SIGMA RECEPTORS
Chemistry, Cell Biology and Clinical Implications

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edited by

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Preface

Over the last 30 years, our understanding of σ receptors has undergone a colossal evolution. They began as theoretical entities, then progressed to enigmatic receptors, and finally to identified proteins with important biological functions.

Since the first book on σ receptors was published in 1994, there have been many significant advances in the field. We now know that σ receptors subserve many critical functions in the body and recent studies indicate that they are promising drug development targets for a host of neurological, psychiatric, cardiovascular, ophthalmological, immunological, and gastrointestinal disorders. This book provides a timely update on the medicinal chemistry, cell biology, and clinical implications of σ receptors. It puts the information in a historical perspective to help new comers to the field successfully navigate the confusing early history surrounding these proteins, and it provides a launching point from which future studies and research directions can easily be developed.

The full impact of σ receptors on biological function has yet to be determined. The existing gaps in our knowledge base offer untold opportunities for future research. It is our hope that the information contained in this book will stimulate new, exciting research on σ receptors and ultimately lead to innovative insights into basic biological mechanisms and novel therapeutic advances.

Rae R. Matsumoto
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Chapter 1

σ RECEPTORS: HISTORICAL PERSPECTIVE AND BACKGROUND

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1. HISTORICAL PERSPECTIVE

σ Receptors were first proposed in 1976 by Martin and coworkers based on the actions of SKF-10,047 (N-allylnormetazocine) and related benzomorphans (1). The name σ was in fact derived from the first letter "S" from SKF-10,047 which was thought to be the prototypic ligand for these receptors. Over the next 10 years, a series of studies determined that SKF-10,047 interacts with a number of distinct binding sites (Figure 1-1), leading to much confusion about the true identity and nature of σ receptors during its early history.

σ Receptors were originally thought to be a type of opioid receptor. This belief stemmed from a historic study by Martin and colleagues who evaluated SKF-10,047 and other benzomorphans in morphine-dependent and non-dependent chronic spinal dogs (1). In this groundbreaking study, Martin and colleagues discovered that the physiological actions of the tested compounds fell into three distinct groups. They hypothesized that the differences between the groups stemmed from interactions with different subtypes of opioid receptors (1). Martin and his colleagues proposed a μ subtype which mediated the actions of morphine and related compounds, a κ subtype based on the actions of ketocyclazocine and its grouping, and a σ subtype which was characterized by SKF-10,047 and related compounds. Martin's study employed the use of racemic benzomorphans, a mixture of the (+)- and (-)-isomers of the compounds. Therefore, in later studies, when the isomers of benzomorphans were evaluated separately, it was determined that the (-)-isomers accounted for the vast majority of opioid-mediated

effects. In the case of SKF-10,047, the (+)-isomer was determined in subsequent studies to produce actions that were insensitive to opioid antagonists (2-4), while the (-)-isomer was responsive to opioid antagonists (5,6). It is now accepted that the opioid-mediated actions of (-)-SKF-10,047 are relayed primarily through μ and κ opioid receptors.

During the 1980s, renewed interest in the (+)-isomer of SKF-10,047 occurred when it was determined that it possessed phencyclidine (PCP)-like properties. During this period, the term σ/PCP made its appearance in the literature and many investigators believed that the σ and PCP sites were identical. There was conclusive evidence that (+)-SKF-10,047 interacted with the PCP binding site, which was ultimately determined to be within the ionophore of the N-methyl-D-aspartate (NMDA) receptor (7-11). However, as selective ligands for the NMDA receptor were identified, it became apparent that [^{3}H](+)-SKF-10,047 binding could only be partially displaced using selective NMDA receptor ligands (11). Therefore, it appeared that (+)-SKF-10,047 bound to another site in addition to the ionophore of the NMDA receptor. This other binding site was ultimately identified as the entity that today retains the designation of the σ receptor.

