidotti, I. Hanbauer, T. Hexum, L. Saini, S. Stine and H.-Y. T. Yang. (1981). "Regulation of acetylcholine receptors by endogenous cotransmitters: studies on adrenal medulla." *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **40**: 160-165.
- Coupland, R. E. (1965). "The natural history of the chromaffin cell." Longmans, London. :
- Day, R., I. Lemaire, S. Masse and S. Lemaire. (1985). "Pulmonary bombesin in experimentally induced asbestosis.." *Experimental Lung Res.* **8**: 1-13.
- Dean, D. M., S. Lemaire and B. G. Livett. (1982). "Evidence that inhibition of nicotine-mediated catecholamine secretion from adrenal chromaffin cells by enkephalin, β -endorphin, dynorphin (1-13), and opiates is not mediated via specific opiate receptors." *J. Neurochem.* **38**: 608-614.
- Derome, G., R. Tseng, P. Mercier, I. Lemaire and S. Lemaire. (1981). "Possible muscarinic regulation of catecholamine secretion mediated by cyclic GMP in isolated bovine adrenal chromaffin cells." *Biochem. Pharmacol.* **30**(8): 855-860.
- Doherty, J. D., M. Simonovic, R. So and H. Y. Meltzer. (1980). "The effect of phencyclidine on dopamine synthesis and metabolism in rat striatum." *Eur. J. Pharmacol.* **65**: 139-149.
- Domino, E. F. (1964). "Neurobiology of phencyclidine (Sernyl) a drug with an unusual spectrum of pharmacological activity." *Int. Rev. Neurobiology.* **6**: 303-347.
- Douglas, W. W. (1966). "The mechanisms of release of catecholamines from the adrenal medulla." *Pharmacol. Rev.* **18**(1): 471-480.
- Douglas, W. W. (1968). "Stimulus-secretion coupling: the concept and clues from chromaffin and other cells." *Br. J. Pharmacol.* **34**: 451-474.

Douglas, W. W. and A. M. Poisner. (1965). "Preferential release of adrenaline from the adrenal medulla by muscarine and pilocarpine." *Nature*. **218**: 94-95.

Douglas, W. W. and R. P. Rubin. (1961). "Role of calcium in the secretory response of the adrenal medulla to acetylcholine." *J. Physiol. London*. **159**: 40-59.

Douglas, W. W. and R. P. Rubin. (1968). "The mechanism of catecholamine release from the adrenal medulla and the role of calcium in stimulus secretion coupling." *J. Physiol. London*. **167**: 288-310.

Downes, C. P., P. E. Lewis and M. A. Stone. (1986). "A comparison of the binding of sigma opioids and phencyclidine, and the interaction with antipsychotic drugs in rat brain membranes." *Br. J. Pharmacol.* **88**: 231-237.

Dumont, M. and S. Lemaire. (1984). "Opiate receptors in bovine adrenal medulla." *Can. J. Physiol. Pharmacol.* **62**: 1284-1291.

El-Fakahany, E., A. T. Eldefrawi, D. L. Murphy, L. F. Agayo, D. J. Triggle, E. X. Albuquerque and M. E. Eldefrawi. (1984). "Interactions of phencyclidine with crayfish muscle membranes, sensitivity to calcium channel antagonists and other drugs." *Mol. Pharmacol.* **1985**: 369-378.

Eldefrawi, A. T., R. Miller, D. L. Murphy and M. E. Eldefrawi. (1982). "[³H]Phencyclidine interactions with the nicotinic acetylcholine receptor channel and its inhibition by psychotropic, antipsychotic, opiate, antidepressant, antibiotic, antiviral and antiarrhythmic drugs." *Mol. Pharm.* **22**: 72-81.

Eldefrawi, M. E., E. F. El-Fakahany, D. L. Murphy, A. T. Eldefrawi and D. J. Triggle. (1982). "High affinity binding of phencyclidine (PCP) to crayfish muscle. Displacement by calcium antagonists." *Biochem. Pharmacol.* **31**: 2549-2552.

Evans, D. H., E. Erdelyi, E. Weber and J. D. Barchas. (1983). "Identification of pro-opiomelanocortin derived peptides in the bovine adrenal medulla." *Science*. **221**: 957-958.

