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Sigma Receptors: Biology and Function*

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I. Pharmacology of Sigma-binding Sites

A. Historical Perspective

Sigma receptors were postulated in 1976 by Martin et al. (1976) to account for the actions of (+/−)-SKF 10,047 (N-allyl-normetazocine) and related racemic benzomorphans. These compounds produce a spectrum of behaviors in the dog referred to as canine delirium and have psychotomimetic effects in humans (Haertzen, 1970; Keats and Telford, 1964). Great interest in the hypothesis of Martin et al. concerning sigma receptors led to intense scrutiny of (+/−)-SKF 10,047. Ten years of further research revealed that (+/−)-SKF 10,047 binds to three types of receptors: (−)-SKF 10,047 binds primarily to mu and kappa opiate receptors (Mangan et al., 1982); (+)-SKF 10,047 binds to PCP‡ receptors (Zukin et al., 1986; Zukin, 1982) and to a unique site that retains the designation sigma receptor (cf. Quirion et al., 1987). Sigma receptors have also been called haloperidol-sensitive sigma receptors, etorphine-inaccessible sigma receptors, and naloxone-inaccessible sigma receptors

‡ Abbreviations: PCP, phencyclidine; SAR, structure-activity; OHBQ, octahydro[*f*]benzoquinoline; DTG, 1,3-di-*o*-tolylguanidine; R_1, R_2, receptor points; UV, ultraviolet; DM, dextromethorphan; PC12 cells, pheochromocytoma cells; Az-DTG, azido-DTG; DIGIT, 1-(2-methyl-4-isothiocyanatophenyl)-3-(2-methyl-phenyl)guanidine; Metaphit, 1-[1-(3-isothiocyanatophenyl)cyclohexyl]piperidine; G protein, guanine nucleotide-binding protein; Gpp(NH)p, nonhydrolyzable guanosine triphosphate analog; PPI, phosphoinositide; NPY, neuropeptide Y; NMDA, N-methyl-D-aspartic acid; PAG, periaqueductal gray; 3-PPP, 3-(3-hydroxyphenyl)-N-(1-propyl)piperidine; 5HT, 5-hydroxytryptamine; TCP, 1-[1-(2-thienyl)cyclohexyl]piperidine; BD614, (+/−)-*cis*-N-methyl-N-[2-(3,4-dichlorophenyl)ethyl]-2-(1-pyrrolidinyl)cyclohexylamine; ChTG, N-(*o*-tolyl)-N′-cyclohexyl guanidine; AdTG, N-(*o*-tolyl)-N′-(adamant-1-yl)guanidine; DPG, N,N′-di-(phenyl)guanidine; AdIpG, N-(2-iodophenyl)-N′(adamant-1-yl)guanidine; DAG, N,N′(di-(adamant-1-yl))guanidine.

(McLean and Weber, 1988; Su, 1982; Tam, 1983; Walker et al., 1988).

B. Properties of Sigma Receptors

1. A non-opiate pharmacology. Sigma receptors were originally thought to be a type of opiate receptor, but two subsequent findings convincingly demonstrate that it is not: (*a*) whereas opiate receptors are enantioselective for the (−)-isomers of opium-derived narcotics, narcotic antagonists, and their congeners, sigma receptors are enantioselective for the (+)-isomers and (*b*) naloxone is ineffective against both the in vivo and the in vitro effects of sigma ligands (Brady et al., 1982; Iwamoto, 1981; Katz et al., 1985; Slifer and Balster, 1983; Vaupel, 1983). Therefore, it is clear the sigma receptor is not a type of opiate receptor.

2. Differences between sigma and phencyclidine receptors. In earlier papers investigators asserted that sigma receptors were identical with PCP receptors based on the displacement of [^{3}H]PCP binding by the prototypic sigma ligand (+)-SKF 10,047 (Mendelsohn et al., 1985; Zukin et al., 1984). For this reason, sigma receptors were sometimes called "sigma opiate/PCP receptors" (Zukin et al., 1984). However, the drug selectivity pattern of [^{3}H](+)-SKF 10,047 differs from that of [^{3}H]PCP showing that these substances bind to different receptors. For example, antipsychotic drugs (such as haloperidol) potently displace [^{3}H](+)-SKF 10,047 binding, but they are weak or inactive against [^{3}H]PCP binding (Tam 1983; Tam and Cook, 1984). Conversely, PCP is weak against [^{3}H]haloperidol binding (Tam and Cook, 1984; Downs et al., 1986; Itzhak, 1987).

Sigma and PCP receptors may also be differentiated by their distinct anatomical distributions, because [^{3}H](+)-SKF 10,047 and [^{3}H]PCP-binding sites are concentrated in different brain areas (Gundlach et al., 1985; Largent et al., 1984; McLean and Weber 1988; Sircar et al., 1986; Gundlach et al., 1986). Tam (1985) pointed out other differences between [^{3}H]PCP binding and [^{3}H](+)-SKF 10,047 binding: the sensitivity of [^{3}H]PCP binding to sodium ions and the low affinity and small stereoselectivity shown by PCP receptors toward (+)-SKF10,047 and (+)-ethylketocyclazocine. It is clear from these findings that [^{3}H](+)-SKF 10,047 binds to two distinct sites: a haloperidol-sensitive site (subsequently called the sigma receptor) and a PCP-sensitive site (subsequently called the PCP receptor; Quirion et al., 1987).

3. Potent binding of antipsychotic drugs to sigma receptors. Radioligand-binding studies reveal that many antipsychotic drugs bind to sigma receptors with high affinity. Haloperidol is among the most potent inhibitors of [^{3}H](+)-SKF 10,047 binding, with a K_i of 4 nM (Tam and Cook 1984; Itzhak, 1988). Other antipsychotic drugs that possess moderate (K_i < 1000 nM) to high potency include perphenazine, (−)-butaclamol, acetophenazine, trifluoperazine, molindone, pimozide, thioridazine, and chlorpromazine (Tam and Cook 1984). The connection between sigma receptors and antipsychotic drugs was further strengthened by the finding that [^{3}H]haloperidol binding is strongly reduced by the sigma ligands (+)-SKF10,047, (+)-pentazocine, and (+)-cyclazocine (Tam and Cook, 1984). In fact, the sigma ligand (+)-pentazocine displaces [^{3}H]haloperidol from its binding sites in guinea pig brain about 10 times more potently than the dopamine ligand spiperone (Tam and Cook, 1984).

At first glance, these findings could lead to the conclusion that [^{3}H](+)-SKF 10,047 labels dopamine receptors. However, this cannot be the case because the K_i values of dopamine and apomorphine for [^{3}H](+)-SKF 10,047 are >10,000 nM, and [^{3}H](+)-SKF 10,047-binding sites only sparsely populate dopamine-rich areas such as the basal ganglia (Gundlach et al., 1986; McLean and Weber, 1988; Sircar et al., 1986).

C. Structure-Activity Relationships for Sigma Receptor Binding

As shown in table 1, many compounds have been tested for their ability to displace sigma radioligands. However, until recently, few systematic SAR studies of sigma receptor activity have been conducted. Largent et al. (1987) examined a large series of compounds to find structural determinants for sigma receptor affinity. In two SAR studies (Wikstrom et al., 1987; Van de Waterbeemd et al., 1987), a series of OHBQ and 3-phenylpiperidines was analyzed at the sigma site. Manallack and coworkers (Manallack et al., 1988; Manallack and Beart, 1987) examined structurally unrelated sigma ligands and described distinct receptor models for both PCP and sigma-like drugs. These studies revealed a wide degree of tolerance for both stereochemical and topographical demands for the sigma receptor.

Several classes of compounds bind to sigma receptors (fig. 1; table 1). These classes include: (*a*) the sigma "opiates," including benzomorphans, such as pentazocine (fig. 1, *1*: R = 3,3-dimethylallyl), and morphinans such as dextrallorphan (fig. 1, *6*: R_1 = H, R_2-allyl); (*b*) analogs of (+)- and (−)-*cis*-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide (fig. 1, *8*; such as the *cis* isomers of U50,488, R_1 = R_2 = Cl); (*c*) arylcyclohexylamines, including PCP (fig. 1, *2*: R_1 = H, R_2, R_3 = pentamethylene); (*d*) N,N′-diaryl-substituted guanidines such as DTG (fig. 1, *7*: R_1 = R_2 = 2-tolyl); (*e*) phenylpiperidines, such as (+)-1-propyl-3-(3-hydroxyphenyl)piperidine [(+)-3-PPP] (fig. 1, *3*: R_1 = *n*-propyl, R_2 = 3-OH;) and OHBQs (fig. 1, *4*: R_1 = R_2 = H); (*f*) steroids, including progesterone (fig. 1, *10*); (*g*) butyrophenones, including the neuroleptic haloperidol (fig. 1, *5*: R_1 = Cl, R_2 = F); and (*h*) (+)- and (−)-*cis*-N-[2-(3,4-dichlorophenyl)ethyl]-N-methyl-2-(1-pyrrolidinyl) cyclohexylamine [fig. 1, (+)- and (−)-9, R = Me] and congeners.

Initially, we will focus on the SAR relationships that occur within each class and then examine common features that appear within unrelated classes of sigma li-

TABLE 1

*Sigma-binding affinities of various drugs**

	[^{3}H](+)-SKF 10,047		[^{3}H]-(+/−)-EKC	[^{3}H]-PENT	[^{3}H]-DTG		[^{3}H]-3-PPP	
	Guinea pig	Rat	Guinea pig	Guinea pig	Guinea pig	Rat	Guinea pig	Rat
Opiates								
(+/−)-Pentazocine	18†		23‖		69¶	992†††		23#
(+)-Pentazocine				3.2***	43¶		8¶	
(−)-Pentazocine				85.8***	135¶		81¶	29#
(+/−)-Cyclazocine	36†		67‖	26.4**				96#
(+)-Cyclazocine		350††			365¶		47¶	111#
								280††
(−)-Cyclazocine		430††			2,600¶		1,000¶	408#
								1,500††
(+/−)-SKF 10,047								373#
(+)-SKF 10,047	48†	50††	32‖	61.2***	625¶	867†††	93¶	340#
								130††
(−)-SKF 10,047	1,800‡	1,100††	1,000‖	>3,000**	4,000¶		2,850#	1,350#
								1,500††
Dextrallorphan	14**						18**	
3-Methoxydextrallorphan	14**						36**	
(+)-Ethylketocyclazocine	55‡		42‖				36**	
Dextrorphan		400‡‡				916†††		3,400‡‡
Sufentanil	94‡							
Dextrallorphan	13.2**			13.5**	96.2**			154#
Levallorphan				>3,000**				1,780#
(+/−)-Bremazocine	195‡		190‖					
U50,488	696‡		520‖		1,350¶			
Dextromethorphan	245**							768#
N-nordextromethorphan	1,169**						506**	
(+)-Thebaine	593**						1,322**	
Butorphanol	2,300‡							
(+/−)-Tifluadom	6,600‡							
(−)-Ethylketocyclazocine	19,000‡		14,000‖					
Nalorphine	80,000‡							
Nalbuphine	>100,000‡							
(+)-Dihydrocodeinone	>10,000**							
(+)-14-OH-codeinone	>10,000**							
(+)-Nordihydrocodeinone	>10,000**					>10,000†††		
Dynorphin-(1-9)	>10,000‡							
Dynorphin-(1-13)	>10,000‡							
Dynorphin-(1-17)					>10,000¶			
[Leu^5]-Enkephalin					>10,000¶			
β-Endorphin				>6,000**	>10,000¶			
Levorphanol								>10,000#
(−)-Nalorphine			13,000‖					
DADLE	>10,000‡			>6,000**				>10,000#
α-,β-Neo-Endorphin								>10,000#
Morphine	>100,000‡				>10,000¶			
Oxymorphone	100,000‡							
(+/−)-Naloxone			>100,000‖		>10,000¶			>10,000#
(−)-Naloxone	>100,000†							
(+)-Naloxone	>100,000†							
Dihydromorphine								>10,000#
Metorphamide					>10,000¶			
DAGO				>6,000**				
Antipsychotics								
Haloperodol	4†		87††		5¶	3.1	17¶	2.4#
	10.4**			3.3***	18.9**		3.8**	
Perphenazine	12†				42¶		21¶	24#
Fluphenazine	17†			25.7**				62#
(−)-Butaclamol	38†	250††			530¶		183¶	157#
	96**			83.4*	378**			63††
Acetophenazine	54†							
Trifluoperazine	67†				345¶			
Molindone	124†							
Pimozide	144†							508#
Thioridazine	174†		235‖					
Chlorpromazine	180†				1,475¶			
Triflupromazine	214†				605¶			
Spiperone	1,090†			>3,000**	690¶			632#
(+)-Butaclamol	1,300†		1,400‖	2,334**	2,150¶		2,100¶	1,800#
	2,119**				2,124**			

TABLE 1—Continued

	[^{3}H](+)-SKF 10,047		[^{3}H]-(+/−)-EKC	[^{3}H]-PENT	[^{3}H]-DTG		[^{3}H]-3-PPP	
	Guinea pig	Rat	Guinea pig	Guinea pig	Guinea pig	Rat	Guinea pig	Rat
Thiothixene	1,400†							
Loxapine	1,600†							
Clozapine	11,400†		18,000‖					
Sulpiride	>10,000¶¶							
Potential antipsychotics								
Rimcazole (BW 234U)	450§§				1,400¶¶			1,460‖‖
BMY 14802					120¶¶			74‖‖
BMY 13980								60‖‖
Remoxipride								60‖‖
Tiospirone					233¶¶			60‖‖
Cinuperone (HR 375)								130‖‖
WY47384								80‖‖
Antidepressants								
Amitriptyline					300¶			
Imipramine					520¶			
Desipramine					4,000¶			
Nortryptyline					2,000¶			
Ipindole								>10,000#
Antihistamines								
Pyrilamine			110‖					
Promethazine			190‖					
Chlorpheniramine			560‖					
Cimetidine					>10,000¶			
PCP-related								
PCP	560‡‡		2,400‖		1,050¶		1,000¶	711#
	512**			1,813***	2,632**		1,014**	2,900‡‡
MK-801	>10,000¶¶	860‡‡		>6,000***				
TCP	1,100¶¶	240‡‡						1,200‡‡
TCM	5,300‡‡							>10,000‡‡
Dexoxadrol	3,700‡‡							1,880#
								29,000‡‡
Levoxadrol	4,100††							2,080#
								6,900‡‡
Steroids								
Progesterone	268§							
Testosterone	1,014§							
Desoxycorticosterone	938§							
11β-Hydroxyprogesterone	1,535§							
Pregnenolone sulfate	3,196§							
Corticosterone	4,074§							
Dopaminergics								
Dopamine	>100,000†			>6,000**	>10,000¶			>10,000#
Apomorphine	>31,000†			>6,000***	>10,000¶			>10,000#
Lisuride								>10,000#
(+)-Amphetamine	>10,000†							>10,000#
Cocaine					>10,000¶			>10,000#
Sulpiride	>100,000¶¶							
Adrenergics								
Propranolol			320‖					
Clonidine					>10,000¶			
Phentolamine								>10,000#
Serotinergics								
Minanserin			630‖					
5-Hydroxytryptamine				>6,000**	>10,000¶			
LSD								>10,000#
Cholinergics								
Atropine			>10,000‖	>6,000**				
Hexamethonium					>10,000¶			>10,000#
Decamethonium								>10,000#
Scopolamine					>10,000¶			>10,000#
Amino acids and related drugs								
γ-Aminobutyric acid				>6,000**	>10,000¶			>10,000#
Bicuculline					>10,000¶			>10,000#
Diazepam					>10,000¶			
Guanidines								
DTG	15.1**	89‡‡		28.4***	28¶	16.9†††	53¶	19‡‡
					21.7**		20.5**	
AdipG					6.2¶¶			
AdTG					7.6¶¶			

TABLE 1—Continued

	[^{3}H](+)-SKF 10,047		[^{3}H]-(+/−)-EKC	[^{3}H]-PENT	[^{3}H]-DTG		[^{3}H]-3-PPP	
	Guinea pig	Rat	Guinea pig	Guinea pig	Guinea pig	Rat	Guinea pig	Rat
DAG					11.8¶¶			
AdChG					12.5¶¶			
ChTG					13¶¶			
DChG					86¶¶			
DXG					90¶¶			
NH_2-DTG					280¶¶			
DPG					397¶¶			
Bridge-DPG					>100,000			
DMG					>100,000			
Guanabenz					4,600¶			
Peptides								
Angiotensin II								>10,000♯
Bradykinin								>10,000♯
Cholecystokinin -4, -8, -33								>10,000♯
								>10,000♯
Neurotensin								>10,000♯
Pancreatic polypeptide		118##						
Neuropeptide Y		9.82##						
Peptide YY		4.87##						
Channel blockers								
Tetrodotoxin								>10,000♯
Picrotoxin								>10,000♯
Other								
(+)-3-PPP				3.3***	76¶		33¶	30♯
					138**		17.9**	
(−)-3-PPP	110**			185**	280¶		235¶	155♯
					383**			
BD614						277†††	2‡‡‡	
Rauwolscine								>10,000♯
Cannabinol								>10,000♯
Δ^9-Tetrahydrocannabinol								>10,000♯

* Results are K_i values expressed in nM, brain. In some cases K_i values were derived from IC_{50} values and K_d of labeling ligand. Abbreviations: LSD, lysergic acid diethylamide; 8-OH-DPAT, 8-hydroxydipropylaminotetralin; 3-PPP, 3-(3-hydroxyphenyl)-N-(1-propyl)piperidine; PCP, phenycyclidine; DADLE, [D-Ala^2, D-Leu^5]enkephalin; DAGO, [D-Ala^2, MePhe^4, Gly-ol^5]enkephalin; DTG, N,N′-di(*o*-tolyl)guanidine; AdlpG, N-(2-iodophenyl)-N′(adamant-1-yl)guanidine; AdTG, N-(*o*-tolyl)-N′-(adamant-1-yl)guanidine; DAG, N,N′(di(adamant-1-yl)guanidine; AdChG, N-(cyclohexyl)-N′-(adamant-1-yl)guanidine; ChTG, N-(*o*-tolyl)-N′-(cyclohexyl)guanidine; DChG, N,N′-(dicyclohexyl)guanidine; DXG, N,N′-di(2,6-methylphenyl)guanidine; NH_2-DTG, N-(*o*-tolyl)-N′-(4-amino-2-methylphenyl)guanidine; DPG, N,N′-di(phenyl)guanidine; Bridge-DPG, 2-amino-1,3H-dibenzo[*d*,*f*]-[1,3]-diazepine; DMG, N,N′-di(methyl)guanidine; TCM, (1-(2-thienyl)cyclohexylmorpholine; TCP, 1-[1-(2-thienyl)cyclohexyl]piperidine; BD614, (+/−)-*cis*-N-methyl-N-[2-(3,4-dichlorophenyl)ethyl]-2-(1-pyrrolidinyl)cyclohexylamine.

† Tam and Cook (1984).
‡ Tam (1985).
§ Su et al. (1988).
‖ Tam (1983).
¶ Weber et al. (1986).
♯ Largent et al. (1984).
** W. D. Bowen, unpublished data.
†† Contreras et al. (1988a).
‡‡ Contreras et al. (1988b).
§§ Ferris et al. (1986).
‖‖ Largent et al. (1988).
¶¶ Campbell et al. (1989).
Roman et al. (1989).
*** de Costa et al. (1989).
††† Matsumoto et al. (1990).
‡‡‡ de Costa et al. (1990).

gands. A model for the sigma receptor derived from these observations will then be reviewed.

1. Opiate-related compounds. Examination of the structures in fig. 1 suggests that the pharmacophore common to most of the compounds is the 3- and 4-phenylpiperidine moiety. For example, examination of (+/−)-N-allylnormetazocine (SKF 10,047; fig. 1, *1*: R = allyl) and other sigma opioid benzomorphans (1) clearly reveals a 4-phenylpiperidine part structure. A detailed qualitative study of opioid compounds by Largent et al. (1987) revealed that the determinants for sigma receptor activity differ strikingly from the determinants for opiate

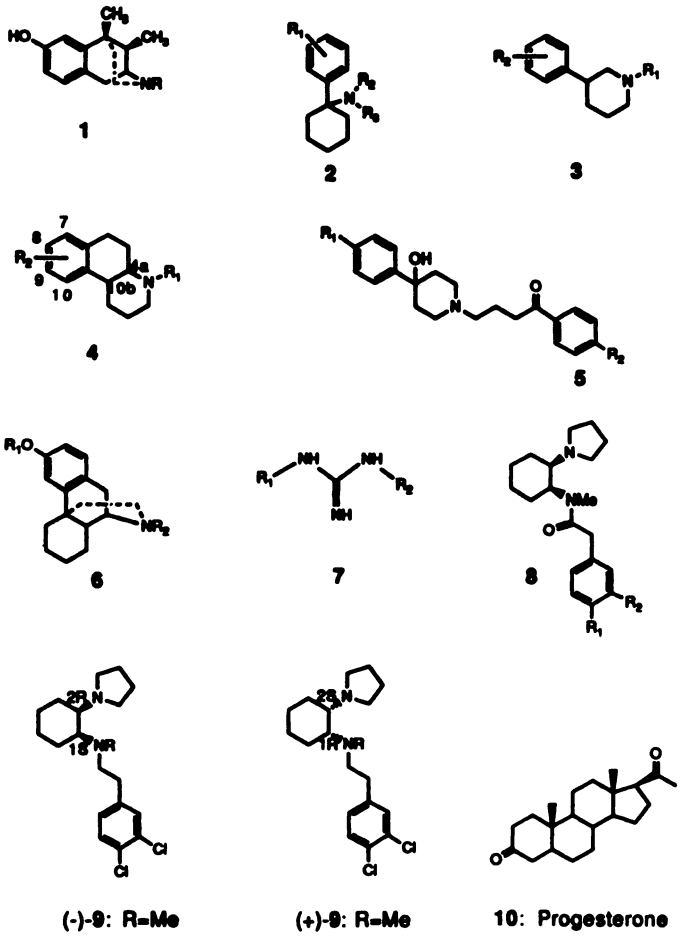


FIG. 1. Structures of selected sigma ligands (see text).

receptors. Intact ringed opiate-related compounds, such as morphine and naloxone, have negligible affinity for sigma receptors. The highest affinity among opioids occurs with the ring C opened analogs lacking a 4,5-epoxide ring, or benzomorphans (fig. 1, *1*). Also, the morphinans (fig. 1, *6*), which lack C ring substituents and 4,5-epoxide bridge, show high affinity for sigma receptors. With opiate-related compounds, the sigma receptor (at least the major subtype) displays reverse stereoselectivity to the classical opiate receptors. The (+)-benzomorphans (fig. 1, *1*) display highest affinity for sigma receptors followed closely by the more bulky (+)-morphinans (fig. 1, *6*).

Of greater importance than relative bulk of the molecule is relative lipophilicity of certain regions of the molecule. Removal of the epoxy bridge, as well as 3 hydroxy and 6-keto groups of naloxone (IC_{50} vs. [^{3}H](+)-3-PPP > 100,000 nM), results in the greater potency of levallorphan (IC_{50} vs. [^{3}H](+)-3-PPP > 1890 nM). Furthermore, the more lipophilic N-allyl group of levallorphan may account for its greater potency, when compared with levorphanol (IC_{50} > 10,000 nM), which possesses an N-methyl group. Dextrallorphan (fig. 1, *6*: R_1 = H, R_2 = allyl), the "unnatural" or (+)-isomer of levallorphan, exhibits an 11-fold greater affinity for sigma receptors (IC_{50} = 153 nM) than levallorphan itself. However, dextrallorphan shows low but significant affinity for the PCP-binding site (K_i = 5680 nM). Addition of still larger N-alkyl side chains (such as 3,3-dimethylallyl and phenethyl) to dextrallorphan might be expected to result in even higher potency at sigma receptors and reduced potency at the PCP-binding site.

Replacement of the phenolic hydroxyl of dextrallorphan with an O-methyl (fig. 1, *6*: R_1 = Me, R_2 = allyl) either fails to affect affinity at sigma receptors, indicating that any hydrogen bonding at this site in the molecule is not as important for sigma receptor affinity as it is for opiate receptor binding in the (−)- or natural series (unpublished observation). Among the (+)-benzomorphans (fig. 1, *1*), replacement of the N-allyl group of (+)-SKF 10,047 with the more bulky and lipophilic N-3,3-dimethylallyl group of (+)-pentazocine (fig. 1, *1*: R_1 = 3,3-dimethylallyl) results in a 53-fold increase in sigma receptor potency [IC_{50} = 70 nM for (+)-SKF10,047 versus IC_{50} = 1.3 nM for (+)-pentazocine determined in guinea pig brain versus [^{3}H](+)-3-PPP]. A similar potency difference is seen when racemic mixtures of pentazocine and SKF 10,047 are compared (Largent et al., 1987). Furthermore, unlike (+)-SKF 10,047, (+)-pentazocine fails to interact with PCP sites (Rothman et al., 1988). These large differences in comparable potency and selectivity of (+)-pentazocine compared with (+)-SKF 10,047 can only be accounted for by the increased size and lipophilicity of the side chain. Similarly, the more lipophilic pi-bonding N-phenethyl of (+/−)-phenazocine (fig. 1, *1*: R = phenethyl) accounts for its comparable potency to (+/−)-pentazocine at sigma receptors (Largent et al., 1987). In contrast, lipophilic keto groups, as seen in the 8-position in ketocyclazocine and ethylketocyclazocine, result in considerably lowered affinities (1% that of pentazocine). In light of the high affinity and selectivity of (+)-pentazocine for sigma receptors, de Costa et al. (1989b) synthesized [^{3}H](+)-pentazocine as a selective sigma receptor probe.

2. 2-[(1-Pyrrolidinyl)cyclohexyl]benzeneacetamides and 2-(1-pyrrolidinyl)cyclohexylamines. The recently identified sigma receptor activity in the *cis* diastereoisomers of U50,488 (fig. 1, *8*: R_1 = R_2 = Cl) was used in a large SAR study to identify still more potent and selective compounds (de Costa et al., 1989a; 1990). 1*R*,2*S*-(+)-U50,488 exhibited a K_i of 250 nM against [^{3}H](+)-3-PPP, whereas its enantiomer 1*S*,2*R*-(−)-U50,488 exhibited a K_i of 81 nM in guinea pig brain. Although the compounds failed to interact with kappa receptors labeled by [^{3}H]bremazocine and interacted only weakly with kappa receptors labeled by (−)-[^{3}H]U69,593, these diastereomers were selective for the sigma site to the extent that they also failed to interact with PCP or D_2-dopamine receptors, sites that commonly cross-react with sigma ligands (de Costa et al., 1989a).

The 3- and 4-chlorine atoms of the *cis* diastereomers

of U50,488 could be replaced with a naphthyl ring (fig. 1, *8*: R_1,R_2 = benzo) to give compounds with further enhanced potency and selectivity at the sigma receptor (1*S*,2*R*-(−)-enantiomer, K_i = 8.66 nM against [^{3}H](+)-3-PPP; 1*R*,2*S*-(+)-enantiomer, K_i = 1372 nM). Because the compound devoid of lipophilic groups in the 3- and 4-positions (fig. 1, *8*: R_1 = R_2 = H) showed a considerable decrease in potency (IC_{50} = 1155 nM) compared with the 3,4-dichloro- and naphthyl analogs, it appears that lipophilic groups in the 3- and 4-positions on the benzene ring occupy a lipophilic pocket that allows increased binding affinity.