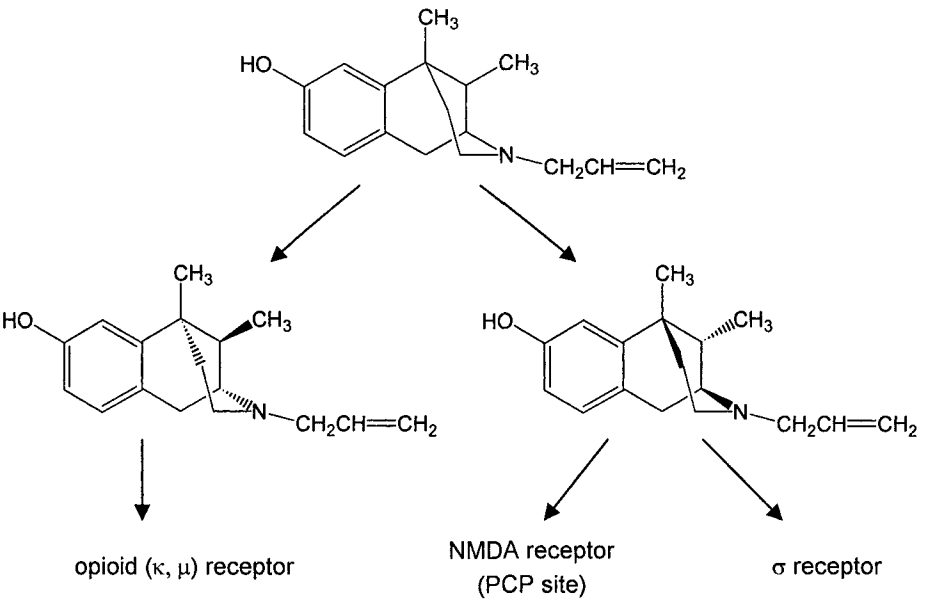

Figure 1-1. Association between different forms of SKF-10,047 and multiple binding sites

During the early 1980s, pioneering studies by Tsung-Ping Su had already begun shedding light on this additional component of [^{3}H]SKF-10,047 binding. These studies ultimately led to the identification of a unique drug selectivity pattern that characterized σ receptors from other known receptors (Table 1-1). Using guinea pig brain homogenates, Su characterized [^{3}H](±)-SKF-10,047 binding sites that were inaccessible to the opioid etorphine (12,13). Radioligand binding to these sites, which are today recognized as σ receptors, could be inhibited by a number of factors including phospholipase C and divalent cations, and exhibited a drug binding profile that was unlike anything characterized at that time (13). In these classic studies, Su demonstrated that σ receptors displayed high affinity for several (+)-benzomorphans including (+)-pentazocine, dextrallorphan, and (+)-cyclazocine (13). These binding sites were distinct from classical opioid receptors because in addition to having reverse stereoselectivity for benzomorphans (i.e. opioid receptors preferentially bind the (-)-isomer), a number of established opiates and opioid peptides failed to display significant affinities for these sites (13). In addition, these etorphine-inaccessible sites also bound neuroleptics such as haloperidol, the antidepressant imipramine, the β-adrenergic blocker propranolol, and the dissociative anesthetic PCP (13). Together, the data collected by Su clearly indicated the existence of a new and previously uncharacterized receptor, which is today recognized as the σ receptor.

Table 1-1. Drug selectivity profile of select compounds for σ receptors

Opioid-Related:		**DA-Related:**		**Other:**	
(+)-SKF-10,047	+++	Haloperidol	++++	PCP	++
(-)-SKF-10,047	+	Fluphenazine	+++	MK-801	---
(+)-Pentazocine	++++	Perphenazine	+++	Propranolol	++
(-)-Pentazocine	++	Chlorpromazine	++	Atropine	---
Dextrallorphan	+++	Pimozide	++	Clonidine	---
(+)-Cyclazocine	++	Molindone	++	Imipramine	++
(+)-EKC	+++	(+)-3PPP	++++	Pyrilamine	+++
Morphine	---	(-)-Butaclamol	+++	Chlorpheniramine	++
Naloxone	---	Clozapine	---	Promethazine	++
β-endorphin	---	Dopamine	---	Cimetidine	---
Leu-enkephalin	---	Apomorphine	---	Histamine	---
Dynorphin (1-13)	---	Amphetamine	---	DTG	+++

Relative affinities based on competition binding studies. ++++ <10 nM; +++ 11-100 nM; ++ 101-1000 nM; + 1001-10,000 nM; --- >10,000. EKC = ethylketocyclazocine. Adapted from refs. (13,14,15,18,19).

