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Psychotomimetic opiate receptors labeled and visualized with (+)-[^{3}H]3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine

(σ receptor/[^{3}H]haloperidol/autoradiography/dopamine autoreceptor/phencyclidine)

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ABSTRACT **3-(3-Hydroxyphenyl)-*N*-(1-propyl)piperidine (3-PPP) has been proposed as a selective dopamine autoreceptor agonist in the central nervous system. This report describes the pharmacology and localization of specific high-affinity binding sites for (+)-[^{3}H]3-PPP in brain. The drug specificity of (+)-[^{3}H]3-PPP binding is identical to that of σ receptors, which may mediate psychotomimetic effects of some opiates. Haloperidol and the opioid derivatives, pentazocine, cyclazocine, and SKF 10,047 are potent inhibitors of (+)-[^{3}H]3-PPP binding. Stereoselectivity is exhibited for the (+) isomers of cyclazocine and SKF 10,047 at the σ site, opposite to the stereoselectivity seen at μ, δ, and κ opiate receptors. (+)-[^{3}H]3-PPP does not label dopamine receptors, as potent dopamine agonists and antagonists are weak inhibitors of binding and the localization of specific (+)-[^{3}H]3-PPP binding sites does not parallel that of dopamine neurons. Discrete localizations of (+)-[^{3}H]3-PPP binding sites in many brain areas including limbic, midbrain, brainstem, and cerebellar regions may explain psychotomimetic actions of opiates and behavioral effects of 3-PPP.**

Psychotomimetic actions of certain opiates in man (1) and parallel behavioral effects in animals have suggested the existence of specific psychotomimetic opiate receptors, designated σ receptors (2). Since these effects are not blocked by opiate antagonists, such as naloxone (3–5), the putative σ sites differ from more classical opiate receptors. It has been suggested that σ sites mediate the psychotomimetic effects of phencyclidine (PCP) or that psychotomimetic effects of opiates reflect influences on unique PCP receptors (6–8). Opiate ligands can label putative σ sites in brain membranes. The neuroleptic drug, haloperidol, is the most potent competitor at these binding sites (9, 10). The drug specificity of these sites correlates with the pharmacological profile of psychotomimetic opiates in behavioral tests (3, 9–11).

Dopamine neurons possess autoreceptors at which dopamine and related agonists inhibit dopaminergic neuronal activity, including dopamine synthesis and release (12). 3-(3-Hydroxyphenyl)-*N*-(1-propyl)piperidine (3-PPP) produces behavioral and biochemical effects in rodents that have been interpreted as reflecting agonist actions at dopamine autoreceptors (13–15). However, direct examination of dopamine autoreceptor function *in vitro* indicates that 3-PPP is quite weak at inhibiting dopamine release or reducing tyrosine hydroxylase activity (16–18).

The present report describes the pharmacology and localization of specific high-affinity binding sites for (+)-[^{3}H]3-PPP in brain. The drug specificity of these binding sites closely resembles that of σ receptors labeled in previous studies with ^{3}H-labeled opiates (9, 10). The pharmacology of the (+)-[^{3}H]3-PPP binding sites differs markedly from that of known dopamine receptor subtypes (19, 20). Autoradiographic studies reveal discrete localizations of (+)-[^{3}H]3-PPP binding sites in limbic regions and in brainstem and midbrain areas regulating motor function. These sites may account for the behavioral effects of 3-PPP as well as the psychotomimetic actions of opiate drugs.

