Steroid Binding at σ-"Opioid" Receptors

T.-P. Su *et al.* reported that several steroids, especially progesterone, can competitively inhibit the in vitro binding of [^{3}H](+)SKF-10,047 [(+)*N*-allylnormetazocine] and of [^{3}H]haloperidol to σ-"opioid" receptors on membranes from guinea pig forebrain and splenocytes (*1*). Some of these steroids that are active at the σ receptor have also been reported to prevent the formation of granulomas in rats (*2*). This led Su *et al.* to propose a new link between the endocrine, nervous, and immune systems and to conclude that σ sites could "mediate some aspects of steriod-induced mental disturbances and alterations in immune functions" (*1*). Although we do not question the experimental observations of Su *et al.*, and their conclusions appear to be consistent with developments in the field of psychoneuroimmunology (*3*), several crucial factors need to be clarified.

1) Contrary to the suggestion of Su *et al.* (*1*), the psychotomimetic and other behavioral actions of (+)SKF-10,047 and other dextrorotatory benzomorphans, like those of phencyclidine (PCP) and ketamine, are more likely to be mediated by blockade of the *N*-methyl D-aspartate receptor (NMDA) (which binds PCP) than by the σ_H receptor (*4–11*). However, none of these steriods displaced [^{3}H]PCP from brain membranes (*1*).

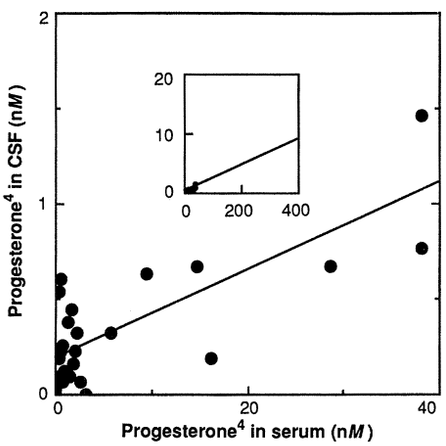

Fig. 1. Linear relation between total progesterone in CSF (ordinate) and in peripheral venous serum (abscissa). The relation was statistically highly significant: $P < 0.001$ ($y = 0.18 + 0.02 \pm 0.003$). Total (free plus protein-bound) progesterone was measured by specific radioimmunoassay (*39*) in matched serum and CSF samples from 25 patients with intact blood-CSF barriers (*12*). (**Inset**) An extrapolation to maximal serum levels of total progesterone, as would be seen in the last trimester of normal human pregancy (*14*). In all these patients the serum levels of steroids, as well as those of albumin, immunoglobulin G, sex hormone binding globulin, and corticosterone binding globulin were within the normal range reported previously by us (*12, 40*) or others (*14, 41*). Similar values for CSF progesterone have been reported (*41*) and correspond to those in human saliva (*42*).

2) We have analyzed the differential permeabilities of the blood–cerebrospinal fluid (CSF) barrier for various classes of blood-borne substances (*12*). The concentrations of progesterone in the CSF were only ≈2% of the total serum concentrations (corresponding to a slope of 0.02 in Fig. 1), a value that agrees with the calculated and measured unbound (that is, free) fraction in serum (*13*). It is generally accepted that only the unbound steroid concentration is of biological relevance (*14*) because only free steroids can, due to their lipophilicity, pass through the membrane of steroid target cells or endothelial cells of the blood-CSF (or blood-brain) barrier (*15*) to reach the central nervous system. Thus, although the total serum levels of progesterone can reach ≈400 n*M* in late pregnancy, as noted by Su *et al.* (*1*), the free serum concentration, which corresponds to the total CSF concentration [extrapolated to be ≈8 n*M* (Fig. 1, inset)], would barely suffice (*16*) to occupy σ_H sites on peripheral splenocytes or on neuronal cells in the brain: the apparent inhibitory constant (K_i) of progesterone was 376 n*M* for σ_H sites on splenocytes and 268 n*M* for σ_H sites on brain membranes (*1*).