The most dramatic increase in sigma receptor-binding affinity occurred when the amide function of the *cis* diastereomers of U50,488 was reduced to a tertiary amine to give diamines 1*R*,2*S*-(+)-9 and 1*S*,2*R*-(−)-9, both members of a new class of highly potent and selective sigma ligands (de Costa et al., 1990). 1*S*,2*R*-(−)-9 exhibited a K_i of 1.3 nM against [^{3}H](+)-3-PPP, representing a 60-fold increase in sigma receptor potency compared to its amide precursor [1*S*,2*R*-(−)-8: R_1 = R_2 = Cl]. Similarly, 1*R*,2*S*-(+)-9 (K_i=6 nM) exhibited a 40-fold increase in potency compared to its amide precursor. These enormous increases in potency for (+)- and (−)-9, when compared with their precursors, can be explained by additional hydrogen bonding from the added amine function. Compounds (+)- and (−)-9 failed to interact with any of the other receptor systems tested, making them the most potent and selective known sigma receptor ligands (de Costa et al., 1990; Bowen et al., 1990b; Matsumoto et al., 1990).

The SAR of compounds related to (+)- and (−)-9 have been extended by investigating the effect of changing the nitrogen substituent. Preliminary results suggest that some loss of affinity occurs by replacing methyl with ethyl, propyl, or cyclopropyl methyl (Radesca et al., 1990). The results of this study indicate that the nitrogen substituent for potent sigma receptor-binding affinity is the methyl group. This result appears to be in contrast to the trend observed with N-alkyl-substituted (+)-benzomorphans and (+)-morphinans.

A clear finding from the SAR studies described above is that sigma receptors and kappa receptors bind the same opiates. However, kappa receptors prefer one isomer, and sigma receptors prefer the other. Whereas kappa opiate receptors bind (−)-benzomorphans, sigma receptors bind (+)-benzomorphans. Examples are (−)-SKF 10,047, (−)-pentazocine, (−)-cyclazocine, and (−)-ethylketocyclazoine, which bind kappa opiate receptors; their (+)-enantiomers bind to sigma receptors. Another example is found with *cis* and *trans* isomers of U50,488. Whereas the *trans* isomers show preference for kappa opiate receptors, the *cis* isomers show preference for sigma receptors. Thus, in two chemically unrelated classes of compounds, different isomers show preference for kappa or sigma receptors. This suggests a possible relationship between the topography of the kappa opiate and sigma receptor-binding sites.

3. Phencyclidine-related compounds. Among the phenylcyclohexylamines (fig. 1, *2*), PCP (fig. 1, *2*: R_1 = H, R_2,R_3 = pentamethylene) exhibits greater affinity for PCP receptors than it does for sigma receptors [K_i = 1,014 nM versus [^{3}H](+)-3-PPP]. Unfortunately, no systematic studies of this compound have been completed at this time. However, recent data (unpublished observations) indicate that decreasing the lipophilicity of the compound by addition of a 3-hydroxy group (fig. 1, *2*: R_1 = 3-OH, R_2,R_3 = pentamethylene) results in loss of affinity for sigma receptors. Similarly, opening of the piperidinyl group as in PCA (fig. 1, *2*: R_1 = R_2 = R_3 = H) results in loss of affinity for both PCP and sigma receptors (K_i versus [^{3}H]DTG = 3133 nM). This is clearly an important area for further investigations because it would appear likely that potent sigma ligands could be developed from this series.

4. Guanidines. Weber et al. (1986) described the high affinity and selectivity of DTG for sigma sites in the brain. The rank order of potency of dopamine receptor agonists, neuroleptics, psychotomimetics, and benzomorphan opiates in displacing [^{3}H]DTG indicated a sigma-binding profile. In contrast, ligands for other receptors were very weak in displacing [^{3}H]DTG binding. Additional work by this group (Campbell et al., 1989) involving congeners of DTG (fig. 1, *7*: R_1 = R_2 = 2-tolyl) has revealed some interesting SAR data. The potency of DTG against displacement of [^{3}H]DTG was doubled (IC_{50} = 28.0 nM for DTG versus IC_{50} = 13.0 nM for ChTG) by replacing one of the aromatic rings of DTG with a cyclohexyl ring to give ChTG (fig. 1, *7*: R_1 = 2-tolyl R_2 = cyclohexyl). This result indicates that both aromatic rings are not necessary for high binding affinity. The fact that the potency is increased when the aromatic ring is replaced with a cyclohexyl ring provides evidence that a more lipophilic group that occupies a greater volume in three-dimensional space is more important for binding than an aromatic group. This is further exemplified by AdTG (fig. 1, *7*: R_1 = 2-tolyl, R_2 = adamantyl) which exhibited an IC_{50} of 7.6 nM against [^{3}H](+)-3-PPP or a 4-fold increase in potency compared with DTG. The ortho methyl groups of DTG are important for improved potency at the sigma receptor, because the desmethyl analog (DPG; fig. 1, *7*: R_1 = R_2 = phenyl) of DTG (IC_{50} = 397 nM) exhibits a 14-fold reduction in potency relative to DTG. The methyl group of AdTG can be replaced with an iodine atom to make AdIpG which exhibits equivalent potency, indicating that the iodine atom substitutes in terms of size and lipophilicity for the methyl group to give high sigma receptor affinity. An attempt to increase the potency of DTG further by adding extra equivalent (ortho) methyl groups to give the tetramethyl analog N,N′-di(2,6-dimethylphenyl)guanidine resulted instead in a reduction in potency (IC_{50} = 90 nM). An

explanation for this effect is likely to be steric interference to binding, by the additional methyl groups.

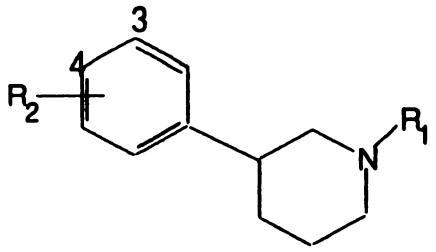
Replacement of both of the aromatic rings of DTG with methyl groups (as in N,N′-di(methyl)guanidine) (fig. 1, *7*: $R_1 = R_2 =$ Me) resulted in total loss of potency ($IC_{50} >$ 100,000 nM); this suggests that at least one aromatic ring is important for sigma receptor activity. However, replacement of both of the aromatic rings of DTG with more bulky and lipophilic adamantyl groups (fig. 1, *7*: $R_1 = R_2 =$ adamantyl) resulted in DAG. This compound showed an increase in affinity relative to DTG (DAG: $IC_{50} =$ 11.8 nM) which suggests that bulky and lipophilic groups are capable of completely substituting for the aryl rings. The minimum number of adamantyl groups required for high binding affinity is one, because the N-adamantyl-N′-cyclohexyl analog showed potency ($IC_{50} =$ 12.5 nM) equivalent to the bis adamantyl analog DAG ($IC_{50} =$ 11.8 nM). Because sigma receptor activity is totally lost with conformational restriction of both aromatic rings as in bridged N,N′-diphenylguanidine ($IC_{50} >$ 100,000 nM), conformational mobility of these aromatic rings is of utmost importance for sigma receptor binding.

5. 3-Phenylpiperidines. In a qualitative SAR study by Largent et al. (1987), a number of compounds which included opioids, neuroleptics, and phenylpiperidine dopaminergics were examined for sigma receptor affinity. In the 3-phenylpiperidine series (fig. 1, *3*), many of the compounds were studied as their individual enantiomers for their ability to compete with [^{3}H](+)-3-PPP in rat brain membranes. Generally, among the 3-PPP analogs (fig. 1, *3*), the *R* enantiomers were more potent than the *S* enantiomers in displacing [^{3}H](+)-3-PPP. Analogs with larger nitrogen substituents exhibited markedly higher affinities for the sigma receptor. Larger nitrogen substituents provide greater hydrophobicity in an analogous fashion to the (+)-benzomorphans (fig. 1, *1*) and (+)-morphinans (fig. 1, *6*). The increase in affinity with increasing size of the R_1 group is seen with both the *R* and the *S* enantiomers of (fig. 1, *3*) (table 2).

The trend for increasing potency of both *R* and *S* enantiomers with increasing size of R_1 is broken with R_1 = isopropyl (see compounds 9 and 10 in table 2), which may indicate differing sensitivities toward steric bulk for the two enantiomers. A 3-OH is better for sigma receptor affinity than a 4-OH. Furthermore, although the phenolic 3-OH is a requirement for the dopamine agonist effects of these compounds, it is not essential for sigma receptor activity, because analogs of (+)-3-PPP possessing 3-F and 3-CF_3 (fig. 1, *3*: R_2 = 3-F or 3-CF_3; compounds 16 and 17 in table 2) are potent sigma ligands. Additionally, the methyl ether derivative (fig. 1, *3*: R_1 = N-propyl, R_2 = OMe) of (+)-3-PPP is twice as potent as the parent compound, indicating that the 3-OH group of (+)-3-PPP is not important for hydrogen-bonding interactions. Of interest is the observation that the N-(1-propyl) analog of MPTP, which does not possess aromatic electron-donating or -withdrawing groups, is still a potent sigma receptor ligand; this suggests that the aromatic substituents may not be important in the 3-phenylpiperidines.

TABLE 2

*Potencies of compounds in a series of phenylpiperidines for inhibition of (+)-[^{3}H]3-PPP binding to rat whole brain membranes**



Compound no.	R_1	R_2	IC_{50} *R*-Enantiomer	IC_{50} *S*-Enantiomer	*S/R*
1, 2	H	3-OH	4570 ± 190	15,600 ± 2300	3.4
3, 4	Me	3-OH	373 ± 66	1910 ± 350	5.1
5, 6	Et	3-OH	111 ± 12	1030 ± 66	9.3
7, 8	*n*-Pr	3-OH	32 ± 2	165 ± 18	5.2
9, 10	*i*-Pr	3-OH	274 ± 9	208 ± 12	0.8
11, 12	*n*-Bu	3-OH	9 ± 0.8	31 ± 5	3.4
13, 14	Pheth	3-OH	8 ± 1.8	16 ± 3	2.0
15	*n*-Pr	4-OH	276 ± 5†		
16	*n*-Pr	3-CF_3	317 ± 2†		
17	*n*-Pr	3-F	49 ± 11†		

* The IC_{50} values are presented as the means (nM) ± SE. The R_1 group represents the nitrogen substituent of the piperidine, and the R_2 group denotes the substituent on the phenyl moiety for the given structure. Abbreviations of substitutions: H, hydrogen; Me, methyl; Et, ethyl; *n*-Pr, *n*-propyl; i-Pr, isopropyl; *n*-Bu, *n*-butyl; Pheth, phenethyl.

† Compounds 15-17 are racemic mixtures. Data from Largent et al. (1987).

Because the above studies indicated that the structural requirements for sigma receptor and dopamine receptor activity are divergent, new compounds with better selectivity for sigma receptors can be designed. For example, the *cis*OHBQs (fig. 1, *4*) with large nitrogen substituents have high sigma receptor affinity, compared to dopamine receptor affinity. In contrast, some of the *trans*OHBQs are very potent dopamine D_2 receptor agonists, while showing little or no affinity for sigma receptors. Furthermore, whereas sigma receptors are insensitive to increased steric bulk at the nitrogen substituent, dopamine receptor agonists have defined directions for their N-alkyl substituents. Because the nonhydroxylated 3-phenylpiperidines lose dopamine receptor affinity, but retain sigma receptor affinity, it was suggested (Largent et al., 1987) that certain of these nonhydroxylated compounds (for example, the fluorinated analog of 3-PPP; compound 17 in table 2) may serve as templates for the development of more selective sigma ligands. In consideration of the number of classes of compounds (e.g., phenylpiperidines, OHBQs, butyrophenones, and phenothiazines) which exhibit interactions with both sigma

and dopamine receptors, it is possible that these two receptors have an evolutionary or functional link.

Sigma receptor affinity (measured as the potency in displacing [^{3}H](+)-3-PPP) is sensitive to pH, a finding consistent with the importance of lipophilicity in the vicinity of the N-alkyl region of the piperidine ring of (+)-3-PPP (Largent et al., 1987). As one would expect from this, [^{3}H](+)-3-PPP binding is enhanced as the pH is increased in the range 7.0–8.9. The effect of pH on [^{3}H](+)-3-PPP binding may be mediated through changes in the charge on both the phenolic group and the nitrogen atom in 3-PPP (fig. 1, *3*: R_1 = N-propyl, R_2 = 3-OH). However, because substituting the 3-OH of (+)-3-PPP with an OMe group results in a doubling of the affinity to sigma sites, it is unlikely that ionization of the phenolic at increased pH values accounts for the changes in affinity. Thus, the increase in affinity at higher pH values is more likely to be be due to deprotonation of a piperidine ring.

6. Steroids. Su et al. (1988a) demonstrated that progesterone (fig. 1, *10*) binds with reasonable affinity (K_i = 268 nM, measured against [^{3}H]SKF 10,047) to guinea pig brain tissue. As shown in table 1, a limited sampling of related compounds revealed that testosterone and desoxycorticosterone bound with affinities of approximately 1 μM. These authors speculated that progesterone may act physiologically at the sigma receptors, serving as an endogenous ligand. However, Schwarz et al. (1989) challenged this idea, countering that it has only modest affinity for sigma receptors and circulates primarily in a bound form. Su et al. maintain that especially during pregnancy sufficient levels might be achieved for endogenous progesterone to achieve significant occupation of sigma sites. Furthermore, the concentrations assumed by Schwartz et al. may be underestimates if one considers the high degree of lipophilicity of progesterone. The assumption that progesterone which is bound to serum proteins is unavailable for receptor binding may also be questioned. Ke and Ramirez (1990) showed that [^{125}I] progesterone conjugated with bovine serum albumin still exhibits binding to membrane receptors. Further research is needed to establish whether sigma receptors may mediate certain central nervous system effects of progesterone.

7. Miscellaneous compounds. The piperazines BMY 14802 and rimcazole bind to sigma receptors and have been investigated for antipsychotic potential (Ferris et al., 1986; Taylor et al., 1990). One reason for the interest in these compounds is that they lack neuroleptic like pharmacological properties. They do not, for example, produce catalepsy in rats (cf. Taylor et al., 1990). However, firm conclusions regarding the function of sigma receptors cannot be inferred from the actions of these compounds. Rimcazole binds only weakly to sigma receptors, with potency estimates as low as in the micromolar range (table 1). Although BMY 14802 is considerably more potent than rimcazole at sigma receptors (K_i against [^{3}H]DTG = 32 nM, table 1), it also binds with moderate affinity to 5HT1a receptors (K_i against [^{3}H]8-hydroxydipropylaminotetralin = 151 nM; Taylor et al., 1990). As discussed in more detail below, these drugs are of considerable clinical interest but are not suitable for use as selective sigma ligands in basic research.

II. Molecular Models of Sigma Receptors

In recent years, several models of the sigma receptor have been proposed. These can be placed into five categories: (*a*) topographic models, (*b*) distinct allosterically coupled binding sites or domains on a single sigma receptor macromolecule, (*c*) multiple subtypes of sigma receptors differing in affinity for various sigma ligands, (*d*) species variations in the sigma receptor, and (*e*) sigma receptors that can exist in high and low affinity states.

A. Quantitative Considerations and Topographic Models

Several attempts have been made to formulate models of the sigma receptor that can explain the SAR data for various classes of sigma ligands. Largent et al. (1987) performed conformational calculations on a total of 10 compounds, which included phenothiazines and other structures, in an attempt to determine the N-(aromatic plane) and N-(polar function) interatomic distances. The calculated minimized conformations of (−)-cyclazocine, *cis*- and trans-clopenthixol, haloperidol (fig. 1, *5*: R_1 = Cl, R_2 = F), and (+)-dexclamol were found to match their X-ray conformations. The results of the study showed that the N-(aromatic plane) distance is not critical for sigma receptor binding because this distance for the compounds varied substantially (0.08–2.9 Å). Furthermore, the N-(midpoint of the aromatic plane) and N-(polar function) distances varied between 4.3–6.4 and 6.5–8.9 Å, respectively. This large degree of variation in the N-(aromatic ring) distances may account for the variety of chemical structures which exhibit reasonable affinity for the sigma receptor.

The study indicated several structural requirements for sigma binding. First, the primary pharmacophore at sigma sites appears to be the 3- or 4-phenylpiperidine moiety, which is present in most compounds showing high affinity for sigma receptors. Second, affinity is greatly influenced by large hydrophobic N-alkyl substituents. Third, compounds from many different structural classes exhibit substantial affinity for sigma receptors, indicating that certain interatomic distances are not subject to rigid constraint (e.g., N to aromatic ring). This lack of rigid constraint is further exemplified by the lack of strict enantioselectivity between the different classes of sigma ligands.

Manallack et al. (1988) performed a study to determine the receptor site topography for both PCP and sigma-like drugs by correlating quantitative conformational, electrostatic potential, and radioreceptor assay results.

The study predicted different receptor site topographies for PCP and sigma receptors, adding additional evidence in support of distinct sigma and PCP receptors as opposed to a sigma/PCP complex (Manallack and Beart 1987, Manallack et al., 1986). *Trans*-4a*R*,10b*R*-9-OH-*n*-Pr-OHBQ (fig. 1, *4*: R_1 = N-propyl, R_2 = 9-OH), a potent sigma ligand that is a conformationally restricted form of (+)-3-PPP, is a relatively rigid molecule. The crystal structure of its tricyclic backbone is considered to be its biologically active conformation. Similarly, the crystal structures of R-(+)-3-PPP, (+)-SKF 10,047 (from the structure of cyclazocine) and haloperidol (fig. 1, *5*: R_1 = Cl, R_2 = F) were used to define low energy conformations of these ligands. The structure of DTG was developed from standard bond lengths and angles. The primary pharmacophore for sigma receptor binding was constructed by using the crystal structure of *trans*-4a*R*,10b*R*-9-OH-*n*-Pr-OHBQ as a template onto which all four of the other structures were superimposed.

Although the sigma site defined by this model accepted many unrelated sigma ligands, it failed to predict the differences in potency of sigma ligands substituted on the aromatic ring. For instance, substitution of a hydroxy group on the 7-, 8-, or 9-positions in the *trans*-4a*R*,10b*R*-9-OH-*n*-Pr-OHBQ series increased potency with the rank order being 8-OH > 9-OH >7-OH. Similar changes to (+)-3-PPP have indicated that the 4-OH derivative is much less potent than (+)-3-PPP. However, this result is inconsistent with the OHBQ series in which the 8-OH group corresponds to the 4-OH group of (+)-3-PPP. Sigma ligands which have substituents other than hydroxy groups include haloperidol, bromoperidol, and (+)-3-(3-fluorophenyl)-N-(*n*-propyl)piperidine. The latter compound is similar in potency to (+)-3-PPP, indicating that a fluoro substituent is an acceptable alternative to a hydroxyl group. Similarly, DTG possesses higher affinity for sigma receptors than its desmethyl analog DPG, presumably due to the conformational restraints imposed by the methyl groups. The greater conformational freedom exhibited by DPG would result in lowered binding affinity because of loss in entropy.

The electrostatic potentials of the five compounds chosen to define the primary sigma receptor model were investigated further to examine the effect of aromatic substitution on receptor binding. The results of the study indicated that haloperidol, the most potent in the series, possessed a deep potential energy well in the region near the chlorine atom. DTG did not produce a potential energy well and (+)-SKF 10,047 and *trans*-4a*R*,10b*R*-9-OH-*n*-Pr-OHBQ produced shallow to negligible potential energy wells in the region of their hydroxy substituents. The higher potency of bromoperidol and haloperidol may be partly explained by their possessing potential energy wells; however, the high potency of DTG cannot be explained this way. Unlike the PCP analogs examined by Manallack et al. (1988) for PCP-binding activity, the sigma analogs examined are all from different structural classes, which makes interpretation of the effects of electrostatic potential subjective rather than quantitative. Thus far, it can be concluded that electronegative substituents on the C-9 position of *trans*-4a*R*,10b*R*-9-OH-*n*-Pr-OHBQ appear to increase sigma receptor-binding affinity.

The increases in sigma receptor potency seen with changing the nitrogen substituent in (+)-benzomorphans, (+)-morphinans, and 3-phenylpiperidines can be explained by the presence of a lipophilic cleft. With the (+)-3-PPP series (table 2), the most potent compounds in the series are those that have the largest nitrogen substituents. The rank order of potency for nitrogen-substituted derivatives of (+)-3-PPP is N-phenethyl > N-butyl > N-propyl > N-ethyl > N-methyl. A similar rank order of potencies has also been demonstrated for *trans*-4a*R*,10b*R*-9-OH-*n*-Pr-OHBQ.

The hypothetical receptor points (R_1, R_2, R_3) for the primary pharmacophore were found to be R_1 (0.00, 3.50, 0.00), R_2 (0.00, −3.50, 0.00), R_3 (6.09, 2.09, 0.00), and N (4.9, −0.12, −1.25). Secondary binding requirements for sigma ligands were proposed with interaction of the nitrogen substituent and aromatic group with lipophilic groups on the receptor. The proposed sigma receptor model differs from the PCP receptor model in (*a*) position of the nitrogen atom, (*b*) direction of the lone pair vector, and (*c*) secondary binding requirements. These differing quantitative SARs of PCP and sigma ligands allow definition of discrete receptors, as well as the design of novel PCP and sigma ligands of high potency and selectivity. In figs. 2 and 3 the hypothetical primary pharmacophore formulated with the secondary binding requirements is illustrated.

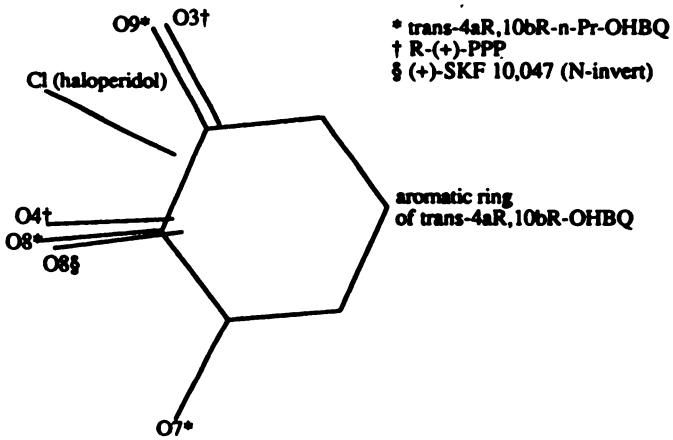


FIG. 2. Diagrammatic representation of the location of various aromatic substituents of molecules from the benzomorphan, phenylpiperidine, and OHBQ drug classes. For reference purposes, the phenyl ring of (*trans*)-(4a*R*,10b*R*)-9-OH-*n*-Pr-OHBQ and the carbon-oxygen or carbon-chloride bonds for each molecule are shown. Optimal sigma receptor affinity appears to require electronegative aromatic substituents residing in the 08–09 region of (*trans*)-(4a*R*,10b*R*)-9-OH-*n*-Pr-OHBQ. From Manallack et al., 1988.

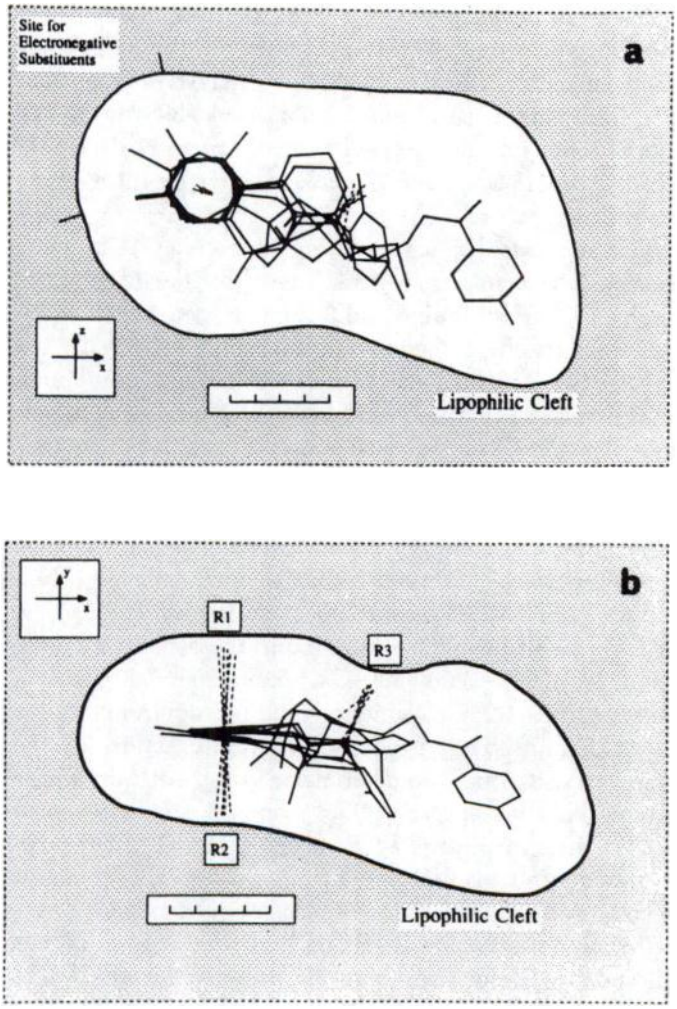


FIG. 3. Diagrammatic representation of the sigma receptor model detailing the position of receptor points R_1, R_2, and R_e, lipophilic clefts, and a site for electronegative substituents. *Dashed line*, R_1-R_2 and N-R_e vectors. Hydrogen atoms have been deleted for clarity. Each unit on the scale bar represents 1 Å. *a*, Sigma receptor model viewed down the y axis, showing the molecules haloperidol, (*trans*)-(4a*R*,10b*R*)-9-OH-*n*-Pr-OHBQ, (*R*)-(+)-[^{3}H]-3-PPP, DTG, and (+)-SKF 10,047 in their best fit low energy conformations to the primary pharmacophore. *b*, Sigma receptor model viewed down the z axis, detailing the position of the receptor points R_1, R_2, and R_e and the lipophilic cleft. From Manallack et al., 1988.

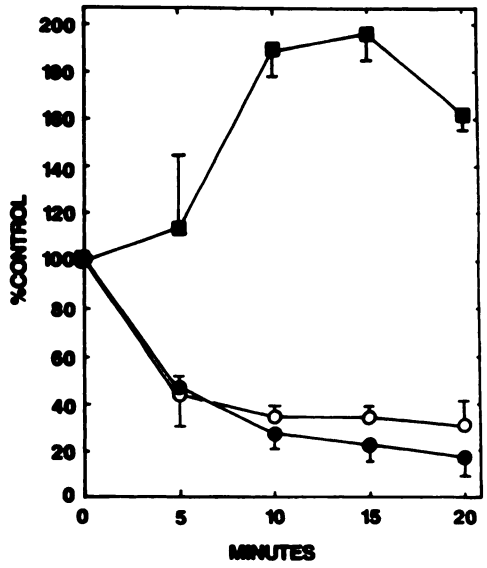


FIG. 4. Effect of UV irradiation on binding of ligands to the sigma receptor. Rat brain membranes were irradiated using a high intensity UV lamp of 254 nm. Aliquots were taken at each time point and pelleted. After resuspension in the assay buffer, the ability of the membranes to bind [^{3}H]DTG (●), [^{3}H](+)-3-PPP (○), and [^{3}H](+)-SKF 10,047 (■) was determined. Values are expressed as percentages of the specific binding of time-zero (nonirradiated) controls and are the averages of two experiments carried out in duplicate ± SEM. Time-zero specific binding: [^{3}H]DTG, 1472 ± 296 cpm; [^{3}H](+)-3-PPP, 1462 ± 404 cpm; [^{3}H](+)-SKF 10,047, 299 ± 36 cpm. Nonspecific binding for each ligand was determined in the presence of 10 μM (+/−)-cyclazocine. Identical results were obtained when 1 μM haloperidol was used to determine specific binding of [^{3}H]DTG and [^{3}H](+)-3-PPP. Irradiation had no effect on nonspecific binding. Reprinted from Bowen et al., 1989a.