MATERIALS AND METHODS

(+)-[^{3}H]3-PPP and (−)-[^{3}H]3-PPP (98 and 110.6 Ci/mmol, respectively; 1 Ci = 3.7×10^{10} becquerels) were prepared by S. Hurt (New England Nuclear). [^{3}H]Haloperidol (18 Ci/mmol) was also supplied by New England Nuclear. The enantiomers of 3-PPP were provided by H. Hall (Astra, Sweden). The (±), (+), and (−) isomers of *N*-allylnormetazocine (SKF 10,047) were donated by D. Jasinski (National Institute on Drug Abuse, Baltimore, MD). Samples of (±)-, (+)-, and (−)-cyclazocine and (±)- and (−)-pentazocine were from Sterling-Winthrop Research Institute (Rensselaer, NY). Dexoxadrol and levoxadrol were obtained from Upjohn. Dextrallorphan was supplied by Hoffman-La Roche. All other reagents were obtained from commercial sources.

In membrane-homogenate binding studies, fresh or frozen whole brains (male Sprague–Dawley rats, 150–250 g) were homogenized in 25 vol of ice-cold 50 mM Tris buffer (pH 7.7 at 25°C) and centrifuged at 45,000 × *g* for 10 min at 4°C. The pellet was then suspended in fresh buffer and recentrifuged. This procedure was repeated once more before membranes were finally suspended in an appropriate volume of 50 mM Tris buffer (pH 8.0 at 25°C; incubation buffer) for use in binding assays. Storage of the homogenate at −70°C for up to 4 wk did not alter binding activity. In a final assay volume of 0.5 ml, 2–4 nM (+)-[^{3}H]3-PPP (or [^{3}H]haloperidol) was incubated in the presence of various concentrations of unlabeled drug with the equivalent of 5–6 (or 2–3) mg (original wet weight) of tissue for 120 (or 45) min at room temperature. Nonspecific binding was defined as that remaining in the presence of 5 μM (or 10 μM) (±)-pentazocine. Similar levels of nonspecific binding were obtained with 5 μM (+)-3-PPP or 0.5 μM haloperidol. Binding of [^{3}H]haloperidol was always assessed in the presence of 25 nM spiperone to prevent binding of the ligand to D_2 dopamine receptors. Incubations were terminated by the addition of 2.5 ml of ice-cold 10 mM Tris buffer (pH 7.7 at 25°C) and filtration under vacuum through glass fiber filters (Schleicher & Schuell no. 32; pretreated with 0.5% polyethylenimine). Filters were washed with two [(+)-[^{3}H]3-PPP] or three ([^{3}H]haloperidol) consecutive 5-ml aliquots of washing buffer. The total time taken for the filtration/washing procedure was less than 10 sec. Radioactivity remaining on the filters was measured by liquid scintillation spectrometry at 60% efficiency. Saturation binding data were analyzed by Scatchard analysis, while

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Abbreviations: 3-PPP, 3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine; SKF 10,047, *N*-allylnormetazocine; PCP, phencyclidine.

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IC_{50} values and Hill coefficients were calculated from drug competition data using an iterative curve-fitting program (21). $K_{0.5}$ values were obtained using the Cheng–Prusoff equation and are reported instead of K_i values as no assumption is made regarding the competitivity of the drug interactions (22, 23).

Autoradiographic studies were carried out using slide-mounted rat and guinea pig brain sections prepared as described (24). Brain sections (6–10 μm) were first incubated for 15 min in 50 mM Tris buffer (pH 8.0 at 25°C) at room temperature, then incubated for 45 min in the presence of 8 nM (+)-[^{3}H]3-PPP. Adjacent sections included 5 μM (+)-3-PPP or 1 μM haloperidol in the incubation mixture to measure nonspecific binding. After two consecutive 4-min washes in buffer, slides were rinsed in distilled water and dried under a stream of cold dry air. Autoradiograms were generated by apposition of slides to tritium-sensitive film ([^{3}H]Ultrofilm; LKB) for 4–6 wk at 4°C (25).