- Fagg, G. E. (1987). "Phencyclidine and related drugs bind to the activated N-methyl-D-aspartate receptor-channel complex in rat brain membranes." *Neurosci. Lett.* 76: 221-227.
- Fagg, G. E., A. C. Foster and A. H. Gagnon. (1986). "Excitatory amino acid synaptic mechanisms and neurological function." *Trends Pharmacol. Sci.* 7: 357-363.
- Fagg, G. E. and A. Matus. (1984). "L-Glutamate receptor heterogeneity: labeling of distinct receptor sub-types using radioligand binding techniques." *J. Receptor Res.* 4: 202-207.
- Feldberg, W. and G. P. Lewis. (1964). "The action of peptides on the adrenal medulla. Release of adrenaline by bradykinin and angiotensin." *J. Physiol. London.* 171: 98-104.
- Feldberg, W., B. Mintz and H. Tsudzimura. (1934). "The mechanism of nervous discharge of adrenaline." *J. Physiol. London.* 81: 286-304.
- Ferris, R. M., F. L. M. Tang, K. J. Chang and A. Russel. (1986). "Evidence that the potential antipsychotic agent rimcazole (BW234U) is a specific, competitive antagonist of sigma sites in brain." *Life Sci.* 38: 2329-2337.
- Fischer, S. K., R. W. Holz and B. W. Agranoff. (1981). "Muscarinic receptors in chromaffin cell cultures mediate enhanced phospholipid labelling but not catecholamine secretion." *J. Neurochem.* 37: 491-497.
- Foster, A. C. and E. H. F. Wong. (1987). "The novel anticonvulsant MK-801 binds to the activated state of the N-methyl-D-aspartate receptor in rat brain." *Br. J. Pharmacol.* 91: 403-409.
- Fujita, T., S. Kobayashi and M. Osaka. (1974). "Symposium on gastrointestinal hormones. Source cells and secretion mechanisms of gastrointestinal hormones." *J. Jpn. Soc. Intern. Med.* 63(9): 1014-1020.
- Gonzalez, M. C., A. R. Artalejo, C. Montiel, P. P. Hervas and A. G. Garcia. (1986). "Characterization of a dopaminergic receptor that modulates adrenal catecholamine release." *J. Neurochem.* 47: 382-388.

Greenberg, A. and O. Zinder. (1982). "alpha and beta receptor control of catecholamine secretion from isolated adrenal medulla cells." *Cell Tissue Res.* **226**: 655-665.

Gundlach, A., B. L. Largent and S. H. Snyder. (1986a). "Autoradiographic localization of sigma receptor binding sites in guinea pig and rat central nervous system with (+)3H-3-(3-hydroxyphenyl)-N-(1-propyl)piperidine." *Neuroscience.* **6(6)**: 1757-1770.

Gundlach, A. L., B. L. Largent and S. H. Snyder. (1986b). "Phencyclidine (PCP) receptors: autoradiographic localization in brain with the selective ligand, [3H]TCP." *Brain Research.* **386**: 266-279.

Haertzen, C. A. (1970). "Subjective effects of narcotic antagonists cyclazocine and nalorphine on the Addiction Research Center Inventory (ARCI)." *Psychopharmacologia.* **18**: 366-377.

Hattori, T and H. C., Fibiger. (1982). "On the use of lesions of afferents to localize neurotransmitter receptor sites in the striatum." *Brain Research.* **238**: 245-250.

Healy, D. P., A. R. Maciejewski and M. P. Printz. (1985). "Autoradiographic localization of [125I]angiotensin II binding sites in the rat adrenal gland." *Endocrinology.* **116**: 1221-1223.

Helle, K. B. and G. Serck-Hanssen. (1975). "The adrenal medulla: a model for studies of hormonal and neuronal storage and release mechanisms." *Mol. Cell. Biochem.* **6**: 127-146.

Hexum, T. D., I. Hanbauer, S. Govoni, H.-Y. T. Yang and E. Costa. (1980). "Secretion of enkephalin-like peptides from canine adrenal gland following splanchnic nerve stimulation." *Neuropeptides.* **1**: 137-142.

Hillarp, N.-A. and B. Nilson. (1953). "The isolation of a granule fraction from the suprarenal medulla, containing the sympathomimetic catecholamines." *Acta. Physiol. Scand.* **29**: 251-263.

Holets, V. and R. Elde. (1982). "The differential distribution and relationship of serotonergic and peptidergic fibers to sympathoadrenal neurons in the intermediolateral cell column of the

rat: a combined retrograde axonal transport and immunofluorescent study." *Neuroscience*. 7(5): 1155-1174.

Honey, C. R., Z. Miljkovic and J. F. Macdonald. (1985). "Ketamine and phencyclidine cause a voltage-dependent block of responses to L-aspartic acid." *Neuroscience Letters*. 61: 135-139.

Hsu, L. L., R. C. Smith, C. Rolsten and D. E. Leelavathi. (1980). "Effects of acute and chronic phencyclidine on neurotransmitter enzymes in rat brain." *Biochem. Pharmacol.* 29: 2524-2526.

Hughes, J. (1981). "Peripheral opiate receptor mechanisms." *Trends Pharmacol. Sci.* 2: 21-24.

