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ACCELERATED COMMUNICATION

Multiple Affinity Binding States of the σ Receptor: Effect of GTP-Binding Protein-Modifying Agents

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SUMMARY

The σ receptor, which is labeled with (+)-[^{3}H]3-(3-hydroxyphenyl)-*N*-1-(propyl)piperidine [(+)-[^{3}H]3-PPP], is a site that binds several psychotomimetic opiate benzomorphans and certain antipsychotics, such as haloperidol. In order to elucidate the mechanisms involved in σ receptor ligand binding, equilibrium binding analysis and kinetics of association and dissociation of the relatively selective σ receptor ligand (+)-[^{3}H]3-PPP were determined in rat brain membranes in the absence and presence of 5′-guanylylimidodiphosphate [Gpp(NH)p]. In the absence of Gpp(NH)p, (+)-3-PPP, cyclazocine, pentazocine, and (+)-SKF 10047 bind to high and low affinity sites (K_H = 1.3–7.5 nM; K_L = 84–500 nM), as determined by computer assisted analysis of the inhibition of (+)-[^{3}H]3-PPP binding by the σ ligands. The antipsychotics haloperidol and chlorpromazine inhibit (+)-[^{3}H]3-PPP binding in a manner indicating interaction with a single state of the receptor. Gpp(NH)p (0.1 mM) abolished the high affinity binding component of the σ agonist-like compounds tested but had no effect on the affinities of the antipsychotics for the receptor. Gpp(NH)p decreased the association rate of (+)-[^{3}H]3-PPP binding 5-fold and also converted the biexponential dissociation kinetics of the ligand, observed in the absence of Gpp(NH)p, to a rapid monophasic dissociation process. Pretreatment of membranes with *N*-ethylmaleimide and pertussis toxin inhibited (+)-[^{3}H]3-PPP binding and abolished the effect of Gpp(NH)p on the σ ligand binding. These findings indicate that the σ receptor is capable of existing in two discrete states, having high and low affinity for σ agonist-like drugs. The regulation of the high affinity binding state by GTP-binding protein-modifying agents suggests its coupling to GTP-binding protein(s).

Psychotomimetic opiate benzomorphans, such as SKF 10047, cyclazocine, and pentazocine, have been postulated to elicit their effects by activation of the σ receptors, which are distinct from the classical μ, δ, and κ opioid receptors (1). Initial characterization of the receptors for psychotomimetics suggested coidentity between the binding site for the psychotropic agent PCP and the dextrorotatory isomer of SKF 10047 (2, 3). However, more recent studies revealed that (+)-SKF 10047 binds in rat and guinea pig brain membranes to at least two distinct receptor sites, which are designated as σ/haloperidol and σ/PCP receptors (4–6). The σ/haloperidol receptor is highly sensitive to the psychotomimetic opiates, the neuroleptic haloperidol, and the dopamine autoreceptor agonist (+)-3-PPP but has relatively low sensitivity for PCP analogs (4, 6, 7). In contrast, the σ/PCP receptor, which is labeled with either (+)-SKF 10047 or with the potent PCP analog PCP-3-OH, is insensitive to haloperidol (7, 8). Binding of [^{3}H]PCP-3-OH is associated with an additional low affinity binding site selective for PCP analogs (7, 8) that is coupled to the *N*-methyl-D-aspartate-ion channel complex (9).

The characterization of the drug specificity and the unique regional distribution of the σ/haloperidol receptor (hereafter referred to as the σ receptor) in the mammalian central nervous system was greatly facilitated by using the relatively selective and potent σ ligand (+)-[^{3}H]3-PPP (4, 10–12). In an attempt to further distinguish between the properties of the σ and PCP receptors, we have recently found that σ receptor binding, unlike PCP receptor binding, is highly sensitive to GTP and its stable analog Gpp(NH)p (13).

The effects of guanine nucleotides are mediated through specific G proteins, which are thought to have a pivotal role in signal transduction mechanisms involving different second messenger systems (for review, see Refs. 14–16). The formation of the complex consisting of agonist, receptor and a G protein and the changes in receptor affinity during the uncoupling of the receptor from a G protein have been the focus of numerous receptor neurotransmitter studies (14–16).