RESULTS

Characteristics of (+)-[^{3}H]3-PPP and [^{3}H]Haloperidol Binding. (+)-[^{3}H]3-PPP binds saturably and with high affinity to rat whole brain membranes (Fig. 1*A*). In typical experiments using 3 nM (+)-[^{3}H]3-PPP total binding is 2700 cpm, while nonspecific binding assayed in the presence of 5 μM (±)-pentazocine is 400 cpm. At room temperature, binding of (+)-[^{3}H]3-PPP reaches equilibrium after 60–90 min and it is fully reversible in the presence of a 100-fold excess of unlabeled ligand or a 1:100 dilution with buffer. Specific binding of (+)-[^{3}H]3-PPP is linear with tissue concentration between 2 and 40 mg (wet weight) of tissue/ml and is absent in boiled tissue. Scatchard analysis of saturation data indicates the presence of a single population of binding sites with an apparent K_d of 30 ± 1.5 × 10^{-9} M and a maximal number of binding sites (B_{max}) of 31 ± 2.5 pmol/g (wet weight) of tissue (n = 9).

Using rat whole brain homogenates, total binding of 2.5 nM [^{3}H]haloperidol (in the presence of 25 nM spiperone) is typically 2300 cpm, while nonspecific binding measured in the presence of 10 μM (±)-pentazocine is 1000 cpm. The K_d of [^{3}H]haloperidol is 2.0 ± 0.3 × 10^{-9} M and the B_{max} is 30 ± 1.5 pmol/g (wet weight) of tissue (n = 3) (Fig. 1*B*).

Pharmacology of (+)-[^{3}H]3-PPP and [^{3}H]Haloperidol Binding. The drug specificities of (+)-[^{3}H]3-PPP and [^{3}H]haloperidol binding are similar, with the same absolute and relative potencies of drugs obtained in drug competition studies using the two ligands (Table 1). Haloperidol is the most potent drug against both ligands, with an affinity of 2–3 × 10^{-9} M. In addition to 3-PPP, other potent drugs include the psychotomimetic opiates, cyclazocine, pentazocine, and SKF 10,047, and the phenothiazines, perphenazine and fluphenazine. The binding sites are quite different from D_2 dopamine receptors in that stereospecificity toward the isomers of butaclamol is reversed. At D_2 receptors (+)-butaclamol is several hundred times more potent than (−)-butaclamol, whereas at both (+)-[^{3}H]3-PPP and [^{3}H]haloperidol sites the (−) isomer is about 10 times more potent than the (+) isomer. Moreover, spiperone, pimozide, and (+)-butaclamol, which have potencies in the low nanomolar range at D_2 dopamine receptors, display $K_{0.5}$ values of greater than 0.5 × 10^{-6} M at the σ site. The dissimilarity of these sites from dopamine receptors is further indicated by the failure of dopamine, apomorphine, and the dopaminergic ergot lisuride to inhibit binding.

Stereoselectivity for the binding site is apparent for the isomers of 3-PPP with the affinity for the (+) isomer being 3- to 5-fold greater. The binding sites for (+)-[^{3}H]3-PPP and [^{3}H]haloperidol display opposite stereospecificity from classical opiate receptors, where the (−) isomer of opiates is generally more potent (26). Dextrallorphan, a (+) isomer, is about 10 times more potent than the corresponding (−) isomer, levallorphan. Likewise, (+)-cyclazocine is substantially more potent that (−)-cyclazocine and a similar reversed stereoselectivity is apparent for SKF 10,047. Interestingly, the racemic mixtures of cyclazocine and SKF 10,047 are approximately equipotent with their respective (+) isomers (see also ref. 9), while (−)-pentazocine is equipotent with (±)-pentazocine. (+)-[^{3}H]3-PPP binding is not inhibited by high concentrations of naloxone, levorphanol, dihydromorphine, and [D-Ala^2-D-Leu^5]enkephalin, which are extremely potent at classical opiate receptors.