Hunter, E. and S. Greenwood. (1962). "Preparation of iodine-131 labelled human growth hormone of high specific activity. ." *Nature*. 194: 495-496.

Itzhak, Y., J. M. Hiller and E. J. Simon. (1984). "Characterization of specific binding sites for [3H](d)-N-allylnormetazocine in rat brain membranes." *Mol. Pharmacol.* 27: 46-52.

Iwamoto, E. T. (1981). "Locomotor activity and antinocioception after putative mu, kappa, and sigma opioid receptor agonists in the rat: influence of dopaminergic agonists and antagonists." *J. Pharmacol. Exp. Ther.* 217: 451-460.

Johnson, J. W. and P. Ascher. (1987). "Glycine potentiates the NMDA response in cultured mouse brain neurons." *Nature*. 325: 529-531.

Johnson, K. M. (1983). "Partial characterization of specific binding of [3H]-N-allylnormetazocine (SKF-10047) to rat brain membranes." *Res. Commun. Subst. Abuse*. 4: 33-47.

Johnson, K. M. and K. C. Oeffinger. (1981). "The effect of phencyclidine on dopamine metabolism in the mouse brain." *Life Sci.* 28: 361-369.

Johnson, K. M., A. I. Sacaan and L. D. Snell. (1988). "Equilibrium analysis of [3H]TCP binding: effects of glycine, magnesium and N-methyl-D-aspartate agonists." *Eur. J. Pharmacol.* 152: 141-146.

Jones, S. M., L. D. Snell and K. M. Johnson. (1987). "Inhibition by phencycline of excitatory amino acid-stimulated release of

neurotransmitter in the nucleus accumbens." *Neuropharmacology*. **26**: 173-179.

Jones, S. M., L. D. Snell and K. M. Johnson. (1989). "Characterization of the binding of radioligands to the N-Methyl-D-Aspartate, Phencyclidine, and Glycine receptors in buffy coat membranes." *J. Pharmacol. Methods*. **21**: 161-168.

Kamikubo, K., H. Murase, M. Murayama, K. Miura, M. Nozaki and K. Tsurumi. (1986). "Opioid and non-opioid binding of beta-endorphin to bovine adrenal medullary membranes." *Reg. Peptides*. **15**: 155-162.

Kao. (1985). "Muscarinic receptors on bovine chromaffin cells mediate a rise in cytosolic calcium that is independent of extracellular calcium." *J. Biol. Chem.* **260**: 2019-2022.

Kataoka, Y., Y. Gutman, A. Guidotti, P. Panula, J. Wroblewski, D. Cosenza-Murphy, J. Y. Wu and E. Costa. (1984). "Intrinsic GABA-ergic system of adrenal chromaffin cells." *Proc. Natl. Acad. Sci. USA*. **77**: 7473-7475.

Katz, J. L., R. D. Spealman and R. D. Clark. (1985). "Stereoselective behavioral effects of N-allylnormetazocine in pigeons and squirrel monkeys." *J. Pharmacol. Exp. Ther.* **232**: 452-461.

Kayaalap, S. O. and N. H. Neff. (1979). "Muscarinic cholinergic receptors of bovine adrenal medulla." *Neuropharmacol.* **18**: 909-911.

Kayaalp, S. O. and N. H. Neff. (1979). "Cholinergic muscarine receptors of bovine adrenal medulla." *Neuropharmacology*. **18**: 909-911.

Keats, A. S. and J. Telford. (1964). "Narcotic antagonists as analgesics. Clinical aspects." *Adv. Chem. Ser.* **45**: 170-176.

Kinouchi, K., S. Maeda, K. Saito, R. Inoki, K. Fukumitsu and I. Yoshiya. (1989). "Effects of pentazocine and other opioids on the potassium-evoked release of [3H]noradrenaline from guinea pig cortical slices." *Eur. J. Pharmacol.* :

Kirshner, N. (1969). "Storage and secretion of adrenal catecholamines." *Adv. Biochem. Psychopharmacol.* **1**: 71-89.

