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Steroid Binding at σ Receptors Suggests a Link Between Endocrine, Nervous, and Immune Systems

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Specific σ binding sites have been identified in the mammalian brain and lymphoid tissue. In this study, certain gonadal and adrenal steroids, particularly progesterone, were found to inhibit σ receptor binding in homogenates of brain and spleen. The findings suggest that steroids are naturally occurring ligands for σ receptors and raise the possibility that these sites mediate some aspects of steroid-induced mental disturbances and alterations in immune functions.

IN 1976, MARTIN AND CO-WORKERS postulated the existence of σ receptors to account for the psychotomimetic effects of certain benzomorphan opioids (*1*). Specific haloperidol-sensitive σ receptor binding sites were first identified in preparations of the guinea pig brain by using [^{3}H]*N*-allylnormetazocine (SKF-10,047) as a radioactive ligand (*2*). In more recent studies, tritiated ethylketocyclazocine, haloperidol, 3-(3-hydroxyphenyl)-*N*-(1-propyl) piperidine, and 1,3-di(2-tolyl)guanidine have been used to label σ receptors (*3*). Data from autoradiographic and biochemical studies suggest that the σ receptors are not D_2 dopamine receptors, for which most antipsychotic drugs, including haloperidol, have a high affinity (*3, 4*). There is also a distinction between the σ and phencyclidine (PCP) receptors (*5, 6*), although many drugs bind to both sites. The fact that prototypic σ receptor ligands and PCP alter mood and produce hallucinations suggested that the endogenous σ system may play a role in the etiology of human psychosis (*7, 8*). Nonetheless, the physiological role of the σ receptor remains unknown.

The search for endogenous σ receptor ligands has yielded guinea pig brain extracts with selective activity at σ receptors (*8, 9*). Partial purification and chemical characterization indicated that the active material is nonpeptidic and has a mass of 300 to 700 daltons (*8, 9*). The material potentiated electrically stimulated contractions of the guinea pig vas deferens in vitro, a bioassay for σ receptor ligands (*8–10*).

The recent discovery of σ receptors on human peripheral blood lymphocytes and in the rat spleen (*11*) and the hypothesis that σ receptors mediate psychotomimetic responses suggested that an endogenous σ ligand might affect immune function, cause psychosis, and alter mood. Gonadal and adrenal steroids have molecular weights that are in the approximate range of σ-active brain extracts (*8–10*). They can also influence humoral and cell-mediated immunity (*12*) and appear to have a central nervous system action, with complex effects on behavior and mood (*13, 14*). We therefore examined the interaction of σ receptors in the brain and spleen with 20 of the representative gonadal and adrenal steroids. Two putative σ receptor antagonists, haloperidol (*10*) and BW 234U (*15*), were also tested. We report here that progesterone, testosterone, desoxycorticosterone, and several other steroids are potent ligands at σ receptors in the brain and spleen. The potencies of these steroids in binding to σ receptors are also compared with published findings on their efficacies in preventing granuloma formation in rats (*16*).

Brain and spleen tissue was obtained from male Hartley guinea pigs (300 to 400 g). Animals were killed by carbon dioxide asphyxiation, and homogenates were prepared from the excised tissues (*17*). Two-milliliter aliquots of the brain homogenates were incubated with varying concentrations of the inhibitors and 2 n*M* *d*-[^{3}H]SKF-10,047 [23 Ci/mmol; New England Nuclear (NEN)]. Specific binding was defined as that