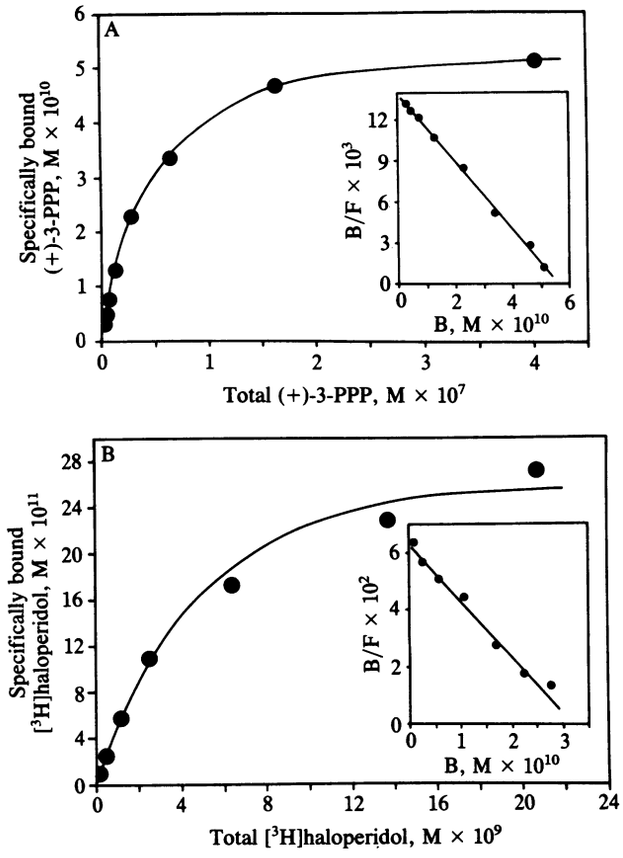

FIG. 1. Equilibrium saturation binding of (+)-3-PPP and haloperidol to rat brain membranes. (*A*) Membranes [5 mg (wet weight)] were incubated with 3 nM (+)-[^{3}H]3-PPP and various concentrations (1–400 nM) of (+)-3-PPP in 0.5 ml of 50 mM Tris buffer (pH 8.0) for 120 min at room temperature. (*B*) Membranes [3 mg (wet weight)] were incubated with various concentrations (0.1–21 nM) of [^{3}H]haloperidol in 0.5 ml of 50 mM Tris buffer (pH 8.0) for 45 min at room temperature. For each ligand, results are from a single typical experiment and values are the mean of duplicate determinations. (*Insets*) Scatchard plots of the saturation binding data. B and F, bound and free ligand, respectively.

The psychotomimetic anesthetic PCP displays a $K_{0.5}$ of 0.8 × 10^{-6} M at (+)-[^{3}H]3-PPP sites and a $K_{0.5}$ of about 1.0 × 10^{-6} M at [^{3}H]haloperidol sites. These potencies are somewhat less than the affinity of this drug at sites labeled with [^{3}H]PCP. The PCP-like drugs dexoxadrol and levoxadrol do not exhibit stereoselectivity for (+)-[^{3}H]3-PPP sites.

The slopes of competition curves for various drugs against (+)-[^{3}H]3-PPP and [^{3}H]haloperidol binding differ considerably, although the Hill coefficients for any given drug are similar for both ligands (Table 1). Shallow curves are obtained for the opiate drugs with Hill coefficients of 0.5–0.8, while neuroleptic drugs such as perphenazine and haloperidol display Hill coefficients of close to 1.0, as do the isomers of 3-PPP. The low Hill coefficients for the psychotomimetic opi-

Table 1. Potencies and Hill slopes of drugs competing for specific (+)-[^{3}H]3-PPP and [^{3}H]haloperidol binding to rat brain membranes