B. Allosteric Models

1. Allosteric interactions between (+)-benzomorphan- and non-benzomorphan-binding domains. In a markedly different approach to modeling the sigma receptor, Bowen et al. (1989a) found evidence supporting a model of distinct, allosterically coupled binding domains for non-benzomorphan sigma ligands and sigma-related (+)-benzomorphans. Studies of the sensitivity of rat brain sigma receptors to UV irradiation revealed unusual binding interactions of the various radiolabeled sigma probes. As shown in fig. 4, irradiation of membranes with 254 nm light produced a time-dependent decrease in the binding of both [^{3}H]DTG and [^{3}H](+)-3-PPP, an effect that was largely due to a decrease in the B_{max}. On the other hand, the binding of [^{3}H](+)-SKF 10,047 was markedly enhanced by irradiation, due to an increase in the binding affinity. The data suggest that benzomorphan and non-benzomorphan sigma ligands interact with different sites on the receptor macromolecule that can be distinguished by their different sensitivities to UV light.

Other studies also yielded results consistent with an allosteric model. Scatchard analysis (fig. 5) of labeled [^{3}H](+)-3-PPP binding revealed that, when unlabeled DTG or haloperidol are added to the incubation, an increase in the K_d occurs without a change in the B_{max}; this pattern is consistent with competitive inhibition. On the other hand, when the experiment is performed in the presence of unlabeled (+)-opiates, both the K_d and the B_{max} are decreased, a finding suggestive of uncompetitive inhibition. Thus, the non-benzomorphans DTG and haloperidol are competitive inhibitors of [^{3}H](+)-3-PPP binding, whereas the benzomorphans (+)-SKF 10,047 and (+)-pentazocine are uncompetitive inhibitors. Uncompetitive inhibition is consistent with allosteric coupling of a benzomorphan-binding site to a non-benzomorphan-binding site.

A third line of evidence favoring an allosteric model was again derived from studies of membranes that were

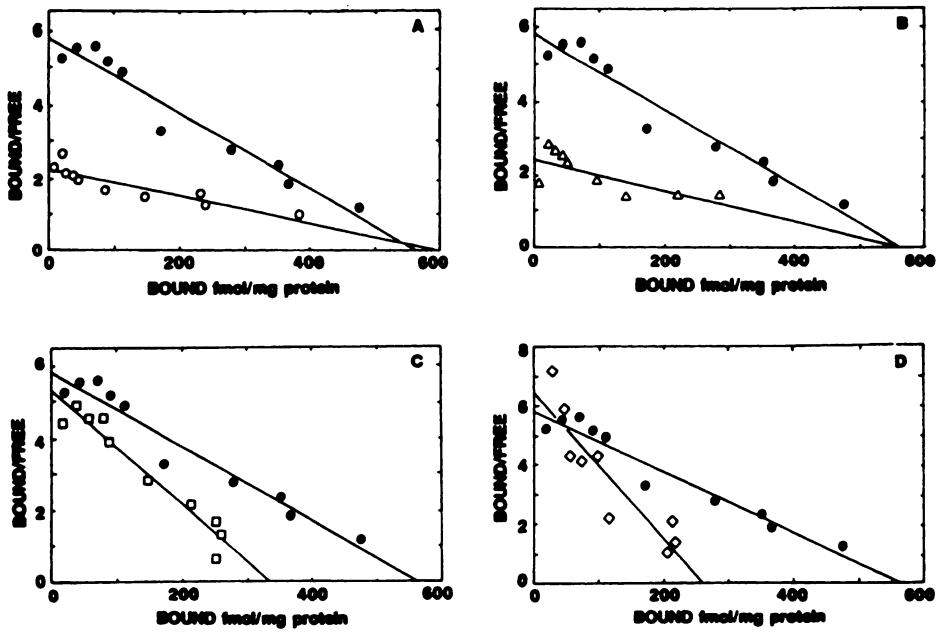


FIG. 5. Determination of the mechanism by which unlabeled sigma ligands inhibit binding of [^{3}H](+)-3-PPP. Scatchard analysis of [^{3}H](+)-3-PPP binding was carried out in the absence (*closed circles*) and the presence (*open symbols*) of a fixed concentration of the specified unlabeled competing ligand using nonirradiated membranes. Non-specific binding was determined in the presence of 1 μM haloperidol. The binding of [^{3}H](+)-3-PPP alone (●) was repeated for comparison in all 4 panels. As illustrated in *A* and *B*, the increase in the apparent K_d of [^{3}H](+)-3-PPP in the presence of 50 nM unlabeled DTG (○) or 10 nM unlabeled haloperidol (△) demonstrates competitive inhibition of binding. By contrast, as illustrated in *C* and *D*, the simultaneous alterations in apparent K_d and B_{max} of [^{3}H](+)-3-PPP induced by addition of unlabeled 300 nM (+)-SKF 10,047 (□) or 50 nM (+)-pentazocine (◇) demonstrates uncompetitive inhibition of binding, supporting an allosteric model of at least one subtype of sigma receptor. Reprinted from Bowen et al., 1989a.

irradiated with UV light. This treatment markedly reduced the ability of DTG and (+)-3-PPP to inhibit binding of [^{3}H](+)-SKF 10,047 but had no effect on the potency of (+)-pentazocine and (+)-SKF 10,047. Similar results have been obtained with [^{3}H](+)-pentazocine. These findings imply that the binding site of the neuroleptic type compounds was disrupted by the UV light, while the binding site for the (+)-opiate ligands remained intact.

A model (fig. 6) consistent with all the data cited above is one in which benzomorphans bind to a domain on the receptor macromolecule that is resistant to the effects of UV light. This domain is allosterically coupled to a binding domain for non-benzomorphans. The non-benzomorphan domain is sensitive to UV irradiation, perhaps because of the presence of a UV-sensitive residue such as tryptophan.

An allosteric model may have implications for an endogenous sigma ligand. The marked enhancement in [^{3}H](+)-SKF 10,047 binding following UV irradiation was intriguing. Conceivably, an endogenous sigma ligand normally occupies the neuroleptic like binding site and inhibits the binding of (+)-opiates. In such a case, disruption of the neuroleptic site by UV irradiation would remove the inhibition, thereby increasing the binding of (+)-opiate ligands.

2. Allosteric interactions of antitussive and anticonvulsant compounds with sigma sites. Musacchio and coworkers (Musacchio et al., 1989a,b; Klein et al., 1989; Klein and Musacchio, 1989; Canoll et al., 1989) identified a binding site for the non-opioid antitussive DM in guinea pig, rat, and mouse brain. [^{3}H]DM binds to high and low affinity sites with K_d values of 57 nM and 24 μM, respectively (Klein and Musacchio, 1989). Prototypic sigma ligands, such as haloperidol, (+)-pentazocine, and (+)-SKF 10,047, displace [^{3}H]DM from the high affinity site with a rank order of potency that is typical of the sigma

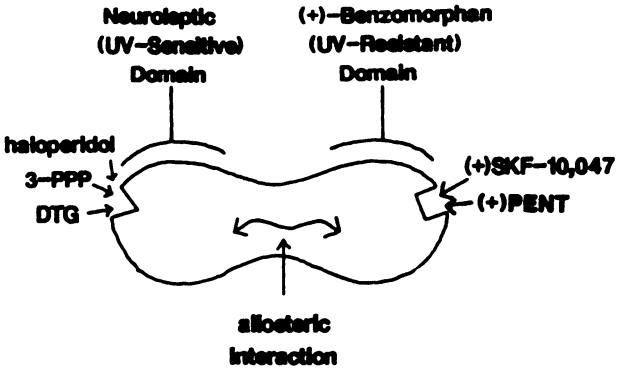


FIG. 6. Schematic of an allosteric model of sigma-binding sites hypothesized by Bowen et al. (1988a). Various sigma ligands may interact with either a neuroleptic binding site or (+)-benzomorphan-binding site on a sigma receptor macromolecule. Ligands that interact with the same site exhibit competitive inhibition; ligands interacting with different sites inhibit binding by an allosteric (noncompetitive) interaction. The neuroleptic site is readily destroyed by UV irradiation, whereas the (+)-benzomorphan site is relatively unaffected.

site (Klein et al., 1989). Similarly, there is a high correlation between the potency of compounds in displacing [^{3}H](+)-3-PPP and [^{3}H]DM (table 3). These data support the contention of Musacchio et al. that the sigma site and the high affinity DM-binding site are the same entity.

Support for an allosteric model of sigma sites comes from the effect of antitussive and anticonvulsant compounds on binding of [^{3}H]DM (Musacchio et al., 1989b). The anticonvulsants phenytoin and ropizine produce dose-dependent enhancement of [^{3}H]DM binding to guinea pig brain membranes. The increase in binding is biphasic, shows an inhibitory phase after a peak of enhancement, and appears to be related to a 3-fold increase in binding affinity without a change in the number of sites. Both ropizine and phenytoin were shown to slow the dissociation of [^{3}H]DM. These results suggest that [^{3}H]DM binds to a macromolecule that contains a distinct but allosterically coupled binding site for ropizine and phenytoin.

Perhaps the strongest evidence that sigma sites are identical with at least one of the [^{3}H]DM sites characterized by Musacchio and coworkers is the finding that [^{3}H](+)-3-PPP binding shows allosteric modulations that are similar to those of [^{3}H]DM. As shown in fig. 7, ropizine and phenytoin enhanced binding of [^{3}H] (+)-3-PPP to guinea pig brain membranes (Musacchio et al., 1989b). The effect was biphasic and occurred over a nearly identical dose range of both ropizine and phenytoin. This phenomenon is quite dramatic; ropizine caused nearly a 3-fold increase in the binding affinity of [^{3}H] (+)-3-PPP without a change in the number of sites. These results support a model in which the sigma receptor macromolecule possesses allosterically coupled binding sites for (+)-opiates, neuroleptics, and certain antitussives and anticonvulsants.

TABLE 3

*Effect of dextromethorphan and sigma ligands on the binding of [^{3}H] DM and (+)-[^{3}H]3-PPP to guinea pig brain homogenate**

DRUG	[^{3}H]DM		(+)-[^{3}H]3-PPP	
	K_i1 (nM)	K_i2 (μM)	K_i1 (nM)	K_i2 (μM)
Dextromethorphan	57	24	37	0.78
Caramiphen	9.5	0.7	9.4	15
Carbetapentane	11.3		11	
(+)-3-PPP	27	3	25.4	0.93
(+)-SKF 10,047	44	3.3	40	0.9
(−)-SKF 10,047	2,620	386		
Opipramol	0.41	0.16	0.92	
Cinnarizine	22	0.40	28.9	
Quinidine	1,090	225	20,050	
Haloperidol	1.4	4.5		
(+)-Pentazocine	1.9	9.9		
(−)-Pentazocine	71	220		
(+)-Cyclazocine	11	67		
(−)-Cyclazocine	395	12		
(−)-Butaclamol	81	9		
(+)-Butaclamol	290			

* Data from Musacchio et al. (1989b).

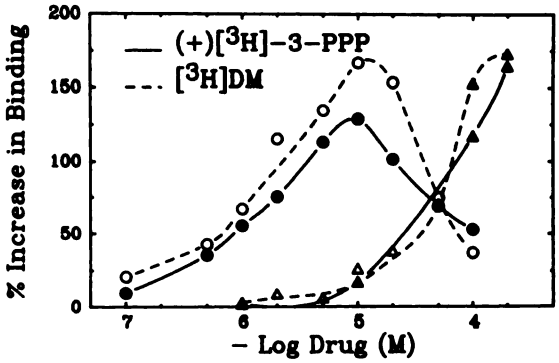


FIG. 7. Effects of ropizine and phenytoin on the specific binding of [^{3}H](+)-3-PPP (*solid lines*) and [^{3}H]DM (*dashed lines*) in guinea pig brain. Increasing concentrations of ropizine (*circles*) and phenytoin (*triangles*) enhanced the binding of both radioligands suggesting (*a*) a common binding site for DM and sigma ligands and (*b*) the presence of allosteric modulation of this site by certain antitussives. Reprinted from Musacchio et al. (1989b) with permission of the authors.

The model of Musacchio et al. has received some support from physiological studies showing that relatively low doses of DM or carbetapentane potentiate the anticonvulsant activity of phenytoin by decreasing its ED_{50} (Tortella and Musacchio, 1986; Choi and Peters, 1987). These findings imply that sigma ligands and DM would produce similar pharmacological effects, because they presumably induce the same conformation of the receptor. Whether the antitussive, anticonvulsant, and neuroprotective properties of DM are shared by sigma ligands is an important line of inquiry.

C. Species Differences in Sigma Sites

Several studies have shown differences in sigma receptors among species. Sigma receptors from rat and guinea pig brain exhibit marked differences in their sensitivity to the effects of UV irradiation (Bowen et al. 1989a; Bowen and Hellewell, 1988): (*a*) [^{3}H]DTG and [^{3}H](+)-3-PPP binding to guinea pig brain membranes is decreased by irradiation but at a much less rapid rate than in rat brain membranes, (*b*) the marked increase in [^{3}H] (+)-SKF 10,047 binding following UV irradiation observed in rat brain is not observed in guinea pig brain membranes, and (*c*) [^{3}H](+)-pentazocine binding is increased by irradiation in rat brain membranes but decreased in guinea pig brain (unpublished observation). The different sensitivities of guinea pig and rat brain sigma receptors suggest that they differ in composition or position of UV-sensitive amino acid residues.

Allosteric interactions also differ among species. Musacchio and coworkers reported that the allosteric enhancement of [^{3}H]DM binding by the anticonvulsant diphenylhydantoin or the antitussive, noscapine, observed in guinea pig brain does not occur in rat or mouse brain (Craviso and Musacchio, 1983b). From this it appears that the rat and mouse brain receptor do not possess the allosteric site that regulates binding of [^{3}H] DM. These workers also reported differences in the

ability of carbetapentane, caramiphen, and dextrorphan to displace [^{3}H]DM from rat, guinea pig, and mouse brain membranes (Craviso and Musacchio, 1983b).

Prototypic sigma ligands also exhibit different binding affinities in rat and guinea pig brain. Whereas [^{3}H]DTG binds with similar affinity in both species, [^{3}H](+)-3-PPP binds with 3.5-fold lower affinity in rat brain (Bowen and Hellewell, 1988; Bowen et al., 1989a; Hellewell and Bowen, 1990). There is an even more pronounced loss of affinity of (+)-morphinans and (+)-benzomorphans in the rat. The affinities of these drugs in the rat brain are 4- to 30-fold lower than in the guinea pig brain (table 1; Bowen and Hellewell, 1988; Matsumoto et al., 1990). It appears that either sigma receptors are different in rats and guinea pigs or the brains of these two species have different proportions of sigma receptor types that discriminate (+)-morphinans and (+)-benzomorphans (see below).

When these data are viewed as a whole, it is apparent that sigma receptor structure or organization must differ in important ways among species. As a consequence, we must assume the presence of concomitant species differences in the in vivo pharmacology and function of sigma receptors.

D. Multiple Sigma Receptor Types

Studies showing different binding profiles in various species and tissues and mathematical analysis of binding data within particular tissues have led investigators to hypothesize multiple forms of sigma receptors. Bowen and coworkers (Bowen and Hellewell, 1988; Hellewell and Bowen, 1990) reported binding of the prototypic sigma ligands [^{3}H]DTG and [^{3}H](+)-3-PPP to rat PC12 cells. PC12 cells are a tumor cell line derived from the rat adrenal medulla which attains the phenotype of sympathetic neurons when stimulated with nerve growth factor (Greene and Tischler, 1976). These cells exhibit many of the properties of neurons in culture, including neurite formation and expression of several neurotransmitter receptors (Guroff, 1985).

The presence of sigma-binding sites in PC12 cells was first suggested by the relatively large number of high affinity binding sites for [^{3}H]DTG and [^{3}H](+)-3-PPP (K_d = 23.7 and 86.3 nM, respectively). For some compounds, the typical sigma-binding profile was observed. Binding of [^{3}H]DTG and [^{3}H](+)-3-PPP is potently displaced by haloperidol, weakly displaced by PCP, and unaffected by high concentrations of apomorphine and MK-801, further evidence of a relationship between this site and sigma receptors. Similar results were obtained by Yang et al. (1989) using [^{3}H](+)-3-PPP. In addition, binding of [^{3}H]TCP is undetectable, demonstrating absence of PCP receptors (Hellewell and Bowen, 1990).

However, the PC12 sigma-like site differs from the guinea pig brain sigma site in its substantially lower affinity for (+)-morphinans and (+)-benzomorphans (Bowen and Hellewell, 1988; Hellewell and Bowen, 1990). For example, in PC12 cells, (+)-pentazocine has a K_i of 1000 nM, and (+)-SKF 10,047 and dextrallorphan both have K_i values >10,000 nM (versus [^{3}H](+)-3-PPP; table 4). The corresponding values in guinea pig brain are, respectively, 1.2, 62.5, and 16.1 nM. Similar results were obtained when [^{3}H]DTG was used as probe (table 4). The PC12 site also differs from the guinea pig site in possessing greater affinity for (−)-benzomorphans than (+)-benzomorphans (table 4). Consistent with the low affinity of (+)-benzomorphans, binding of 5 nM [^{3}H](+)-SKF 10,047 and [^{3}H](+)-pentazocine cannot be detected in membranes from these cells.

Affinity labeling studies in which [^{3}H]Az-DTG was used (see below) also revealed differences in the sigma-like binding sites of guinea pig brain and PC12 cells (Hellewell and Bowen, 1988; 1990). This probe labeled a polypeptide of 25 kDa in membranes from guinea pig brain but labeled polypeptides of 18 and 21 kDa in PC12 cell membranes (fig. 8). Taken with the ligand-binding data, these results argue for the existence of different molecular forms of the sigma receptor.

The existence of sigma-like sites with low affinity for (+)-benzomorphans in various tissue sources raises the question of how sigma sites are to be defined. In recent years, sigma sites have been defined pharmacologically by their high affinity for haloperidol and (+)-benzomorphans and their low affinity for PCP-related compounds. According to this definition, the sites described above could not be considered sigma sites. However, when other prototypic sigma ligands are taken into account, the marked overlap in their pharmacologic properties suggests a close relationship. As a result it appears that the current definition is inadequate and should be modified. In light of this, the terminology "sigma-1" and "sigma-2" was suggested for the guinea pig brain traditional sigma site and the PC12 cell sigma-like sites, respectively (Hellewell and Bowen, 1990). The properties of these putative sigma receptor types are summarized in table 5, and the apparent biological correlates to these two sites are discussed below. Because PC12 cells appear to lack the sigma-1 receptor, this may be an ideal system in which to study the properties and function of the sigma-2 site.

The tissue variation in stereoselectivity ratio with enantiomeric pairs of opiates (when [^{3}H]DTG or [^{3}H](+)-3-PPP are used as sigma receptor probes) may be due to varying proportions of sigma-1 and sigma-2 sites. Furthermore, it is interesting that (−)-benzomorphans have equal affinity at the putative sigma-1 and sigma-2 sites (table 4). This might explain observations in which (−)-opiates produce steep displacement curves (Hill coefficients of unity) against [^{3}H](+)-3-PPP or [^{3}H]DTG, whereas (+)-benzomorphans often produce shallow or biphasic curves (Largent et al., 1984; Matsumoto et al., 1990). (−)-Benzomorphans would not differentiate the sigma-1 and sigma-2 sites labeled by these probes.

TABLE 4

*Affinities of various ligands at sigma receptors of PC12 cells and guinea pig brain**

Ligand	K_i (nM) vs. [^{3}H](+)-3-PPP		K_i (nM) vs. [^{3}H]DTG	
	Guinea pig	PC12	Guinea pig	PC12
Haloperidol	3.8 ± 2.9	24.3 ± 14	18.9 ± 8	48.7 ± 16
DTG	20.5 ± 3.6	14.6 ± 5	21.7 ± 1.9	21.2 ± 4
(+)-Pentazocine	1.2 ± 0.2	1000 ± 263	169 ± 30	1402 ± 51
(−)-Pentazocine	87.0 ± 14	82.2 ± 15	ND	ND
(+)-SKF10,047	62.5 ± 11.6	No inhibn[1]	419 ± 53	No inhibn[1]
(−)-SKF10,047	4657 ± 506	4581 ± 102	ND	ND
Dextrallorphan	16.1 ± 1.8	No inhibn[2]	96.2 ± 18	No inhibn[2]
3-Methoxy-dextrallorphan	32.1 ± 8.9	1212 ± 588	226 ± 9	1500
KCR 12-83.1	138.4 ± 15.1	5255 ± 1062	911 ± 156	No inhibn[2]
PCP	1014 ± 38	285 ± 2.4	2632 ± 737	331 ± 53
MK 801	No inhibn[1]	No inhibn[1]	ND	ND
Apomorphine	No inhibn[1]	No inhibn[1]	ND	ND
(−)-Sulpiride	No inhibn[1]	No inhibn[1]	No inhibn[1]	No inhibn[1]

* [^{3}H](+)-3-PPP (3 nM) or [^{3}H]DTG (5 nM) was incubated with various concentrations of unlabeled ligand (0.05–10,000 nM). Data were analyzed using the iterative curve fitting program CDATA (EMF Software Inc., Baltimore, MD). The Cheng-Prusoff equation was then used to calculate apparent K_i from the IC_{50} value. Values are the averages ± SEM from two or three experiments, each carried out in duplicate. Abbreviations: No inhibn[1] and no inhibn[2], the compound produced <30% inhibition of control binding at 10,000 and 5,000 nM, respectively; ND, not determined. KCR 12-83.1, (+)-N-cyclopropylmethyl-nordihydrocodeinone. Data from Hellewell and Bowen (1990).

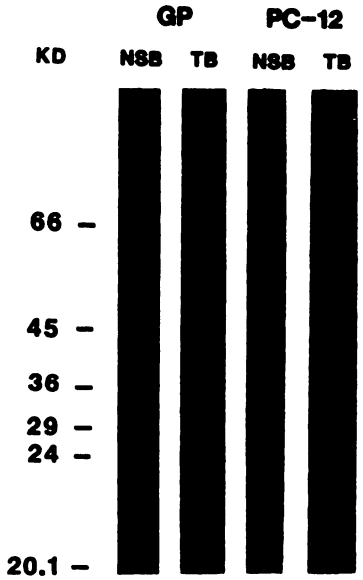


FIG. 8. Photolabeling of guinea pig (*GP*) brain membranes and PC12 cell membranes with [^{3}H]Az-DTG. Membranes from guinea pig brain or PC12 cells were photolabeled with 10 nM [^{3}H]Az-DTG, solubilized, and analyzed by polyacrylamide gel electrophoresis. *Ordinate*, positions of molecular weight markers in kilodaltons (KD): bovine albumin, 66; egg albumin, 45; glyceraldehyde-3-phosphate dehydrogenase, 36; carbonic anhydrase, 29; trypsinogen, 24; soybean trypsin inhibitor, 20.1. *NSB*, the nonspecific binding control; *TB*, total binding. [^{3}H]Az-DTG labels a 25-kDa polypeptide in guinea pig brain. By contrast, [^{3}H]Az-DTG labels an 18-kDa and a 21-kDa polypeptide in PC12 cells. The differing molecular weight polypeptides correspond with differing pharmacological profiles of sites in these tissues, supporting the hypothesis of multiple sigma receptor subtypes. Reprinted from Hellewell and Bowen, 1990.

Binding of sigma ligands to certain peripheral tissues of the rat also suggests the possibility of multiple forms of sigma-like binding sites. Hellewell et al. (1990) investigated the binding of [^{3}H]DTG, [^{3}H](+)-3-PPP, and [^{3}H](+)-pentazocine to membranes of rat liver. [^{3}H]DTG and [^{3}H](+)-3-PPP bound to sites with K_d values of 17.9 and 51.9 nM. The two probes labeled the same number of sites, with a density much higher than guinea pig brain; i.e., approximately 11,500 fmol/mg protein. Competition of various ligands against [^{3}H]DTG revealed the following rank order of potency: DTG = (−)-pentazocine > fluphenazine > (+)-3-PPP > haloperidol > (−)-3-PPP > dextrallorphan > (−)-SKF 10,047 > (+)-pentazocine = (+)-SKF 10,047. Opioid peptides, (−)-sulpiride, apomorphine, and MK-801 were inactive. This ligand selectivity is similar to that observed in PC12 cells as described above (Hellewell and Bowen, 1990).

The selective sigma probe, [^{3}H](+)-pentazocine, bound to hepatic membranes with K_d = 7.5 nM and exhibited a B_{max} that was only 25% of that for [^{3}H]DTG and [^{3}H](+)-3-PPP. The K_d determined by direct Scatchard analysis is in marked contrast to the K_i of 1058 nM determined by competition with [^{3}H]DTG. Furthermore, when [^{3}H](+)-pentazocine was used as probe, competition studies revealed the typical sigma ligand selectivity observed in guinea pig brain, including potent displacement by haloperidol, DTG, and (+)-3-PPP. These data suggest that [^{3}H]DTG and [^{3}H](+)-3-PPP label sites with both high and low affinity for (+)-opiates in this tissue. These sites may be synonymous with the sigma-1 and sigma-2 sites of guinea pig brain and PC12 cells, respectively, as proposed by Hellewell and Bowen (table 4). In the nomenclature scheme proposed in table 5, [^{3}H](+)-pentazocine would selectively label the sigma-1 site in the liver, giving the ligand selectivity profile observed in guinea pig brain. [^{3}H]DTG and [^{3}H](+)-3-PPP would label both sigma-1 and sigma-2 sites. Because the B_{max} of [^{3}H](+)-pentazocine is only 25% that of [^{3}H]DTG and [^{3}H](+)-3-PPP, it appears that the majority of binding in liver is to the sigma-2 site.

TABLE 5
*Properties of putative sigma receptor types**

Property	Sigma-1	Sigma-2
Ligand affinities		
Haloperidol	High	High
DTG	High	High
(+)-3-PPP	High	High to moderate
(+)-Benzomorphans and (+)-morphinans	High to moderate	Low to negligible
PCP	Low	Moderate
MK-801	Negligible	Negligible
Apomorphine	Negligible	Negligible
(−)-Sulpiride	Negligible	Negligible
Stereoselectivity for benzomorphans	(+) > (−)	(−) > (+)
Molecular weight [sodium dodecyl sulfate polyacrylamide gel electrophoresis (kDa)]	25	18–21

* From the model developed by Hellewell and Bowen (1990).

Affinity labeling of liver membranes with [^{3}H]Az-DTG (see below) reveals labeling of two distinct polypeptides (Hellewell et al., 1990). One polypeptide (25 kDa) is comparable to the protein labeled in guinea pig brain, whereas the other (21.5 kDa) is comparable to the proteins labeled in PC12 cells. This confirms the presence of two [^{3}H]DTG-binding sites in the liver. Notably, labeling of the higher molecular weight polypeptide can be completely blocked by 100 nM dextrallorphan, whereas labeling of the lower molecular weight band is not affected. This strongly supports the notion that the 25-kDa protein represents the high affinity (+)-opiate-binding site (sigma-1) and the lower molecular weight polypeptide represents the low affinity (+)-opiate site (sigma-2).

Based on differing drug selectivity profiles, Musacchio et al. (1988) have also hypothesized multiple types of [^{3}H]DM-binding sites in guinea pig liver, kidney, and adrenal medulla. The anticonvulsants carbetapentane and caramiphen potently inhibited [^{3}H]DM binding to guinea pig brain and kidney medulla membranes but were virtually inactive in liver and kidney cortex. Adrenal sites were sensitive to carbetapentane, although not as sensitive as the brain sites. In contrast, the antidepressant drug opipramol potently inhibited [^{3}H]DM binding in brain, liver, and adrenal tissues. This suggests that the receptors from these tissues have different molecular forms. In view of the evidence that the brain contains [^{3}H]DM-binding sites that appear to be identical with sigma receptors, these results with peripheral tissues may suggest heterogeneity of sigma sites. The finding of high densities of sigma-like sites in peripheral tissues with characteristics different from the brain sites may suggest the existence of peripheral- and central-type sigma sites, analogous to the benzodiazepine receptor (Braestrup and Squires, 1977).