3) The finding that progesterone displaced [^{3}H](+)SKF-10,047 but not [^{3}H]PCP suggests specificity, that is, the selectivity of the steriod for the σ_H site relative to the PCP site on the NMDA receptor. However, recent studies (*17–23*) have reported similar phenomena, from which we infer that at micromolar concentrations many steroids can modulate in vitro postsynaptic membrane-bound neurotransmitter receptors or presynaptic neurotransmitter uptake mechanisms. Figure 2 illustrates an example from our experience with the use of various binding modulators [steroids, cations, guanosine triphosphate (GTP) analogs, drugs] to "probe" structural differences between σ_H and PCP sites on NMDA receptors and μ and δ opioid receptors, respectively (*24*). Such chemically and functionally diverse substances as the dopamine D_2 receptor agonist 2-bromo-α-ergocryptine, the steroids 17α- and 17β-estradiol, and cations can inhibit the binding of the μ-opioid receptor–selective ligand [^{3}H][D-Ala^2, MePhe^4, Gly-ol^5]-enkephalin (DAGO) to rat brain membanes. In all but one of the diverse examples mentioned above, a single and specific mechanism can not explain the particular selective activity or receptor specificity exhibited by a steroid (*17–22*). A notable exception is the interaction with nanomolar affinity and structural stereo specificity, of certain progestins with the γ-aminobutyric acid A ($GABA_A$) receptor (*23*). This effect explains the sedative and hypnotic property of many steroids, particularly progesterone congeners (*25*). Otherwise, however, a likely common denominator for relative nonspecific actions of steroids might be their lipid solubility: exchanges between steroid and membrane-embedded cholesterol or other mechanisms might lead to membrane perturbations such as alterations in membrane fluidity, which in turn might afffect receptor affinity (*26*). In any event, binding inhibition assays should be cautiously interpreted (*27*). Recently, the inability to distinguish between various types of receptor interaction on the basis of inhibition of equilibrium binding alone has been pointed out (*28*). Demonstration of [^{3}H]progesterone binding as well as of the reciprocal displaceability by unlabeled (+)SKF-10,047 (or the other σ_H-selective ligands haloperidol, 3-PPP, and DTG) would be required to demonstrate that progesterone acts as a competitive inhibitor of (+)SKF-10,047 at the σ_H receptor (*29–33*). Finally, if progesterone interacts with σ_H sites, its agonist and antagonist properties should be assessed.

4) The in vitro σ-inhibitory activity of the steroids on splenocytes (*1*) appears difficult to reconcile with their in vivo granuloma formation–inhibiting activity (*2*). The lymphocytic population within σ_H receptor-containing splenocytes belongs predominantly to the B lineage, whereas that of xenograph-elicited granulomas belongs mainly to the T lineage (*34*). Even though there are also σ_H receptors on peripheral blood lymphocytes (*35*), which are mainly T cells (*34*), several lines of evidence speak against the hypothesis put forward by Su *et al.* (*1*). First, the fact that dexamethasone (which is a potent glucocorticoid agonist) and corticosterone were active, while androgens and estrogens were not (*2*), suggests that the inhibition of granulomas was mediated through glucocorticoid receptors (*2*), which are present in all cell lineages of the immune system (*36*). Second, the fact that the three "anti-inflammatory" steroids cited in (*1*) were progesterone, 11-desoxycorticosterone, and corticosterone is consistent