Drug	(+)-[^{3}H]3-PPP			[^{3}H]Haloperidol		
	n	$K_{0.5}$, $\times 10^{-9}$ M	n_H	n	$K_{0.5}$, $\times 10^{-9}$ M	n_H
(+)-3-PPP	9	30 ± 1.5*	1.02 ± 0.04	6	58 ± 8	0.79 ± 0.07
(−)-3-PPP	5	155 ± 17	1.01 ± 0.03	5	162 ± 24	0.81 ± 0.04
Haloperidol	5	2.4 ± 0.4	0.89 ± 0.07	3	2.0 ± 0.3†	0.99 ± 0.01
(+)-Butaclamol	6	1800 ± 130	0.88 ± 0.03	6	955 ± 190	1.03 ± 0.07
(−)-Butaclamol	6	157 ± 19	0.76 ± 0.02	6	85 ± 10	1.08 ± 0.09
Perphenazine	6	24 ± 3	0.90 ± 0.04	6	13 ± 1	0.90 ± 0.05
Fluphenazine	4	62 ± 3	0.93 ± 0.10	—		
Spiperone	6	632 ± 42	0.92 ± 0.05	—		
Pimozide	5	508 ± 31	1.24 ± 0.04	—		
(±)-SKF 10,047	4	373 ± 13	0.63 ± 0.05	4	516 ± 75	0.80 ± 0.04
(+)-SKF 10,047	4	343 ± 33	0.58 ± 0.03	4	319 ± 55	0.53 ± 0.03
(−)-SKF 10,047	4	1350 ± 110	0.75 ± 0.05	4	1860 ± 400	0.93 ± 0.04
(±)-Cyclazocine	7	96 ± 5	0.58 ± 0.01	6	180 ± 41	0.64 ± 0.04
(+)-Cyclazocine	4	111 ± 13	0.50 ± 0.02	4	149 ± 20	0.53 ± 0.02
(−)-Cyclazocine	4	408 ± 64	0.76 ± 0.04	5	504 ± 49	0.74 ± 0.04
(±)-Pentazocine	8	23 ± 2	0.75 ± 0.04	6	43 ± 8	0.80 ± 0.09
(−)-Pentazocine	5	29 ± 6	0.77 ± 0.05	—		
Dextrallorphan	4	154 ± 21	0.50 ± 0.01	5	78 ± 12	0.75 ± 0.06
Levallorphan	4	1780 ± 130	0.68 ± 0.05	5	1060 ± 70	0.83 ± 0.01
Dextromethorphan	5	768 ± 68	0.75 ± 0.02	—		
Phencyclidine	8	711 ± 84	0.66 ± 0.01	6	956 ± 135	0.84 ± 0.04
Dexoxadrol	4	1880 ± 510	0.88 ± 0.02	—		
Levoxadrol	4	2080 ± 490	0.98 ± 0.02	—		

Drugs that did not inhibit specific (+)-[^{3}H]3-PPP binding included ($K_{0.5} > 10 \times 10^{-6}$ M) phentolamine, iprindole, (+)-amphetamine, cocaine, levorphanol, bradykinin, and cholecystokinin (CCK-4, -8, -33), as well as ($K_{0.5} > 100 \times 10^{-6}$ M) dopamine, apomorphine, lisuride, naloxone, dihydromorphine, [D-Ala^2-D-Leu^5]enkephalin, α and β neo-endorphin, hexamethonium, decamethonium, scopolamine, rauwolscine, picrotoxin, bicuculline, γ-aminobutyric acid, amrinone, tetrodotoxin, lysergic acid diethylamide, cannabinol, (+)-Δ9-tetrahydrocannabinol, angiotensin II, and neurotensin. Values given are mean ± SEM of the number of experiments (n) shown. IC_{50} values and Hill coefficients (n_H) were determined from data obtained using six (triplicate) or nine (duplicate) concentrations of drug, by computer-assisted iterative curve fitting (21). $K_{0.5}$ values are calculated from IC_{50} values according to the Cheng–Prusoff equation (22) using apparent K_D values for (+)-[^{3}H]3-PPP and [^{3}H]haloperidol of 30 and 2 $\times 10^{-9}$ M, respectively. Results are from experiments using 2–4 nM (+)-[^{3}H]3-PPP (or [^{3}H]haloperidol, in the presence of 25 nM spiperone) with 5–6 (or 2–3) mg (original wet weight) of tissue incubated for 120 (or 45) min at room temperature. Nonspecific binding of (+)-[^{3}H]3-PPP (or [^{3}H]haloperidol) was measured in the presence of 5 (or 10) μM (±)-pentazocine.