The unique pattern of binding that was initially reported by Su was subsequently corroborated and extended in William Tam's laboratory, first using [^{3}H](±)-ethylketocyclazocine and [^{3}H]SKF-10,047 to bind naloxone-inaccessible sites in the rat central nervous system, and then using [^{3}H](+)-SKF-10,047 and [^{3}H]haloperidol in the guinea pig brain (14,15). Tam confirmed that σ receptors bound a number psychotomimetic opioids (e.g. SKF-10,047, ethylketocylazocine, pentazocine, cyclazocine, bremazocine), the β-blocker propranolol, and the dissociative anesthetic PCP (14,15). In addition, Tam greatly expanded the list of neuroleptics that were shown to bind σ receptors with nanomolar affinity (e.g. haloperidol, perphenazine, fluphenazine, molindone, pimozide, thioridazine, chlorpromazine), and revealed that H1 antihistamines also interacted with these sites (e.g. pyrilamine, promethazine, chlorpheniramine) (14,15). Moreover, Tam demonstrated that the binding profile of drugs at σ receptors differed from the pattern of binding when using [^{3}H]PCP and [^{3}H]spiperone to label NMDA and dopamine receptors (14,15). Together, the studies of Su and Tam identified a unique drug selectivity pattern which characterized the binding sites that are now recognized as σ receptors.

Soon thereafter, more selective radioligands were identified for σ receptors. [^{3}H](+)-3-(3-hydroxyphenyl)-N-(1-propyl)piperidine (3PPP) was successfully used by a number of groups to discriminate σ receptors in binding studies from interactions with opioid, NMDA or dopamine receptors, which were problematic for historic radioligands such as [^{3}H]SKF-10,047 and [^{3}H]haloperidol (16). However, (+)-3PPP was also a presynaptic dopamine autoreceptor agonist (17), necessitating the search for even more selective compounds which could be used as radiolabeled probes to study σ receptors. A crucial breakthrough occurred with the introduction of [^{3}H]di-o-tolylguanidine (DTG) by Eckard Weber's group, the first truly selective radioligand for σ receptors (18). Subsequently, [^{3}H](+)-pentazocine was identified as another selective radioligand for σ receptors; this probe selectively binds to the σ_1 subtype (see below for additional information about σ receptor subtypes) (19,20). The availability of selective radioligands for σ receptors represented a major milestone for the field, and firmly established σ receptors as a viable topic for research.

In contrast to the early history of σ receptor research which was defined by pharmacological studies, the revolution in molecular and cell biology has greatly altered the way in which science is approached. Although its impact on the σ receptor field has been relatively slower than in some other areas, significant advances have been made. Foremost among these achievements was the cloning of the first σ receptor (σ_1 subtype), which is described in more detail in subsequent chapters of this book. This information led to insights about the structure and function of the receptor, and its relationship

to other known proteins. In addition, the development of cell and molecular biological-based probes allowed investigators to further elucidate σ receptor function; other chapters in this volume detail our current knowledge in these areas.

Table 1-2. Characteristics of σ_1 and σ_2 receptors

Feature	σ_1 Receptor	σ_2 Receptor	Reference
Physical Characteristics:			
Size	25-29 kDa	18-22 kDa	
Sequence [a]	AF030199 (mouse) AF004218 (rat) U75283 (human)	n.d.	
Tissue Expression:			
Brain	High	High	
Heart	High	Low	
Liver	High	High	
Spleen	High	Low	
GI tract	High	High	
Putative Agonists (Ki in nM):			
DTG	74 ± 15	61 ± 13	20
CB-184	7,436 ± 308	13 ± 2	49
Igmesine	n.d.	n.d.	
(+)-Pentazocine	7 ± 1	1361 ± 134	20
PRE-084	n.d.	n.d.	
Pregnenolone	n.d.	n.d.	
SA4503	17 ± 2 [b]	1784 ± 314 [b]	121
(+)- SKF-10,047	29 ± 3	33,654 ± 9,409	20
Putative Antagonists (Ki in nM):			
BD1047	0.9 ± 0.1	47 ± 0.6	33
BD1063	9 ± 1	449 ± 11	33
BMY 14802	60 [b]	230 [b]	50
Lu 28-179	17 [b]	0.12 [b]	50
NE-100	2 ± 0.3 [b]	85 ± 33 [b]	32
Panamesine	n.d.	n.d.	
Progesterone	n.d.	n.d.	
(±)-SM 21	>1000	67 ± 8	122
SR 31742A	n.d.	n.d.	