There is evidence that guinea pig brain contains two types of sigma receptors. Results from ligand-binding surface analysis led Rothman and coworkers (Reid et al., 1988) to argue for the existence of two classes of binding sites for [^{3}H]DTG, termed "site 1" and "site 2" (table 6). Although site 1 and site 2 have comparable affinities for DTG, there are marked differences in the affinity of the two sites for (+)-benzomorphans and (+)-morphinans. Site 1 binds (+)-benzomorphans and (+)-morphinans with high affinity, whereas site 2 has very low affinity for these compounds. Also, although possessing high affinity for both sites, haloperidol binds with a lower affinity to site 2. (+)-3-PPP binds with only moderate affinity to site 2. Binding of (−)-opiates at these sites has not yet been investigated. A surprising finding from

TABLE 6
*Inhibitory dissociation constants of test drugs for DTG site 1 and site 2 in guinea pig brain**

Drug	Site 1 (K_d ± SEM)	Site 2 (K_d ± SEM)	Site 2/ site 1
Haloperidol	0.30 ± 0.01	36.1 ± 1.6	120
(+)-Pentazocine	2.0 ± 0.06	456 ± 11	228
R-(+)-PPP	5.1 ± 0.3	442 ± 34	86
Carbetapentane	5.2 ± 0.3	1523 ± 85	292
BD738	6.4 ± 0.1	188 ± 6	29.4
BD737	8.0 ± 0.3	502 ± 29	62.8
Fluzphenazine	7.6 ± 0.5	440 ± 30	58
Dextrallorphan	8.4 ± 0.4	1861 ± 94	221
Perphenazine	8.9 ± 0.5	429 ± 27	48
DTG	11.9 ± 0.1	37.6 ± 0.6	3
S-(−)-PPP	30.5 ± 0.6	1544 ± 37	50
KCR-11-240.1	33.5 ± 1.4	1399 ± 68	42
BMY 14802	41.4 ± 2.0	728 ± 52	18
(+)-SKF 10,047	44.8 ± 1.6	4263 ± 190	95
BD446	47.4 ± 3.0	1383 ± 96	29
(−)-Butaclamol	47.4 ± 2.9	3646 ± 312	77
Caramiphen	65.3 ± 3.8	2864 ± 224	44
(+)-Cyclazocine	77.6 ± 6.4	1238 ± 108	16
Buspirone	95.7 ± 2.3	744 ± 19	8
Dextromethorphan	121 ± 6	53503 ± 3962	442
BD445	126 ± 7	4144 ± 265	33
KCR-12-83.1	154 ± 4	18245 ± 657	118
KCR-12-84.1	179 ± 11	29258 ± 2081	163
Dextrorphan	202 ± 10	11386 ± 719	56
Levallorphan	721 ± 20	13686 ± 422	19
KCR-11-239.1	1245 ± 85	18705 ± 1373	15
KCR-12-69.1	9064 ± 1289	> 1 mM	> 110

* From Rothman et al. (1990).

this study was that DTG and haloperidol accelerated dissociation of [^{3}H]DTG from both sites 1 and 2 (Rothman et al., 1990). These data add to the growing body of evidence that sigma sites are allosterically modulated (Bowen et al., 1989a; Musacchio et al., 1989b).

Guinea pig brain, therefore, appears to contain sigma sites that differ mainly in their affinity for (+)-opiates. It will be interesting to learn whether the low affinity (+)-opiate site of guinea pig brain is the same as that described in PC12 cells and liver (sigma-2). Failure of [^{3}H]Az-DTG to photolabel a polypeptide in the 21-kDa molecular weight range (fig. 8) and the relatively low affinity for (+)-3-PPP (table 6) suggest that this may not be the case. Further studies will be needed to determine the relationship between these low affinity, sigma-like sites.

E. High and Low Affinity States of the Same Receptor

Another possible model of the sigma receptor is one in which the receptor can exist in interconvertible high and low affinity states. This possibility is discussed below in the section on coupling of sigma receptors to G proteins.

F. Summary

At the present time, there does not appear to be a unifying hypothesis capable of reconciling these different topographic and structural models of the sigma receptor. However, the evidence clearly points to interaction of sigma ligands with a heterogeneous population of sites. It is also important to note that one model does not necessarily preclude another (i.e., allosteric models versus multiple receptor models versus multiple state models). For example, it is conceivable that there are at least two sigma receptor macromolecules, one of which consisting of distinct allosterically coupled binding sites and the other consisting of a single ligand binding domain. Superimposed on these general schemes might be subtle species or tissue differences in the structure of receptor proteins that might affect the ligand-binding profiles.

Additional studies in the area of quantitative SAR relationships will allow further refinement of predictions regarding topographical features of the ligand receptor-combining site(s) (fig. 3). Elucidation of overall structural features, such as possible subunit composition and organization, must await purification and characterization of the receptor proteins. Progress toward this latter goal is discussed in the next section.

III. Physical and Chemical Properties of Sigma Receptors

Molecular characterization of sigma sites has proceeded slowly. However, steady progress is being made and the data have confirmed that the sigma site is a protein and that it differs from the PCP receptor.

A. Proteinaceous Nature of the Sigma Receptor

Converging lines of evidence from several laboratories support the protein nature of sigma-binding sites. First, sigma-binding sites appear to be heat labile. Binding to sigma sites labeled by both [^{3}H]SKF 10,047 (Su, 1982) and [^{3}H]DM (Craviso and Musacchio, 1983a) was significantly reduced following heat treatment of guinea pig brain membranes. Second, the pH optimum for both [^{3}H](+)-3-PPP and [^{3}H]DM binding was between pH 8.0 and 8.9 (Craviso and Musacchio, 1983a; Largent et al., 1987). For [^{3}H]DM sites in guinea pig brain, there was a sharp decrease in specific binding below pH 8.0 and a gradual decline above pH 8.5 (Craviso and Musacchio, 1983a). For [^{3}H](+)-3-PPP sites in rat brain, there was an increase over the pH range 7.0–8.9 with a fairly steep increase between pH 8.0 and 8.9; higher pH was not tested. The increases in binding were due to decreases in the K_d and are attributed to the protonation state of the ligand (Largent et al., 1987). Third, proteases decreased the binding of both [^{3}H]SKF 10,047 and [^{3}H]DM to guinea pig brain membranes. [^{3}H]SKF 10,047 binding was significantly reduced by trypsin and phospholipase C (Su, 1982). Similarly, incubation of membranes with trypsin or alpha-chymotrypsin attenuated [^{3}H]DM binding (Craviso and Musacchio, 1983a). Also, sulfhydryl reagents significantly reduced sigma binding (Craviso and Musacchio, 1983a). The sensitivity of these binding sites to temperature, pH, and protein-modifying agents strongly suggest that these sites are proteins.

B. Affinity Labeling and Molecular Weight Determination

Affinity labeling has been a useful tool in characterizing sigma sites, as it has with other receptor systems. Kavanaugh et al. (1988) developed a radiolabeled azido derivative of the selective sigma ligand, DTG. In the dark, [^{3}H]Az-DTG binds to guinea pig brain sigma sites with properties nearly indistinguishable from those of [^{3}H]DTG. Irradiation of the ligand-receptor complex with light of 366 nm produced covalent attachment of the ligand to the sigma receptor. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed labeling of a single polypeptide of 29 kDa. Labeling of this band was blocked by haloperidol but not by PCP, suggesting that this protein is a component of the sigma-binding site and not the PCP receptor.

When photolabeled membranes were solubilized with sodium cholate and subjected to chromatography on Sepharose Cl-6B, radioactivity was associated with a major protein peak with Stokes radius of 4.6 nm. This corresponded to an approximate molecular weight of 150,000 Da (uncorrected for bound detergent). Kavanaugh et al. (1988) suggested that the lack of higher molecular weight proteins on sodium dodecyl sulfate-polyacrylamide gel electrophoresis indicated that the 29-kDa polypeptide is part of a larger native protein com-

plex. No disulfide bridges appeared to be involved in stabilizing the structure because the same 29-kDa protein was observed under both reducing and nonreducing conditions.

[^{3}H]Az-DTG also labels a 29-kDa polypeptide in NCB-20 cells (Adams et al., 1987a). NCB-20 cells have been shown to have sigma receptors with pharmacological characteristics similar to those of guinea pig brain (Largent et al., 1986; Kushner et al., 1988). Thus, the pharmacological similarity between sigma receptors from NCB-20 cells and guinea pig brain also extends to their physical properties. The labeling of a 29-kDa protein in membranes of both guinea pig brain and NCB-20 cells lends support to the notion that this protein is the binding site of the sigma receptor, or at least a protein near the binding domain of the receptor.

Hellewel and Bowen (1990) used [^{3}H]Az-DTG to study the physical properties of sigma receptors from rat PC12 cells. In contrast to the guinea pig brain, which yielded a single polypeptide of 29 kDa, PC12 cells yielded two polypeptides with molecular weights of 18 and 21 kDa. It is unlikely that the lower molecular weight proteins were generated by proteolysis, because similar results were obtained in the presence of protease inhibitors. As discussed above, PC12 cells possess a sigma-like binding site that differs from the guinea pig brain receptor in having low affinity for sigma-related morphinans and benzomorphans. The lower molecular weight of the sigma site in PC12 cells is thus consistent with the different pharmacology of this receptor and strengthens the notion that there may be multiple types of sigma receptors.

Sigma receptors have also been examined with several isothiocyanate probes. The isothiocyanate moiety is a chemically reactive electrophilic group that may be attacked by amine- or thiol-type nucleophiles at or near the ligand-binding site of the receptor, forming thiourea or dithiocarbamate links, respectively. Adams et al. (1987b) synthesized and characterized an isothiocyanate derivative of DTG, DIGIT. Competition studies in which [^{3}H]DTG was used revealed high affinity binding of DIGIT to sigma sites (K_i = ~28 nM). Membranes pretreated with DIGIT showed virtually no sigma-binding sites even after repeated washing, suggesting covalent attachment to the receptor. DIGIT acylates sigma receptors with an IC_{50} of 50 nM. Furthermore, the effect was selective for sigma sites, because binding of ligands for PCP, dopamine D_2, benzodiazepine, and mu-opioid receptors was unaffected. DIGIT produced competitive inhibition of [^{3}H]DTG binding, which is quite surprising because noncompetitive inhibition would be expected of an agent that covalently occludes the ligand-binding site.

Bluth et al. (1989) used another irreversible isothiocyanate ligand, Metaphit, to study the sigma receptor. Metaphit was previously shown to produce irreversible blockade of PCP receptors in rat brain (Contreras et al., 1986). The effect on sigma receptors was investigated because of the known interaction of some PCP-related compounds with sigma sites. Pretreatment of guinea pig brain membranes with Metaphit produced a concentration-dependent loss of sigma binding that was not reversed by repeated washing. Residual receptor occupation due to failure to remove noncovalently bound Metaphit cannot account for this effect, because equivalent concentrations of PCP could be efficiently removed from the receptor by the washing procedure. These data thus suggest that Metaphit irreversibly labels sigma receptors.

Metaphit had a differential effect on the binding of various radiolabeled sigma ligands. The order of sensitivity to Metaphit was [^{3}H]DTG > [^{3}H](+)-3-PPP >> [^{3}H](+)-SKF 10,047, with half-maximal loss of binding occurring at 2, 10, and 50 μM, respectively. In fact, [^{3}H]DTG binding was more sensitive to Metaphit than was the binding of [^{3}H]TCP, suggesting that Metaphit is more potent at sigma than at PCP receptors. The differential sensitivity of [^{3}H]DTG and [^{3}H](+)-3-PPP compared to [^{3}H](+)- SKF 10,047 again seems to reflect the different modes of interaction of benzomorphans and non-benzomorphans with sigma receptors.

Surprisingly, like DIGIT, Metaphit was a competitive inhibitor of the binding of sigma ligands. This is in contrast to effects on [^{3}H]TCP binding, in which Metaphit produced the expected noncompetitive inhibition in the same membranes. Reid et al. (1990) showed that several other electrophilic derivatives of PCP-related compounds also irreversibly bound to sigma sites with varying degrees of potency.

From their studies of several isothiocyanate derivatives of (+)-3-PPP, Grayson et al. (1988) added to the list of sigma receptor acylators that form covalent attachment but inhibit binding competitively. Although there is no unequivocal explanation for the several demonstrations of this phenomenon, a plausible explanation is that the nucleophile may lie close to, but outside of, the ligand-binding site. This arrangement could allow the ligand to attach in such a way that it is free to move in and out of the binding site. This, in turn, would allow a competing ligand to enter the binding site when it is present in sufficient concentration. The differential effect of acylation on the various classes of sigma ligands may reflect the complexities of the structural organization of sigma receptors as described above (i.e., the existence of allosteric binding sites on the sigma macromolecule or the existence of multiple types of receptors). Although a clear understanding of the mode of interaction of isothiocyanates with sigma receptors is presently lacking, further study of these compounds may provide important clues to the organization and structure of sigma receptors and may provide novel long-acting sigma receptor antagonists.

C. Solubilization and Purification of Active Receptors

Purification of active sigma receptors must follow solubilization in active form. Kavanaugh et al. (1989) solu-

bilized sigma receptors from guinea pig brain using sodium cholate. The solubilized receptors possess binding properties that are similar to those of intact brain membranes. [^{3}H]DTG and [^{3}H](+)-3-PPP bound to solubilized preparations with K_d values of 31 and 33 nM, respectively. Competition of [^{3}H]DTG with a series of compounds revealed a ligand selectivity pattern indistinguishable from that of brain. Size exclusion chromatograpy on Sepharose Cl-6B showed that the binding activity has an estimated Stokes radius of 8.7 Å. Similar results were obtained with membranes solubilized after photolabeling with [^{3}H]Az-DTG. However, this high molecular weight may reflect aggregation of receptors or association with other membrane proteins or detergent micelles. Further work will be needed to exclude these possibilities.

Sigma receptors have also been solubilized from rat and bovine cerebellum by Arnold et al. (1988). These investigators reported solubilization with 3-(3-cholamidopropyl)dimethylammonio-1-propanesulfonate (CHAPS) and a 6000-fold purification on an affinity column derivatized with a 3-PPP analog. After reconstitution into lipid vesicles, the purified material showed the characteristic ligand selectivity of the sigma site: high affinity for haloperidol and DTG, preference for the (+)-isomer of SKF 10,047, and preference for the (−)-isomer of butaclamol. Ligands for other receptors, including PCP receptors, had little or no affinity. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed two polypeptides of 63 and 65 kDa. This differs from the 29-kDa band photolabeled with [^{3}H]Az-DTG in guinea pig brain and may again suggest species differences in sigma sites, as described above. Further studies must be carried out to investigate this possibility.

IV. Sigma Receptors and Signal Transduction Mechanisms

A. Coupling of Sigma Receptors to Guanine Nucleotide-binding Proteins

G proteins play a central role in several types of signaling mechanisms (Gilman, 1987). In some systems, the first step in the cascade of biochemical events, from the formation of a transmitter-receptor complex to membrane conductance changes, is the coupling of the receptor to a G protein. G proteins play a role in cyclic adenosine monophosphate-related systems, PPI turnover, direct coupling to some ion channels, arachidonic acid-derived systems, and protein translocation (Gilman, 1987; Casey and Gilman, 1988).

Itzhak and coworkers (Itzhak and Khouri, 1988; Itzhak, 1989) obtained evidence that sigma receptors interact with G proteins. As observed with other G protein-coupled receptors, guanosine triphosphate and Gpp(NH)p inhibited the binding of [^{3}H](+)-3-PPP to rat brain membranes. Binding of [^{3}H](+)-SKF 10,047 was also inhibited but was less affected than [^{3}H](+)-3-PPP binding. Guanosine monophosphate and adenosine triphosphate had no effect on [^{3}H](+)-3-PPP binding, demonstrating specificity for G protein-active guanine nucleotides. In the absence of Gpp(NH)p, (+)-SKF 10,047 displaced [^{3}H](+)-3-PPP in a biphasic manner from high and low affinity sites. Gpp(NH)p eliminated the high affinity phase, suggesting conversion to a low affinity state (Itzhak and Khouri, 1988). Further studies revealed that Gpp(NH)p had a similar effect on displacement of [^{3}H](+)-3-PPP by (+)-3-PPP, pentazocine, and cyclazocine. However, haloperidol displaced [^{3}H](+)-3-PPP from a single state and was not affected by guanine nucleotides (Itzhak, 1989). Displacement by chlorpromazine was also not affected.

Kinetic studies revealed that Gpp(NH)p decreased the association rate of [^{3}H](+)-3-PPP by about 5-fold. Dissociation experiments demonstrated biexponential dissociation of [^{3}H](+)-3-PPP. Inclusion of Gpp(NH)p eliminated the slower phase, leaving only the rapid monophasic component intact (Itzhak, 1989). This suggested that the formation of a high affinity receptor state is prevented by guanine nucleotides.

Other agents known to affect receptor-G protein coupling also inhibited [^{3}H](+)-3-PPP binding. Treatment of rat brain membranes with either N-ethylmaleimide (a nonselective agent) or pertussis toxin (which selectively alters G proteins) significantly decreased [^{3}H](+)-3-PPP binding (Itzhak, 1989). These reagents also eliminated the effect of Gpp(NH)p on [^{3}H](+)-3-PPP binding. These results are similar to those obtained with other G protein-coupled receptors where these reagents are believed to cause uncoupling of the receptor from the G-protein unit. Because pertussis toxin adenosine diphosphate ribosylates Gi and Go, coupling to these particular G proteins is suggested.

Taken together, these results strongly suggest that the sigma receptor labeled by [^{3}H](+)-3-PPP can exist in a high and low affinity state, with the high affinity state coupled to a G protein. This has important implications for the function of sigma sites, because it suggests that sigma receptors are involved in signal transduction. Evidence for this is discussed in the next section.

The failure of guanine nucleotides to alter haloperidol binding suggests that it is an antagonist at sigma sites (because antagonist binding is not sensitive to guanine nucleotides, Gilman, 1987), whereas (+)-benzomorphans and (+)-3-PPP would be agonists. However, this contradicts the results from several functional systems discussed below. The reason for the apparent discrepancy is not clear but may be related to the presence of multiple sigma-binding sites. Conceivably, haloperidol is an antagonist at one of the sites and an agonist at the other. This question clearly calls for further investigation.

B. Modulation of Phosphoinositide Turnover

Although the biochemical systems acted on by sigma receptors remain largely unknown, certain modulatory

actions of sigma ligands on second messengers are now well established. Bowen and coworkers investigated the effects of sigma ligands on PPI turnover (Bowen et al., 1988a; 1989b; Bowen et al., 1990b; Bowen and Tolentino, 1990). Sigma ligands did not significantly alter PPI turnover at concentrations up to 100 μM in a rat brain synaptoneurosome preparation; although at higher concentrations there was slight depression of basal inositol phosphate production with some ligands. Thus, sigma ligands do not directly stimulate PPI turnover in the manner of a number of brain neurotransmitters.

Although sigma ligands do not have direct effects on PPI turnover, they do modulate the actions of ligands for the cholinergic receptor. In rat brain synaptoneurosomes, sigma ligands attenuated the ability of both carbachol and oxotremorine-M to stimulate inositol phosphate production. The effect was dose dependent and occurred at concentrations less than those required to produce the minor changes in basal activity. This effect of sigma ligands was observed for compounds from varied chemical classes. The rank order of potency of sigma ligands at attenuating the PPI stimulatory effects of oxotremorine-M correlated highly (r = 0.92) with their ability to displace [^{3}H](+)-3-PPP from guinea pig brain sigma receptors (Bowen et al., 1990b; fig. 9). A similar correlation was obtained with carbachol as the cholinergic agonist (Bowen et al., 1988a). This constitutes strong evidence for sigma receptor moduluation of the PPI second messenger system. It should be noted that this potency profile is consistent with actions at sigma-1 (table 5) and not sigma-2, because (+)-benzomorphans and (+)-morphinans are in the same potency range as DTG and haloperidol.

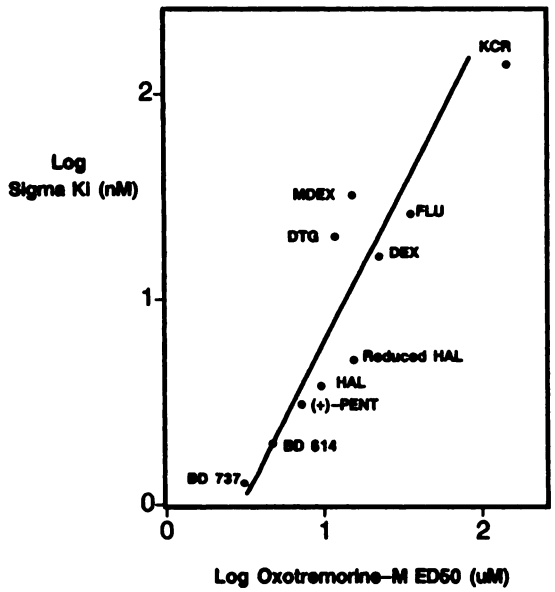


FIG. 9. Correlation between sigma-binding affinity and potency at inhibiting the cholinergic PPI response. Sigma-binding affinity was determined using [^{3}H](+)-3-PPP in guinea pig brain membranes. Similar results are obtained if carbachol (rather than oxotremorine-M) is used to stimulate the response. The least squares correlation coefficient is 0.92. Reprinted from Bowen et al., 1990b. Abbreviations: KCR, (+)-N-cyclopropylmethyl-nordihydrocodeinone; MDEX, 3-methoxydextrallorphan; FLU, fluphenazine; DEX, dextrallorphan; Red. HAL, reduced haloperidol; HAL, haloperidol; (+)-PENT, (+)-pentazocine.

The only sigma compound tested that gave anomalous results was (+)-3-PPP. This compound produced 40% inhibition at concentrations up to 100 μM and produced little additional inhibition at concentrations up to 1 mM (Bowen et al., 1990b). (+)-3-PPP also exhibited anomalous effects in other assays of sigma receptor function, as discussed in more detail below.

Sigma ligands also attenuated the action of norepinephrine via the α_1-adrenergic receptor, but at concentrations 15-fold greater than those needed to affect cholinergic activity (Bowen et al., 1990b). The differential sensitivity of cholinergic and adrenergic stimulated PPI turnover suggests receptor specificity in the effect and argues against actions on a component common to both systems. Consistent with this notion, sigma ligands did not appear to affect incorporation of myoinositol into the inositol phospholipid pool. They also did not affect hydrolysis of inositol trisphosphate, an effect that would decrease accumulation of inositol-1-phosphate, the inositol phosphate monitored in the studies described above (Bowen et al. 1989b). Sigma ligands blocked formation of all three inositol phosphates, suggesting blockade of phospholipase C activation.

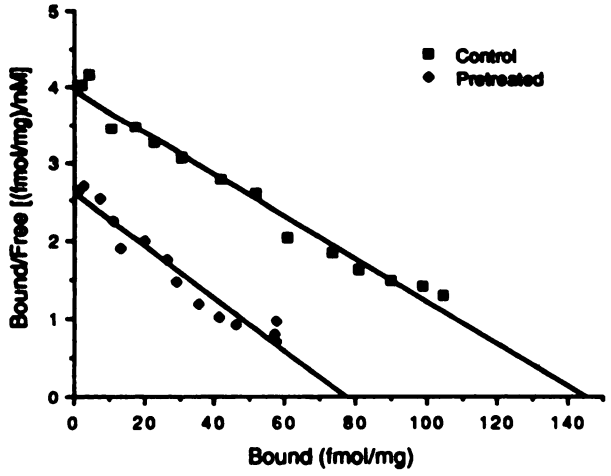
The effects on both cholinergic and adrenergic activity were noncompetitive, with sigma ligands reducing the maximal stimulation (Bowen et al., 1990b). This suggests that the effect does not occur through a simple competitive interaction of sigma ligands with cholinergic or adrenergic receptors. All of the active sigma ligands possess micromolar affinity for cholinergic receptors labeled by [^{3}H]oxotremorine-M. However, direct receptor antagonism was ruled out by a series of pretreatment experiments which demonstrated that sigma inhibition of the cholinergic PPI response could be observed when there was no occupation of cholinergic receptors by a sigma ligand (Bowen et al., 1990b). An interesting observation in the course of these studies was that despite the order of magnitude lower binding affinity of sigma compounds at cholinergic receptors labeled by [^{3}H]oxotremorine-M, the rank order of affinity was very similar to that at sigma receptors labeled by [^{3}H](+)-3-PPP (r = 0.89; Bowen et al., 1990b). This suggests some relationship between sigma and cholinergic receptor topography and deserves further study.

Further investigation of the mechanism of sigma action demonstrated that activation of sigma-binding sites affects cholinergic receptor binding (Bowen et al., 1990b; Bowen and Tolentino, 1990). Pretreatment of synaptoneurosomes with the selective sigma ligand (+)-pentazocine resulted in a decrease in the binding of [^{3}H] oxotremorine-M. This was due to a marked decrease in the B_{max} with no change in the K_d (Bowen et al., 1990b)

(fig. 10). However, the pretreatment did not affect the binding parameters of the membrane-permeant cholinergic ligand [^{3}H]quinuclidinyl benzylate ([^{3}H]QNB) (Bowen and Tolentino, 1990). This suggested that sigma ligands heterologously desensitize the cholinergic PPI response by causing internalization of cholinergic receptors. A mechanism involving receptor internalization is consistent with the observation that sigma ligands decrease the maximal stimulation produced by cholinergic agonists. More work will be needed to verify this and to determine what signal is produced by activation of the sigma receptor.

It should be noted that, despite nanomolar binding affinities at sigma receptors, micromolar concentrations of sigma ligands are required to affect the PPI system. This might, at first glance, be somewhat disturbing. However, it is also true that micromolar concentrations of oxotremorine-M are required to stimulate PPI turnover, despite nanomolar affinity at cholinergic receptors (K_d = 39 nM). This is also true for some other agonists linked to second messenger systems. Consequently, the discrepancy between sigma-binding affinity and effective dose may be indicative of coupling of sigma receptors to a signaling system.

Recently, several neurotransmitters have been shown to inhibit agonist-stimulated PPI turnover in a heterologous manner (Linden and Delahunty, 1989). The sigma receptor may thus be a member of a growing family of receptors that are coupled to negative modulation of the PPI response. It remains to be seen whether sigma receptors affect other PPI-linked receptor systems. The current state of understanding is, however, sufficient to conclude that sigma ligands may produce certain effects in vivo by modulating the efficacy of transmitters that signal through the PPI system. These findings add to the body of evidence that sigma-binding sites are functional entities.



Control: K_d = 39 nM; B_{max} = 151 fmol/mg P

Pretreated: K_d = 32 nM; B_{max} = 78 fmol/mg P

FIG. 10. Reduction in the density of muscarinic receptors in synaptoneurosomes following pretreatment with (+)-pentazocine. Intact synaptoneurosomes were pretreated with 50 μM (+)-pentazocine under conditions identical with those used in PPI assays. After the membranes were washed and resuspended in fresh medium that was free of (+)-pentazocine, [^{3}H]oxotremorine-M binding was examined. Scatchard analysis revealed that pretreatment with (+)-pentazocine markedly decreases the B_{max} of muscarinic binding. The K_d is relatively unaffected by pretreatment with sigma ligands. These findings suggest that sigma ligands affect cholinergic induced PPI turnover by causing internalization of cholinergic receptors. Reprinted from Bowen et al., 1990b.