*Values given are K_d and n_H from (+)-3-PPP saturation experiments.

†Values given are K_d and n_H from haloperidol saturation experiments.

ates may indicate a multicomponent binding model, as has been demonstrated for β-adrenergic and classical opiate receptors, where agonists show shallow competition curves with ^{3}H-labeled antagonists but not with ^{3}H-labeled agonists (26).

Binding of (−)-[^{3}H]3-PPP to rat whole brain homogenates has also been evaluated. This ligand has a lower affinity for the binding site, with specific binding levels approximately 50% of total binding. However, specific binding levels are adequate to evaluate drug potencies and the drug specificity for sites labeled with (−)-[^{3}H]3-PPP is essentially the same as that observed with (+)-[^{3}H]3-PPP (data not shown).

Autoradiographic Localization of (+)-[^{3}H]3-PPP Binding Sites. The regional distribution of (+)-[^{3}H]3-PPP binding in homogenates reveals high binding levels in the brainstem, cerebellum, and hypothalamus, with lower levels in the cerebral cortex, corpus striatum, and olfactory tubercle. Regional studies of (+)-[^{3}H]3-PPP and [^{3}H]haloperidol binding indicate essentially the same regional variations (data not shown).

Autoradiographic studies provide details of (+)-[^{3}H]3-PPP binding site localizations, with a number of areas displaying high densities of receptor-associated grains. All descriptions of (+)-[^{3}H]3-PPP binding site localizations refer to specific grains that can be abolished by incubation with either 5 μM (+)-3-PPP or 1 μM haloperidol. Essentially all (+)-[^{3}H]3-PPP labeling of brain sections is abolished by this treatment. Autoradiography has been carried out in both rats and guinea pigs with similar results; examples of localizations in guinea pig brain are presented in Fig. 2.

Two major groups of structures are labeled by (+)-[^{3}H]3-PPP, one associated with the limbic system and the other related to the brainstem motor regulatory system. Among limbic structures, some of the highest grain densities are apparent in the hippocampal formation. A dense band of grains appears in the pyramidal cell layer, especially in layers CA3 and CA4 (Fig. 2*B*). The granular layer of the dentate gyrus is also labeled, as is the subiculum. Other limbic structures such as the olfactory tubercle and the mamillary bodies have low levels of receptor-associated grains. Most of the cerebral cortex displays low grain densities. However, both the pyramidal cell layer of the pyriform cortex and the superficial cell layers of the cingulate cortex, which is functionally associated with the limbic system, show a dense layer of grains (Fig. 2 *A* and *B*).

Within the midbrain, the periaqueductal grey and the dorsal raphe nucleus, structures associated with the limbic system, display high grain densities. Most of the hypothalamus possesses high grain densities, while closely adjacent portions of the thalamus, such as the posterior thalamic nuclei group are low in grains. However, some portions of the thalamus, such as the zona incerta, do possess high grain densities. The medial and lateral septal nuclei and the diagonal band of Broca are heavily labeled. The caudate putamen and nucleus accumbens, areas associated with dense dopaminergic innervation, have low (+)-[^{3}H]3-PPP grain densities, although labeling is present in the zona compacta of the substantia nigra, which contains the cell bodies of dopamine neurons (Fig. 2 *A* and *B*).