See Appendix A for chemical names of compounds. [a] Accession numbers for representative sequences. [b] IC_{50} in nM. n.d. = affinities for specific subtypes not determined; existing affinity information based on binding to both subtypes.

2. σ RECEPTOR SUBTYPES AND SPLICE VARIANTS

There are two well established subtypes of σ receptors, which have been designated σ_1 and σ_2. These receptor subtypes can be distinguished from one another based on their molecular weights, tissue distribution, and drug selectivity patterns. Select features of these two subtypes and compounds that are commonly used as agonists and antagonists at σ receptors are summarized in Table 1-2.

The σ_1 subtype has been cloned from a number of species including mouse, rat, guinea pig, and human (21-25; see Chapter 5). This subtype is predicted to be a 223 amino acid protein with at least one transmembrane-spanning region (26,27). It is widely expressed in a number of tissues, including heart and spleen where the expression of the σ_1 subtype appears to predominate over the σ_2 subtype (28,29). σ_1 Receptors appear to translocate during signaling and are linked to the modulation or production of intracellular second messengers (see Chapter 8). In addition, σ_1 receptors can associate with other proteins, including ankyrin B, heat shock protein 70 (hsp70), heat shock conjugate protein (hsc 70), glucose-related protein (GRP78/BiP), and potassium channels (26,30,31). To study their function, (+)-pentazocine is commonly used as a selective agonist at σ_1 receptors, and selective antagonists such as 1-[2-(3,4-dichlorophenyl)ethyl]-4-methyl piperazine (BD1063) and N,N-dipropyl-2-[4-methoxy-3-(2-phenylethoxy) phenyl]ethylamine (NE-100) are also available (32,33). In addition, sequence-specific antibodies, antisense oligodeoxynucleotides, and a σ_1 knockout mouse have been developed to further delineate the functions of this receptor subtype (34-46). Many of these functions are described further in the chapters that follow.

The σ_2 subtype appears to be a distinct physical entity from the σ_1 receptor. Comparisons of their sizes based on affinity labeling studies indicated that the σ_2 subtype is slightly smaller than the σ_1 receptor (47,48). The sequence of the σ_2 receptor has not yet been determined, although considerable progress has been made in this area in recent years. In contrast to σ_1 receptors that readily translocate, σ_2 receptors appear to be lipid raft proteins that affect calcium signaling via sphingolipid products (see Chapter 11). Unfortunately, there are no truly selective σ_2 receptor agonists and antagonists. (+)–1R,5R-(E)-8-(3,4-dichlorobenzylidene)-5-(3-hydroxy-phenyl)-2-methylmorphan-7-one (CB-184), one of the more selective σ_2 agonists, also interacts with μ opioid receptors (49). 1'-[4-[1-(4-fluorophenyl)-1H-indol-3-yl]-1-butyl]spiro[isobenzofuran-1(3H),4'-piperidine (Lu 29-179), one of the more selective σ_2 antagonists, interacts with dopamine D_2 and α-adrenergic receptors (50), while (±)-tropanyl 2-(4-

chlorophenoxy)butanoate [(±)-SM-21] interacts with dopamine transporters (unpublished data). Therefore, to study σ_2 receptor function, nondiscriminating σ ligands such DTG have been used in systems that are enriched in the σ_2 subtype; alternatively, nonselective σ_2 compounds have been used in systems that are enriched in σ receptors. The development of truly selective experimental tools with which to manipulate σ_2 receptors will greatly enhance understanding of their function.

In addition to σ_1 and σ_2 receptors, numerous papers have cited evidence in support of additional subtypes (e.g. 29,51-53). However, these putative subtypes have not yet been well characterized and will therefore not be described here in detail.