V. Functions of the Sigma Receptor

The radical modifications of Martin's original hypothesis of sigma opiate receptors (1976) raise many questions about the pharmacology and function of this system. Several questions must be addressed: (*a*) Is the sigma-binding site a true biologically active receptor, an (inactive) acceptor site, or some other entity (e.g., ion channel or enzyme)? (*b*) Assuming that the sigma site is a biologically functional receptor, are the ligands for this site acting as agonists or antagonists? (*c*) What are the functions of the sigma receptor? (*d*) Are some of the actions of antipsychotic drugs that are traditionally ascribed to dopamine receptors mediated instead by sigma receptors?

Until recently, finding answers to these questions has been hampered by the lack of selective ligands for the sigma receptor. For example, conclusions about the functions of the sigma receptor based on the actions of SKF 10,047 must be viewed with caution because it acts at a number of receptors. Consequently, a reexamination of the functions of sigma receptors using more selective ligands has been an important recent thrust.

A. Putative Endogenous Sigma Ligands

To prove beyond any doubt that a binding site is a true receptor requires identification of the neurotransmitter. Although this has not yet occurred, several laboratories have isolated substances from brain extracts that inhibit the binding of sigma radioligands. Contreras et al. (1987a,b) reported on a polypeptide isolated from porcine brain designated β-endopsycosin, and Sonders et al. (1986) reported on a low molecular weight material from bovine brain extracts that inhibits sigma binding. Su and colleagues (1986; 1988) reported on a possible endogenous ligand (molecular weight ~500 Da) from guinea pig brain that inhibits [^{3}H](+)-SKF 10,047 binding but not [^{3}H]PCP binding.

In what may be a crucial discovery, Roman et al. (1989) reported that two endogenous peptides, NPY and peptide YY, have high affinity for rat brain sigma receptors labeled with [^{3}H](+)-SKF 10,1047. In fact, with IC_{50} values of 9.8 and 4.9 nM respectively, these compounds were 2.5–5 times more potent than haloperidol in the rat. The distribution of NPY in rat brain (Allen et al., 1983) corresponds reasonably well with the distribution of sigma receptors in guinea pig (Gundlach et al., 1986;

McLean and Weber, 1988), except in the nucleus accumbens where there is a great deal of NPY but very few sigma sites. NPY may be the most abundant peptide in the central nervous system (Adrian et al., 1983; Tatemoto 1982), and the possibility that it serves as the endogenous ligand for sigma receptors is a very exciting prospect that needs further investigation.

Although they have not identified a chemical structure, Chavkin and coworkers have presented a strong case for the existence of an endogenous sigma ligand in a hippocampal slice preparation maintained in vitro (Neumaier and Chavkin, 1989; Connor and Chavkin, 1990). Depolarization of physiologically intact slices by focal electrical stimulation, veratridine, or potassium reduced the binding of [^{3}H]DTG or [^{3}H](+)-3-PPP in the slice. As shown in fig. 11, the effect was calcium dependent and dissipated over a 90-min period, findings consistent with the investigators' conclusion that depolarization induced the release of an endogenous sigma ligand that then inhibited the binding of sigma radioligands.

Evidence that the inhibition of binding is caused by the release of an endogenous sigma ligand is found in the selective effects of electrically stimulating different regions of the hippocampus. Connor and Chavkin (1990; personal communication) found that stimulation of the mossy fibers or the perforant path produced the strongest reduction in binding. The perforant path and the mossy fibers interact mainly with granule cells in the dentate gyrus and with pyramidal cells in CA1 and CA3, areas that are rich in sigma receptors. By contrast, stimulation of the stratum radiatum or the alvius/oriens regions of CA1, loci where stimulation should not have widespread effects on major fiber pathways in the hippocampus, had little effect. The high degree of specificity observed in these experiments provides solid evidence that the observed effects represent a physiologically significant process.

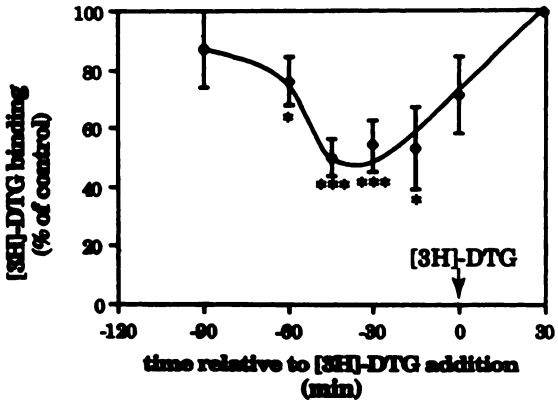


FIG. 11. Time course of depolarization-induced displacement of [^{3}H] DTG binding to hippocampal slices. *Abscissa*, times of depolarization relative [^{3}H]DTG addition (*arrow*); *ordinate*, percentage of control-specific binding (undepolarized slices). The initation of veratridine-induced depolarization was varied, but the period of depolarization was held constant by terminating the action of veratridine with the addition of tetrodotoxin 30 min later. Depolarization induced a transient reduction of [^{3}H]DTG binding suggestive of release of an endogenous ligand that inhibited the binding of the radiolabel. Reprinted from Neumaier and Chavkin (1989) with permission of the authors. *, $P < 0.05$; ***, $P < 0.001$.

In addition to being confined to particular loci within the hippocampus, this response shows considerable pharmacological specificity. Inhibition of sigma binding following perforant path stimulation, but not mossy fiber stimulation, is blocked by CNQX, an antagonist at the kainate and quisqualate excitatory amino acid receptors. The effect of mossy fiber stimulation is blocked by AP4, a selective NMDA receptor antagonist. The different pharmacological susceptibilities of the different pathways in the hippocampus adds further support to the proposed inhibition of binding through release of an endogenous neurotransmitter for the sigma receptor.

An interesting feature of these investigations is the possible significance of the stimulation parameters necessary to produce an inhibition of binding. Chavkin's group found that high frequency stimulation (1-s trains of 50-Hz stimulation, one train every 10 s, 300 μA) was effective in causing up to 50% inhibition of binding after 30 min. However, constant stimulation at the rate of 5 Hz, which produces the same number of pulses over the 30-min stimulation period, was not effective. These findings corroborate reports from a number of laboratories (DeGroat et al., 1984; DeGroat and Kawatani, 1985; Lundberg and Hökfelt, 1986; Lundberg et al., 1986) showing that the rate of stimulation is an important variable in determining whether or not certain neurotransmitters are released. Notably, peptides have consistently required relatively high rates of stimulation for release to occur, raising the speculation that the endogenous sigma ligand may be a peptide. Because NPY is abundant in the hippocampus (Allen et al., 1983), further work with this preparation may reveal whether it is released under these conditions and whether it can affect sigma binding in this preparation.

Although Connor and Chavkin (1990) have cautioned that these data may reflect voltage-induced alterations in receptor conformation leading to changes in binding of the radioligand, there are several reasons why this seems much less likely than inhibition of binding due to release of a neurotransmitter or modulator. As this group has noted, (*a*) depolarization alone does not inhibit sigma binding and (*b*) concentrates of the superfusion medium contain a factor that inhibits sigma binding. Furthermore, the lack of the effect in the presence of tetrodotoxin or in the absence of calcium argues convincingly for an action of an endogenous sigma ligand. These experiments constitute the best evidence to date that an endogenous sigma ligand is secreted by neurons in the central nervous system.

B. Receptor Regulation

Chronic haloperidol treatment in rats differentially affects sigma-binding sites labeled by (+)-opiate-related

and non-opiate-related probes, providing further evidence for the existence of multiple types of sigma-binding sites. Furthermore, these alterations suggest the mode of action of ligands at the sigma receptor and support the biological relevance of sigma receptors.

Although both sites are down-regulated/desensitized by chronic haloperidol treatment, the magnitude and time course of the changes differ. Binding sites labeled by either [^{3}H]DTG or [^{3}H](+)-3-PPP are depressed following 10–21 days of chronic haloperidol treatment (fig. 12) (Bremer et al., 1989; Matsumoto et al., 1989b). However, after longer periods of treatment (up to 60 days), the B_{max} and K_d values compensate to maintain the level of radioligand bound (Matsumoto et al., 1989b).

In contrast to the transient down-regulation/desensitization observed with [^{3}H]DTG and [^{3}H](+)-3-PPP, (+)-opiate binding to sigma sites using [^{3}H](+)-pentazocine as the radiolabeled probe is virtually eliminated with haloperidol. This effect, observed in the same batch of membranes as the [^{3}H]DTG binding, occurs fairly quickly (seen after 5 days of treatment) and is long-lasting (binding is still depressed after 60 days of chronic treatment)(Matsumoto et al., 1989b). A similar elimination of binding to sigma sites labeled by (+)-opiates was observed in mice using [^{3}H](+)-SKF 10,047 as the radioligand (Itzhak and Alerhand, 1989). These results may reflect differences in the regulation of the putative sigma-1 and sigma-2 receptors (table 5), because (+)-opiates would selectively label the sigma-1 site. Alternatively, there may be differential effects on allosterically coupled sites for (+)-benzomorphan- and non-benzomorphan-related ligands (Bowen et al., 1989a).

The ability of haloperidol to down-regulate/desensitize sigma receptors appears to be related to its sigma-binding properties because dopamine receptors were up-regulated in the same animals (Itzhak and Alerhand, 1989; Matsumoto et al., 1989b). Furthermore, PCP receptors were unaffected by chronic haloperidol treatment (Bremer et al., 1989; Itzhak and Alerhand, 1989) and chronic PCP treatment had no effect on binding to sigma receptors labeled by [^{3}H](+)-SKF 10,047 (Itzhak and Alerhand, 1989). Although the molecular mechanisms that underlie these changes are still in question, the selective regulation of the sigma site by its ligands provides further evidence that they are unique physical entities that are physiologically relevant.

The only study to date that appears to contradict the above interpretation of the data involves the apparent up-regulation of sigma receptors following subchronic treatment (up to 5 days) with the sigma ligand, rimcazole (Beart et al., 1989). In the study by Beart et al., subchronic treatment with rimcazole produced a simultaneous increase in B_{max} and K_d of [^{3}H](+)-3-PPP-labeled sites. Because of the 30% increase in B_{max}, the authors referred to the change as up-regulation. However, there was a simultaneous 97% decrease in the affinity of the sites.

C. Anatomical Distribution of Sigma Receptors

Sigma receptors have been labeled and visualized with various radiolabeled ligands using receptor autoradiographic procedures similar to those detailed by Herkenham and Pert (1982) (Aanonsen and Seybold, 1989; Gundlach et al., 1986; McLean and Weber, 1988; Walker et al. 1990; Sircar et al., 1986). As shown in table 7, sigma receptors are unevenly distributed throughout many brain areas. The procedures that have led to the identification of multiple sigma-binding sites have yet to be applied to the autoradiographic procedures; consequently, nothing is known about any differences that may exist between the regional distributions of the subtypes.

1. Cellular localization of sigma binding. As shown in fig. 13, sigma receptors are highly concentrated in gray matter regions, with little binding occurring in white matter areas. Conspicuously high levels are found in many areas populated by large neuronal cell bodies (e.g., supraoptic nucleus, Purkinje cell layer of the cerebellum, pyramidal cell layer of the hippocampus, cranial nerve nuclei, and the red nucleus). The location of sigma receptors relative to neuronal cell bodies was further examined in the hippocampus by Gundlach et al. (1986). They found that quinolinic acid lesions caused a marked loss of binding in the hippocampal pyramidal cell layer, whereas lesions of the entorhinal cortex and lateral septum, major inputs to the hippocampus, had no effect on the density of [^{3}H](+)-3-PPP binding. These results suggest that sigma receptors in this region are localized on pyramidal cells rather than on the terminals of input neurons.

However, some binding may occur on axons or terminals because 6-hydroxydopamine lesions of the corpus striatum caused a loss of sigma binding. Because the caudate is a terminal region for the ascending midbrain dopamine system, the reduced binding (approximately 20%) was attributed to sigma binding to dopamine nerve terminals.

The subcellular localization of sigma receptors has also been investigated (Craviso and Musacchio, 1983a; McCann et al., 1989). Craviso and Musacchio (1983a) showed that [^{3}H]DM binding displaceable by 10 μM unlabeled DM is found in the nuclear, mitochondrial, synaptic plasma membrane, and microsomal fractions of guinea pig brain. However, high affinity [^{3}H]DM binding (presumably synonymous with sigma) is found only in the microsomal fraction. [^{3}H]DM binding in the other fractions was of low affinity and high capacity. Subfractionation of the microsomal fraction showed that [^{3}H]DM binding closely paralleled the distribution of the smooth endoplasmic reticulum marker, the reduced form of nicotinamide adenine dinucleotide-cytochrome c reductase. Similar results were obtained by McCann et al.

TABLE 7

*Regional distribution of binding in guinea pig brain for various sigma radioligands**

Region	Labeling ligand			Ratios		
	(+)-3-PPP (fmol/mg protein)	DTG (fmol/mg tissue)	(+)-Pent (fmol/mg tissue)	DTG/3-PPP	Pent/3-PPP	DTG/Pent
Cortex		88				
Frontal	85		152	1.0	1.8	0.58
Frontoparietal	94					
Pyramidal cell layer	125					
Entorhinal	84		138		1.6	
Cingulate (anterior)	88		205		2.3	
Cingulate (posterior)	190		338		1.8	
Basal ganglia						
Caudate putamen	73	69	191	0.95	2.6	0.36
Nucleus accumbens	70		177		2.5	
Globus pallidus	77		63		.81	
Septal area						
Lateral septal nucleus	130	150	236	1.2	1.8	0.63
Medial septal nucleus	223	198	249	0.89	1.1	0.80
Dorsal diagonal band	182	213	315	1.2	1.7	0.68
Septohypothalamic nucleus	217					
Ventral pallidum	138		206		1.5	
Septofimbrial nucleus	116					
Hippocampal formation						
Stratum pyramidale	144					
Stratum radiatum	46		123		2.7	
Dentate gyrus	126	98	329	0.77	2.6	0.30
Subicilum	144		235		1.6	
Amygdala						
Central nucleus	107		183		1.7	
Medial nucleus	108					
Anterior cortical nucleus	98					
Thalamus						
Habenula	184	220		1.2		
Medial geniculate nucleus	93		76		0.82	
Lateral geniculate nucleus	100	126				
Reticular nucleus	206					
Anteromedial nucleus	123					
Ventral thalamic nucleus	76					
Zona incerta	155	129	323	0.83	2.1	0.4
Hypothalamus						
Anterior hypo. area	182	184		1.0		
Paraventricular nucleus	150	296		2.0		
Lateral preoptic area	161	192		1.2		
Medial preoptic area	221	213	274	0.96	1.2	0.78
Supraoptic nucleus	221	252		1.1		
Mamillary nucleus	107					
Lateral mamillary nucleus	160		399		2.5	
Med. forebrain bundle	134					
Midbrain						
Superior colliculus	159	117	269	0.74	1.7	0.44
Inferior colliculus	118	68		0.58		
Red nerve	291	158	432	0.54	1.5	0.37
Substantia nigra	172	86	279	0.5	1.6	0.31
Interpeduncular nucleus	121	123	279	1.0	2.3	0.44
Lateral tegmental nucleus	182					
Dorsal tegmental nucleus	207	173	601	0.84	2.9	0.29
Anterior pretectal area	325		323		0.99	
Central gray	268	144	476	0.54	1.8	0.30
Dorsal raphe nucleus	251	209		0.83		
Deep mesencephalic nucleus	178		247		1.4	
Cranial nerve nuclei						
Oculomotor nucleus	392	309	590	0.79	1.5	0.52
Motor trigeminal nucleus	523	178	597	0.34	1.1	0.3
Facial nucleus	802	215	541	0.27	0.67	0.4
Medial vestibular nucleus	275		487		1.8	
Superior vestibular nucleus	305					
Nucleus ambiguus	301	349		1.2		
Hypoglossal nucleus	784	279		0.36		

TABLE 7—Continued

Region	Labeling ligand			Ratios		
	(+)-3-PPP (fmol/mg protein)	DTG (fmol/mg tissue)	(+)-Pent (fmol/mg tissue)	DTG/3-PPP	Pent/3-PPP	DTG/Pent
Pons						
Pontine central gray	245					
Pontine reticular nucleus	373					
Pontine area	162					
Reticulotegmental nucleus	376					
Locus coeruleus	241	226		0.94		
Cuneiform nucleus	262					
Gigantocellular ret. nucleus	242					
Paragiganto. ret. nucleus	251					
Ventral parabrachial nucleus	291					
Dorsal parabrachial nucleus	201					
Medulla						
Deep cochlear nucleus	126					
Ventral cochlear nucleus	250		309		1.2	
External cuneate nucleus	319					
Parvocellular ret. nucleus	171					
Cerebellum						
Purkinje layer	330	230	515	0.7	1.6	0.45
Molecular layer	104		173		107	
Granular layer	191	117	319	0.61	1.7	0.37
Nucleus interpositus	134		327		2.4	

* Data from the following sources: [^{3}H](+)-3-PPP from Gundlach et al. (1986); [^{3}H]DTG from McLean and Weber (1988); [^{3}H](+)-pentazocine (Pent) from Walker et al. (1990).

(1989) using [^{3}H](+)-SKF 10,047 as a sigma receptor probe and Wong et al. (1990) using [^{3}H]DTG.

These findings have led to the speculation that sigma sites may represent a type of drug-metabolizing enzyme or other nonreceptor-related protein (McCann et al., 1989). However, although liver microsomes metabolized [^{3}H]DM in the presence of the reduced form of nicotinamide adenine dinucleotide, brain microsomes failed to do so (Craviso and Musacchio, 1983a). Because brain microsomes were shown to metabolize other drugs, these investigators argued that [^{3}H]DM does not bind to a drug-metabolizing enzyme in the brain. Thus, the significance of the microsomal localization of sigma receptors is not clear. It is conceivable that these microsomal sites represent nascent receptors undergoing synthesis or transport, as is believed to be the case with other receptors (Wamsley et al., 1984).

2. Regional distribution of sigma receptors in the central nervous system. McLean and Weber (1988) noted that sigma receptors are concentrated in (*a*) brainstem areas that primarily subserve motor functions, (*b*) certain limbic structures, (*c*) some predominantly sensory areas, and (*d*) brain areas associated with endocrine function. As illustrated in fig. 14, sigma receptors are more concentrated in motor areas than in limbic areas. The distribution in the motor system is marked by the high densities found in brainstem motor circuits. For example, the cerebellum and its closely associated circuits, the red nucleus, inferior olive, and locus coeruleus, are all rich in sigma receptors. Furthermore, sigma binding is found in cranial nerve nuclei that are rich in motor neurons (facial, motor trigeminal, hypoglossal, and oculomotor), as well as in the anterior horn of the spinal cord. These data form one of several lines of evidence for a function of the sigma receptor in motor function.

In the cat, sigma receptors are concentrated in an area of the substantia nigra termed the densocellular zone (Graybiel et al., 1989). This subdivision of the pars compacta projects selectively to regions in the caudate (striosomal compartment) that receive inputs from certain limbic structures such as the amygdala, midline thalamus, and the prefrontal and insular cortex. A concentration of sigma receptors at an interface between the limbic and motor system, together with the motor effects of sigma ligands, suggests that sigma receptors may modulate behavioral responses differently under different affective conditions.

Several limbic structures are labeled by sigma radioligands. These areas include the cingulate cortex, lateral and medial septum, hippocampus, hypothalamus, parts of the limbic thalamus, habenula, and anterodorsal nucleus. The presence of sigma receptors in limbic systems might suggest a role of sigma receptors in emotion and memory.

Sigma receptors are found in certain areas that are clearly related to sensory processing. Most notable among these is the heavy labeling of dorsal root ganglia by [^{3}H](+)-3-PPP (Gundlach et al., 1986). The dorsal lateral geniculate and anterior pretectal areas (associated with visual information processing) are also heavily labeled by [^{3}H]DTG.

Although the brain distribution of sigma receptors is unique, some associations with the distribution of cholinergic neurons (reviewed by Sofroniew et al., 1985) are

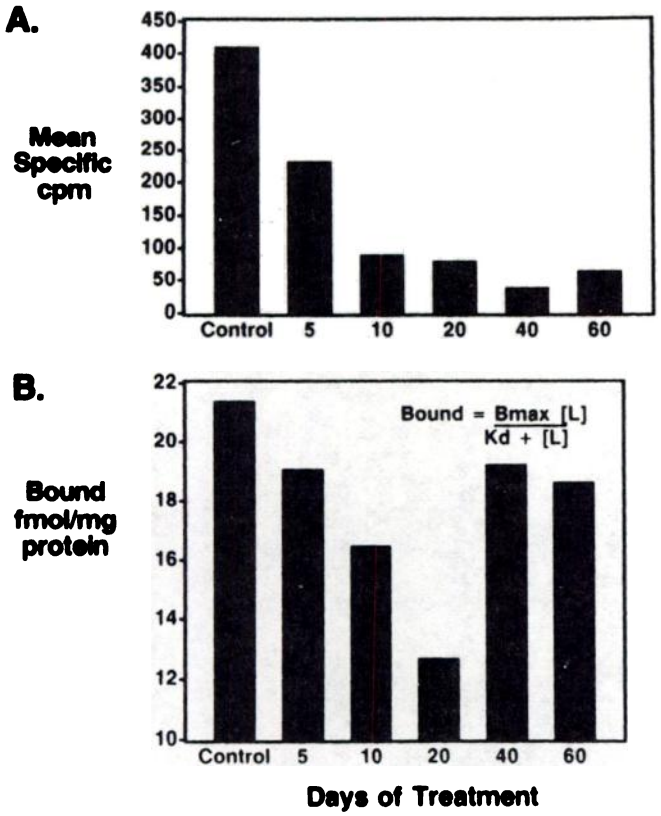


FIG. 12. Down-regulation/desensitization of sigma receptors by chronic administration of haloperidol. Rats were given 5 mg/kg once daily for the times shown. *A*, [^{3}H](+)-pentazocine binding is rapidly and profoundly reduced by chronic administration of haloperidol. Whether the obeserved changes are due to a decreased affinity or number or both was impossible to determine because the level of binding was so low. *B*, Chronic administration of haloperidol produced time-dependent alterations in both affinity and density. In this summary of the data, the binding parameters were used to calculate the amount of ligand bound at a subsaturating concentration of 3 nM. As shown, the changes were all in the direction of down-regulation/desensitization. These data further demonstrate the differences between neuroleptic like compounds (DTG) and (+)-opiates in their interactions with sigma receptors. Data from Matsumoto et al., 1989b, 1990.

notable. For example sigma receptors are rich in cranial nerve motor nuclei, spinal ventral horns, dorsal diagonal band of Broca, and septal region, all of which possess cholinergic neurons. These two receptor systems do not overlap completely, however, because the caudate, which is rich in acetylcholine, has low levels of sigma receptors.

Sigma receptors are found in many areas of the brain associated with endocrine function. The heavy labeling over the supraoptic and paraventricular nuclei within the hypothalamus suggests that sigma receptors participate in the regulation of vasopressin (and/or dynorphin) secretion. Dense labeling was also found in the adenohypophysis (Gundlach et al. 1986; Wolfe et al. 1989), suggesting regulation of anterior pituitary hormones. Using [^{3}H](+)-3-PPP, Jansen et al. (1990) demonstrated high levels of sigma receptors in the rat pineal gland, again linking sigma receptors to endocrine function. The relation of sigma receptors to endocrine function is further supported by the presence of sigma receptors in many peripheral endocrine tissues discussed below.

3. *Species differences in the regional distribution of sigma receptors.* The above discussion focussed mainly on guinea pig because several complete studies have been conducted in that species. However, it is clear from other work that sigma receptors occur in many different species including sea anemone, smooth dogfish, goldfish, frog, turtle, chicken, squirrel monkey, and man (Vu et al., 1990; Weismann et al., 1988). The conservation of sigma receptors across species suggests important functions for this site.

The regional distribution of sigma receptors differs among species to some extent. For example, a comparison of the autoradiographs from rats and guinea pigs reveals markedly greater sigma binding in cerebellum (relative to other areas) in guinea pig compared to rat (Gundlach et al. 1986) . By contrast, compared to the guinea pig, humans apparently have even higher levels of sigma binding in cerebellum relative to other structures, being the most densely labeled structure in humans (Weissman et al., 1988). The human brain is also marked by having relatively high levels of sigma binding in the nucleus accumbens and cortex, a pattern not observed in lower animals (Weissman et al., 1988; Tam and Zhang, 1988).

4. *Distribution of sigma receptors in the periphery.* Sigma receptors are quite rich in many peripheral tissues. Peripheral binding of sigma ligands might have important implications for side effect profiles when considering potential therapeutic actions of sigma drugs. As discussed earlier, the sigma receptors found in some tissues differ in their drug selectivity patterns, raising questions about nomenclature and hypotheses of subtypes. For the present purposes, we will continue with the broad definition.

Wolfe et al. (1989) using [^{3}H]haloperidol, [^{3}H]DTG, and [^{3}H](+)-3-PPP, found sigma-binding sites in the adrenal gland, the testis, and the ovary, with the highest density in the ovary. The reasonably high affinity of progesterone for sigma receptors (Su et al., 1988a) provides further support for this view. The localization of sigma receptors in the endocrine system is consistent with the varied effects of (+)-SKF 10,047 and PCP on endocrine function, especially in light of the failure to detect PCP receptors in endocrine organs with either [^{3}H]TCP or [^{3}H]MK-801 (Wolfe et al., 1989). These findings raise the possibility that some of the endocrine effects of neuroleptics, previously attributed to actions at dopamine D_2 receptors, may be mediated by sigma receptors.