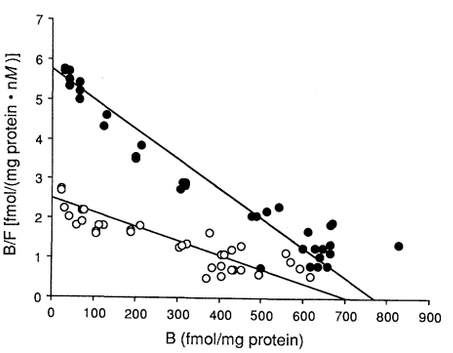

Fig. 1. Scatchard plot of *d*-[^{3}H]SKF-10,047 binding to cerebral σ receptors in the absence (closed symbols) and presence (open symbols) of 800 n*M* progesterone. Increasing concentrations of *d*-[^{3}H]SKF-10,047 (5 to 800 n*M*) were incubated, in the absence and presence of progesterone, with guinea pig brain homogenates in a total volume of 2 ml. Each determination was performed in duplicate and was repeated four times. Data from all four determinations were combined for statistical analysis by the LIGAND computer program (*19*).

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which was displaced by 0.1 m*M* unlabeled *d*-SKF-10,047. The rest of the procedures were the same as those described elsewhere (*18*). Phencyclidine receptor binding was assayed with 1 n*M* ^{3}H-labeled *N*-(1-[2-thienyl]cyclohexyl)piperidine ([^{3}H]TCP) (47.6 Ci/mmol; NEN), as described previously (*18*). Portions (500 μl) of spleen homogenates were incubated in a final volume of 3 ml of 0.05*M* tris-HCl buffer (*p*H 8.0), inhibitor, 50 n*M* spiperone, and 1 n*M* [^{3}H]haloperidol (8 Ci/mmol; NEN). Specific binding was defined as that which was inhibited by 0.1 m*M* unlabeled *d*-SKF-10,047. After 2 hours of incubation at 25°C, bound radioactivity was separated from the unbound by reduced pressure filtration with GF/C filters (Whatman). Two 5-ml washes with ice-cold buffer were used to remove unbound radioactivity. Radioactivity was measured spectrometrically by a Tri-Carb 460 CD and a Beckman LS 2800 liquid scintillation counter (50 to 51% counting efficiency) for the brain and spleen assays, respectively. The LIGAND computer program (*19*) was used in this study for statistical analysis of the binding isotherms. IC_{50} values (concentrations required to inhibit 50% of specific binding of the radioactive ligand) were estimated from log-logit plots with the use of at least three to four inhibitor concentrations producing 20 to 80% inhibition of total specific binding. Inhibition constants (K_i) for each inhibitor were calculated from the IC_{50} values by using the Cheng-Prusoff equation (*20*). The mean values of the dissociation constants (K_d) (±SEM) were 134 ± 9 n*M* ($n = 4$) for *d*-[^{3}H]SKF-10,047 in the brain and 0.3 ± 0.04 n*M* ($n = 4$) for [^{3}H]haloperidol in the spleen.

Progesterone was the most potent inhibitor of *d*-[^{3}H]SKF-10,047 binding to σ receptors in the brain (Table 1), with a K_i value of about 268 n*M*, which was comparable to values obtained with many putative σ ligands in the same assay (*2*). Although all estrogenic hormones tested (estriol, estrone, and estradiol) were inactive, testosterone was active and was about equipotent to the adrenocortical steroid desoxycorticosterone (K_i values about 1000 n*M*, Table 1). Pregnenolone was inactive in the brain σ receptor assay. Pregnenolone sulfate, however, had a K_i of about 3200 n*M*. Two potent ligands for the cytosolic progesterone receptor, promegestone and RU 27987 (*21*), were inactive in the σ receptor binding assay (Table 1).

A rank order of potencies in inhibiting σ receptor binding was obtained in the spleen similar to that in the brain (Table 1). Again, progesterone was the most potent inhibitor, with a K_i of about 376 n*M*. Testosterone and desoxycorticosterone were approximately equipotent but less potent than progesterone (K_i values around 2000 n*M*). The potency of BW 234U appeared to be slightly higher in the spleen σ assay than in the brain. A K_i about equal to that of progesterone was observed for BW 234U in the spleen.