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ABBREVIATIONS: PCP, phencyclidine; (+)-3-PPP, (+)-3-(3-hydroxyphenyl)-*N*-1-(propyl)piperidine; (+)-SKF 10047, (+)-*N*-allylnormetazocine; NEM, *N*-ethylmaleimide; PT, pertussis toxin; Gpp(NH)p, 5′-guanylylimidodiphosphate; G protein, GTP-binding protein.

The present study was undertaken to determine whether the σ receptor is coupled to G protein(s) and to elucidate the molecular events associated with the formation of a σ ligand-receptor complex. The effects of Gpp(NH)p on competition experiments and kinetics of (+)-[^{3}H]3-PPP binding in rat brain membranes were examined. In addition, the effects of NEM and PT were assessed. Our results provide evidence suggesting the existence of two molecular states of the σ receptor, the high affinity state being coupled to GTP-sensitive binding protein(s).

Materials and Methods

(+)-[^{3}H]3-PPP (99 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Unlabeled (+)-3-PPP, haloperidol and chlorpromazine were purchased from Research Biochemicals (Natick, MA.). Pentazocine and cyclazocine were obtained from the National Institute on Drug Abuse Research Technology Branch. Gpp(NH)p, pertussis toxin, and NEM were purchased from Sigma Chemical Co. (St. Louis, MO).

Binding assays were performed essentially as previously described (7). Male Sprague Dawley rats (200–220 g) were decapitated and whole brains were homogenized in 15 volumes of ice-cold Tris·HCl buffer (50 mM; pH 7.7) and centrifuged at 45,000 × *g* for 15 min at 0–4°. Pellets were resuspended in the buffer, centrifuged once more (45,000 × *g*; 15 min), and then resuspended in 6 volumes of sucrose (0.32 M). Membrane aliquots were frozen at −80° until needed. For typical competition binding assays, membranes were resuspended in 10 volumes of Tris·HCl buffer (10 mM; pH 7.7), and 1-ml samples in triplicate were incubated with 1–1.2 nM (+)-[^{3}H]3-PPP in the absence and presence of either unlabeled (+)-3-PPP or pentazocine (10 μM) in order to determine specific ligand binding. Based on the kinetic binding experiments performed in this study, the incubation time at 25° was 60 min for routine assays. The effect of Gpp(NH)p in competition assays was assessed following addition of the nucleotide (0.1 mM) at the onset of the reaction. Similarly, to assess the association kinetics of (+)-[^{3}H]3-PPP in the presence of Gpp(NH)p, the nucleotide was added coincident with the radiolabeled ligand. Dissociation experiments were carried out following incubation of the radiolabeled ligand with the membranes for 60 min, a time period that allowed equilibrium to be reached. To initiate dissociation, an excess of unlabeled pentazocine or (+)-3-PPP (10 μM) (both resulted in very similar results) was added, and samples of 1 ml were filtered, at various time intervals, through Whatman GF/B filters presoaked in 0.03% polyethyleimine and were washed twice with ice-cold Tris·HCl buffer (10 mM). The effect of Gpp(NH)p on the dissociation rate was assessed following incubation of the membranes with the nucleotide (0.1 mM) during the association step (60 min). Previous studies have shown that prolonged incubation of Gpp(NH)p with membrane preparations (up to 2 hr) results in minimal (less than 5%) hydrolysis of the analog (17).

Rate constants for dissociation were calculated from the following equation (18):

$$[LR]_t/[LR]_0 \equiv Ae^{-k_{-1}t} + (1 - A)e^{-k'_{-1}t}$$

where $[LR]_t$ and $[LR]_0$ denote the concentration of ligand bound at time t and zero, respectively, A is the fraction of sites having a first-order dissociation rate constant of k_{-1} and $(1 - A)$ is the fraction of sites having a first-order dissociation constant k'_{-1}. The program is solved for the floating parameters, A, k_{-1}, and k'_{-1}. If k'_{-1} and k_{-1} did not vary significantly, and A or $(1 - A)$ was a small fraction, a homogeneous system was assumed.