Sigma ligands also bind to tissues of the immune system. Su et al. (1988b) and Wolfe et al. (1988) showed that sigma ligands bind with high affinity to spleen and to human peripheral blood leukocytes. Thus, endogenous or exogenous sigma ligands could play some role in immune responses. The binding of progesterone to sigma

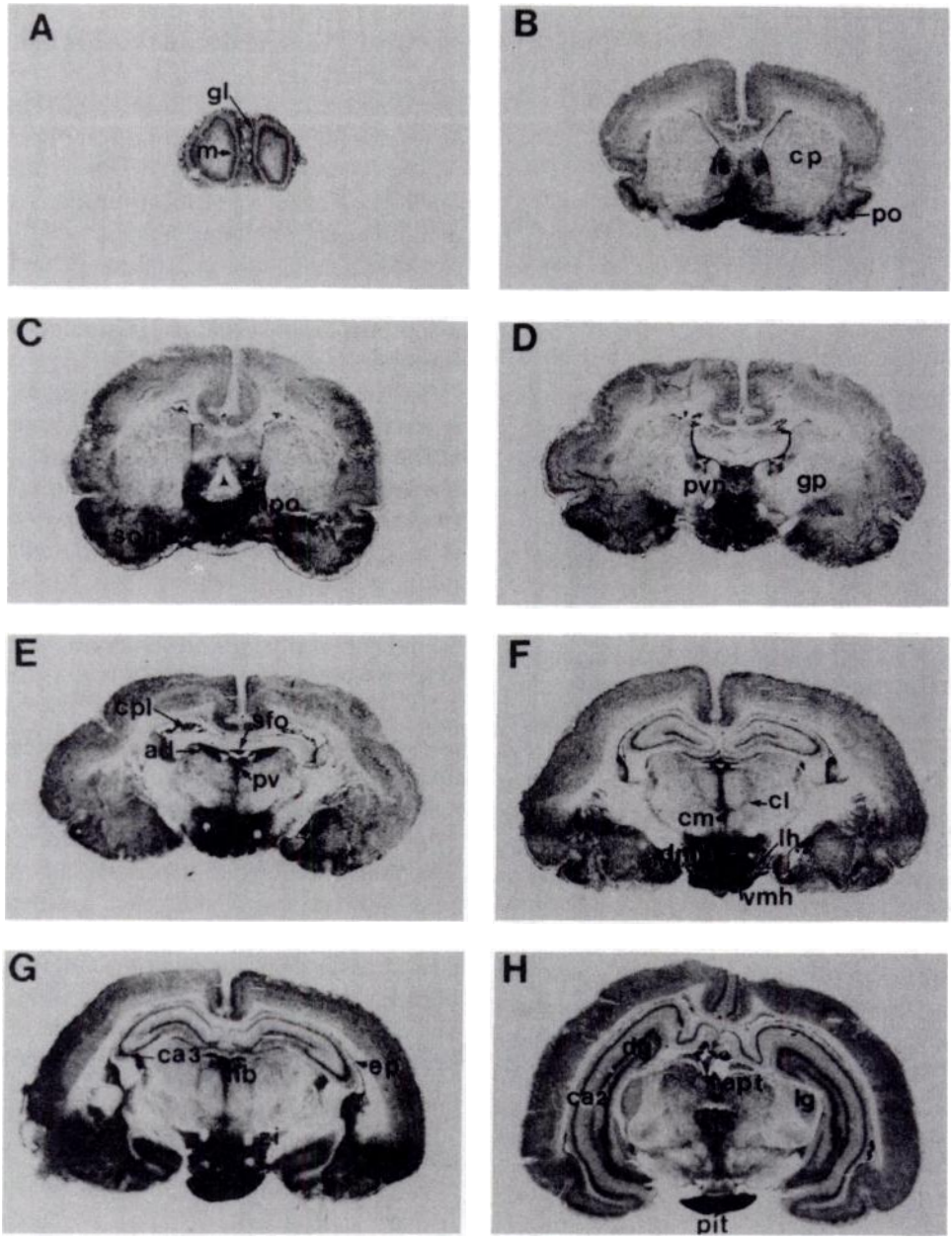


FIG. 13. Autoradiographic distribution of sigma receptors labeled with [^{3}H]DTG in coronal sections of guinea pig brain. Abbeviations: ad, anterodorsal n. thalamus; lg, lateral geniculate; ah, anterior hypothalamus; lh, lateral hypothalamus; apt, anterior pretectal n.; ls, lateral septum; CA2, field CA2 of Ammons horn; m, mitral cell layer; CA3, field CA3 of Ammons horn; mh, medial habenula; cg, central gray; mpo, medial preoptic area; ci, centrolateral thalamic n.; nts, nucleus tractus solitarius; cm, central medial thalamic n.; pit, pituitary gland; cp, caudate putamen; pk, Purkinje cell layer; cpl, choroid plexus; pn, pontine nuclei; db, diagonal band of Broca; po, primary olfactory cortex; dg, dentate gyrus; pv, paraventricular thalamic n.; dm, dorsal motor n. vagus; pvn, paraventricular n. hypothalamus; dmh, dorsomedial n. hypothalamus; rn, red nucleus; dr, dorsal raphe; rs, retrosplenial cortex; dt, dorsal tegmentum; sc, superior colliculus; ep, ependymal cells; sfo, subfornical organ; gl, glomerular layer; sn, substantia nigra; gp, globus pallidus; so, superior olive; hg, hypoglossal n.; v, vestibular n.; ic, inferior colliculus; vmh, ventromedial hypothalamus; io, inferior olive; zi, zona incerta; ipn, interpeduncular n.; 3n, oculomotor n.; lc, locus coeruleus. From McLean and Weber (1988).

receptors of spleen (Su et al., 1988a,b) suggests that hormones might modulate immune functions via sigma receptors. The high concentrations of sigma receptors and absence of PCP receptors in human peripheral blood leukocytes led Wolfe et al. (1988) to suggest that PCP may exert its immunosuppressant effects via interaction with sigma receptors.

Sigma-like binding sites have been detected in both hepatic and renal tissue. Musacchio et al. (1988) investigated these tissues using [^{3}H]DM and Samovilova et al. (1988) studied [^{3}H](+)-SKF 10,047 binding in rat liver membranes. [^{3}H](+)-SKF 10,047 was displaced with a ligand selectivity similar to sigma sites of guinea pig brain labeled with [^{3}H](+)-3-PPP. These results are consistent with the results of Hellewell et al. (1990) when another (+)-benzomorphan probe, [^{3}H](+)-pentazocine,

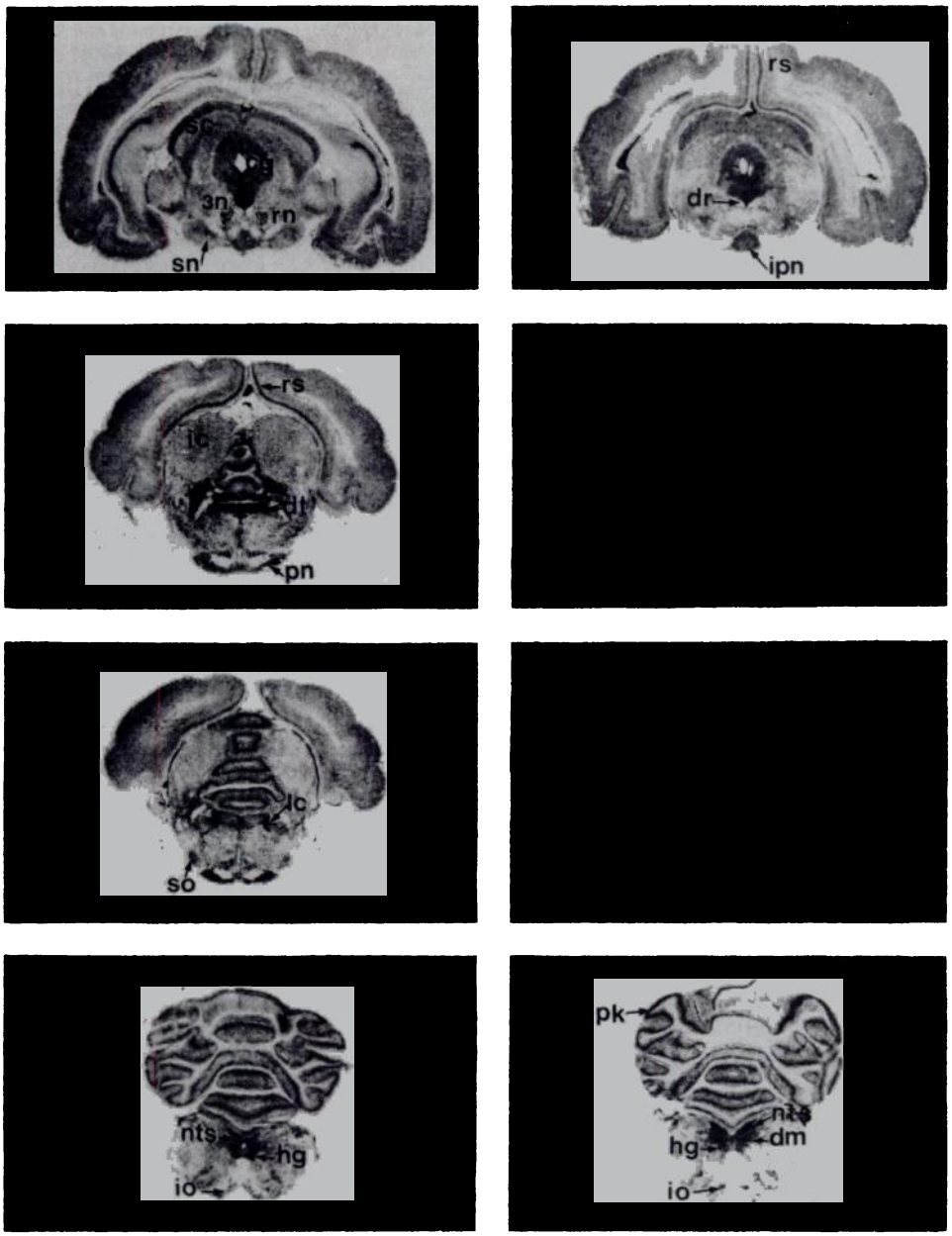


FIG. 13.

was used to label hepatic sites. However, as described above, a different ligand selectivity pattern suggesting the presence of sigma-1 and sigma-2 sites is revealed when either [^{3}H]DTG or [^{3}H](+)-3-PPP is used to label hepatic sigma-like sites (Hellewell et al., 1990).

As mentioned earlier, very high levels of sigma binding occur in the liver as evidenced by the B_{max} of either [^{3}H]DTG or [^{3}H](+)-3-PPP (Hellewell et al., 1990). In fact, the levels are nearly 10 times higher in liver compared to guinea pig brain. Although the function of sigma-like binding sites in liver is unknown, the high levels of binding suggest a role in hepatic physiology. Alternatively, as noted above, these findings may reflect binding of sigma ligands to a metabolic enzyme. Samovilova et al. (1987) reported extraction from porcine liver of a low molecular weight, protease-resistant inhibitor of hepatic [^{3}H](+)-SKF 10,047 binding. Further characterization of this substance may help elucidate this function.

D. Electrophysiological Effects

Although in a number of electrophysiological studies the actions of sigma compounds have been examined, only one study clearly links the electrophysiological changes to a sigma-like binding site. In most of the studies only a limited number of compounds were examined, some of the sigma ligands were nonselective, and most of the preparations contained a plethora of receptor types with which nonselective sigma ligands interact. Although we cannot be certain that sigma receptors mediated the effects observed in these experiments, these studies nonetheless suggest some common properties of sigma compounds.

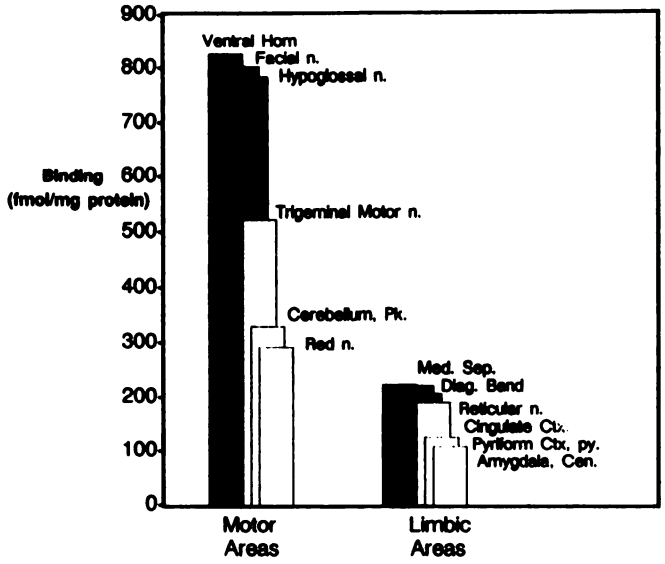


FIG. 14. Autoradiographic distribution of sigma receptors (labeled with [^{3}H](+)-3-PPP) in motor areas compared to limbic areas of the brain. Abbreviations: cerebellum, pk, Purkinje cell layer of cerebellum; cingulate ctx, cingulate cortex; pyriform ctx, py, pyramidal cell layer of pyriform cortex; Med. Sep., medial septum; Diag. Band, dorsal diagonal band of Broca; Amygdala, cen., central nucleus of the amygdala. Data from Largent et al., 1986.

1. Intracellular electrophysiology and effects on ion channels. a. EFFECTS ON NCB-20 CELLS. The only preparation in which the electrophysiological effects of a series of sigma ligands correlated with binding potency is the NCB-20 cell line investigated using whole-cell patch clamp by Bell et al. (1988). These investigators found that a tonic potassium current was blocked by numerous sigma ligands. The potencies of the drugs in producing this effect correlated highly ($r = 0.96$) with their affinities at a sigma-like site in these cells (Wu et al., 1990). However, this is not the typical sigma receptor because the order of potency was haloperidol > BMY 14802 > (−)-pentazocine > (+)-pentazocine > (−)-SKF 10,047 > (−)-3-PPP > (+)-SKF 10,047 > (+)-3-PPP > PCP > TCP. Because the site exhibits a low affinity for (+)-SKF 10,047 (K_d = 6997 nM), and because its stereoselectivity is the reverse of that shown by the sigma receptor as currently defined [(−)-opiates are more potent than (+)-opiates in the NCB-20 cells], it may be identical with the sigma-2 site discussed above (table 5), which shows a similar binding profile. This finding may thus provide the first known function of a newly identified subtype of the sigma receptor.

b. SIGMA RECEPTORS AND CALCIUM CHANNELS. Several lines of evidence suggest a possible relationship of sigma receptors (and/or [^{3}H]DM sites) and calcium channels. Klein et al. (1985) demonstrated inhibition of [^{3}H]DM binding to guinea pig brain membranes by the calcium antagonists prenylamine, cinnarizine, and hydroxyzine with K_i values of 17, 22, and 46 nM, respectively. Dihydropyridine (nifedipine)- and benzothiazepine (diltiazem)-type calcium channel blockers were much less potent. Carpenter et al. (1988) further showed that micromolar concentrations of DM and dextrorphan inhibit potassium-stimulated calcium uptake into rat brain synaptosomes (N-type channels) and PC12 cells (L-type channels). In both systems, DM was more potent than dextrorphan, arguing against the involvement of PCP/NMDA receptors. Furthermore, the concentrations required are higher than would be expected for actions at either high affinity [^{3}H]DM sites or PCP/NMDA receptors, and PC12 cells have been shown to lack PCP receptors (Hellewell and Bowen, 1990; Yang et al., 1989). Inorganic calcium channel blockers such as Li^{2+}, Ni^{2+}, and Cd^{2+} selectively accelerated dissociation of [^{3}H]DTG from binding site 2 (table 6), suggesting an association of site 2 with calcium channels (Rothman et al., 1990). Taken together, these results suggest a possible link between sigma-like sites with low affinity for (+)-opiates and modulation of calcium channels. These results are particularly interesting in view of the existence in PC12 cells (Hellewell and Bowen, 1990) and brain (Reid et al., 1988) of sigma-like sites with low affinity for (+)-opiates (tables 4 and 6). This warrants further investigation with other sigma ligands.

c. CONDUCTANCE CHANGES NOT CLEARLY RELATED TO SIGMA BINDING. Using intracellular recordings, Galligan et al. (1989) found that DTG and (+)-SKF 10,047 inhibited acetylcholine-induced depolarization of guinea pig myenteric neurons. However, several structurally related compounds with no sigma-binding affinity were equipotent. Therefore, it is unclear at this time whether DTG and (+)-SKF 10,047 acted through a non-sigma mechanism or whether the other compounds produced a similar effect through an independent mechanism.

Similarly, Bobker et al. (1989) found that DTG and (+)-3-PPP inhibited ligand-activated hyperpolarizations in three separate preparations: the guinea pig submucosal plexus, the dorsal raphe, and the locus coeruleus. However, haloperidol, which has high affinity for sigma receptors, was inactive. The authors thus questioned a sigma-mediated mechanism but also raised the possibility that the lack of effect of haloperidol may be related to its affinity for other receptor types [e.g., dopaminergic and adrenergic sites (Seeman 1981; Peroutka et al. 1977)].

An interaction between sigma ligands and certain types of potassium channels is also suggested by the ability of sigma ligands to inhibit potassium-stimulated efflux of rubidium from rat cortical synaptosomes, an effect that is thought to involve potassium channels (Fletcher et al., 1989). Kennedy and Henderson (1989a) also described an inhibition of two potassium currents (the M current and a fast, calcium-activated potassium current) in voltage clamp studies in the mouse hypogastric ganglion.

2. Effects of sigma ligands on the firing of neurons in the red nucleus and cerebellum. Iontophoretic application

of sigma ligands onto neurons in the red nucleus inhibits the firing of these cells (Matsumoto and Walker, 1988a,b; fig. 15). Both DTG and (+)-pentazocine inhibit the firing of rubral neurons in a dose-dependent and reversible manner. These actions occur in the absence of local anesthetic effects (however, see Malouf et al. 1988) and are more frequent in the red nucleus than in the surrounding reticular formation (an area containing a lower density of sigma-binding sites). As in behavioral studies, (+)-3-PPP apparently acts in the red nucleus, at least in part, through an unidentified non-sigma mechanism (Matsumoto et al., 1990; Matsumoto and Walker, 1988b). However, the inhibitory actions of DTG and (+)-pentazocine in the red nucleus may be mediated through sigma receptors because the ligands are relatively selective for sigma-binding sites, the red nucleus is virtually devoid of the receptors with which nonselective sigma ligands interact, and the compounds are more efficacious in areas containing a higher density of sigma-binding sites. However, it must be recognized that not enough compounds have been tested to establish the role of sigma receptors in these effects.

Similarly, application of sigma ligands in the cerebellum tends to inhibit the firing of these neurons. Micropressure ejection of the relatively selective sigma ligand, DTG, onto Purkinje cells inhibits the firing of these neurons in a dose-dependent and reversible manner (Kim et al., 1989). This effect appears to rely on the presence of endogenous norepinephrine because destruction of noradrenergic terminals with 6-hydroxydopamine reduces the efficacy of DTG (Kim et al., 1989). Although a connection to sigma receptors cannot be clearly established from an examination of a single drug, these findings are suggestive of possible sigma receptor function in the cerebellum.

3. Effects of sigma ligands on midbrain dopamine neurons. The effects of sigma ligands on midbrain dopamine neurons have been the focus of numerous studies. Intravenous application of DTG, (+)-pentazocine, and (+)-3-PPP all inhibit A9 dopamine neurons (Steinfels and Tam, 1988; Steinfels et al., 1989; Clark et al., 1985a). Iontophoretic application of (+)-pentazocine and (+)-3-PPP onto these cells during intracellular and extracellular recordings suggest that at least some of these effects are due to direct actions on dopamine neurons (Clark et al., 1985a; Steinfels et al., 1989).

The effects of BMY 14802 and rimcazole on midbrain dopamine neurons are considerably more difficult to interpret because these compounds are nonspecific and because many different receptor types are found in the A9 and A10 region of the brain. In contrast to the inhibitory effects of DTG, (+)-pentazocine, and (+)-3-PPP, intravenous application of the BMY 14802 increases the firing rate of A9 dopamine neurons (Steinfels and Tam, 1988). Furthermore, BMY 14802 reversed the inhibition produced by (+)-3-PPP (Steinfels and Tam, 1988), although the effects of BMY 14802 do not appear to result from a direct interaction with dopamine receptors (Wachtel and White, 1988). Whether the effects of BMY 14802 are due to actions at sigma receptors, however, remains unresolved.

Although the effects of rimcazole, another atypical antipsychotic with affinity for sigma receptors, have been tested by a number of laboratories, the data are difficult

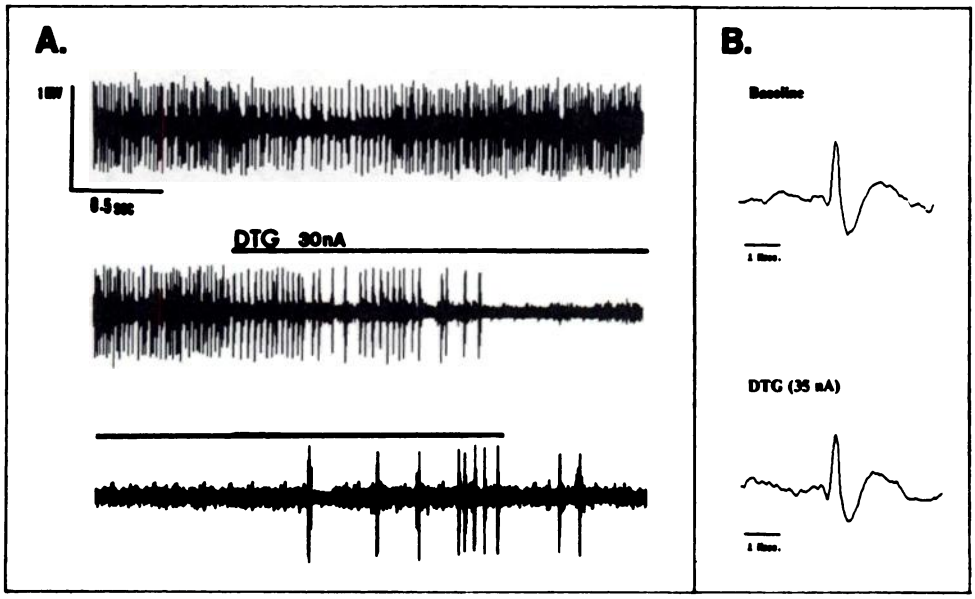


FIG. 15. Inhibition of firing of a red nucleus neuron by iontophoretic application of the sigma ligand DTG. *A*, Action potentials of a rubral neuron before, during, and immediately after an application of DTG. *B*, Expanded record of action potentials from a rubral neuron before drug application (*top*) and during a period when DTG produced a 37% inhibition of firing rate compared to baseline values. These data indicated that DTG inhibits the firing of rubral neurons without visible effects on the amplitude or duration of the action potentials. Reprinted from Matsumoto and Walker, 1988a.

to interpret. Acute and chronic administration of rimcazole preferentially alters the spontaneous firing rate of A10 over A9 cells (Piontek and Wang, 1986). However, intravenous administration of rimcazole has no consistent effects on the firing rate of A9 dopamine neurons and fails to alter apomorphine-induced inhibitions (Piontek and Wang, 1986; Steinfels and Tam, 1988). The compound has also been reported to antagonize the effects of (+)-SKF 10,047 on A10 neurons, but careful examination of the dose-response curves suggests that rimcazole is a partial agonist/antagonist (Ceci et al., 1988). Taken together with the relatively weak affinity of rimcazole for sigma receptors, it is difficult at this time to evaluate which of the observed effects may have been mediated through sigma receptors.

Although more studies are needed before the electrophysiological effects described above can be attributed to actions at sigma receptors, two general trends occur: (*a*) local application of sigma ligands predominantly inhibits the firing of spontaneously active neurons and (*b*) in vitro studies sometimes reveal inactivation of potassium conductances. These trends pose an apparent contradiction because inactivation of potassium conductances would result in cellular excitation, not cellular inhibition. It appears that the differences between these sets of findings coincides with whether the data were obtained from in vivo or in vitro preparations. The alterations in potassium conductances are observed in vitro, where the ability of sigma ligands to modulate ongoing actions of classical neurotransmitters is minimized. By contrast, the in vivo inhibitions occur in preparations that show tonic activity from active excitatory inputs. Conceivably, sigma ligands negatively modulate excitatory neurotransmitters, in the same manner seen biochemically with cholinergic ligands. If so, further studies using modeling approaches coupled with extracellular recording and intracellular studies of interactions between sigma ligands and excitatory neurotransmitters may be fruitful.

E. Peripheral Nervous System Actions

1. Sigma actions on the guinea pig ileum. In what is perhaps the most clearly defined sigma action on peripheral tissues, Campbell et al. (1989) showed that sigma ligands block electrically or 5HT-induced contractions of the isolated guinea pig ileum/myenteric plexus preparation. As shown in figs. 16 and 17, these effects were concentration dependent, highly correlated with sigma-binding affinity, and appeared to be due to inhibition of the stimulated release of acetylcholine from the preparation. Many compounds from several different chemical classes were tested in this assay and a series of very strong correlations between sigma binding and efficacy within chemical classes were found. However, several compounds were well off the regression line, including (+)- and (−)-3-PPP and chlorpromazine. As a result, the overall correlation using least squares linear regression was not particularly impressive (with all compounds r = 0.37). However, the correlation value from a Spearman rank correlation, which is less affected by extremes in the distribution of data, was close to 0.7. Not surprisingly, if the outliers are omitted the correlation is very close to unity. These data thus provide support for the notion that sigma receptors modulate cholinergic neurotransmission in the guinea pig ileum. The weak actions of (+)-3-PPP in this system are difficult to explain but coincide with a similar lack of efficacy in the modulation

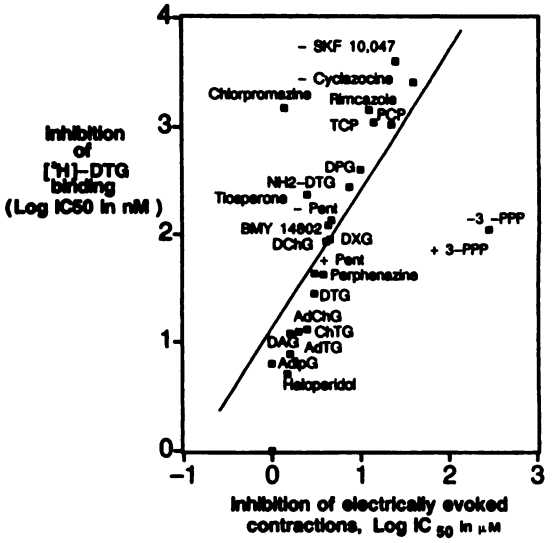


FIG. 16. Correlation between sigma-binding potency (measured in guinea pig brain using [³H]DTG) and potency in inhibiting electrically evoked contractions of the guinea pig ileum. A high correlation between sigma-binding affinity and potency in this bioassay is found with the exception of (+)- and (−)-3-PPP and chlorpromazine. Abbreviations: +pent, (+)-pentazocine; DXG, N,N-di(2,6-methiphenyl)guanidine; DChG, N,N′-(dicyclohexyl)guanidine; AdChG, 5,N-(cyclohexyl)-N′-(adamant-1-yl)guanidine. Data from Campbell et al., 1989.

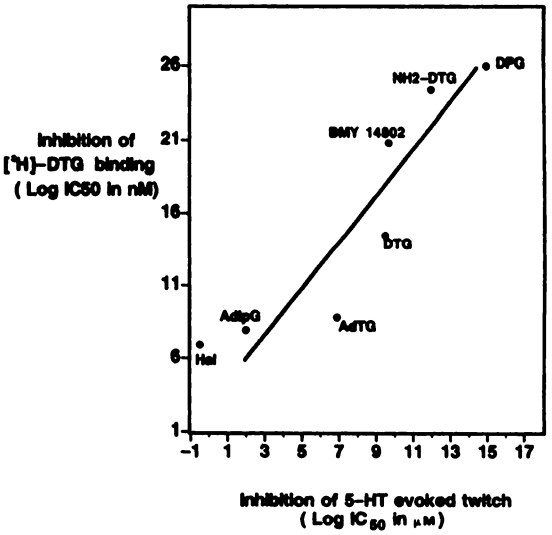


FIG. 17. Correlation between sigma-binding potency (measured in guinea pig brain using [³H]DTG) and potency in inhibiting serotonin-evoked contractions of the guinea pig ileum. As with the electrically evoked twitch, inhibitory potency in the bioassay is highly correlated with sigma-binding potency. Abbreviations: Hal, haloperidol. Data from Campbell et al., 1989.

of PPI turnover (Bowen et al., 1990b), and in the production of postural changes following rubral microinjections (Matsumoto et al., 1990).

2. Sigma actions on other peripheral tissues. In a separate study, Campbell et al. (1987) reported that (+)-3-PPP potentiates electrically induced contractions of the mouse vas deferens by enhancing release of norepinephrine from the preparation. Kennedy and Henderson (1989b) observed a similar potentiation but only in the presence of sulpiride which was needed to block an inhibitory effect of (+)-3-PPP alone. Other sigma ligands that had this effect included (+)-SKF 10,047 and haloperidol. However, DTG produced only inhibitory effects, and these required high doses. Kennedy and Henderson (1989b) noted that, although these effects may reflect a sigma action, the surprising lack of action of DTG raises questions about the mechanism.

A site showing the typical sigma-binding profile is found in the guinea pig vas deferens (Su and Wu, 1990; Vaupel and Su, 1987). In this tissue, certain sigma ligands potentiate electrically stimulated contractions much as they do in the rat. However, the stereoselectivity is the reverse of that seen in binding assays, i.e., (−)-isomers of the opiates are more potent than (+)-isomers. This resembles the low affinity binding site discussed by Hellewell and Bowen (1990) and the electrophysiological effects found in NCB20 cells, which also show this property. However, it is the reverse of the binding profile found in this tissue.