- Kirshner, N. and O. H. Viveros. (1972). "The secretory cycle in the adrenal medulla." *Pharmacol. Rev.* **24**(2): 385-426.
- Kumakura, K., F. Karoum, A. Guidotti and E. Costa. (1980). "Modulation of nicotinic receptors by opiate receptor antagonists in cultured adrenal chromaffin cells." *Nature.* **283**: 489-492.
- Largent, B. L., A. L. Gundlach and S. H. Snyder. (1984). "Psychotomimetic opiate receptors labeled and visualized with (+)-[3H]3-(3-hydroxyphenyl)-N-(1-propyl)piperidine." *Proc. Natl. Acad. Sci. USA.* **81**: 4983-4987.
- Largent, B. L., A. L. Gundlach and S. H. Snyder. (1986). "Pharmacological and autoradiographic discrimination of sigma and phencyclidine receptor binding sites in brain with (+)-[3H]SKF 10,047, (+)-[3H]-3-[3-hydroxyphenyl]-N-(1-propyl)piperidine and [3H]-1-[1-(2-thienyl)cyclohexyl]piperidine." *J. Pharmacol. Exp. Ther.* **238**(2): 739-748.
- Largent, B. L., H. Wikstrom, A. L. Gundlach and S. H. Snyder. (1987). "Structural determinants of sigma receptor affinity." *Mol. Pharmacol.* **32**: 772-784.
- Lemaire, S., L. Chouinard, P. Mercier and R. Day. (1986). "Bombesin-like immunoreactivity in bovine adrenal medulla." *Reg. Peptides.* **13**: 133-146.
- Lemaire, S., G. Derome, R. Tseng, P. Mercier and I. Lemaire. (1981). "Distinct regulations by calcium of cyclic GMP levels and catecholamine secretion in isolated bovine adrenal chromaffin cells." *Metabolism.* **30**(5): 462-468.
- Lemaire, S., M. Dumont, P. Mercier, I. Lemaire and R. Calvert. (1983). "Biochemical characterization of various populations of isolated bovine adrenal chromaffin cells." *Neurochem. Int.* **5**(2): 193-200.
- Lemaire, S., I. Lemaire, D. M. Dean and B. G. Livett. (1980). "Opiate receptors and adrenal medullary function." *Nature.* **288**: 303-304.
- Lemaire, S., B. Livett, R. Tseng, P. Mercier and I. Lemaire. (1981). "Studies on the inhibitory action of opiate compounds in isolated bovine and adrenal chromaffin cells: non-involvement of stereospecific opiate binding sites." *J. Neurochem.* **36**: 886-892.

- Lemaire, S., A. Valette, L. Chouinard, N. Dupuis, R. Day, G. Porthé and J. Cros. (1983). "Purification and identification of multiple forms of dynorphin in human placenta." *Neuropeptides*. 3: 181-191.
- Lever, J. D. (1955). "Electron microscopic observations on the normal and denervated adrenal medulla of the rat." *Endocrinology*. 57: 621-635.
- Livett, B. G., R. Day, R. P. Elde and P. R. C. Howe. (1982). "Co-storage of enkephalins and adrenaline in the bovine adrenal medulla." *Neuroscience*. 7(5): 1323-1332.
- Livett, B. G. and D. M. Dean. (1980). "Distribution of immunoreactive enkephalins in adrenal paraneurons: preferential localization in varicose processes and terminals." *Neuropeptides*. 1: 3-7.
- Livett, B. G., D. M. Dean, L. G. Whelan, S. Udenfriend and J. Rossier. (1981). "Co-release of Leu-enkephalin and catecholamines from adrenal chromaffin cells in culture." *Nature*. 289: 317-319.
- Livett, B. G., V. Kozousik, F. Mizobe and D. M. Dean. (1979). "Substance P inhibits nicotinic activation of chromaffin cells." *Nature*. 278: 256-257.
- Loo, P. S., A. F. Braunwalder, J. Lehmann, M. Williams and M. A. Sills. (1987). "Interaction of L-glutamate and magnesium with phencyclidine recognition sites in rat brain: evidence for multiple affinity states of the phencyclidine/N-methyl-D-aspartate receptor complex." *Mol. Pharmacol.* 32: 820-830.
- Lozovsky, D., C. F. Saller, M. A. Bayorh, C. C. Chiueh, K. C. Rice, T. R. Burke Jr. and I. J. Kopin. (1983). "Effects of phencyclidine on rat prolactin, dopamine receptor and locomotor activity." *Life Sci.* 32: 2725.
- Luby, E. D. and B. D. Cohen. (1959). "Comparison of a new schizophrenomimetic drug-Sernyl." *AMA Arch. Neurol. Psych.* 81: 363-369.
- Luby, E. D., J. S. Gottlieb, B. D. Cohen, G. Rosenbaum and E. F. Domino. (1962). "Model psychoses and schizophrenia." *Am. J. Psychiatry.* 119: 61-67.