Treatment of brain membranes with NEM (0.5 mM) was carried out at 37° for 30 min. Dithiothreitol (5 mM) was added to stop the reaction and membranes were then centrifuged (45,000 × *g*; 15 min), washed with Tris·HCl buffer (50 mM; pH 7.7), and centrifuged again. A parallel washing procedure was performed in control membrane preparations. PT treatment was carried out as described previously (19, 20). PT (50 μg/250 μl) was preactivated at 25° for 1 hr in 50 mM dithiothreitol. ADP-ribosylation of the membrane proteins was carried out in a final volume of 0.1 ml of 100 mM Tris·HCl buffer (pH 7.7) containing 10 mM thymidine, 1 mM EDTA, 1 mM L-α-dimyristoyl phosphatidylcholine, 1 mM ATP, 1 mM NAD, 5 μg of preactivated PT, and 1.1–1.3 mg of membranes. The reaction was carried out for 60 min at 25° and then (+)-[^{3}H]3-PPP (8 nM) was added and the incubation was continued for 60 min (25°). To determine the effect of PT, control binding was assessed in the ribosylation buffer in the absence of PT. The effect of Gpp(NH)p on the binding of (+)-[^{3}H]3-PPP to PT-pretreated membranes was determined following addition of the nucleotide coincident with the radiolabeled ligand.

The data were analyzed by using the LIGAND curve-fitting and receptor binding analysis program, Version 2.3.10 (21, 22). This weighted, nonlinear, model-fitting computer program distinguishes as many as three distinct binding sites. The fit for a specific number of sites was compared using an F test incorporated in the program and a $p < 0.05$ to be considered a significantly better fit for the model tested.

Results

Competition studies. Several compounds considered to be σ receptor ligands were examined for their ability to compete for (+)-[^{3}H]3-PPP binding in rat brain membranes in the absence and presence of Gpp(NH)p (0.1 mM). In the absence of Gpp(NH)p the LIGAND computer-fitted curves for unlabeled (+)-3-PPP competing for (+)-[^{3}H]3-PPP (1–1.2 nM) binding indicated the best fit for a two-site model over a one-site model ($F = 12$; $p = 0.003$). The affinity constants for the high and low affinity sites are $K_H = 1.3 \pm 0.1$ nM and $K_L = 84 \pm 5$ nM, respectively. The proportion of sites in the high and low affinity states is 1.6:1 (Fig. 1 and Table 1). In the presence of 0.1 mM Gpp(NH)p (Fig. 1), analysis of the binding data indicated the best fit for a one-site model, the two-site model being unacceptable. The K_i value obtained is 72 ± 6 nM, which is very similar to the K_L value for the low affinity binding state observed in the absence of Gpp(NH)p (84 ± 5 nM). The total number of (+)-3-PPP binding sites under these conditions is