In summary, it appears that sigma receptors mediate the inhibition of electrically and 5HT-stimulated contractions of the guinea pig ileum. In other peripheral tissues, some sigma ligands have effects, but the actions are not entirely consistent with actions at the high affinity site described by Tam (1983; 1984) and Su (1982). It is clear from the binding data described above that more than one sigma-binding site exists, and it may be that these peripheral tissue preparations will provide the means to understand the physiological actions of sigma ligands at the subtypes of the receptor.

F. Role of Sigma Receptors in the Central Nervous System

1. Glucose utilization. In a single study the effects on glucose utilization of ligands that bind to sigma receptors were examined. Puppa and London (1989) investigated regional glucose utilization following injection of a single dose of (+)-pentazocine, BMY 14802, and rimcazole. Extensive pharmacological characterization of the effects are lacking, and the latter two of these ligands have certain limitations discussed above. Nevertheless, the marked overlap between anatomical distribution of the changes and the distribution of sigma receptors supports the investigators' conclusion that occupation of sigma receptors leads to changes in glucose utilization. Furthermore, the observation that (+)-pentazocine produced milder effects than BMY 14802 is consistent with the tendency for neuroleptic like compounds to possess greater potency than (+)-opiates in rats. Significant alterations in glucose utilization were observed in cerebellum, hippocampus, paraventricular hypothalamic nucleus, some cranial nerve nuclei, and several other sigma-rich areas. However, certain areas that are rich in sigma receptors, such as the oculomotor nucleus, supraoptic nucleus, and locus coeruleus, did not exhibit significant changes in glucose utilization. Although more work is needed to firmly establish a role of sigma receptors in these effects, the findings are important because they imply a physiological function for sigma receptors in the central nervous system and point to anatomical loci that may be good candidates for future investigations of the physiological and behavioral effects of sigma ligands.

2. Sigma ligands as discriminative stimuli. Holtzman (1989) found that animals trained to discriminate DTG from saline, generalized to a variety of ligands [including certain (+)- and (−)-opiates and PCP-like drugs]. However, these animals failed to generalize to (−)-butaclamol or to haloperidol, both of which bind potently to sigma receptors. Steinfels et al. (1988) found that (+)-pentazocine-trained animals generalized (+)-SKF 10,047. However, they also generalized to PCP, although the effect was incomplete. Balster (1989) found that animals trained to discriminate (+)-SKF 10,047 from saline generalize well to PCP-related compounds but not at all to the sigma compounds DTG, (+)-ketocyclazocine, (−)-butaclamol, and haloperidol. This finding was recently replicated by Singh et al. (1990). It is perhaps not surprising that the pattern of results from these studies is not particularly clear, because in every case it was necessary to use drugs that bind to several different types of receptors. Thus, the failure of haloperidol to generalize to (+)-pentazocine is not surprising, because it binds to certain classes of dopamine, adrenergic, and serotonergic receptors in addition to sigma receptors (Seeman, 1981). Likewise, (+)-SKF 10,047, which potently binds to PCP receptors should not be expected to display a particularly sigma-like profile. It appears that definite conclusions regarding the stimulus properties of the sigma receptor may require the development of more selective agonists and antagonists.

3. Open field behavior. Several attempts to identify sigma-mediated behaviors have been published in recent years (Contreras et al, 1988b; Iwamoto 1989). These studies were not conducted with enough compounds to produce the correlations needed to provide a reasonable connection to the sigma receptor. However, they did produce some interesting findings. Contreras et al. (1988b) reported that DTG (ED_{50} = 55 nmol, intracerebroventricularly) produced a pattern of stereotyped behavior and ataxia that was indistinguishable from that produced by PCP and MK-801, a drug that binds selectively to the PCP/NMDA receptor complex. Because these behaviors have traditionally been related to the

PCP receptor, it is tempting to conclude that DTG produced some effects through the PCP site. However, these results are more difficult to interpret because DTG was the most potent of the three drugs, although the weakest at the PCP site. Together with the drug discrimination data, these data may indicate similar actions through distinct PCP and sigma sites. More work is needed to clarify the nature of these effects.

Iwamoto (1989) hypothesized a "sigma syndrome" that occurs following sensitization of animals by five daily systemic injections of the opiate (−)-SKF 10,047. An injection of (+)-butaclamol in these animals results in a marked increase in locomotor activation, sideways circling, and backward walking. These effects are antagonized by compounds with sigma affinity, hypothesized by Iwamoto to be sigma antagonists (rimcazole, (+/−)-BMY 14802, and haloperidol) but not sulpiride, a dopamine D_2 antagonist lacking activity at the other receptors having affinity for haloperidol. A more detailed pharmacological analysis of this phenomen should reveal whether, in fact, the sigma system becomes hyperactive in (−)-SKF 10,047-treated animals.

Anatomical studies revealed high levels of sigma binding in the PAG (Gundlach et al., 1986; McLean and Weber, 1988), an area involved in defensive behaviors. One function of the circuitry in the PAG is to regulate startle responses to strong acoustic stimuli (Davis, 1989), presumably through its connections to the nucleus of the lateral lemniscus (part of the primary acoustic circuitry; Davis et al., 1982). Recent data suggest that sigma receptors are part of this modulatory circuit.

The role of sigma receptors in this circuit was investigated by presenting powerful acoustic stimuli (115 db, 3840 Hz) to rats and quantifying the resulting startle response. Microinjections of both DTG and (+)-pentazocine into the PAG markedly amplified responses to these tone bursts (Jones and Walker, unpublished observations). Clearly, more work is necessary to establish whether these responses are mediated by sigma receptors, because only two compounds have been tested. If an unequivocal role of sigma receptors can be established in this paradigm, it would suggest that sigma receptors play a role in defensive behaviors. In addition to startle, the PAG is involved in a number of behaviors related to acoustic startle, including pain, fear, escape behavior, freezing behavior, and defensive behavior (Liebman et al., 1970; Jacquet and Lajtha, 1974; Jacquet et al., 1977; Edwards and Adams, 1974)—behaviors that share the property of being responses to threat. Hence, although microinjections of sigma ligands into the PAG do not appear to alter pain sensitivity (Matsumoto and Walker, unpublished data), more work may establish a role in defensive behavior evidenced by the modulation of startle responses.

4. Role of sigma receptors in posture and movement. Anatomical and physiological evidence argue for important functions of sigma receptors in the regulation of movement and posture. The highest concentrations of sigma receptors in the brain occur in areas that are intimately linked to movement: the cerebellum, red nucleus, superior colliculus, spinal ventral horn, and various cranial nerve nuclei (facial, hypoglossal, trigeminal motor oculomotor, trochlear, and abducens; Gundlach et al., 1986; Largent et al., 1984; McLean and Weber, 1988). The locus coeruleus, which influences cerebellar function, is one of the most heavily labeled hindbrain structures (Gundlach et al., 1986; McLean and Weber, 1988). Lower levels of sigma binding occur in the substantia nigra and striatum (Graybiel et al., 1989; Gundlach et al., 1986). It follows from this distribution that sigma receptors would influence the motor system, a notion supported by physiological studies.

a. MOTOR ACTIONS OF SIGMA LIGANDS IN THE RED NUCLEUS. The red nucleus has proven to be a fruitful site for investigating the potential role of sigma receptors in motor control, because it contains high concentrations of sigma receptors but very low densities of other receptors that have affinity for some sigma ligands (dopamine, opiate, PCP, 5HT, or α-adrenergic receptors; Boyson et al., 1986; Pazos and Palacios, 1985; Quirion et al., 1981; Sircar and Zukin, 1988). As shown in fig. 18, microinjections of a variety of sigma ligands in the red nucleus, including (+)-SKF10,047, (+)-pentazocine, BD614, dextrallorphan, DTG, and haloperidol, resulted in quantifiable dystonia (torticollis) in rats, whereas non-sigma controls, such as (+)-nordihydrocodeinone [a structural homolog of (+)-opiate sigma ligands with low affinity for sigma receptors] did not (Matsumoto et al., 1990). Similarly, a series of ligands for other receptors that are weak or inactive at sigma receptors failed to produce dystonia. The list includes the dopamine D_1 antagonist SCH 23390,

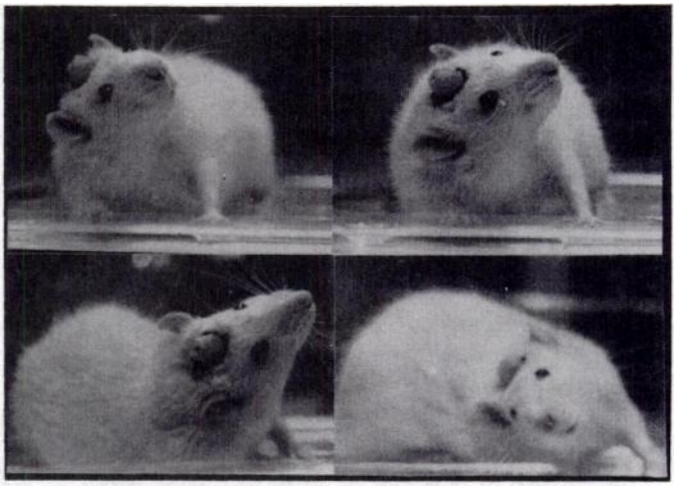


FIG. 18. Appearance of a rat at various times following microinjection of DTG (and other sigma ligands) in the red nucleus. Times after injection: *top left*, 1 min; *top right*, 5 min; *bottom left*, 15 min; *bottom right*, 30 min. The eye ipsilateral to the injections site faces upward; the limbs are affected as well especially in the later periods. The effect dissipates within 90 min. From Walker et al., 1988.

the dopamine D_2 antagonist (−)-sulpiride, the $5HT_{1a}$ agonist 8-hydroxydipropylaminotetralin, PCP, and the atypical antipsychotic clozapine (Matsumoto et al., 1990; Walker et al., 1988).

As illustrated in fig. 19, sigma receptor-binding affinity (defined by [^{3}H]DTG binding in rat brain) correlated highly with potency in this behavioral assay, for those compounds that had sufficient activity to derive an ED_{50}. This highly significant correlation ($r = 0.94$) suggested that sigma receptors mediate the dystonic actions of these ligands in the red nucleus. The only compound with high binding affinity that failed to produce consistent effects in this system was (+)-3-PPP, a compound that also lacked efficacy in other assays in which high correlations were found between sigma binding and potency, i.e., the guinea pig ileum and PPI turnover (Bowen et al., 1990b; Campbell et al., 1989). These findings thus support the biological relevance of sigma-binding sites and suggest the same mode of action at the receptor for the compounds tested. These data are also consistent with sigma actions in the guinea pig ileum and PPI turnover where haloperidol, DTG, and the (+)-opiates all had similar actions, presumably acting as agonists.

Further support for the notion that sigma receptors mediate the effects observed in these experiments stem from developmental studies of this phenomenon. Sigma-binding parameters were determined in young adult rats (2–3 months old) and middle-aged rats (5–6 months old). Scatchard analyses revealed that the density and affinity of receptors labeled with [^{3}H]DTG was markedly greater in the young adult rats (Matsumoto et al., 1989a). A similar pattern of differences in behavioral sensitivity to sigma ligands was observed. Compared to older animals, young adult rats showed an approximately 300% greater alteration in head angle following microinjection of DTG into the red nucleus and, again, an approximately 300% greater circling response following microinjection into the substantia nigra (Matsumoto et al., 1989a). Hence, the greater number and affinity of sigma receptors in younger rats is consistent with the greater physiological effects of sigma ligands in these animals.

These age-related differences were further examined in younger rats by Hemstreet et al. (1990). Male rats between the ages of 30 and 90 days showed marked variations in sigma binding characterized by a U-shaped function with a nadir at approximately 75 days. As shown in fig. 20, a similar pattern of responsiveness to rubral microinjections of DTG in littermates resulted in a correlation of 0.87 between sigma binding at various ages and the potency of DTG in producing dystonia. Thus, the extent of dystonia correlated not only with the potency of the drug for sigma receptors but also with the sensitivity of the receptors at different developmental stages.

It appears that the drugs microinjected into the red nucleus had direct effects on this structure, because iontophoretic application of sigma ligands inhibits these cells. The alterations in posture produced by the sigma-mediated inhibition of rubral neurons are not particularly surprising because other means of inhibiting rubral neurons also cause postural changes. For example, transient torticollis is produced by rubral microinjections of

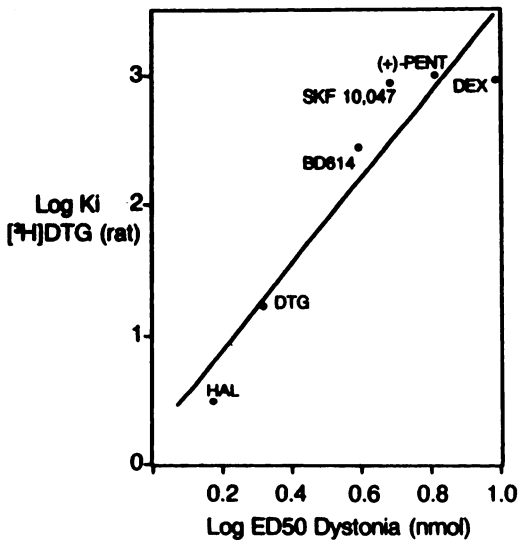


FIG. 19. Relationship between potency in displacing [^{3}H]DTG from rat brain membranes and ED_{50} in producing torticollis following rubral microinjections. The significant correlation ($r = 0.94$) between binding affinity and potency in this test suggests that sigma receptors may mediate the actions of these compounds. Abbreviations: (+)-PENT, (+)-pentazocine; DEX, dextrallorphan; HAL, haloperidol. Reprinted from Matsumoto et al., 1990.

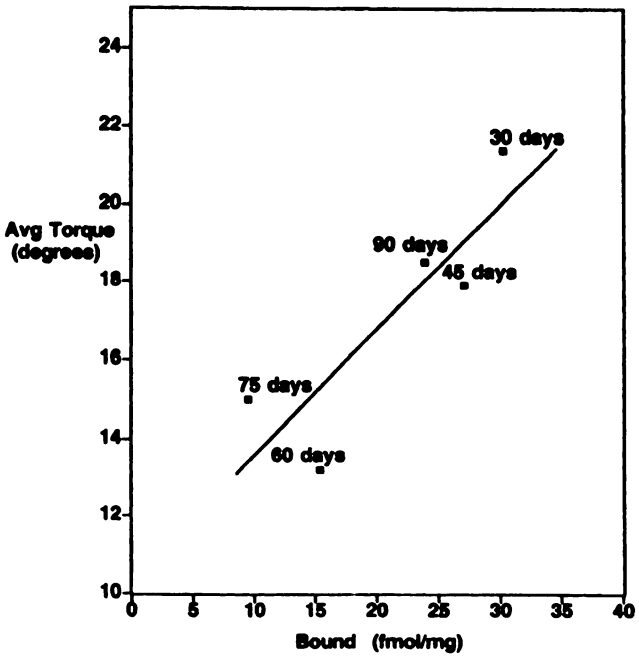


FIG. 20. Relationship between specifically bound [^{3}H]DTG membranes from rats of various ages and the degree of torticollis produced by a microinjection of DTG in the red nucleus. Higher levels of sigma binding at various ages correlated highly with efficacy of DTG in producing the behavioral effect; $r = 0.87$. Data from Hemstreet et al., 1990.

the inhibitory γ-aminobutyric acid mimetic muscimol (Matsumoto and Walker, unpublished observations), and a similar although long-lasting change in head posture was induced in primates with rubral lesions (Battista et al., 1976; Carpenter, 1956). These findings indicate that inhibition of the red nucleus, either permanently (by lesion) or temporarily (by microinjecting inhibitory substances), leads to changes in the motor system. In fact, experiments dating back to the time of Sherrington have suggested that an important function of the red nucleus is the maintenance of muscle tone (Massion et al., 1967; Ingram et al., 1934).

Sigma receptors on neurons in the magnocellular division of the red nucleus appear to mediate the effects of sigma drugs on posture. This area receives its major input from the nucleus interpositus of the cerebellum (Dekker, 1981; Massion, 1967). It affects movement through the efferent projection of the rubrospinal tract to the intermediate levels of the spinal cord, the nucleus interpositus, and the lateral reticular nucleus (Brown, 1974; Flumerfelt and Hrycyshyn, 1985; Massion, 1967; Robinson et al., 1987). Although current investigators have stressed the role of the red nucleus in the regulation of distal and proximal musculature (Ghez, 1975; Gibson et al., 1985a,b; Kennedy, 1987; McCurdy et al., 1987), it also influences axial structures, e.g., the neck muscles, through rubrospinal projections to the cervical cord (Huisman et al., 1981). The anatomical, electrophysiological, and behavioral evidence thus suggest that sigma receptors in the red nucleus function as part of a circuit that regulates muscle tone.

b. MOTOR ACTIONS OF SIGMA LIGANDS IN THE SUBSTANTIA NIGRA. This presence of sigma receptors in the substantia nigra also suggests their role in voluntary movement. This possibility has been confirmed in behavioral experiments, which showed that sigma ligands act in the nigra to increase motor activity (Goldstein et al., 1989). Nigral microinjections of (+)-pentazocine and DTG induced significant contralateral circling, yet this effect did not occur in animals with 6-hydroxydopamine lesions of ascending dopamine tracts (Goldstein et al., 1989). It thus appeared that the circling behavior was mediated mainly by sigma-stimulated release of dopamine.

c. DOPAMINE-RELEASING ACTION OF SIGMA LIGANDS. Biochemical data also suggest that sigma ligands induce the release of dopamine from central neurons. Berkowitz (1974) found that (+)-pentazocine decreases brain levels of dopamine in animals treated with the dopamine synthesis inhibitor α-methyl-tyrosine. Because this effect cannot be attributed to decreased synthesis, a dopamine-releasing action appears likely. This interpretation receives support from the finding that unilateral microinjections of (+)-pentazocine in the substantia nigra produce circling behavior that is reversed by destruction of the nigrostriatal dopamine system with 6-hydroxydopamine (Goldstein et al., 1989).

BMY 14802 was also found to decrease dopamine levels in rats and to increase the levels of the dopamine metabolite 3,4 dihydroxyphenylacedic acid (Taylor et al., 1990). These findings suggest that it caused the release of dopamine, although an alteration in synthesis cannot be ruled out from these observations.

(+)-3-PPP nearly doubled the rate of spontaneous release of [^{3}H]dopamine from preloaded striatal slices (Arbilla and Langer, 1984). In contrast, it slightly reduced the release of dopamine induced by electrical stimulation (Arbilla and Langer, 1984). These findings were originally interpreted as reflecting a dopamine autoreceptor agonist action of (+)-3-PPP. However, because (+)-3-PPP is approximately 150 times more potent at sigma receptors than at dopamine receptors, a sigma action in these assays is certainly possible. Some support for this notion stems from the weak effects of (−)-sulpiride in this experiment, because this compound is a potent dopamine D_2 antagonist lacking sigma activity.

In the striatal slice preparation described above, it has been found that the spontaneous release of dopamine is reduced by the presence of tetrodotoxin or the absence of calcium (Dismukes and Mulder, 1977; Giorguieff et al., 1977). These findings were surprising because they suggest an action potential-stimulated release in a preparation free of external stimulation (Starke, 1978). This, together with the inhibitory electrophysiological effects of sigma compounds and the inhibition of electrically evoked dopamine release by (+)-3-PPP, raises many questions about the basis for these effects.

d. THE (+)-3-PPP ENIGMA. The effects discussed above typify the confusing effects of (+)-3-PPP. Apart from its behavior in binding assays, the effects of (+)-3-PPP relative to the sigma receptor may be best characterized as mysterious. In all three assay systems discussed above in which binding affinity of a series of ligands correlates well with assay potency (PPI turnover, guinea pig ileum, and rubral dystonia), (+)-3-PPP has shown neither clear agonist nor antagonist actions. This is a serious problem in view of the high sigma-binding affinity of (+)-3-PPP and cannot be ignored.

It is possible that the sigma actions of (+)-3-PPP are masked by its actions at other receptors. Developed by Hjorth and coworkers, (+)-3-PPP was originally thought to be a dopamine autoreceptor agonist (reviewed by Clark et al., 1985b). (+)-3-PPP produced marked suppression of locomotor activity, an effect that was blocked by pretreatment with haloperidol. Because the binding of agonists to dopamine autoreceptors inhibits dopaminergic transmission, resulting in profound effects on motor function, the inhibition of locomotor activity produced by (+)-3-PPP, along with its various other in vivo effects, was thought to result from an agonist action at dopamine autoreceptors (Clark et al., 1985b). Subsequent

binding studies with [^{3}H](+)-3-PPP showed its marked affinity for sigma receptors and its relatively low binding affinity for dopamine receptors (IC_{50} against [^{3}H]dopamine in rat striatal membranes = 5 μM, cf. Seeman, 1981; IC_{50} against [^{3}H]spiperone = 1.7 μM, Wikstrom et al., 1987).

In spite of the relatively poor affinity of (+)-3-PPP for [^{3}H]dopamine-binding sites in vitro, Clark et al. (1985b) have maintained that (+)-3-PPP does act at dopamine autoreceptors, and certain behavioral and electrophysiological effects of this compound support this claim (Clark et al., 1985a; Essman and Woods, 1988). Although its affinity for dopamine receptors is low, there may be sufficient binding for some dopaminergic activity. This idea receives some support from the instances when the actions of (+)-3-PPP have been antagonized by sulpiride, a dopamine D_2 antagonist that lacks activity at sigma receptors (cf. Clark et al., 1985b). Actions through other receptors have also been suggested. For example, (+)-3-PPP has also been found to inhibit phospholipid breakdown through the α_1-adrenergic receptor and the muscarinic receptor (Fowler and Thorell, 1987).

Because a number of reports have brought the specificity of (+)-3-PPP to question, its failures to produce the expected actions in sigma biological assays must be interpreted with caution. And certainly, these failures in themselves do not negate the data suggesting that the sigma receptor is biologically relevant. At the same time, it must be recognized that understanding the nature of the biological effects of sigma receptor occupation by (+)-3-PPP is necessary if we are to gain a full understanding of the sigma receptor.

VI. Clinical Implications and Possibilities

Following the discovery that sigma receptors bind antipsychotic drugs came the expected interest in the possible clinical significance of sigma ligands. Here the question of which effects of antipsychotic drugs may be mediated by sigma receptors becomes the central focus. The high concentration of sigma receptors in the motor system immediately raised the issue of the motor side effects of antipsychotic drugs. Simultaneously, the antipsychotic activity of sigma-active drugs such as haloperidol, coupled with the sigma-activity of rimcazole (a putative antipsychotic), raised the important question of the possibility of novel sigma-binding antipsychotic drugs. Unfortunately, however, few human data are available, so the following discussion is necessarily based on animal models of these disorders.

A. Sigma Receptors and Movement Disorders in Man

Before discussing the evidence for a role of sigma receptors in movement disorders, brief descriptions of the clinical features of the relevant diseases are presented along with current models of the pathophysiology thought to underlie these states. Although connections between sigma receptors and several movement disorders can be established, this discussion is limited to the two most prominent candidates: dystonia and tardive dyskinesia.

1. Background. a. DYSTONIA. Dystonias are disorders involving sustained, involuntary muscle contractions that result in abnormal posture and interfere with normal motor function (Jankovic and Fahn, 1988). They can be focal, as in torticollis (in which the neck involuntarily rotates), or they can be progressive and generalized, as in torsion dystonia (in which the whole body slowly becomes contorted, an affliction leading to premature death). The vast majority of dystonias are idiopathic (Marsden 1982). Because autopsy and neuroimaging studies fail to reveal a consistent anatomical abnormality, most investigators assume that the primary dysfunction is neurochemical. Torsion dystonia is familial, and recent findings showing the location of the defective gene offer hope for a better understanding of its biological basis (Ozelius et al., 1989; Muller and Kupke, 1990).

The most common symptomatic dystonias result from the administration of neuroleptics, either as acute dystonic reactions or tardive dystonia (Burke et al., 1982; Roos and Bruyn, 1988). Acute dystonic reactions tend to occur primarily in young men and usually develop days or weeks after initiation of neuroleptic therapy (Roos and Buruma, 1984). These are temporary but dramatic focal dystonic reactions, usually involving profound torticollis and an otherwise rare ocular dystonia known as oculogyric crisis, in which the eyes show extreme and persistent ocular deviation (Jankovic and Fahn, 1988; Roos and Bruyn, 1988; Roos and Buruma, 1984). Tardive dystonia occurs following chronic treatment (months to years) of neuroleptics; as with tardive dyskinesia, symptoms often begin after withdrawal of the drug (Burke et al., 1982). Although less severe than acute dystonic reactions, tardive dystonia frequently is permanent and difficult to treat.

Because the blockade of dopamine receptors was previously the only known function of neuroleptics, and because anecdotal cases suggested a connection to the basal ganglia, the cause of dystonia has been assumed to involve lesions of the nigrostriatal pathway or a related structure, such as the pallidum. However, there have been few findings to support this idea. Most patients with lesions in the basal ganglia show no evidence of dystonia (Roos and Bruyn, 1988). Conversely, most cases of dystonia are not accompanied by anatomical or neurochemical changes within the basal ganglia. In fact, a number of cases have been associated with lesions in the brainstem (Gibb et al., 1988; Jankovic and Patel, 1983; Jankovic and Ford, 1983; Lang and Sharpe, 1984; Leenders et al., 1986; Day et al., 1986). For example, the association of rubral lesions with dystonia in man has been recognized (Castaigne et al., 1981; Jankovic et al., 1987; Smith, 1975), and in macaques, rubral lesions are associated with marked torticollis (Battista et al., 1976;

Carpenter, 1956). The cerebellum, which is intimately linked to the red nucleus anatomically, has also been implicated in human dystonia (Fletcher et al., 1988). Similarly, in rats, lesions of the locus coeruleus, a structure that exerts significant modulatory influences over the cerebellum, also produce dystonia. The brainstem is again implicated in the hereditary mutant mouse model of dystonia (Stanley et al., 1983), which is also known to be associated with lesions within the cerebellum and brainstem.

b. TARDIVE DYSKINESIA. Tardive dyskinesia is a syndrome that follows chronic use (usually months or years) of neuroleptics, involving choreiform movements, predominantly of the face, mouth, and tongue (Tolosa and Alom, 1988; Tanner, 1986). Symptoms occur after withdrawal of the neuroleptic and can be ameliorated by replacement of the drug, although this merely masks the symptoms of the disorder. In contrast to acute dystonic reactions, tardive dyskinesia is most common in older women (Kane et al., 1988). Like tardive dystonia, it tends to be permanent, and it is difficult to treat.

Tardive dyskinesia is commonly thought to result from the up-regulation of dopamine receptors known to be caused by neuroleptic drugs. This is thought to enhance the effects of physiological dopamine secretion, leading to hyperkinesias after the neuroleptics are discontinued. Although it provides a simple explanation for the emergence of dyskinesias, this model has never found convincing proof, despite many attempts to establish its validity.

Gerlach (1985) pointed out some failures to support a dopaminergic model, including the following: (*a*) no significant differences in dopamine D_1 or D_2 receptors have been found in the postmortem brains of schizophrenics with tardive dyskinesia, compared to a similarly treated group that did not develop the disorder (Cross et al., 1985); (*b*) in endocrinological studies, patients with tardive dyskinesia show a decrease, rather than the predicted increase, in sensitivity to dopaminergic drugs (Ettigi et al., 1976; Tamminga et al., 1977); (*c*) Parkinsonism, which involves the loss of nigrostriatal dopamine neurons, and tardive dyskinesia sometimes occur simultaneously in the same patient (Tanner, 1986); (*d*) *l*-DOPA has minimal or no effect on the severity of tardive dyskinesia (Gerlach and Casey, 1983); (*e*) some neuroleptics that cause tardive dyskinesia do not appear to cause dopamine hypersensitivity (Christensen et al., 1981); and (*f*) the time course of dopamine hypersensitivity in animals treated with neuroleptics does not correlate with the time course of tardive dyskinesia in patients (Christensen, 1981). Although these findings could be debated on an individual basis, taken as a group, they raise serious doubts about the dopamine theory of tardive dyskinesia.