- Lundberg, J. M., B. Hamberger, M. Schultzberg, T. Hokfelt, P.-O. Granberg, S. Efendick, L. Terenius, M. Goldstein and H. Luft. (1979). "Enkephalin and somatostatin-like immunoreactivity in human adrenal medulla and pheochromocytoma." *Proc. Natl. Acad. Sci. USA.* **76**: 4079-4082.
- Maayani, S. and H. Weinstein. (1980). "'Specific binding" of [3H]-phencyclidine: artifacts of the rapid filtration method." *Life Sci.* **26**: 2011-2022.
- MacDonald, J. F., J. H. Schneiderman and Z. Milijkovic. (1986). "Excitatory amino acids and epilepsy, in "Advances in experimental biology and medicine"." (eds. R. Schwarcz and Y. Ben-Ari). **203**: 425-437. Plenum Press, New York.
- Malave, A., J. L. Borowitz and G. K. W. Yim. (1983). "Block by phencyclidine of acetylcholine and barium induced adrenal catecholamine secretion." *Life Sci.* **33**: 511-516.
- Maragos, W. F., D. C. M. Chu, J. I. Greenamyre, J. B. Penny and A. B. Young. (1986). "High correlation between the localization of [3H]TCP binding and NMDA receptors." *Eur. J. Pharmacol.* **123**: 173-174.
- Marley, P. D. and B. G. Livett. (1985). "Neuropeptides in the autonomic nervous system." *CRC Crit. Rev. Clin. Neurobiol.* **1**: 201-283.
- Martin, J. R., M. H. Berman, I. Krawsum and S. F. Small. (1979). "Phencyclidine-induced stereotyped behaviour and serotonergic syndrome in rat." *Life Sci.* **24**: 1699-1704.
- Martin, W. R., C. G. Eades, J. A. Thompson, R. E. Huppler and P. E. Gilbert. (1976). "The effects of morphine-and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog." *J. Pharmacol. Exp. Ther.* **197**: 517-532.
- McGoodall, C. (1951). "Studies of adrenaline and noradrenaline in mammalian heart and suprarenals." *Acta. Physiol. Scand. Suppl.* :
- Mendelsohn, L. G., V. Kalra, B. G. Johnson and G. A. Kerchner. (1985). "Sigma opioid receptor: characterization and co-identity with the phencyclidine receptor." *J. Pharmacol. Exp. Ther.* **233**(3): 597-602.

Mendelsohn, L. G., G. A. Kerchner, V. Kalra, D. M. Zimmerman and J. D. Leander. (1984). "Phencyclidine receptors in rat brain cortex." *Biochemical Pharmacol.* **33**(22): 3529-3535.

Meyer, J. S., F. Greifenstein and M. Devault. (1959). "A new drug causing symptoms of sensory deprivation." *J. Nerv. Ment. Disease.* **129**: 54-61.

Morgenstern, F. S., H. R. Beech and B. M. Davies. (1962). "An investigation of drug induced sensory disturbances." *Psychopharmacologia.* **3**: 193-201.

Nabeshima, T., K. Ishikawa, K. Yamaguchi, H. Furukawa and T. Kameyama. (1987). "Phencyclidine-induced head-weaving observed in mice after ritanserin treatment." *Eur. J. Pharmacol.* **139**: 171-178.

Nabeshima, T., H. Kamei and T. Kameyama. (1988). "A role played by sigma receptors in the conditioned suppression of motility in mice." *Psychopharmacology.* **94**: 515-520.

Nabeshima, T., S. P. Sivam, C. Y. Tai and I. K. Ho. (1982). "Development of dispositional tolerance to phencyclidine by osmotic mini pump in the mouse." *J. Pharmacol. Methods.* **7**: 239-253.

Okubo, M., K. Kaku, T. Kaneko and N. Yanaihara. (1985). "Effects of bombesin and gastrin releasing peptide on catecholamine secretion from rat adrenal gland in vitro." *Endocrinol. Jpn.* **32**: 21-27.

Pechnick, R. N., R. George, R. J. Lee and R. E. Poland. (1985a). "The effects of the acute administration of phencyclidine hydrochloride (PCP) on the release of corticosterone, growth hormone and prolactin in the rat." *Life Sci.* **38**: 291.

Pechnick, R. N., R. George and R. E. Poland. (1985b). "Identification of multiple opiate receptors through neuroendocrine responses. I. Effects of agonists." *J. Pharmacol. Exp. Ther.* **232**: 163-167.

Pert, C. B. and S. H. Snyder. (1973). "Opiate Receptor: demonstration in nervous tissue." *Science.* **179**: 1011-1014.

Petersen, R. C. and R. C. Stillman. (1978). "Phencyclidine Abuse: an appraisal." *NIDA Research Monograph Ser.* **21**: 77-92.

Porter, J. H., J. L. Wiley and R. L. Balster. (1988). "Effects of phencyclidine-like drugs on punished behavior in rats." *J. Pharmacol. Exp. Ther.* **248**(3): 997-1002.

Purifoy, J. A. and R. W. Holz. (1984). "The effects of ketamine, phencyclidine, and lidocaine on catecholamine secretion from cultured bovine adrenal chromaffin cells." *Life Sci.* **35**: 1851-1857.