In addition to subtypes of σ receptors, there is evidence for splice variants. Thus far, only the σ_1 subtype has been sequenced. Therefore, information about splice variants is currently limited to this subtype. There are at least two truncated versions of the σ_1 receptor (54,55), and the extent to which these splice variants affect physiological functions are as yet unknown. However, studies examining σ_1 receptor polymorphisms in disease states have begun (56-61). The results have been mixed, but available nascent data support the possibility that these polymorphisms have functional consequences (58).

3. ENDOGENOUS LIGAND(S)

The conclusive identification of an endogenous ligand for σ receptors has yet to be achieved. The following sections summarize data supporting the existence of an endogenous ligand for these receptors, and raise the possibility of multiple such compounds. This would be consistent with the structural diversity of synthetic ligands that are known to interact with σ receptors.

3.1 Evidence from binding studies

Receptor binding studies to identify known endogenous ligands with significant affinity for σ receptors have been employed by a number of investigators. Although the vast majority of known endogenous compounds exhibit low to negligible affinities for σ receptors (see Appendix B), some activity has been reported. These possible candidates are described below in further detail.

Su and coworkers were the first to suggest that some neurosteroids serve as endogenous ligands for σ receptors. In particular, progesterone was shown to exhibit nanomolar affinity for σ receptors in guinea pig brain and spleen (62). The interaction of progesterone with brain σ receptors was competitive in nature (increase in K_d, but not B_{max} of [^{3}H](+)-SKF-10,047 binding), suggesting that progesterone binds to the same portion of the receptor as classical σ ligands (62). To further confirm that progesterone interacts with σ receptors, competition binding studies using [^{3}H]progesterone revealed a drug selectivity pattern that was consistent with σ receptors (63,64). The ability of progesterone to bind to σ receptors was subsequently confirmed in a number of laboratories (21,54,65-67). Other neurosteroids with micromolar affinities for σ receptors have also been reported (21,62,64,67), but it is unclear whether all of these steroids produce physiological actions through σ receptors. The limited functional studies that are available nevertheless indicate that some of them act as agonists at σ receptors (e.g. pregnenolone), while others act as antagonists (e.g. progesterone) (68).

Neuropeptide Y has been reported to have significant affinity for σ receptors (69). However, subsequent efforts to confirm this interaction have been unsuccessful (see Appendix B). It therefore does not appear that neuropeptide Y is an endogenous ligand for σ receptors.

A number of investigators have shown that divalent cations significantly inhibit radioligand binding to σ receptors. These divalent cations include magnesium, calcium, manganese, zinc, cadmium, copper (13,70,71). Some of these cations appear to preferentially target the σ_1 subtype while others target the σ_2 subtype. The effects of zinc on σ_2 receptors is particularly noteworthy because binding studies were also performed under physiological conditions in which zinc was released from hippocampal slices by depolarization and shown to displace [^{3}H]DTG, but not [^{3}H](+)-pentazocine binding in the slice, suggesting that it may be an endogenous ligand for σ_2 receptors (71). Further studies are needed to fully evaluate the conditions under which these candidates may serve as endogenous ligands for σ receptors.

3.2 Evidence from fractionation studies

A classical strategy for identifying an endogenous ligand is to extract it from the tissues in which it acts. Su and colleagues were among the first to report putative endogenous ligands for σ receptors, which they collectively named sigmaphins (72). These sigmaphins were isolated from guinea pig brain extracts and the partially purified fractions were shown to displace

binding to σ receptors in a competitive manner (72). Since there was a loss of binding after trypsin digestion, the compounds were thought to be peptides (72). However, to date, the active compounds have not been fully purified and identified.

Soon afterward, O'Donoghue and his colleagues also reported a putative endogenous ligand for σ receptors, which they isolated from extracts of porcine brain (73). The active material was also believed to be a peptide because pronase, a general proteolytic enzyme, could abolish its binding (73). In addition, its absorbance spectrum suggested that it contained phenylalanine residues (73). Additional studies to further purify and characterize the material have not been reported.

The high densities of σ receptors in the liver provided the impetus to search for an endogenous σ ligand in this tissue. A substance was extracted from porcine liver that binds to σ receptors (74). In contrast to the brain-derived compounds, this substance did not appear to be a peptide since it was resistant to pronase digestion (74). In addition, the liver-derived substance was thermostable, soluble in both water and organic solvents, and had a molecular weight of less than 1000 Da (74). However, full purification was not achieved.