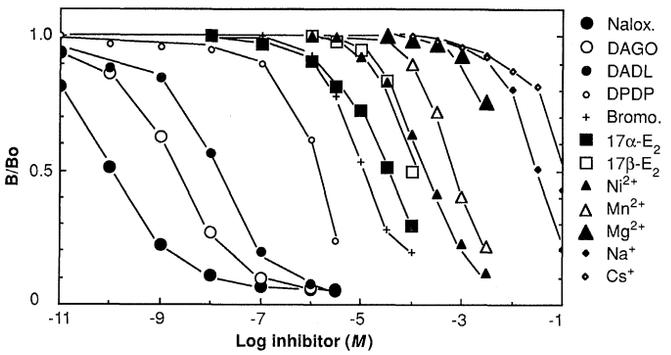

Fig. 2. Influence of various ligands [naloxone, [D-Ala^2,MePhe^4,Gly-ol^5]-enkephalin (DAGO), [D-Ala^2, D-Leu^5]enkephalin (DADL), [D-penicillamine^2, D-penicillamine^5]-enkephalin (DPDP)], drugs, steroids, mono- and divalent cations on binding of the μ opioid–selective ligand [^{3}H]-DAGO (0.5 n*M*) brain membranes (1 mg of protein per assay tube). These binding asays are described elsewhere (*24*), but were similar to those given by Su *et al.* (*1*). Similar results were obtained when the δ-opioid selective [^{3}H]DADL was used, except that the rank order of the opioid ligands was reversed. Only Mn^{2+} and Mg^{2+} ions discriminated between μ and δ sites: the binding of the δ-selective ligands [^{3}H]DADL and [^{3}H]DPDP was enhanced by 1 m*M* Mn^{2+} and Mg^{2+} (*24*), whereas that of the μ-selective ligand [^{3}H]DAGO was inhibited (as shown). All of these cations also suppressed the binding of the σ ligands [^{3}H]SKF-10,047, [^{3}H]haloperidol, [^{3}H]3-PPP ((+)3-[3-hydroxyphenyl]-*N*-(1-propyl)piperidine) and, to a greater extent, the PCP ligands [^{3}H]PCP and [^{3}H]TCP (1-[1-(2-thienyl)cyclohexyl]piperidine). As observed by others (*43*), 17α-estradiol was the most potent steroid so far found in displacing μ and δ ligands. Less potent or not inhibitory at all were all other steroids tested. The rank order at a fixed concentration of 100 μ*M* (the upper level of steroid solubility in assay) was 11-desoxycorticosterone ≥ 11-desoxycortisol ≥ prednisolone ≧ 4-androstene ≥ androsterone ≥ aldosterone ≥ testosterone ≥ 17α-hydroxyprogesterone ≥ 5α-dihydrotestosterone ≥ dexamethasone ≥ dehydroepiandrosterone ≥ progesterone ≥ danazol ≥ digoxin, whereby the binding inhibition found with 11-desoxycorticosterone was only ≈25%. The same pattern was seen when a nonselective opiate agonist like [^{3}H]etorphine or an opiate antagonist like [^{3}H]naloxone was used. In all these assays 100 μ*M* phenol, L- or D-tyrosine, (−)- or (+)-isoproterenol were without effect. That 17α-estradiol was ten times more potent than 17β-estradiol or 17α-ethinylestradiol points to the greater importance of the stereoconfiguration of the C17 moiety of the steroid D-ring (17α-hydroxyl group) as compared with the aromatic character of the steroid A ring for interaction with the opioid receptors or their surrounding lipid environment, respectively. Nalox., naloxone. Bromo., 2-bromo-α-ergocryptine.

with their direct biosynthetic relationship and hence with their similar structural properties (they are all C21 steroids). Finally, the fact that the glucocorticoid receptor has considerable affinity for all three of these steroids (*36*) makes it likely that progesterone can efficiently substitute for corticosterone, the major glucocorticoid hormone in the rat (*36*), which is consistent with the concept that antinflammatory actions are mediated by glucocorticoid receptors rather than by σ_H sites. Nonetheless, σ_H sites on lymphocytes might be implicated in possible alterations of immune functions under conditions of drug abuse (*37*). Likewise, can toxic concentrations of exogenously administered steroids produce psychiatric disorders (*38*)?

In conclusion, we agree that endocrine, nervous, and immune systems are functionally linked and that progesterone and other steroids can act to communicate among these systems. However, when one considers that steroids have an "affinity" for σ_H sites two orders of magnitude below their biologically active concentrations in any extracellular aqueous compartment of the body, the involvement of σ_H receptors and the specific role attributed to them (*1*) in this link seems questionable.

S. SCHWARZ
Immunoendocrinology Research Group, Austrian Academy of Sciences, and Department of General and Experimental Pathology, Faculty of Medicine, University of Innsbruck, A-6020 Innsbruck, Austria
P. POHL
Department of Neurology, Faculty of Medicine, University of Innsbruck
G.-Z. ZHOU
Laboratory of Theoretical and Physical Biology, National Institute of Child Health and Human Development, Bethesda, MD 20892