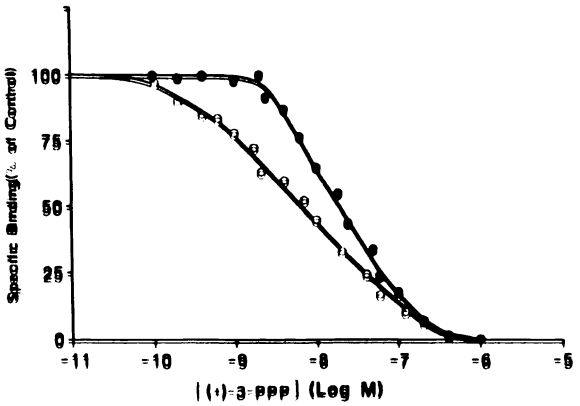

Fig. 1. Competition of unlabeled (+)-3-PPP for (+)-[^{3}H]3-PPP (1–1.2 nM) binding in rat brain membranes in the absence (○) and presence (●) of Gpp(NH)p (0.1 mM). Competition assays were carried out as described in Materials and Methods. Nonspecific binding determined in the presence of either pentazocine or unlabeled (+)-3-PPP (10 μM) was very similar and represented 18–21% of the total binding. The LIGAND computer-fitted curves for the inhibition of the radiolabeled ligand in the absence of Gpp(NH)p resulted in the best fit for a two-site model ($F = 12$; $p = 0.003$). In the presence of Gpp(NH)p, the best fit was for a one-site model. Results are presented as the percentage of the specific binding obtained in the absence or presence of Gpp(NH)p and are the mean of four experiments (SE ≤ 5% of mean values), each carried out in triplicate.

TABLE 1

Affinity constants of σ receptor ligands for (+)-[^{3}H]3-PPP binding sites in rat brain membranes and the proportion of high and low affinity binding states in the absence and presence of Gpp(NH)p

Competition binding assays in rat brain membranes were carried out using 1–1.2 nM (+)-[^{3}H]3-PPP and 10 μM pentazocine for the determination of nonspecific binding, as described in Materials and Methods. K_H and K_L denote the affinity constants for binding of the drug to high and low affinity forms of the receptor, respectively, and K_i denotes the affinity constant for binding to a single state of the receptor. R_H and R_L are the proportions of the receptor in each state and R represents a single state of the receptor. The equilibrium binding parameters of (+)-[^{3}H]3-PPP under the various conditions were input into the LIGAND program for the calculation of the binding parameters of the drugs tested. Results represent the mean ± standard error of three or four separate experiments.

Drug	−Gpp(NH)p				+Gpp(NH)p	
	K_H	R_H	K_L	R_L	K_i	R
	nM	%	nM	%	nM	%
(+)-3-PPP	1.3 ± 0.1	60 ± 4	84 ± 5	40 ± 3	72 ± 6	100
Pentazocine	2.1 ± 0.2	53 ± 3	191 ± 6	47 ± 4	125 ± 7	100
Cyclazocine	7.5 ± 0.4	63 ± 4	520 ± 12	37 ± 3	535 ± 11	100
(+)SKF 10047[a]	2.2 ± 0.2	48 ± 5	180 ± 7	52 ± 5	145 ± 9	100
Haloperidol			1.8 ± 0.01	100	1.4 ± 0.01	100
Chlorpromazine			650 ± 22	100	680 ± 15	100

[a] Taken from Ref. 13.

47 ± 2 pmol/g of tissue, which is comparable to the total number of (+)-3-PPP binding sites detected in the absence of Gpp(NH)p (R_H = 3.8 ± 0.2 and R_L = 44.2 ± 1.5 pmol/g of tissue).

Previous studies have shown that several σ receptor agonist-like compounds inhibit (+)-[^{3}H]3-PPP binding in a manner yielding a Hill coefficient between 0.4 and 0.6 (7, 10). Computer-fitted curves for the competition of pentazocine (Fig. 2) and cyclazocine for (+)-[^{3}H]3-PPP indicated the best fit for a two-site model ($p < 0.001$). In the presence of Gpp(NH)p, however, the best fit observed was for a one-site model, whereas the two-site model was unacceptable. Table 1 summarizes the affinity constants of the compounds tested and the percentage of high and low affinity sites under the various conditions. For comparison purposes, the results observed previously (13) with (+)-SKF 10047 are also presented in Table 1.

Haloperidol and chlorpromazine have been previously reported to interact with the σ receptors (4, 6, 7). Analysis of the inhibition of (+)-[^{3}H]3-PPP binding by haloperidol revealed that the inhibition curves do not fit better for a two-site model than for a one-site model ($p = 0.1$). Chlorpromazine inhibition curves (Fig. 2) could be fitted for a one-site model, whereas a two-site model was unacceptable. In the presence of 0.1 mM Gpp(NH)p, no significant change in the inhibition curves of chlorpromazine (Fig. 2) and haloperidol was observed. The K_i values of the two antipsychotics, in the absence and presence of Gpp(NH)p, were virtually identical (Table 1). These findings support the notion that haloperidol and chlorpromazine act as antagonists at the σ receptor.