In summary, fractionation studies demonstrated the existence of multiple endogenous extracts that bind to σ receptors, although none of them have been fully purified. Since these earlier efforts, there have been significant advances in the development of selective tools to label σ receptors and improved nuclear magnetic resonance and mass spectroscopy technologies to facilitate renewed efforts to discover endogenous σ ligands.

3.3 Evidence from physiological studies

In an elegant series of studies, Chavkin and coworkers demonstrated the release of endogenous ligands with σ-binding properties from hippocampal slices under physiologically relevant conditions. In these studies, fresh hippocampal slices were preloaded with a radioligand to occupy σ receptors. When the brain sections were depolarized using potassium chloride or veratridine, the radioligand that was bound to σ receptors was displaced in a time- and calcium-dependent manner, suggesting that depolarization caused the release of endogenous σ ligands (75). Electrical stimulation of the perforant path and/or mossy fibers, but not other tested regions, of the hippocampus produced similar effects (76), indicating that endogenous σ ligands could be released from specific circuits. Together, the data indicate the existence of σ-binding substances in the brain that can be liberated under conditions that cause neurotransmitter release.

Table 1-3. Representative imaging studies of σ receptors in the nervous system

Species	Tissue	Probe	Reference
Guinea pig	Brain	[^{3}H](+)-3PPP	16, 78, 105
	Brain	[^{3}H]DTG	18, 79, 139
	Brain	[^{3}H]Dextromethorphan	123
	Brain	[^{3}H](+)-Pentazocine (σ_1)	136, 131
	Brain	[^{3}H]DuP 734	126
	Brain	[^{3}H]NE-100 (σ_1)	132
	Brain	In situ (σ_1)	37
	Spinal cord	[^{3}H](+)-3PPP	78
Rat	Brain	[^{3}H](+)-3PPP	78
	Brain	[^{3}H](+)-SKF-10,047	133
	Brain	[^{3}H]DTG	18
	Brain	[^{3}H]DTG (σ_2)	77
	Brain	[^{3}H]Lu 28-179 (σ_2)	134
	Brain	[^{3}H](+)-Pentazocine (σ_1)	77
	Brain	[^{3}H]Nemonapride	135
	Brain	Antibody (143-162) (σ_1)	34
	Brain	Antibody (138-157) (σ_1)	43
	Brain	[^{11}C]SA6298	130
	Brain	[^{11}C]SA4503	94
	Brain	[^{18}F]fluoroethyl SA4503	124
	Pineal gland	[^{3}H]DTG	127
	Spinal cord	[^{3}H](+)-3PPP	78, 90
Mouse	Brain	[^{3}H](+)-SKF-10,047	133
	Brain	[^{3}H](+)-Pentazocine (σ_1)	131
	Brain	[^{11}C]SA6298	130
	Brain	[^{18}F]fluoroethyl SA4503	124
	Brain	In situ (σ_1)	37
Cat	Substantia nigra	[^{3}H]DTG	125
	Brain	[^{11}C]SA6298	130
Primate	Brain	[^{3}H](+)-3PPP	87
	Brain	[^{18}F]fluoroethyl SA4503	124
Human	Brain	[^{3}H]DTG	128
	Brain	[^{3}H]Lu 28-179 (σ_2)	134
	Cerebellum	[^{3}H]DTG	88
	Hippocampus	[^{3}H]DTG	129

4. ANATOMICAL DISTRIBUTION

σ Receptors are present throughout the body and knowledge about their localization can provide clues about their physiological functions. This section summarizes the distribution of σ receptors and their possible implications.

4.1 Nervous system

Following the first reports of σ receptors in the brain, numerous research groups have mapped their distribution in the central nervous system (Table 1-3). σ Receptors are found in the brain and spinal cord, where they subserve a variety of physiological functions.