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4. R. Quirion *et al.*, *Trends Neurosci.* **10**, 444 (1987); P. C. Contreras *et al.*, *Mol. Neurobiol.* **1**, 191 (1987). The PCP site on the NMDA receptor [previously termed the common σ/PCP receptor (*5*)] corresponds to the low affinity (+)SKF-10,047 binding site. However, PCP binding sites might also be linked with structures other than the NMDA channel (*6*) and psychotomimetic reactions might not solely be mediated by PCP on the NMDA receptor-ionophor complex (*7*). The high-affinity (+)SKF-10,047 binding site to which Su *et al.* are referring (*1*) is termed the σ_H site because it also binds haloperidol with high affinity, unlike the PCP site on the NMDA receptor. Other σ_H-selective ligands are (−)butaclamol, (+)3-PPP, DTG (1,3-ditolyguanadine) and BW-234U (rimcazole), whereas TCP (thienylphencyclidine) and MK-801 have no affinity for the σ_H site (*4, 7, 8*). The relationship of the σ_H site or the PCP site on the NMDA receptor to the σ opioid receptor originally postulated by Martin *et al.* (*9*) is not yet established. The σ_H site may participate in the control of motor activity rather than of mental functions (*10*). A radically different view of the role of the σ_H site has been lately proposed, indicating that the σ_H site may represent a membrane-bound microsomal enzyme involved in metabolic breakdown of σ drugs (*11*).
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16. 800 n*M* progesterone was shown (*1*) to be required to lower the apparent affinity of [^{3}H](+)SKF-10,047 by a factor not greater than 2. The fact that CSF levels of cortisol (which normally reaches total serum levels of ≈600 n*M*, similar to progesterone levels in late pregnancy) were found to be on average 25 n*M* is consistent with the extrapolation made for progesterone (Fig. 1, inset). The averaged CSF levels of all other steroids were below 2 n*M*, even in patients with disturbed blood-CSF barriers (*12*). Since corticosterone binding globulin, the major progesterone carrier in serum, rises from ≈300 n*M* to ≈600 n*M* during pregnancy (*14*), the likelihood is small that the serum binding capacity becomes saturated, even at progesterone levels of 400 n*M*. Hydrophilic steroid sulfates were found to have CSF-serum ratios ≈100 times lower than those of the lipophilic steroids (*12*), making an in vivo interaction with σ_H receptors even more unlikely.
17. Pertinent examples are the inhibition by progesterone of [^{3}H]quinuclidinyl benzylate binding to muscarinic receptors (estrogens are ineffective) (*18*), the inhibition of extraneuronal catecholamine uptake by virtually all steroids (*19*), the presynaptic effect of 17β-estradiol in converting striatal D_2-dopamine receptors into a low-affinity agonist binding state (*20*), the insulin resistance syndrome due to cortisol excess–induced diminished insulin receptor affinity of postreceptor defects (*21*) and, similarly, the anti-inflammatory potency of some glucocorticoids that in a certain experimental setting correlates with their potency to diminish the affinity of membrane receptors for tumor necrosis factor (*22*).
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augmented [^{3}H]flunitrazepam binding to GABA-benzodiazepine receptor–Cl^- channel complexes [see M. D. Majewska, N. L. Harrison, R. D. Schwartz, J. L. Barker, S. M. Paul, *Science* **232**, 1004 (1986); N. L. Harrison, M. D. Majewska, J. W. Harrington, J. L. Barker, *J. Pharmacol. Exp. Ther.* **241**, 346 (1987); K. W. Gee, W.-C. Chang, R. E. Brinton, B. S. McEwen, *Eur. J. Pharmacol.* **136**, 419 (1987), A. L. Morrow, P. D. Suzdak, S. M. Paul, *ibid.* **142**, 483 (1987); S. Schwartz-Giblin, M. Canonaco, B. S. McEwen, D. W. Pfaff, *Neuroscience* **25**, 249 (1988)]. By comparison, pregnenolone-sulfate behaved as a $GABA_A$ antagonist [M. D. Majewska and R. D. Schwartz, *Brain Res.* **404**, 355 (1987)].
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27. Consider that, as exemplified in Fig. 2, the slopes of [^{3}H]DAGO binding inhibition isotherms in the presence of the various nonopiate "competitors" were quite similar, that the inhibitory constant (IC_{50}) of 17β-estradiol was equal to that of Ni^{2+} ions, and that the stereospecificity was the opposite of what one would expect if estrogens were of physiological importance to opioid receptors (17α-estradiol was ten times more potent than the natural hormone 17β-estradiol).
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29. This would be of interest because such a hydrophobic pocket (accepting anesthetics or steroids, respectively) on neurotransmitter receptors is hypothetical (*30*) in contrast with allosteric cation binding sites that are typical for a variety of receptors and transmembrane signaling components (*31*). Since there is only anectotal evidence on low-affinity (K_i >100 nM_2) steroid binding sites on synaptosomal membranes (*32*), the most parsimonial interpretation for the mode of action of "naturally" occurring (≈0.1 to 1 n*M*) concentrations of steroid hormones in any compartment of the body is the "classical" one: a steroid, owning to its lipophilicity, permeates the cell membrane, subsequently activates high-affinity (K_i ≈0.1 to 1 n*M*) cytosolic or nuclear receptors and thus ultimately leads to enhanced or silenced transcription via 5′-upstream–located steroid response elements of steroid-responsive genes. Compatible with this mode of action are also all of the known steroid-induced behavioral effects ["changes in mood and psychological parameters" in humans (*1*) and lordosis behavior in rodents as two examples], namely, transcriptional regulation of expression of the various components involved in interneuronal chemical signalling [such as, neurotransmitter receptor molecules, the subunits of the G proteins, the various postreceptor effector units, such as adenylate cyclase, and possibly ion channels as well (*33*)].
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Response: Schwarz *et al.* write that psychotomimetic and other behavioral actions of σ opioids, including *d*-*N*-allylnormetazocine (*d*-SKF-10,047), are more likely mediated by phencyclidine (PCP) receptors than by σ receptors. While some behavioral responses to dextrorotatory benzomorphans are mediated through PCP and other receptors, others appear to be linked specifically to σ receptors. For example, rats trained to discriminate *d*-pentazocine show *d*-pentazocine–appropriate responses to other σ ligands in 100% of trials, but they generalize to PCP in only 50% of trials (*1*). In this paradigm, PCP shows about only one-tenth the potency of *d*-SKF-10,047. Activation of A_{10} mesolimbic dopamine neurons by *d*-SKF-10,047 is blocked by rimcazole (*2*), a selective ligand with negligible potency at PCP receptors (*3*). Furthermore, at least seven potential antipsychotic agents that were efficacious in the preclinical tests share the ability to bind with high affinity to σ receptors (*4*). These drugs have negligible affinity at PCP receptors (*4*). Finally, drug-induced locomotor stimulation, which is selectively antagonized by putative σ antagonists such as haloperidol, BMY-14,802, and rimcazole, appears to be mediated through central activation of σ receptors (*5*).