Quirion, R., D. A. Dimaggio, E. D. French, P. C. Contreras, J. Shiloach, C. B. Pert, H. Everist, A. Pert and T. O'Donohue. (1984). "Evidence for an endogenous peptide ligand for the phencyclidine receptor." *Peptides.* **5**: 967-973.

Quirion, R., R. P. Hammer, M. Herkenham and C. B. Pert. (1981). "Phencyclidine (angel dust)/sigma "opiate" receptor: visualization by tritium sensitive film." *Proc. Natl. Acad. Sci. USA.* **78**(9): 5881-5885.

Quirion, R. and C. B. Pert. (1982). "Certain calcium antagonists are potent displacers of [3H]phencyclidine(PCP) in rat brain." *Eur. J. Pharmacol.* **83**: 153-156.

Quirion, R., K. C. Rice, P. Skolnick, S. Paul and C. B. Pert. (1981). "Stereospecific displacement of [3H]phencyclidine (PCP) receptor binding by an enantiomeric pair of PCP analogs." *Eur. J. Pharmacol.* **74**: 107-108.

Rahwan, R. G. and J. L. Borowitz. (1973). "Mechanisms of stimulus-secretion coupling in Adrenal medulla." *J. Pharmacol. Sci.* **62**(12): 1911-1923.

Reynolds, I. J. and R. J. Miller. (1988). "Multiple sites for the regulation of the N-methyl-D-aspartate receptor." *Mol. Pharmacol.* **33**: 581-584.

Rogers, C. A., D. Cecyre and S. Lemaire. (1989). "Presence of sigma and PCP-like receptors in membrane preparations of bovine adrenal medulla." *Biochem. Pharmacol.* **33**(15): 2467-2472.

Role, L. W., S. E. Leeman and R. L. Perlman. (1981). "Somatostatin and substance P inhibit catecholamine secretion from isolated cells of guinea pig adrenal medulla." *Neuroscience.* **6**: 1813-1821.

Rossier, J., D. M. Dean, B. G. Livett and S. Udenfriend. (1981). "Enkephalin congeners and precursors are synthesized and released by primary cultures of adrenal chromaffin cells." *Life Sci.* **28**(7): 781-789.

Rothman, R. B., V. Bykov, A.-H. Newman, A. E. Jacobson and K. C. Rice. (1988). "Interaction of enantiomeric pairs of opiates with phencyclidine binding sites in rat brain : Identification of (+)pentazocine as a ligand potentially suitable for imaging sigma binding sites using positron emission tomography." *Neuropeptides.* **12**: 1-5.

Saini, L. and A. Guidotti. (1982). "Opiate receptor-mediated inhibition of catecholamine release in primary cultures of bovine adrenal chromaffin cells." *J. Neurochemistry.* **39**: 1669-1676.

Saller, C. F., R. L. Zerbe, M. A. Bayorh, D. Lozovsky and I. Kopin. (1983). "Phencyclidine suppresses plasma prolactin levels." *Eur. J. Pharmacol.* **83**: 309-311.

Schneider, A. S., T. C. Hollis and S. Lemaire. (1979). "Rapid rise in cyclic GMP accompanies catecholamine secretion in suspensions of isolated adrenal chromaffin cells." *Life Science.* **24**: 1389-1394.

Schultzberg, M., T. Hokfelt, J. M. Lundberg, L. Ternius, L.-G. Elfin and R. P. Elde. (1978). "Enkephalin-like immunoreactivity in nerve terminals in sympathetic ganglia and adrenal medulla and in adrenal medullary gland cells." *Acta. Physiol. Scand.* **103**: 475-477.

Serck-Hanssen, G. (1974). "Effects of theophylline and propranol on acetylcholine-induced release of adrenal medullary catecholamines." *Biochem. Pharmacol.* **23**: 2225-2234.

Shannon, H. E. (1981). "Evaluation of Phencyclidine Analogs on the Basis of Their Discriminative Stimulus Properties in the Rat." *J. Pharmacol. Exp. Ther.* **216**: 543-551.

Shannon, H. E. (1982). "Pharmacological analysis of the phencyclidine-like discriminative stimulus properties of narcotic derivative stimulus properties of narcotic derivatives in rats." *J. Pharmacol. Exp. Ther.* **222**: 146-151.

Shannon, H. E. (1983). "Pharmacological evaluation of N-allylnormetazocine (SKF-10047) on the basis of its discriminative stimulus properties in the rat." *J. Pharmacol. Exp. Ther.* **225**: 144-152.

Shepard, D. M. and G. B. West. (1953). "Hydroxytryptamine in medulla of sheep, ox and cow." *J. Physiol. (London)*. **120**: 15-19.

Showalter, C. V. and W. E. Thornton. (1977). "Clinical pharmacology of phencyclidine toxicity." *Am. J. Psychiatry*. **134**: 1234-1238.