The highest concentrations of σ receptors in the brain are found in brainstem motor nuclei. Cranial nerves such as the facial, hypoglossal, and trigeminal contain particularly high levels of σ receptors (77-79). Other constituents of brainstem motor circuits including the cerebellum, red nucleus, and inferior olive are also enriched in σ receptors (77-79). This pattern of distribution provided compelling evidence for a role for σ receptors in motor function, which was confirmed in early functional studies (80,81). The basal ganglia also contain moderate levels of σ receptors (77-79). Lesion studies showed that σ receptors are localized on substantia nigra pars compact neurons (78), and this distribution is consistent with the ability of σ receptor agonists to stimulate motor behavior via nigrostriatal dopaminergic pathways (82-85). Consistent with the enrichment of σ_2 receptors in the substantia nigra (77), these receptors were the first subtype to be implicated in motor function (85). Over time, accumulated data from anatomical and functional studies have supported the involvement of both σ_1 and σ_2 subtypes in motor function (77,84-86).

Significant levels of σ receptors are also found in limbic regions of the brain. The localization of σ receptors in the dentate gyrus and pyramidal cell layer of the hippocampus (77-79) are supportive of their role in learning and memory which are described in additional detail in Chapters 9 and 12. Moreover, the hippocampus, and particularly the dentate gyrus, is enriched in the σ_1 subtype (77), which has been implicated in the modulation of cognitive behaviors (Chapter 12). The presence of σ receptors in the olfactory bulb and other limbic and paralimbic areas such as the frontal cortex, cingulate, hippocampus, and amygdala further suggests that they may modulate affective states (79,87). This is consistent with their apparent role in depression and other mood disorders, which are described in further detail in Chapter 14.

Neuroendocrine areas are also enriched in σ receptors. The supraoptic and paraventricular areas of the hypothalamus, which send projections to the pituitary, contain significant densities of σ receptors, as does the adenohypophysis (78,79,88). Other hypothalamic areas also contain significant concentrations of σ receptors (79), and this region of the brain is particularly enriched in the σ_1 subtype (77). Although the anatomical distribution of σ receptors is highly suggestive of a role for σ receptors in the release of hormones from the pituitary, systematic functional studies to address this question have not been conducted. However, the ability of SKF-10,047 to raise plasma corticosterone levels in a naloxone-independent manner (89) supports this possibility. The additional presence of σ receptors in endocrine organs (see below), further indicates that this may be a fertile area for future research.

In contrast to the negligible levels of σ receptors in most sensory regions of the brain, several regions of the visual system contain significant densities of σ receptors (79). These regions include the lateral geniculate and superior colliculus (79). Together with recent reports of σ receptors in the eye (see below), additional studies to further examine the role of σ receptors in visual function are also needed.

The gray matter of the spinal cord contains extremely high densities of σ receptors (78). The motor subserving ventral horn of the spinal cord is especially enriched in these receptors (78,90), which is consistent with a role for these receptors in motor control. In addition, a sensory role for these receptors is suggested by their expression in dorsal root ganglion (78,90). Since the central gray in the midbrain also contains high densities of σ receptors (78,79), it is conceivable that σ receptors modulate sensory pain transmission. The role of σ receptors in pain is described in further detail in Chapter 16.

4.2 Peripheral organs

In addition to their presence in the nervous system, σ receptors are found in a variety of peripheral organs. The early evidence for the existence of σ receptors in the periphery came from binding studies in tissue homogenates, which were sometimes followed by autoradiographic studies to determine discrete localization in tissue slices. More recently, evidence for the existence of σ receptors in peripheral organs has also come from imaging studies in live organisms and Northern blot analysis against transcripts for the σ_1 subtype. The widespread distribution of σ receptors in the body suggests that they perform an essential physiological function.

The heart contains significant levels of σ receptors. Homogenate binding studies indicate that over 80% of the σ receptors in the heart are of the σ_1 subtype (28). These receptors are present on both the parasympathetic neurons that innervate the heart and the cardiac myocytes themselves (28,91-93). In myocytes, σ receptors influence contractility, calcium influx, and beating rate (28,93). In intracardiac neurons, the σ_1 and σ_2 subtypes affect neuronal excitability by modulating calcium and potassium channels, respectively (91,92). The overall effects of these influences on physiological parameters of cardiovascular function are still unclear.

There are several reports of σ_1 receptors in the lung (94-97). It is unclear whether σ_2 receptors are also present. The role of σ receptors in the lung has thus far been unexplored.

The highest levels of σ receptors in the body have been reported in the liver. Both the σ_1 and σ_2 subtypes are present (24,48,97). However, the function of σ receptors in the liver is currently unknown. Early studies hypothesized that they might have a cytochrome P450-like role, but this was not supported by experimental data (98-100).

