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Drug Specificity of Pharmacological Dystonia

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(+)-Benzomorphans Dextrallorphan Dystonia Neuroleptics (+)-Pentazocine Posture Red nucleus (+)-SKF 10,047 Sigma receptors

PREVIOUS studies implicated sigma receptors in the regulation of movement and posture. This hypothesized function of sigma receptors is based in part on the high density of sigma receptors in many areas of the brain that control movement (e.g., cerebellum, red nucleus, various cranial nerve nuclei) and the motor effects elicited by intracerebral injections of sigma ligands (12, 13, 31). In a previous study, we showed that marked postural asymmetries (characterized by torsional movements of the head) could be produced by unilateral microinjections of sigma ligands [1,3-di-o-tolylguanidine (DTG), haloperidol or (+)-SKF 10,047] into the red nucleus of rats (31). Although the study suggested that rubral sigma receptors were involved in the control of posture and movement, a complete pharmacological evaluation of the role of rubral sigma receptors in dystonia was difficult due to the lack of selective ligands.

Recent work suggested that a number of (+)-opiates bind preferentially to sigma binding sites over PCP receptors (10, 24, 27, 28). In this study, we explored their dystonic effects following intrarubral microinjection in the rat. The drug specificity of the pharmacological dystonia was examined by 1) correlating the behavioral potencies of the active compounds with their binding affinities to sigma receptors in the rat brain using [^{3}H]DTG as the radioligand and 2) evaluating the responses elicited by intrarubral injections of a number of non-sigma compounds.

METHOD

Animals

Male Sprague-Dawley rats from Charles River Laboratories (Boston, MA) were acclimated to the animal colony for at least four days prior to surgery and preparation of the membranes. The animals were subject to automatically controlled lighting (lights on 0800 to 1900 hours) and uniform temperature (23°C). Food and water were provided ad lib.

Drugs

(±)-8-Hydroxy-dipropylaminotetralin hydrobromide (8-OH-DPAT), (+)-3PPP hydrochloride, S(−)-sulpiride, and (+)-SCH23390 hydrochloride were purchased from Research Biochemicals (Natick, MA). (+)-Pentazocine succinate and (+)-SKF 10,047 hydrochloride were obtained from the National Institute of Drug Abuse (Dr. Rao Rapaka, Rockville, MD). 1,3 Di-o-tolylguanidine acetate (DTG) was crystallized from a saturated acetic acid solution of the free base (Aldrich, Milwaukee, WI) as described previously (31). A modification of the method of Schnider and Grussner (25) was used to synthesize dextrallorphan hydrobromide. (+)-Nordihydrocodeinone hydrochloride was synthesized via the NIH Opiate Total Synthesis (22). [^{3}H]1,3-Di-o-tolylguanidine (DTG; 52.3 Ci/mmol) was obtained from Dupont/New

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England Nuclear (Boston, MA). Haloperidol was purchased from Sigma (St. Louis, MO).

Ligand Binding Assays

Binding studies were carried out using the crude P_2 fraction of rat brain (minus cerebellum), prepared as described previously (2). Membranes were incubated with 3 nM [^{3}H]DTG and 500 μg of membrane protein in a total volume of 0.5 ml of 50 mM Tris-HCl, pH 8.0. Incubations were carried out for 120 min at 25°C. Nonspecific binding was determined in the presence of 1 μM haloperidol. Assays were terminated by dilution with 5 ml ice-cold 10 mM Tris-HCl, pH 8.0 and vacuum filtration through glass fiber filters (Schleicher and Schuell, Keene, NH). Filters were then washed twice with 5 ml ice-cold 10 mM Tris-HCl, pH 8.0. Filters were soaked in 0.5% polyethyleneimine for at least 30 min at 25°C prior to use. Scintillation counting was performed with a Packard Model 4450 scintillation spectrometer after overnight extraction of counts from the filters using Ecoscint (National Diagnostics, Manville, NJ).

Head Torque Behavioral Assay

Surgery. Three to seven days before behavioral testing, each animal (245–350 g, N=152) was anesthetized with 50 mg/kg sodium pentobarbital and mounted in a Narishige stereotaxic apparatus. A guide cannula, constructed from 24 gauge thinwall stainless steel tubing, was implanted with its tip 4.0 mm above the left red nucleus of each animal [coordinates: 2.9 mm anterior, 1.1 mm lateral, 4.0 mm ventral, from lambda and the skull surface (21)]. Cannulae were secured with stainless steel screws and dental acrylic. Stainless steel stylets kept the cannulae sealed except during drug infusion.