Except for testosterone, all steroids previously reported as active in anti-inflammatory tests (*16*) were also active in the σ receptor binding assay (Table 1). Steroids that were inactive in the σ receptor assay, including all estrogenic compounds studied, were also reported to be inactive in the anti-inflammatory tests.

Progesterone inhibited *d*-[^{3}H]SKF-10,047 binding to cerebral σ receptors in a competitive manner (Fig. 1). In the presence of 800 n*M* progesterone, the K_d for *d*-[^{3}H]SKF-10,047 binding to σ receptors was increased by almost twofold, from 134 to 284 n*M* (Fig. 1). The increase was statistically significant ($P < 0.001$; LIGAND, *F*-test). In contrast, B_{max} was not significantly altered [Fig. 1; 770 fmol per milligram of protein without progesterone versus 702 fmol per milligram of protein in the presence of the steroid ($P = 0.371$; LIGAND, *F*-test)]. A one-site model was found to be a best fit for *d*-[^{3}H]SKF-10,047 binding to σ receptors both in the absence and in the presence of progesterone (Fig. 1).

All steroids examined in the present study were essentially inactive in the PCP receptor binding assay. Less than 20% inhibition by all steroids was observed at concentrations of up to 10,000 n*M* (assays repeated twice, each in quadruplicate) in incubations containing 1 n*M* [^{3}H]TCP. Most putative ligands for the σ and PCP receptors have shown some degree of cross-reactivity at the two receptors (*2, 3, 6*). However, in the present study, progesterone, the most potent steroid inhibitor of σ receptors examined, showed negligible affinity for PCP receptors, suggesting an important difference between the two receptor systems.

There are several lines of evidence that the σ receptors to which progesterone and other steroids bind are distinct from cytosolic steroid receptors. First, the homogenates used in the present study were crude membrane fractions rather than cytosolic preparations. In addition, potent ligands at cytosolic progesterone receptors, such as promegestone, estradiol, and RU 27987 (*21*), were inactive in the σ receptor assay (Table 1). Furthermore, the equilibrium K_d values of the steroids examined are in the range of 0.1 to 10 n*M* at cytosolic steroid receptors (*22*) but are much higher at σ receptors. The σ receptors also appear to be distinct from the steroid receptors previously identified in a brain synaptosome preparation, since estradiol, which was a potent ligand in the synaptosomal study (*23*), was essentially inactive in the σ receptor assay (Table 1).

Concentrations of steroid hormones in plasma may be sufficient to cause significant interactions at σ receptors. Progesterone concentrations in human plasma range from 30 n*M* during the latter part of the menstrual cycle to about 450 n*M* in late pregnancy (*24*). These values correspond to an approximately 10 to 60% fractional occupancy at σ receptors both in brain and spleen homogenates. Because of the high lipophilicity of progesterone, it is likely that progesterone at

Table 1. Potencies and Hill slopes of steroids and putative σ antagonists (*10*) in inhibiting σ receptor binding in brain and spleen homogenates: comparison to previous data from anti-inflammatory test (*16*). *d*-[^{3}H]SKF-10,047 (2 n*M*) and [^{3}H]haloperidol (1 n*M*) were used to label σ receptors in the brain and spleen, respectively. Values represent means ± SEM for the number of assays indicated in parentheses. K_i values of the following steroids exceeded 10,000 n*M* in the brain σ receptor assay and, therefore, were not tested in the spleen: estriol (NAI), estrone 3-hemisuccinate (NAI), 17β-estradiol-17-hemisuccinate (NAI), 6α-methyl-17α-hydroxyprogesterone acetate, megestrol acetate, hydrocortisone (NAI), Reichstein's substance S, 4-pregnene-11β, 17α-diol-3,20-dione, 11α-hydroxyprogesterone, 17α-hydroxyprogesterone (NAI), digitoxigenin, 5-pregnen-3β-ol-20-one (pregnenolone) (NAI), promegestone, and RU 27987. Of the 14 listed compounds that were inactive in the σ receptor assay, the compounds followed by the abbreviation NAI were inactive in tests of anti-inflammatory activity (*16*).