Kinetic studies. The association kinetics of (+)-[^{3}H]3-PPP to rat brain membranes in the absence and presence of 0.1 mM Gpp(NH)p are presented in Fig. 3. Association of the ligand at 25° reaches equilibrium after 20 min and is stable for at least 90 min. Because (+)-[^{3}H]3-PPP binding in rat brain membranes represents usually less than 10% of the total ligand added (i.e., 3400 ± 150 cpm/15 mg of wet membranes, specific binding, at about 100,000 cpm of ligand added), pseudo-first-

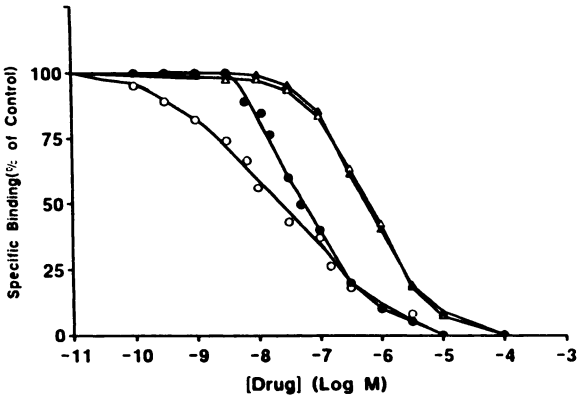

Fig. 2. Competition of pentazocine and chlorpromazine for (+)-[^{3}H]3-PPP binding in rat brain membranes in the absence and presence of Gpp(NH)p. The LIGAND computer-fitted curves for the inhibition of (+)-[^{3}H]3-PPP (1–1.2 nM) binding by pentazocine in the absence of Gpp(NH)p (○) gave the best fit for a two-site model (F = 30; $p < 0.001$), whereas in the presence of Gpp(NH)p (●) the best fit was for a one-site model. Inhibition of (+)-[^{3}H]3-PPP binding by chlorpromazine in the absence (△) and presence (▲) of Gpp(NH)p gave the best fit for a one-site model. Results represent the mean of three experiments (SE < 5% of the mean values in all cases).

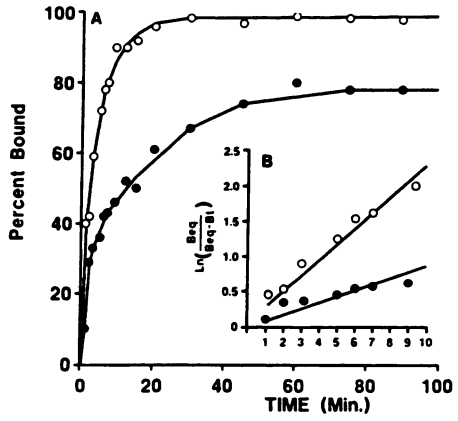

Fig. 3. Association of (+)-[^{3}H]3-PPP binding to rat brain membranes. A, Association time course of 1 nM (+)-[^{3}H]3-PPP was carried out in the absence (○) and presence (●) of Gpp(NH)p (0.1 mM) at 25°, as described in Materials and Methods. Gpp(NH)p was added coincident with the radiolabeled ligand. Results are expressed as the percentage of specific binding at equilibrium in the absence of Gpp(NH)p. Nonspecific binding, determined in the presence of 10 μM pentazocine, did not change over the time period examined. B, Pseudo-first-order association rate constants were calculated (23) in the absence of Gpp(NH)p (○) (K_a = 5.2 × 10^6 M^{-1} min^{-1}) and in its presence (●) (K_a = 1.02 × 10^6 M^{-1} min^{-1}). B_{eq} denotes specific binding at equilibrium and B_t at time t. Results represent a typical experiment, which was repeated three times with similar results (SE < 8% of mean values).