Behavioral testing. Three to seven days after surgery, each rat received a single microinjection of one of the following compounds in the left red nucleus: dextrallorphan (3.72 nmol N=7, 9.3 nmol N=7 or 18.6 nmol N=7), (+)-pentazocine (2.7 nmol N=8, 6.7 nmol N=6 or 13.3 nmol N=7), (+)-SKF 10,047 (3.72 nmol N=7, 9.3 nmol N=9 or 18.6 nmol N=10), (+)-3PPP (9.3 nmol N=7, 18.6 nmol N=5), sulpiride (9.3 nmol N=5), (+)-SCH23390 (9.3 nmol N=7, 18.6 nmol N=4), 8-OHDPAT (9.3 nmol N=6), (+)-nordihydrocodeinone (3.72 nmol N=6, 9.3 nmol N=8 or 18.6 nmol N=7), or DTG plus (+)-3PPP (9.3 nmol/18.6 nmol N=6). In addition, intrarubral injections of the following solutions were used as controls: normal saline (N=8), normal saline pH 5.5 (which equals the lowest pH of any of the drug solutions, N=5), 9.3 nmol saline (N=7), and 9.3 nmol sucrose (N=3).

All drugs were dissolved in saline or water and prepared on the day of testing. The drugs were administered in a volume of 0.5 μl over 72 seconds through a 31 gauge microneedle that was constructed to extend 4.0 mm beyond the tips of the guide cannulae. Following the injection, each rat was placed in a clear plastic chamber (23 × 36 cm). The animals were photographed and torsional deviations of the head were measured using the eyes as a reference at 1, 5, 15 and 30 minutes after the injection. Each rat was tested only once in order to minimize damage to brain tissue.

Histology. Rats were sacrificed by an overdose of sodium pentobarbital and perfused intracardially with 10% formalin. Brains were fixed in a 30% sucrose-formalin solution and coronal sections (40 μm) were taken throughout the extent of the injection site. The sections were stained with cresyl violet and examined under a microscope to localize injection sites.

Statistical methods. Only subjects with histologically confirmed injection sites in the red nucleus were used in the data analyses. For each rat, the peak angle of head deviation during the 30-minute testing session was used in the data analyses. A one-way analysis of variance (ANOVA) or Student's *t*-test was used to evaluate the data (14). $p<0.05$ was considered statistically significant. Using a 10 degree angle of the head as a criterion response, the dose data were fitted to the logit equation using an iterative algorithm (BMDP AR, BMDP Statistical Software, Los Angeles, CA). ED_{50} values and 95% confidence intervals were derived from the fitted data.

TABLE 1

BINDING AFFINITIES OF VARIOUS COMPOUNDS AT RAT BRAIN SIGMA RECEPTORS AS DETERMINED USING [^{3}H]DTG

Ligand	[^{3}H]DTG IC_{50} (nM)	[^{3}H]DTG K_i (nM)	n
Haloperidol	3.5 ± 0.4	3.1 ± 0.4	(2)
DTG	18.8 ± 1.9	16.9 ± 1.7	(2)
BD614	308 ± 68	277 ± 61	(4)
(+)-SKF 10,047	966 ± 39	867 ± 35	(3)
Dextrallorphan	1021 ± 24	916 ± 22	(2)
(+)-Pentazocine	1106 ± 170	992 ± 152	(5)
(+)-Nordihydro-codeinone	>10,000	>10,000	(2)

Concentrations of various test ligands ranging from 0.05–10,000 nM were incubated with 3 nM [^{3}H]DTG as described in the Method section. IC_{50} values were determined using the iterative curve fitting program CDATA (EMF Software, Inc., Baltimore, MD). Apparent K_i values were then calculated using the Cheng-Prusoff equation (6) and a K_d of 26 nM which was determined in an independent study (18). Values are the averages of the number of experiments shown in parentheses, ±SEM. Each experiment was carried out in duplicate.

RESULTS

Binding of Various Ligands to Sigma Receptors

Table 1 shows the binding affinities of several compounds for [^{3}H]DTG-labelled sigma sites in the rat brain. Haloperidol and DTG bound with high affinity to sites labelled by [^{3}H]DTG. The (+)-benzomorphan-related compounds, (+)-SKF 10,047, (+)-pentazocine, and dextrallorphan displaced [^{3}H]DTG with affinities in the high nanomolar range. It should be noted that in contrast to the curves obtained with haloperidol and DTG, the (+)-opiate curves were very shallow, suggesting displacement of [^{3}H]DTG either from multiple receptor sites or from an allosteric site on the receptor (2). The novel compound, BD614 (9), bound with moderate affinity and (+)-nordihydrocodeinone, was devoid of sigma binding activity. Consistent with previous studies, the affinities of the (+)-opiates for rat brain sigma sites were lower than for sites present in guinea pig brain (1) and for sites labelled with [^{3}H](+)-3PPP (17).