A variety of interconnected structures involved in regulat-

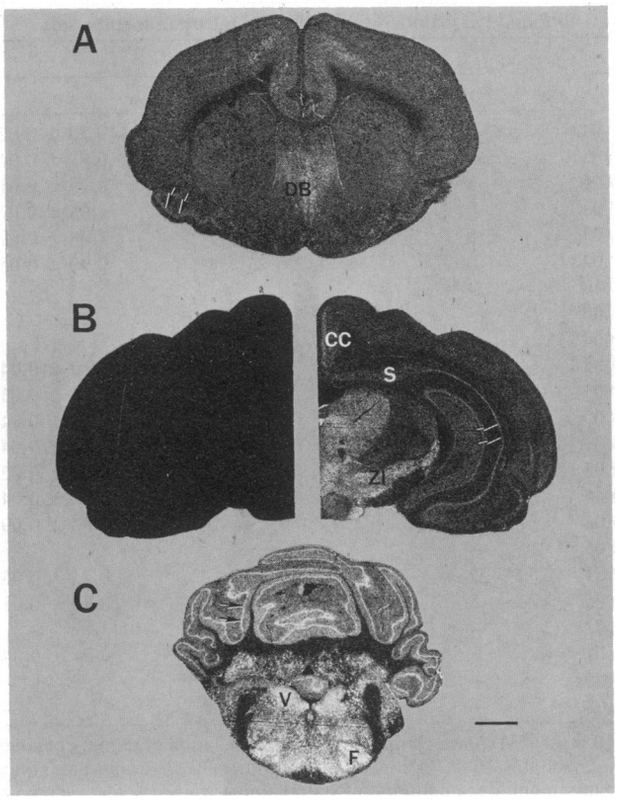

FIG. 2. Autoradiograms of (+)-[^{3}H]3-PPP binding sites in coronal sections of guinea pig brain. Brain sections (6 μm) were incubated with 8 nM (+)-[^{3}H]3-PPP and apposed to tritium-sensitive film for 5 wk before development. Increased grain density is indicated by increased whiteness in the photograph. (*A*) High grain densities are present in the diagonal bond of Broca (DB) and nearby septal regions, the pyramidal cell layer of the pyriform cortex (arrows), and deep cell layers of the medial cortex. (*B*) *Left*, addition of 5 μM (+)-3-PPP to the incubation eliminates all specific labeling; *right*, highest grain densities are present in the pyramidal cell layer of the hippocampus (arrows), zona incerta (ZI), subiculum (S), and throughout medial areas of the midbrain–thalamic junction. Superficial cell layers of the cingulate cortex (CC) also have high grain densities. (*C*) Highest grain densities are present in the Purkinje cell layer of the cerebellum (arrows), the facial nucleus (F), vestibular nuclei (V), and throughout the deep cerebellar nuclei. Moderate grain densities are present throughout the remaining areas of the cerebellar cortex and the brainstem, with an absence of labeling in white matter areas. (Bar = 2 mm.)

ing motor behavior display high grain densities. Several motor nuclei in the brainstem, such as the hypoglossal and facial nuclei and nucleus ambiguus, as well as the paramedian, parvocellular, and paragigantocellular reticular nuclei, have high levels of localized labeling. The cerebellum and related nuclei in the brainstem also have high receptor densities. Within the cerebellum a dense band of grains is associated with the Purkinje cell layer. Grain densities are also high in several of the deep cerebellar nuclei. Several of the vestibular nuclei of the brainstem, which receive synaptic input from the deep cerebellar nuclei, also display high concentrations of (+)-[^{3}H]3-PPP binding sites (Fig. 2*C*).

DISCUSSION

In the present study, pharmacological and autoradiographical data demonstrate that (+)-[^{3}H]3-PPP labels high-affinity binding sites in brain that appear identical to σ receptors. Previously, σ binding sites in brain membranes have been labeled using [^{3}H]SKF 10,047 (9) and [^{3}H]ethylketocyclazocine (10) in paradigms in which binding of the ligands to conventional (μ, κ, and δ) opiate receptors was blocked by appropriate drugs. As haloperidol was the most potent competitor at these σ sites, Tam (27) has used [^{3}H]haloperidol to label such sites, a result confirmed in the present study.