order association constants were calculated (23) (K_a = 5.2 × 10^6 M^{-1} min^{-1}) (Fig. 3B). In the presence of Gpp(NH)p, added coincident with (+)-[^{3}H]3-PPP, the association rate was significantly decreased by about 5-fold (K_a = 1.02 × 10^6 M^{-1} min^{-1}) (Fig. 3B). In order to determine whether the association of Gpp(NH)p with the G protein affected the association kinetics of the ligand-receptor complex, membranes were preincubated with Gpp(NH)p for 60 min (25°) before the addition of (+)-[^{3}H]3-PPP (1.2 nM). Under these conditions, the pseudo-first-order association constant was 9.8 × 10^5 M^{-1} min^{-1}, which is not significantly different from the K_a obtained under conditions where the nucleotide was added coincident with the radiolabeled ligand.

Fig. 4 represents the effect of Gpp(NH)p on the dissociation kinetics of (+)-[^{3}H]3-PPP. The dissociation curve of (+)-[^{3}H]3-PPP, in the absence of Gpp(NH)p, gave the best fit for a biexponential decay, determined by nonlinear regression. Results in Fig. 4 represent the dissociation that was initiated by addition of excess unlabeled (+)-3-PPP (10 μM). Very similar results were observed if pentazocine (10 μM) was added to initiate the dissociation (data not shown). These results further confirm the existence of two affinity states of the σ receptor. Inclusion of Gpp(NH)p at the onset of the association step of (+)-[^{3}H]3-PPP eliminates the slow dissociation component of (+)-[^{3}H]3-PPP binding (Fig. 4) and results in a monophasic dissociation curve with $t_{1/2}$ of 6.8 ± 0.2 min. These data suggest that the presence of Gpp(NH)p during the association step prevents the formation of the high affinity-slow dissociation complex and the result is manifested by a rapid monophasic dissociation process. The dissociation constant (K_d) of (+)-[^{3}H]3-PPP, calculated from the kinetic experiments in the presence of Gpp(NH)p, is 98 ± 4 nM, which is in good agreement with the K_L value observed from the equilibrium competition experiments (84 ± 5 nM) (Table 1).

Effects of NEM and PT. The sulfhydryl reagent NEM has

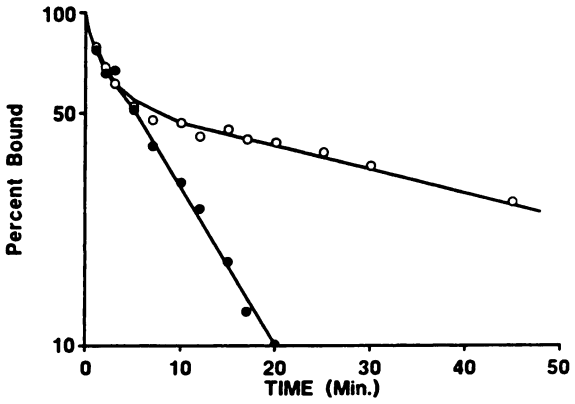

Fig. 4. Dissociation of (+)-[^{3}H]3-PPP from rat brain membranes. Membranes were incubated with (+)-[^{3}H]3-PPP (1 nM) in the absence (○) and presence (●) of Gpp(NH)p for 60 min at 25°. At time 0, unlabeled (+)-3-PPP (10 μM) was added to initiate dissociation, and samples of 1 ml were filtered at the indicated time intervals. Results are expressed as the percentage of specific binding at time 0 in the absence (○) and presence (●) of Gpp(NH)p. Nonspecific binding, determined in the presence of unlabeled (+)-3-PPP (10 μM), did not change over the time period examined. In the absence of Gpp(NH)p, the best fit was for a biexponential decay, determined by nonlinear regression. In the presence of the nucleotide, the *line* represents a fit for a monoexponential decay, because the equation for a biexponential decay (see Materials and Methods) did not fit significantly better. Results represent the mean of three separate experiments (SE < 6% of mean values).