2. *Role of sigma receptors.* a. SIGMA RECEPTORS AND IDIOPATHIC DYSTONIA. The absence of anatomical lesions in idiopathic dystonia leads naturally to the conclusion that it is caused by neurotransmitter or neurotransmitter receptor imbalances. The presence of the sigma receptor in structures that control movement and posture (particularly in the red nucleus, a site implicated in the pathogenesis of dystonia in both animals and man) provides indirect evidence for a link between sigma receptors and dystonia. Supporting data stem from the profound motor effects induced when sigma ligands are microinjected into the red nucleus of animals. The dystonia provoked by these drugs provides among the first structure-function relationships of an endogenous receptor that could account for this idiopathic condition.

Both the mutant mouse and the rubral lesion model of dystonia are compatible with a sigma theory of idiopathic dystonia. However, both preparations deviate from human dystonia in having identifiable lesions (Stanley et al., 1983). In contrast, Lorden et al. (1988) have a strain of rats that are free of any identifiable anatomical lesions; yet, these animals develop lethal dystonia. To further test the sigma hypothesis of idiopathic dystonia, the brains of these dystonic rats were analyzed for the concentration and binding characteristics of sigma receptors. This study revealed a 500% decrease in the affinity of sigma receptors (labeled with [^{3}H]DTG) in dystonic rats compared with their unaffected littermates and a 200% increase in the number of sites was also found (Bowen et al., 1988b).

Previous studies had revealed markedly abnormal glucose utilization in dystonic rats, including brainstem structures (Brown and Lorden, 1989); glutamate decarboxylase utilization in the cerebellum is also abnormal (Oltmans et al., 1988). Yet extensive investigations of these rats have shown no abnormalities in dopamine, acetylcholine, serotonin, or adrenergic receptors (Lorden et al., 1988). To date, the marked change in sigma receptors is one of the most profound alterations observed in these animals and, taken together with other data, suggests that sigma receptors may be involved in the pathogenesis of idiopathic dystonia. This model of dystonia is attractive because it offers a potential means of pharmacotherapeutic intervention. Analysis of sigma receptor affinity and number in postmortem brains of dystonic patients is of critical importance. Unfortunately, very few brains from patients who suffered from idiopathic dystonia are available for study.

b. SIGMA RECEPTORS AND NEUROLEPTIC INDUCED DYSTONIAS. The discovery of potent sigma binding by neuroleptics suggests a novel pathogenetic basis for neuroleptic induced dystonia. The high concentration of sigma receptors in the oculomotor, abducens, and trochlear nuclei, all subserving eye movements, provides a putative anatomical substrate for the oculogyric crises seen in acute dystonic reactions. Furthermore, the torticollis in rats caused by intrarubral microinjection of sigma receptor ligands raises the possibility that sigma

receptors mediate neuroleptic induced dystonic movements.

The differences between the typical and atypical neuroleptic drugs also support a sigma hypothesis of neuroleptic induced movement disorders. Typical neuroleptics are the traditional antipsychotic drugs that cause catalepsy in rats and movement disorders in humans. Two drugs (clozapine and sulpiride) have been found to exert significant antipsychotic effects in humans with little or no liability for producing motor dysfunctions (Miller and Jankovic, 1990; Achiron et al., 1990; Gerlach et al., 1974; Matz et al., 1974; Lindstrom et al., 1988; Friedman et al., 1987). These drugs have become known as the atypical antipsychotics. More recently, some investigators have designated other novel drugs as atypical because they do not produce catalepsy in rats. Unfortunately, however, because the connection between catalepsy in rats and movement disorders in humans is largely a matter of conjecture, and because clinical data are lacking, judgment should be reserved on the classification of these compounds.

Nevertheless, clozapine and sulpiride, the only atypical antipsychotic drugs for which there is a solid base of human data, bind with reasonable affinities to dopamine receptors but poorly to sigma receptors (Lindstrom, 1988; Friedman et al., 1987, Rupniak et al., 1984). This suggests that sigma binding is important in the production of movement disorders.

Reserpine, a drug that depletes dopamine and exerts Parkinsonian and mild antipsychotic effects, also shows no evidence of acute or tardive dystonia (Tarsy and Baldessarini, 1976). Only those antipsychotic drugs that show high or moderate sigma-binding affinity are associated with the development of dystonic reactions; antipsychotic drugs specific for dopamine receptors show no such behavior.

c. SIGMA RECEPTORS AND TARDIVE DYSKINESIA. Several lines of indirect evidence raise the possibility that sigma receptors are involved in tardive dyskinesia. The high concentration of sigma receptors in the facial and hypoglossal nuclei would provide an anatomical basis for the tendency of tardive dyskinesia to involve oral-buccal and lingual movements. Neither clozapine nor sulpiride, each of which binds to dopamine but not to sigma receptors, is commonly associated with tardive dyskinesia (Lindstrom 1988; Friedman et al., 1987; Jenner and Marsden, 1979; Rupniak et al., 1984). It must be noted that the study of tardive dyskinesia has been problematic from the outset, because it is difficult to induce dyskinesias in animals, even with typical neuroleptics that commonly produce the effect in humans. The motor effects of sigma ligands in the substantia nigra (Goldstein et al., 1989) suggest a possible physiological basis for dyskinesias in humans and further suggest a possible role of sigma receptors in Parkinson's syndrome. The possibility that mutual interactions between sigma and dopamine receptors are responsible for other neuroleptic induced movement disorders remains an important avenue for further study.

d. METABOLISM OF HALOPERIDOL TO SIGMA-ACTIVE/DOPAMINE-INACTIVE METABOLITES. Haloperidol has about equal affinity for sigma and dopamine receptors (Bowen et al., 1990a). However, this compound may induce greater and more prolonged actions through sigma receptors, because it produces metabolites that are still very active at sigma receptors but weak at dopamine receptors. Studies in which the binding of haloperidol and its metabolites to dopamine D_2 and sigma receptors was compared revealed that reduced haloperidol has only moderate affinity for dopamine D_2 receptors but is still very potent at sigma receptors. Because reduced haloperidol accumulates in the brains of chronically treated humans (Korpi et al., 1984), it is likely that sigma activity would eventually become dominant.

The relatively slow accumulation of sigma-active/dopamine-inactive metabolites of haloperidol in humans corresponds to the time course of actions of this drug. For example, certain motor disorders and the antipsychotic actions of haloperidol do not occur immediately after drug administration. Although many explanations for this phenomenon could be offered, it is conceivable that the delay results from the time required to accumulate sufficient plasma levels of sigma-active metabolites. The presence of sigma-active metabolites further suggests that individual differences in the metabolism of haloperidol to sigma-active or inactive metabolites may underlie some of the variation in the motor or psychotropic effects of haloperidol.

A serious question for the sigma hypothesis of motor disorders is why sigma-active compounds used clinically, e.g., DM and (+)-pentazocine [found in Talwin, (+/−)-pentazocine], have not caused movement disorders in humans. Conceivably, more than one receptor is involved in neuroleptic induced dystonias so that simultaneous stimulation and/or blockade of both dopamine and sigma receptors might be necessary for their induction. This seems plausible because none of the selective ligands for any of the receptors that typically bind neuroleptics (dopamine, adrenergic, serotonergic, sigma) are proven to cause movement disorders on their own. Furthermore, under certain conditions, synergies do occur among ligands for different receptors (e.g., dopamine D_1 + D_2 or D_1 + $5HT_{1a}$; Dall'Olio et al., 1989; Gessa et al., 1985; Molloy and Waddington, 1985). Studies of the propensity for mixtures of selective compounds [e.g., sulpiride plus (+)-pentazocine] to produce movement disorders in primates, compared to the individual substances, would be valuable.

Another plausible explanation for the failure of systemic administration of (+)-pentazocine and DM to produce movement disorders is based on the multiplicity of sigma-binding sites in the brain. It is possible that only

those drugs that bind to the UV-susceptible (neuroleptic) site or the PC12-like subtype [sigma-2, which is insensitive to (+)-opiates] produce movement disorders. The marked difference in the actions of chronically administered haloperidol on the neuroleptic versus (+)-opiate sites (fig. 12) and the reduced efficacy of (+)-opiates in producing dystonia in rats provide some support for this view.

In summary, multiple lines of indirect evidence support the hypothesis that sigma receptors mediate some of the motor effects of antipsychotic drugs. These include the anatomical distribution of sigma receptors, the motor effects of sigma ligands in rats, altered sigma binding in mutant dystonic rats, the dopamine-releasing action of sigma ligands, and the formation of sigma-active/dopamine-inactive metabolites of haloperidol. However, these data are indirect, and clinically used sigma ligands do not produce movement disorders, raising questions about the nature of this relationship. Further investigation will be required to establish a role of sigma receptors in motor disorders. In particular, sigma binding and haloperidol metabolism in the brains of patients treated with neuroleptics should be studied. Studies of the motor effects of sigma-inactive antipsychotics (e.g., sulpiride) are needed. Finally, studies of the motor effects of new antipsychotic drugs with significant sigma affinity (such as BMY 14802) are needed.

3. Sigma receptors and psychosis. For many years, the dominant theory of the biological basis of schizophrenia has been the dopamine theory, which asserts that dopamine hyperactivity (broadly defined) is the underlying cause of the disorder (Snyder et al., 1974; Losonczy et al., 1987). Although recent data have offered considerable support for this notion, certain aspects of the disease cannot be accounted for by dopamine dysfunction. The more recently discovered common property of neuroleptic drugs (binding to sigma receptors) raises the possibility that sigma interactions mediate some of the antipsychotic effects of neuroleptics. The distribution of sigma receptors in limbic areas known to be involved in cognition and emotion supports this view.

The development of rimcazole (BW234U) has provided some experimental support for a role of sigma receptors in schizophrenia. Behavioral studies in rats suggested that this drug may possess antipsychotic efficacy independent of classic anti-dopaminergic effects (Ferris et al., 1986), and in preliminary clinical trials, some evidence of antipsychotic efficacy was found (Chouinard and Annable, 1984; Davidson et al., 1982; Ferris et al., 1982). However, it should be noted that the clinical studies were not double-blind and that no effect on global improvement was noted in at least one open-label trial (Davidson et al., 1985).

The behavioral effects of rimcazole have been attributed to sigma receptors. However, the activity of rimcazole at sigma receptors is approximately 1% that of haloperidol and 50% that of propranolol (Ferris et al., 1986), a drug with minimal cognitive effects. The affinity of rimcazole for certain other central nervous system receptors casts further doubt upon the role of sigma receptors in its actions (cf. Ferris et al., 1986). Although it is tempting to infer a role for sigma receptors in psychosis from these data, they must be considered no more than preliminary at this time.

The potential antipsychotic agent BMY 14802 also binds to sigma receptors (Taylor and Dekleva, 1987), and it has a greater affinity for the site than rimcazole. However, this compound has never been tested on psychotic patients; its antipsychotic actions have been postulated based on animal models of antipsychotic drug action (Matthews et al., 1986). In particular, this compound was found to reduce the avoidance behavior more effectively than escape behavior in rats (Taylor et al., 1990). Because many antipsychotics have this effect (Worms et al., 1983), these data were taken as evidence for a selective antipsychotic action.

Based on current models of antipsychotic drug-induced movement disorders, Taylor and Dekleva (1988) argued that BMY 14802 will not cause motor effects in man because it lacks activity at dopamine D_2 receptors, fails to induce catalepsy in rats, and reverses trifluperazine-induced catalepsy. Although these findings are indeed encouraging, it must be noted that motor dysfunctions are produced by all sigma-active antipsychotic drugs for which there is significant clinical experience. Furthermore, the reliability of these animal models is not well established, and the high affinity of BMY 14802 for $5HT_{1a}$ receptors raises questions about the mechanism(s) mediating its effects. This is problematic for the inhibition of trifluoperazine-induced catalepsy, because the selective $5HT_{1a}$ agonist 8-hydroxydipropylaminotetralin produces the same effect (McMillen et al., 1989). $5HT_{1a}$ receptors also bind certain neuroleptics (Pedigo et al., 1975; Wander et al., 1987) raising the possibility that this site may mediate certain effects of antipsychotic drugs.

Despite the somewhat suggestive findings provided by rimcazole and BMY 14802, there are substantive lines of evidence that argue against a relationship between sigma binding and antipsychotic efficacy. Although dopamine-binding properties of neuroleptics correlate closely with their antipsychotic effectiveness (Peroutka and Snyder, 1980), no such correlation with sigma binding has been found (see fig. 21 for comparison). Furthermore, drugs such as clozapine and sulpiride show extremely poor binding to sigma receptors yet bind to dopamine receptors and have substantial antipsychotic effects (Tam and Cook, 1984).

Another blow to the sigma receptor hypothesis of schizophrenia came from recent insights suggesting that sigma receptors do not mediate the psychotomimetic actions of prototypic sigma ligands (Musacchio, 1990).

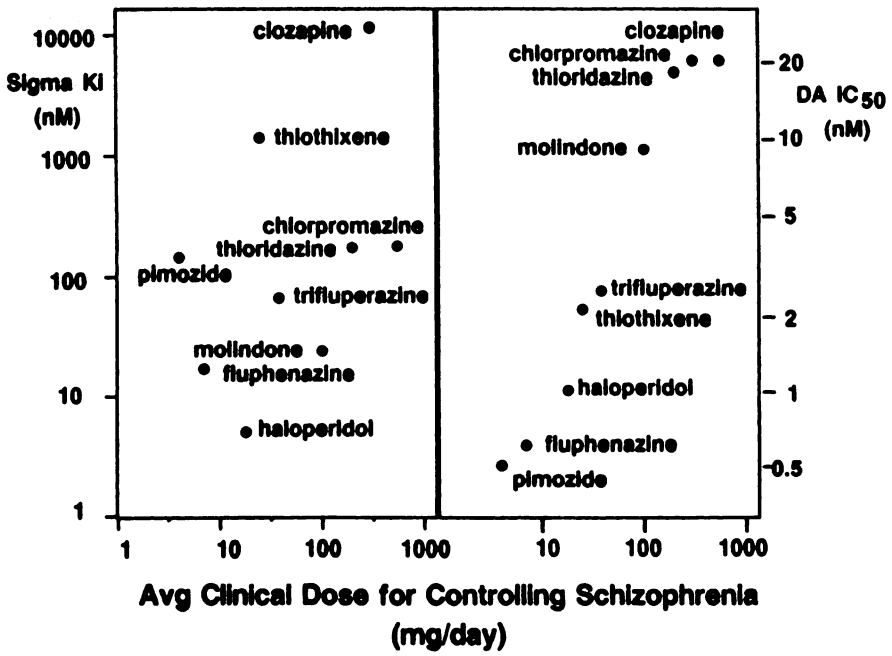


FIG. 21. Correlation between doses of neuroleptics required to control schizophrenia and binding potency at sigma (*left*) and dopamine (*right*) receptors. A strong relationship is found for dopamine but not sigma receptors. Sigma-binding data from Tam and Cook (1984); dopamine-binding data from Seeman (1981).

This is problematic because theories of the underlying basis of psychosis inevitably rest upon psychotomimetic or antipsychotic properties of prototypic ligands. For example, the dopamine theory of schizophrenia is founded on amphetamine psychosis and the antipsychotic activity and dopamine-antagonist action of neuroleptics (Snyder et al., 1974). Similarly, other theories rely on the psychotomimetic effects of phenylethylamines and PCP (Domino and Luby, 1973, Bowers, 1987). Likewise, the psychotomimetic effects of SKF 10,047, together with the sigma actions of this compound and the binding of neuroleptics to sigma receptors, were taken to suggest a possible etiology of schizophrenia and mechanism for antipsychotic drug action.

Although the psychotomimetic actions of SKF 10,047 provided a logical basis for assuming a role of sigma receptors in psychosis, a reevaluation of the data has shown that it is extremely unlikely that sigma receptors mediate the psychotomimetic effects of (+)-opiates. Foremost among these is the lack of psychotomimetic actions of the potent and selective sigma ligand (+)-pentazocine (Bellville and Forrest, 1967; Forrest et al., 1969). Although the psychotomimetic effects of racemic pentazocine have been cited as evidence for sigma-mediated psychotomimetic symptoms in humans, this effect is reversed by naloxone (cf. Martin, 1984), demonstrating mediation by opiate receptors, not sigma receptors. Parallel logic applies to studies of DM. This compound has relatively high affinity for sigma receptors (table 1) and has been widely used in humans for its antitussive properties, yet it does not have psychotomimetic effects even at high doses (Walker and Hunt, 1989). These findings strongly suggest that (+)-opiates do not exert psychotomimetic actions through the sigma receptor. However, these conclusions do not preclude antipsychotic actions of neuroleptics through the sigma-2 site, which has low affinity for (+)-opiates.

If the psychotomimetic effects of (+)-SKF 10,047 are not sigma mediated, then what is their biological basis? Two logical possibilities are kappa-opiate receptors and PCP receptors because (+/−)-SKF 10,047 binds to both types of receptors and both sites apparently mediate psychotomimesis (Pfeiffer et al., 1986; Domino and Luby, 1973).

The role of sigma receptors in psychosis remains ambiguous. On the positive side, the evidence shows that sigma receptors (*a*) are concentrated in limbic areas, (*b*) bind haloperidol and other neuroleptics with high or moderate affinity, and (*c*) bind certain novel potential antipsychotics that are predicted from animal models to lack significant motor effects. On the negative side, the evidence shows that sigma receptors (*a*) are found in highest concentration in motor systems, not limbic areas; (*b*) bind rimcazole only weakly; (*c*) are of questionable significance in the actions of BMY 14802, because the compound binds to $5HT_{1a}$ receptors and has not been tested in humans; and (*d*) do not mediate psychotomimetic effects of (+)-opiates in humans. What is clear is that the establishment of a role of sigma receptors in psychosis could have profound implications both for drug therapy and the prognosis of those afflicted with the disease (Snyder and Largeut, 1989; Sonders et al. 1988). Therefore, this question deserves to receive full treatment before definite conclusions are drawn.

VII. Conclusions

A. Pharmacological Evidence for Biologically Relevant Sigma Receptors

In addition to the accumulating evidence for an endogenous ligand for sigma receptors, there is now considerable pharmacological data supporting its biological relevance. The strongest support is found in the high correlations (≥ 0.70) between sigma-binding affinity and potency in at least four biological assay systems: (*a*) PPI turnover (Bowen et al., 1988a; 1990b), (*b*) stimulated guinea pig ileum (Campbell et al., 1989), (*c*) block of tonic K^+ currents in NCB-20 cells in culture (Bell et al., 1988), and (*d*) torticollis induced by rubral microinjections of sigma ligands (Matsumoto et al., 1990). The compounds used in all of these assays cover a broad range of chemical classes and include the most selective and potent compounds discussed above [e.g., (+)-pentazocine, BD614, DTG, (+)-SKF 10,047, haloperidol].

These functional assays appear to represent the activities of different subtypes of the sigma receptor. Hellewell and Bowen's sigma-1 site (1990), the site that follows the binding profile established initially by Su (1982) and Tam (1983), appears to mediate the effects observed in the guinea pig ileum and in the PPI assay because (+)-opiates were more potent than (−)-isomers and drugs followed the binding profile shown by [^{3}H]DTG and [^{3}H](+)-3-PPP in guinea pig brain.

Considerable evidence now suggests the existence and functional significance of a second sigma site, termed sigma-2 by Hellewell and Bowen (1990) and in this review. (−)-Opiates are more potent than (+)-opiates at this site, although its binding profile is clearly non-opiate. Several laboratories have now encountered this site in several tissues: (*a*) Bowen and coworkers (Hellewell and Bowen, 1990; Helewell et al., 1990) describe it in PC12 cells and rat liver, (*b*) Itzhak's group has seen it in C57 black mouse brain (Kassim et al., 1990), and (*c*) Wu et al. (1990) have also discovered this site in NCB-20 cells using Scatchard analysis and curve fitting. The potency of a series of drugs for this site correlates highly (0.96) with their potency in inhibiting a tonic K^+ conductance in these cells. Another report from studies of guinea pig brain (Reid et al., 1988) shows some of the characteristics of this site, although stereoisomers were not tested.

To date, the demonstrations of high correlations between sigma binding and potency in the rubral/torticollis assay have not used stereoisomers. However, the observation that (+)-opiates are substantially less potent than DTG or haloperidol would suggest actions at the sigma-2 site. Further investigation of the actions of stereoisomers are needed to establish this.

The regulation of sigma receptors by chronic administration of sigma drugs adds further support to the notion that this is a biologically significant receptor. In three different laboratories, the binding of sigma ligands was altered by chronic administration of haloperidol (Bremer et al., 1989; Itzhak and Alerhand, 1989; Matsumoto et al., 1989b). It seems implausible that chronic administration of sigma ligands would alter the expression or affinity of an inactive acceptor.

The demonstration of occupancy of sigma sites following focal electrical stimulation or potassium- or veratridine-induced depolarization provides powerful evidence for an endogenous neurotransmitter for the sigma receptor and strengthens the case for its biological significance (Connors and Chavkin, 1990; Neumaier and Chavkin, 1989). Demonstrations by other laboratories of brain extracts with sigma activity add further support to this notion (Contreras et al., 1987a; Sonders et al., 1986; Su et al., 1986; Su and Vaupel, 1988). Therefore, based on the demonstrations that biological changes follow sigma binding, that the site is regulated by administration of sigma-active ligands, and that an endogenous ligand(s) exists, we conclude that the sigma-binding site is biologically significant.

B. Agonist Actions of Sigma Ligands, at Least at Some Populations of Sigma Receptors

Current evidence suggests that all sigma ligands tested except (+)-3-PPP have similar effects in physiological systems and act as agonists. This view contrasts with the assertions of those investigators (e.g., Deutsch et al., 1988) who have stated or implied that (+)-opiates are agonists at the sigma receptor, whereas haloperidol and structurally related neuroleptic like compounds (such as typical neuroleptics, DTG, rimcazole, and BMY 14802) act as antagonists. The physiological evidence demonstrates that the actions of haloperidol, rimcazole, BMY 14802, and compounds with a similar binding profile (e.g., DTG) are similar to those of the (+)-opiate sigma ligands. For example, DTG, haloperidol, (+)-SKF 10,047, (+)-pentazocine, and dextrallorphan all have the same effect on posture following intrarubral microinjections (Matsumoto et al., 1990; Walker et al., 1988). Using iontophoretic application in the rat, Matsumoto and Walker (1988a,b) found that (+)-pentazocine and DTG both inhibit the firing rate of red nucleus neurons. Bowen and colleagues (1988a; 1990b) showed that DTG, haloperidol, fluphenazine, (+)-pentazocine, and several other sigma ligands all inhibit carbachol-stimulated (and oxotremorine-M-stimulated) PPI turnover in rat synaptoneurosomes. In a recent study similar actions of (+)-SKF 10,047, (+)-pentazocine, DTG, rimcazole, and BMY 14802 on muscle contractions in the guinea pig ileum (Campbell et al., 1989) were also demonstrated. Thus, although a few reports discuss the possibility of sigma antagonist actions of certain compounds (e.g., Deutsch et al., 1988; Ferris et al., 1986), most studies show that these substances produce effects that are similar to those of the presumed agonists.

Stronger evidence favoring agonist actions of the known sigma ligands stems from the down-regulation of

sigma receptors by chronic administration of haloperidol in mice (Itzhak and Alerhand, 1989) and rats (Bremer et al., 1989; Matsumoto et al., 1989b). Although there are instances in which antagonists are suspected of down-regulation of a receptor, the weight of the evidence supports the view that down-regulation accompanies the actions of agonist drugs (cf. Seeman, 1981). Thus, the down-regulation of sigma receptors by haloperidol suggests an agonist action at the sigma receptors. These observations, together with studies establishing similar physiological actions of sigma compounds, imply that all the known active sigma ligands are agonists.

C. Neuromodulatory Actions of Sigma Ligands

From the findings discussed in this review, we may tentatively conclude that sigma ligands act primarily as neuromodulators. We use this term in the manner proposed originally by Barker et al. (1978) to describe substances that act by altering the activity of another transmitter rather than by a direct action of their own. This appears to be a rather common process in the nervous system and it appears likely that many of the actions of sigma ligands are neuromodulatory in nature. In each of the functional assays that appear to be sigma mediated other transmitters appear to be involved: alteration of acetylcholine actions, 5HT actions, and electrically stimulated tissues. The in vivo effects occur in systems in which ample opportunity exists for neuromodulation, i.e., in "spontaneously" active systems such as the red nucleus, cerebellum, and substantia nigra. By contrast, clear sigma actions have been difficult to identify in preparations that are free of external stimulation, such as in brain slice preparations treated with tetrodotoxin. These observations encourage further studies of the ability of sigma ligands to modify the actions of other neuroactive substances.

D. Clinical Implications

The investigation into the clinical applicability of sigma ligands is still in its infancy. One important question is whether the motor effects of sigma ligands in animals reflect processes that underlie certain motor disturbances in acute or chronic antipsychotic drug therapy. Beyond these considerations is the possibility that these drugs might have efficacy in the treatment of certain motor disorders. In particular, motor disturbances in which antipsychotics are used, such as Huntington's chorea, dystonia, and Tourette's syndrome, are candidates. The presence of sigma receptors in the substantia nigra raises the possibility of the use of sigma ligands in treating Parkinson's disease. Clinical trials may not carry great liability, considering the apparent excellent safety margin found in certain sigma compounds [e.g., (+)-pentazocine, DM].

The other clear direction discussed was the possibility of antipsychotic actions of these compounds. The fact that certain antidepressant drugs have marked sigma affinity also raises the possibility that affective disorders might be amenable to therapy with antipsychotic drugs.

E. Future Directions in the Pharmacology of Sigma Receptors

Many questions about the nature and function of the sigma receptor loom before us. Among the most pressing present needs are (*a*) the development of a sigma receptor antagonist, (*b*) the identification of the endogenous ligand(s) for the sigma receptor, and (*c*) identification of the sequence of the sigma-binding protein. At present, only a few assays have been studied in enough detail to make a reasonable connection between efficacy and sigma binding. For each of these, it was necessary to characterize many drugs and carry out correlational analyses to establish the connection to sigma receptors. In addition to the tediousness of this approach, correlational analyses are weak because they cannot establish a causal link. The main risk in this line of investigation stems from the possibility that the sigma site may be an ion channel site (such as the PCP site) or some other entity rather than a neurotransmitter receptor, making the possibility of developing an antagonist quite unlikely. Conversely, the development of an antagonist would add to the data supporting the biological relevance of the sigma site. It is clear, therefore, that a sigma antagonist would overcome several problems, increasing the precision and hastening the progress of research on the function of sigma receptors.

The identification of the putative endogenous sigma ligand(s) is another major hurdle. Until this question is resolved, we cannot be completely secure in referring to sigma-binding sites as receptors. A similar logic applies to the purification and sequencing of the sigma-binding protein, because by comparison to homologous structures this may demonstrate beyond any reasonable doubt the cellular function of this site. In this article, we have freely used the term sigma receptor based on several demonstrations pointing to its biological activity. However, it must be observed that sigma receptors cannot be investigated in their true neurobiological (rather than pharmacological) context until these problems are solved. These questions notwithstanding, this review of the literature demonstrates that in recent years significant advances have taken place in virtually every aspect of the sigma receptor research including medicinal chemistry, molecular pharmacology, signal transduction, and functional correlates.

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