Schwarz *et al.* argue that concentrations of progesterone (extrapolated to be about 8 n*M*) in cerebrospinal fluid (CSF) would be insufficient for appreciable occupation of σ receptors in the brain. But because progesterone is highly lipid-soluble, it would rapidly enter the brain from the plasma and would most likely show a long half-life in the brain before entering the CSF. Therefore, the CSF concentration of progesterone does not necessarily reflect the "available free" concentration in the brain or other tissues. Likewise, although the "available free" plasma concentration of progesterone in the latter part of human pregnancy is about 50 n*M* (*6*), which obviously could still occupy about 12% of σ receptors in the peripheral lymphocytes, the progesterone concentration in the placenta could reach approximately 30 μ*M* or even more (*7*). Additionally, high concentrations of progesterone should be present in the ovary and adrenal gland. These results suggest that progesterone may interact with σ receptors on lymphocytes not only systemically but also when blood cells reach organs, such as the placenta, the ovaries, or the adrenal gland, where there is an abundance of progesterone.

Schwarz *et al.* argue against the specificity of progesterone for σ receptors. We contend that progesterone is relatively more specific for σ than for PCP receptors. We did not imply that binding of progesterone to other sites is impossible. Progesterone occupies 50% of σ receptors at a concentration of 268 n*M*. In figure 2 of Schwarz *et al.*, steroids 17a-E2 and 17b-E2 are shown to "saturate" 50% of the delta opioid receptors, but only at extremely high concentrations (30,000 n*M* and 100,000 n*M*, respectively). Although there may be instances in which specific binding is not pharmacologically meaningful, as is the case with the steroids binding to delta receptors, the interaction of progesterone with σ receptors is not comparable. It would be desirable to demonstrate [^{3}H]progesterone binding to σ receptors, but the abundance of nonspecific binding (resulting from the lipophilicity of progesterone) appears to represent a potential problem.

Although glucocorticoids are antiinflammatory, the mechanism of the glucocorticoid-induced immune responses is largely unknown. We agree that the results in the granuloma formation test by Siiteri *et al.* (*7*) might reflect the involvement of glucocorticoid receptors. However, the correlation of the efficacies of preventing granuloma formation with the affinities of steroids at σ receptors is striking. As Schwarz *et al.* and we noted, PCP is immunosuppressive; and it is likely that PCP exerts immunosuppression through σ receptors rather than through PCP receptors. The relative contribution of glucocorticoid and of σ receptors to the