"uncoupling" effects similar to those produced by GTP in several receptor systems (24–27). Preincubation of rat brain membranes with NEM (0.5 mM) resulted in 30–35% inhibition of (+)-[^{3}H]3-PPP binding. This treatment diminishes significantly the effect of Gpp(NH)p (Table 2). Addition of Gpp(NH)p to NEM-treated membranes reduces (+)-[^{3}H]3-PPP binding by only 4–6%, whereas control binding is reduced by 38 ± 2%.

Bordetella PT has been shown to ADP-ribosylate both the inhibitory G protein (G_i) and an additional G protein, G_o (28, 29). To determine whether σ receptor binding is associated with coupling of the receptor to G_i/G_o regulatory proteins, the effect of PT (5 μg/mg of tissue) on (+)-[^{3}H]3-PPP was assessed. Binding of the radioligand to PT-treated membranes was reduced by 28–34% from control binding, whereas Gpp(NH)p produced only 3–6% inhibition of (+)-[^{3}H]3-PPP binding in PT-treated membranes (Table 2).

Discussion

The major findings of the present study are the identification of two affinity states of the σ receptor and the formation of a high affinity σ-ligand-receptor-G protein complex. In a preliminary study, we reported that binding of the σ ligand (+)-[^{3}H]3-PPP is highly sensitive to GTP and its stable analog Gpp(NH)p, compared with the binding of PCP receptor ligands (13). The specificity of the GTP effect was indicated by the finding that GMP and ATP had no effect on (+)-[^{3}H]3-PPP (13). In the present study, we examined the regulatory effects of Gpp(NH)p on the affinities of several σ receptor agonist-like compounds and postulated antagonists and on the formation and dissociation of the σ ligand-receptor complex.

That the σ receptor exists in two discrete states having high and low affinity for agonist-like compounds is indicated by both competition and kinetic binding experiments. Analysis of the inhibition of (+)-[^{3}H]3-PPP binding by unlabeled (+)-3-PPP, cyclazocine, pentazocine, and (+)-SKF 10047 indicates interaction with high and low affinity binding sites. These findings are consistent with previous studies reporting Hill values significantly less than 1 for the inhibition of (+)-[^{3}H]3-PPP binding by the opiate benzomorphans (7, 10). Moreover, the biexponential nature of the dissociation of (+)-[^{3}H]3-PPP from its receptor further supports the existence of two affinity states of the σ receptor.

TABLE 2

Effect of Gpp(NH)p, NEM, and PT on the binding to the σ receptor

The effect of Gpp(NH)p (0.1 mM) and NEM (0.5 mM) or both on (+)-[^{3}H]3-PPP (1 nM) binding in rat brain membranes (15 mg of wet brain tissue/ml) was assessed as described in Materials and Methods. The effect of PT (5 μg/mg of brain tissue) on the binding of 8 nM (+)-[^{3}H]3-PPP was determined in a final volume of 0.1 ml. Control specific binding for PT assays was determined in 0.1 ml of membranes resuspended in the ribosylation buffer, as described in Materials and Methods. Nonspecific binding was determined in the presence of 10 μM pentazocine. Results represent the mean ± standard error of 16–20 determinations for the Gpp(NH)p effect and 5 or 6 determinations each for the effects of the other reagents tested.

Reagent	Specific binding	
	cpm	%
None (control)	3250 ± 45	100 ± 2
Gpp(NH)p	2015 ± 38	62 ± 2
NEM	2210 ± 85	68 ± 4
NEM + Gpp(NH)p	2115 ± 78	65 ± 4
Ribosylation medium (control)	2513 ± 54	100 ± 2
PT	1733 ± 25	69 ± 2
PT + Gpp(NH)p	1641 ± 31	65 ± 2