The kidney contains both σ_1 and σ_2 receptors (24,48,94,97). The function of σ receptors in the kidney has yet to be determined.

Reproductive organs such as the testis, ovaries, vas deferens, and placenta contain σ receptors (67,101-103). The specific subtypes that are present within these tissues are unclear because the studies were performed under conditions where both σ_1 and σ_2 receptors were labeled (103). Autoradiographic studies to localize receptor distribution were performed in some of these tissues. In the testis, σ binding was highest in the ductuli efferentes and ductus epididymis, with lower levels of binding in the seminiferous tubules (103). In the ovaries, the highest densities of σ receptors were present in maturing follicles (103).

The adrenal gland contains σ receptors. The presence of the σ_1 subtype has been confirmed (103); the extent to which σ_2 receptors are expressed is unclear. Although the function of σ receptors in the adrenal gland has not been studied systematically, they may have a role in the modulation of neurosecretory processes (104).

Similar to the heart, the spleen is enriched in σ_1 receptors (29,94,105). Autoradiographic studies revealed that σ_1 receptors are most densely concentration in T cell zones (29). Together with the presence of σ receptors on immune cells (see below) and the ability of steroids to bind to these receptors (62), the data are supportive of a role for these receptors in immune function (see Chapter 17 for additional details).

The gastrointestinal tract contains significant levels of σ receptors, of both σ_1 and σ_2 subtypes (106). Within the gastrointestinal tract, autoradiographic studies revealed high concentrations of σ receptors in the

mucosa and submucosal plexus (107,108). Labeling was especially dense at the level of the fundus and duodenum (108). The functional relevance of σ receptors in the gastrointestinal tract is described in detail in Chapter 18.

σ_1 Receptors have recently been reported in the eye (109,110). They are found in the iris-ciliary body and retina, including the projecting terminals of the retina to the superior colliculus (110). Specific cell types that contain σ_1 receptor mRNA and protein include: retinal ganglion cells, photoreceptors, and retinal pigment epithelial cells (109). In addition, they are associated with cells in the inner nuclear layer (109). Investigations into the physiological and therapeutic significance of σ receptors on visual function have only just begun. Data thus far indicate that σ receptor agonists can reduce ocular pressure and protect against retinal cell death (111-113).

4.3 Cell types

σ Receptors are found in a variety of cell types that are not components of organs. Naturally-occurring cells such as blood cells and tumor cells contain significant levels of σ receptors. Blood cells that express σ receptors include: peripheral blood leukocytes, granulocytes, lymphocytes, natural killer cells (52,114,115). The functional role of σ receptors on these cells and their implications for treating a variety of immune disorders are described in detail in Chapter 17. Tumor cells also contain high densities of σ receptors, and recent studies report that they are expressed in especially high densities in proliferating tumors (116-120). The implications of σ receptors in tumors are discussed further in Chapters 11 and 17.

In addition to their expression in cells *in situ*, σ receptors have been reported in many different cell lines. These cell types and the subtype(s) of σ receptor that they express are summarized in Appendix C. Many of these cell types have been valuable experimental tools for delineating σ receptor function, and were used in the studies described in subsequent chapters of this book.

5. SUMMARY

The early history of σ receptors is characterized by classical pharmacological approaches which succeeded in defining a unique drug selectivity pattern and anatomical distribution for these proteins. σ Receptors are widely distributed in the body, where they mediate a variety of physiological functions. The chemical diversity of compounds that interact

with σ receptors is vast and includes therapeutically relevant entities including psychotomimetic opiates, neuroleptics, antihistamines, and antidepressants. The recent revolution in molecular biology has provided additional information about σ receptors, including the sequence of one of its major subtypes and a host of experimental tools to aid in selectively deciphering its functions. We now know that σ receptors have important implications for a number of disease states and mounting evidence indicates that they are viable therapeutic targets for medication development. The remaining chapters in this book summarize our current knowledge regarding the medicinal chemistry, cell biological and clinical implications of σ receptors. It is hoped that this information will lay the groundwork for innovative future studies to stimulate new insights into the physiological and therapeutic relevance of σ receptors.

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