Evidence that the sites labeled by (+)-[^{3}H]3-PPP and [^{3}H]haloperidol represent pharmacologically relevant σ receptors derives from comparisons between pharmacological effects in animals and man with the drug specificity of the binding sites. Thus among a large number of opiates, those most potent at σ binding sites are psychotomimetic, while opiates that are not psychotomimetic are extremely weak at these sites despite their very high affinity for classical opiate receptors (1–3, 9–11). Also, (+) isomers of opiate drugs, which lack classical opiate receptor actions, are more potent than the (−) isomers at the σ binding sites and demonstrate "σ-like" behavioral effects (3, 11, 28).

Localization of receptors by autoradiography has helped explain actions of numerous drugs. For example, the classical effects of opiates on pain perception, affective states, pupillary diameter, and respiration (29) and differential effects of μ (30) and κ (31) opiates can be accounted for by receptor localizations. Therefore, the psychotomimetic actions of opiates may be explained by the selective localizations of σ receptors observed in the present study. Limbic structures that regulate emotional behavior such as the hippocampal formation, periaqueductal grey, septal areas, and the raphe nuclei possess the highest densities of σ receptors. If the σ receptors described here are responsible for behavioral effects of psychotomimetic opiates, then receptor antagonists should block behavioral effects of psychotomimetic drugs such as SKF 10,047. Interestingly, E. T. Iwamoto (ref. 32 and personal communication) found that (−)-butaclamol was uniquely potent in blocking behavioral actions of SKF 10,047, suggesting effects on σ receptors.

Sites labeled with [^{3}H]PCP (33–35) differ from the σ receptor labeled by ^{3}H-labeled opiates (9, 10) and by (+)-[^{3}H]3-PPP in the present study. Thus, haloperidol is potent at the σ receptor, while lacking potency at the [^{3}H]PCP binding site. Furthermore, the PCP-like drugs dexoxadrol and levoxadrol are equipotent at the σ receptor, yet dexoxadrol is substantially more potent than levoxadrol at the [^{3}H]PCP site (34) while, in drug discrimination paradigms dexoxadrol, but not levoxadrol, is recognized as PCP (36). However, σ binding sites may have relevance to the actions of PCP in man. Areas of high 2-deoxy-D-glucose uptake following behaviorally active doses of PCP include the hippocampus, subiculum, and cingulate cortex (37), which also contain large numbers of σ receptors. The EC_{50} brain level (0.8–5 μM) for behavioral effects of PCP (38, 39) is close to the $K_{0.5}$ for the drug against (+)-[^{3}H]3-PPP and [^{3}H]haloperidol. Moreover, PCP reduces the firing rate of cerebellar Purkinje cells, an action that is potently reversed by haloperidol (40), which correlates with the localization of σ receptors to the Purkinje cell layer and the potency of haloperidol at σ sites.

The (+)-[^{3}H]3-PPP binding sites described here are not dopamine autoreceptors, since their distribution does not parallel that of dopamine neurons and classical dopamine agents are weak at these sites. However, actions of 3-PPP at σ receptors may account for the effects of the drug on behavior and dopaminergic nerve function. The zona compacta of the substantia nigra, which contains dopamine neurons that project to the corpus striatum, is labeled by (+)-[^{3}H]3-PPP. Furthermore, neurons with σ receptors project to dopaminergic regions. For instance, reticular nuclei and the dorsal raphe nucleus, rich in σ receptors, project to the substantia nigra. σ receptors occurring in locomotor regulatory areas, such as cerebellar, motor, and reticular formation nuclei, may relate to the locomotor effects of 3-PPP.

If the receptors labeled here do explain the dopaminergic behavioral effects of 3-PPP, then drugs with high affinities

for these σ binding sites should have marked influences on 3-PPP-induced behavior. In preliminary studies, SKF 10,047 was more effective in antagonizing the locomotor effects of 3-PPP than those of apomorphine (data not shown). Furthermore spiperone, which is much more potent at dopamine than at σ receptors, is less effective in reversing the locomotor effects of 3-PPP than those of apomorphine (41).

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