Simon, E. J., J. Hiller and I. Edelman. (1973). "Stereospecific binding of the potent narcotic analgesic [3H] etorphine in rat brain homogenate." *Proc. Natl. Acad. Sci. USA*. **70**: 1947-1950.

Sircar, R., R. Nichtenhauser, J. R. Ieni and S. R. Zukin. (1986). "Characterization and autoradiographic visualization of (+)-[3H]SKF-10047 binding in rat and mouse brain: further evidence for phencyclidine/"sigma opiate" receptor commonality." *J. Pharmacol. Exp. Ther.* **237**(2): 681-688.

Smith, A. D. (1968). "Biochemistry of adrenal chromaffin granules. In," *The interaction of drugs and subcellular components in animal cells.* (PN Campbell,ed) London, Churchill. (239-292):

Snell, L. D. and K. M. Johnson. (1985). "Antagonism of N-methyl-D-aspartate induced transmitter release in the rat striatum by phencyclidine-like drugs and its relationship to turning behaviour." *J. Pharmacol. Exp. Ther.* **235**: 50-57.

Snell, L. D. and K. M. Johnson. (1986). "Characterization of the inhibition of excitatory amino acid-induced neurotransmitter release in the rat striatum by phencyclidine-like drugs." *J. Pharmacol. Exp. Ther.* **238**(3): 938-946.

Sonders, M. S., J. F. Keana and E. Weber. (1988). "Phencyclidine and psychotomimetic sigma opiates: recent insights into their biochemical and physiological sites of action." *Trends Pharmacol. Sci.* **11**(1): 37-40.

Stjarne, C. (1972). "The synthesis, uptake, and storage of catecholamines in the adrenal medulla. The effects of drugs. In, *Handbook of Experimental Pharmacology*. Vol. XXXIII,

Catecholamines." (H. Blasko, and E. Muscholl, eds Springer, Berlin): 231-269.

Stojilkovic, S. S., M. L. Dufau and K. J. Catt. (1987). "Receptors and secretory actions of sigma/phencyclidine agonists in anterior pituitary cells." *Endocrinol.* **121**: 2044-2054.

Strittmatter, W. J. (1988). "Molecular mechanisms of exocytosis: the adrenal chromaffin cell as a model system." *Cell. Mol. Neurobiol.* **8**(1): 19-25.

Sturgeon, R. D., R. G. Fessler and H. Y. Meltzer. (1979). "Behavioural rating scales for assessing phencyclidine-induced locomotor activity. Stereotyped behaviour and ataxia in rats." *Eur. J. Pharmacol.* **59**: 169-179.

Su, T. P. (1982). "Evidence for sigma opioid receptor: binding of [3H]SKF-10047 to etorphine-inaccessible sites in guinea-pig brain." *J. Pharmacol. Exp. Ther.* **223**: 284-290.

Su, T. P., E. D. London and J. H. Jaffe. (1988). "Steroid binding at sigma receptors suggests a link between endocrine, nervous, and immune systems." *Science.* **240**: 219-221.

Su, T. P., S. E. Schell, F. Y. Ford-Rice and E. D. London. (1988). "Correlation of inhibitory potencies of putative antagonists for sigma receptors in brain and spleen." *Eur. J. Pharmacology.* **148**: 467-470.

Su, T. P., A. D. Weissman and S.-Y. Yeh. (1986). "Endogenous ligands for sigma opioid receptors in the brain ("sigmaphin"): evidence from binding assays." *Life Sciences.* **38**: 2199-2210.

Tam, S. W. (1983). "Naloxone inaccessible sigma receptor in rat central nervous system." *Proc. Natl. Acad. Sci. USA.* **80**: 6703-6707.

Tam, S. W. (1985). "(+)-[3H]SKF-10047, (+)ethylketocyclazocine, mu, kappa, delta, and phencyclidine binding sites in guinea pig membranes." *Eur. J. Pharmacol.* **109**: 33-41.

Taylor, D. P. and J. Dekleva. (1987). "Potential antipsychotic BMY 14802 selectively binds to sigma sites." *Drug Dev. Res.* **11**: 65-70.