Compound	Brain (*d*-[^{3}H]SKF-10,047)		Spleen ([^{3}H]haloperidol)	
	K_i (n*M*)	*n*H	K_i (n*M*)	*n*H
	Steroids			
Progesterone*	268 ± 20	0.91 ± 0.03 (8)	376 ± 73	0.84 ± 0.12 (3)
Testosterone	1014 ± 92	0.73 ± 0.04 (5)	1715 ± 324	1.03 ± 0.14 (4)
Desoxycorticosterone*	938 ± 53	0.89 ± 0.10 (4)	2477 ± 378	1.55 ± 0.34 (3)
11β-Hydroxyprogesterone	1535 ± 306	0.94 ± 0.10 (4)	5384 ± 361	0.99 ± 0.14 (2)
Pregnenolone sulfate	3196 ± 823	0.97 ± 0.23 (4)	1272 ± 247	0.91 ± 0.17 (3)
Corticosterone*	4074 ± 621	0.59 ± 0.05 (4)	1698 ± 209	1.00 ± 0.00 (2)
	Putative σ antagonists			
Haloperidol	1.47 ± 0.3	1.09 ± 0.07 (4)	0.24 ± 0.01	1.07 ± 0.02 (3)
BW 234U	693 ± 118	0.97 ± 0.14 (4)	355 ± 140	1.20 ± 0.17 (4)

*Starred compounds prevented granuloma formation in female rats (*16*); 11β-hydroxyprogesterone and pregnenolone sulfate were not tested (*16*).

normal physiological concentrations could interact with or even saturate the σ receptors in the brain and spleen.

Given the recent findings of the presence of σ receptors and the absence of phencyclidine receptors in the spleen and peripheral blood leukocytes (*11*) and the fact that PCP interacts with σ receptors, it is conceivable that the immunosuppressive effects of PCP (*25*) might be mediated by σ receptors in lymphoid tissue. The interactions of progesterone and certain other steroids with σ receptors in the spleen suggest that steroids may modify immune function through actions at these receptors. In a study of granuloma formation in female rats (*16*), progesterone, desoxycorticosterone, and corticosterone, which are potent inhibitors of σ receptor binding, were active, whereas compounds that were inactive at σ receptors in the present study, including all estrogenic steroids, also lacked activity in the anti-inflammatory test (Table 1). The only discrepancy between the σ receptor and anti-inflammatory studies was found in testosterone, which had relatively high affinity at σ receptors in the brain and spleen but was inactive in preventing granuloma formation (Table 1). Direct comparison of the relative potencies of the steroids at the σ receptor and in anti-inflammatory tests could not be carried out because the local steroid concentrations in the anti-inflammatory study were not determined (*16*). The striking qualitative correlation, however, supports a possibility that σ receptors may be mediating the anti-inflammatory action of those steroids. The concomitant existence of σ receptors in brain and spleen homogenates is consistent with the presence in neural and peripheral tissue of neurotransmitters or other endogenous substances that mediate immune responses (*26*).

Despite the demonstration that progesterone and certain other steroids bind to σ receptors in the brain and the spleen, the physiological role of the σ receptor remains unknown. Furthermore, activity of these steroids in σ receptor binding assays is inadequate evidence to identify the endogenous cerebral ligand for the σ receptor ("sigmaphin") as a steroid. However, in view of the present findings and observations that steroids can alter immune function (*12, 14, 16*) and cause changes in mood and psychological parameters (*13, 14*), the speculation that such steroid actions may involve σ receptors on neurons and lymphocytes is not unreasonable. Thus, the present findings suggest that interactions of progesterone and other steroids with σ receptors may constitute an important link between the endocrine, immune, and central nervous systems. Putative σ receptor ligands may, therefore, have a wide spectrum of therapeutic applications.

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