Several lines of evidence suggest that the high affinity binding state is coupled to G protein(s), similar to the coupling of several other receptor types to GTP-sensitive binding proteins. First, Gpp(NH)p leads to a decrease in the affinities of (+)-3-PPP and the opiate benzomorphans for the receptor, which is consistent with the hypothesis of destabilization of the high affinity ligand-receptor-G protein complex following exposure to GTP (14–16). These findings support the notion that these compounds may act as agonists at the σ receptor. The finding, however, that haloperidol and chlorpromazine inhibit σ binding in a monophasic manner in the absence of Gpp(NH)p, whereas in its presence the affinities for the receptor do not change, is consistent with other receptor systems where antagonists display usually similar affinity for the two states of the receptor (16). Second, the effect of Gpp(NH)p on the association and dissociation rates of (+)-[^{3}H]3-PPP further supports the hypothesis that the high affinity state of the σ receptor is coupled to G protein(s). (i) The 5-fold decrease in the association rate in the presence of Gpp(NH)p suggests inhibition of the formation of a high affinity receptor complex and acceleration of the dissociation rate. (ii) The transformation from a biexponential dissociation of the ligand-receptor complex in the absence of Gpp(NH)p to a monophasic dissociation process in its presence during the association step implies that Gpp(NH)p prevents the formation of the high affinity-slow dissociation ligand-receptor-G protein complex. Similar findings were reported for the dissociation kinetics of the δ-opioid receptor complex associated with the inhibitory G_i protein (17). Fig. 5 summarizes schematically the possible interactions of postulated σ agonists and antagonists with the receptor, in the absence and presence of Gpp(NH)p.

Several studies have demonstrated that the regulation of opioid agonist binding by GTP is reduced by pretreatment of brain membranes with the alkylating agent NEM (24–26). Similarly, opioid inhibition of adenylate cyclase is also blocked by this reagent (26). Although evidence suggests that NEM acts at multiple sites in the receptor complex (24–26, 30), the reagent may induce uncoupling of the receptor complex similarly to the effects of GTP. The finding that NEM reduces (+)-[^{3}H]3-PPP binding and practically abolishes the effect of Gpp(NH)p on σ ligand binding suggests that both reagents may affect similar site(s) in the receptor complex.

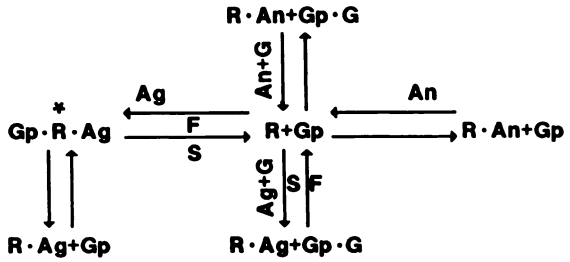

Fig. 5. Schematic model for the interaction of σ ligands with the receptor (adapted partially from Refs. 14–16 and 32). *R* represents the receptor in its uncoupled form, *Gp* a G protein, and *G* Gpp(NH)p. *Ag* and *An* refer to a postulated agonist [i.e., (+)-3-PPP] and antagonist (i.e., haloperidol), respectively. *F* and *S* denote fast and slow kinetic steps, respectively. Binding of the agonist to the receptor results in the formation of a high affinity ligand-receptor-G protein complex (*Gp.R*·Ag*) and a low affinity state receptor complex (*R·Ag*), which does not appear to be regulated by guanine nucleotides. With agonist present, there is an equilibrium between the two states of the receptor. In the presence of Gpp(NH)p, the agonist forms only the low affinity state complex (*R·Ag*), because the high affinity state is destabilized by the interaction of Gpp(NH)p with the G protein (*Gp·G*). Binding of the antagonist is probably associated with the formation of the low affinity state complex only, and thus Gpp(NH)p does not regulate the antagonist receptor complex (*R·An*).

The identity of the G protein or proteins coupled to the σ receptor is not clear, and further studies are required to characterize these regulatory binding proteins. However, the findings that PT inhibits the binding of (+)-[^{3}H]3-PPP and significantly reduces the regulatory effect of Gpp(NH)p may suggest that the G proteins that are coupled to the σ receptor have the characteristics of G_i/G_o regulatory proteins. It has been demonstrated that PT catalyzes specifically the ADP-ribosylation of the G_i and G_o proteins (20, 28, 29), the former regulating the inhibition of adenylate cyclase and the latter regulating presumably neuronal Ca^{2+} channels (31, 32).

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