- Udelsman, R., J.-P. Harwood, M. A. Millan, G. P. Chrousos, D. S. Goldstein, R. Zimlichman, K. J. Catt and G. Aguilera. (1987). "Functional corticotropin releasing factor receptors in the primate peripheral nervous system." *Nature*. **319**: 147-150.
- Unsicker, K., O. Habura-Fluh and U. Zwarg. (1978). "Different types of small granule-containing cells and neurons in the guinea-pig adrenal medulla." *Cell Tiss. Res.* . **189**: 109-130.
- Vaupel, D. B. (1983). "Naltrexone fails to antagonize the sigma effects of PCP and SKF-10,047 in the dog." *Eur. J. Pharmacol.* **92**: 269-274.
- Vignon, J., R. Chicheportiche, M. Chicheportiche, J.-M. Kamenka, P. Geneste and M. Lazdunski. (1983). "[³H]TCP: a new tool with high affinity for the PCP receptor in rat brain." *Brain Res.* **280**: 194-197.
- Vignon, J., V. Pinet, C. Cerruti, J.-M. Kamenka and R. Chicheportiche. (1988). "[³H]N-[1-(2-benzo(B)thiophenyl)cyclohexyl]piperidine ([³H]BTCP): a new phencyclidine analog selective for the dopamine uptake complex." *Eur. J. Pharmacol.* **148**: 427-436.
- Vignon, J., A. Privat, I. Chaudieu, A. Thierry, J.-M. Kamenka and R. Chicheportiche. (1986). "[³H]Thienyl-phencyclidine ([³H]TCP) binds to two different sites in rat brain. Localization by autoradiographic and biochemical techniques." *Brain Research.* **378**: 133-141.
- Vignon, J., J.-P. Vincent, J.-N. Bidard, J.-M. Kamenka, P. Geneste, S. Monier and M. Lazdunski. (1982). "Biochemical properties of the brain phencyclidine receptor." *Eur. J. Pharmacol.* **81**: 531-542.
- Vincent, J. P., D. Cavey, J. M. Kamenka, P. Geneste and M. Lazdunski. (1979). "Interaction of phencyclidines with muscarinic and opiate receptors in the central nervous system." *Brain Research.* **152**: 176-179.
- Viveros, O. H. and S. P. Wilson. (1983). "The adrenal chromaffin cell as a model to study co-secretion of enkephalins and catecholamines." *J. Auton. Nerv. Syst.* **7**: 41-58.
- Von Euler, U. S. (1972). "Synthesis uptake and storage of catecholamines in adrenergic nerves. The effects of drugs." In, "Handbook of Experimental Pharmacology". XXXIII Catecholamines,(H Blashko, E Muscholl)Springer, Berlin: 231-269.

- Wada, A., M. Arita, N. Yanagihara and F. Izumi. (1988). "Binding of [3H] Phencyclidine to adrenal medullary cells: Inhibition of ^{22}Na Influx, ^{45}Ca Influx, ^{86}Rb efflux and catecholamine secretion caused by carbachol and veratridine." *Neuroscience*. **25**(2): 687-696.
- Wada, A., S. Sakuri, S. Kobayashi, N. Yanagihara and F. Izumi. (1982). "Alpha-adrenergic receptors inhibit catecholamine secretion from bovine adrenal medulla." *Brain Res.* **252**: 189-191.
- Wakade, A. R. (1981). "Studies on secretion of catecholamines evoked by acetylcholine or transmural stimulation of rat adrenal gland." *J. Physiol. (London)*. **313**: 463.
- Weber, E., M. Sonders, M. Quarum, S. McLean, S. Pou and J. F. W. Keana. (1986). "1,3-Di(2-[5-3H]tolyl)guanidine: a selective ligand that labels sigma-type receptors for psychotomimetic opiates and antipsychotic drugs." *Proc. Natl. Acad. Sci. USA*. **83**: 8784-8788.
- Wilson, S. P. and N. Kirschner. (1977). "The Acetylcholine receptor of the Bovine Adrenal Medulla. ." *J. Neurochemistry*. **28**: 687-690.
- Wolfe, S. A., S. G. Culp and E. B. De Souza. (1989). "Sigma-receptors in endocrine organs: identification, characterization, and autoradiographic localization in rat pituitary, adrenal, testis, and ovary." *Endocrinol.* **124**(3): 1160-1172.
- Young, G. A. and N. Khazan. (1985). "Differential neuropharmacological effects of mu, kappa, and PCP/sigma receptors, in " Behavioral Pharmacology: the current status"(eds L S Seiden and R L Balster) Alan R. Liss Inc., New York." (131-147):
- Zukin, R. S. and S. R. Zukin. (1981). "Demonstration of [3H]cyclazocine binding to multiple opiate receptor sites ." *Mol. Pharmacol.* **20**: 246-254.
- Zukin, S. R. and R. S. Zukin. (1979). "Specific [3H]phencyclidine binding in rat central nervous system." *Proc. Natl. Acad. Sci. USA*. **76**(10): 5372-5376.
- Zukin, S. R., R. S. Zukin, W. Vale, J. Rivier, R. Nichtenhauser, L. D. Snell and K. M. Johnson. (1987). "An endogenous ligand of the brain

sigma/PCP receptor antagonizes NMDA-induced neurotransmitter release." *Brain Res.* **416**: 84-89.