Effects of Sigma and Non-Sigma Ligands in the Head Torque Assay

A one-way ANOVA revealed that the responses elicited by the various control injections did not differ significantly from one another, $F(3,19)=0.65$, n.s. Therefore, the various vehicles were combined and treated as a single control group in the rest of the analyses. Likewise, Student's *t*-tests revealed that the effects of

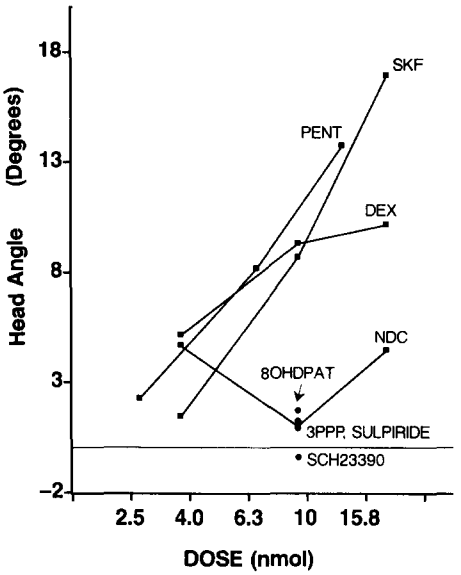

FIG. 1. Postural changes produced by unilateral microinjections of sigma and non-sigma ligands into the red nucleus of rats. For the sake of clarity, the standard error bars are not shown. Note, however, that the standard errors were less than 5 for all of the treatment conditions except the highest dose of 3PPP where the SEM = 9.1. DEX = dextrallorphan, NDC = (+)-nordihydrocodeinone, 8OHDPAT = (±)-8-OH-DPAT, PENT = (+)-pentazocine, 3PPP = (+)-3PPP, SKF = (+)-SKF 10,047. Mean ± SEM of values not shown in the figure: controls (normal saline, 9.3 nmol saline, normal saline pH 5.5, 9.3 nmol sucrose −0.09 ± 1.09), 3PPP (18.6 nmol = 1.0 ± 9.1), and SCH (18.6 nmol = 7.75 ± 3.17). (+)-Pentazocine, dextrallorphan and (+)-SKF 10,047 produced dose-dependent torsional movements of the head. The other compounds were without effect.

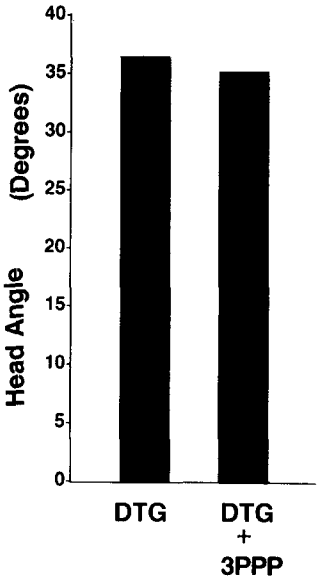

FIG. 2. Failure of (+)-3PPP (18.6 nmol) to attenuate the effects of DTG (9.3 nmol). (+)-3PPP alone does not elicit postural changes although the ligand has high affinity for sigma receptors. (+)-3PPP does not appear to be an antagonist at sigma receptors since it fails to modify the effects of DTG, a selective sigma ligand, when the compounds are coadministered.

(+)-SKF 10,047 did not differ significantly from results previously published by this laboratory (31). Consequently, in order to improve statistical estimates, the data from the two studies were combined.

(+)-Benzomorphans with affinity for sigma receptors produced torsional movements of the head when unilaterally microinjected into the red nucleus of rats (Fig. 1). One-way ANOVAs revealed a significant effect of dose for (+)-pentazocine, $F(3,40) = 8.84$, $p<0.0001$; dextrallorphan, $F(3,40) = 6.50$, $p<0.001$; and (+)-SKF 10,047, $F(3,45) = 12.18$, $p<0.0001$. The overlapping 95% confidence intervals surrounding the ED_{50}s derived from the logit analyses suggest that the (+)-benzomorphans did not differ significantly from one another in potency; ED_{50} values ±95% confidence intervals were: SKF 10,047 = 4.86 ± 4.06; (+)-pentazocine = 6.53 ± 4.08; dextrallorphan = 9.75 ± 9.1. (+)-Nordihydrocodeinone, a structurally related (+)-opiate with negligible affinity for sigma receptors, was without effect, $F(3,40) = 1.10$, n.s.

(+)-3PPP, a ligand with high affinity for rat brain sigma receptors in vitro ($K_d = 96.7$ nM) (2), was also without effect in the head torque assay, $F(2,32) = 0.05$, n.s. Although the combined features of high affinity and lack of efficacy might characterize the actions of an antagonist, (+)-3PPP does not appear to be a sigma receptor antagonist since it failed to attenuate the effects of the selective sigma ligand, DTG (32) ($t = 0.11$, n.s.; Fig. 2).

The selective 5-HT_{1A} agonist, 8-OHDPAT, failed to produce effects that were significantly different from the controls ($t = 0.78$, n.s.). Likewise, significant postural changes were not elicited by the selective dopamine D2 and D1 antagonists, (−)-sulpiride ($t = 0.40$, n.s.) and (+)-SCH23390, $F(2,31) = 1.73$, n.s.

To further assess the role of sigma receptors in these drug-induced dystonias, we correlated the in vivo potencies (ED_{50}s) of sigma drugs with their binding affinities at rat brain sigma receptors. Only three of the drugs tested in the present study produced effects strong enough to obtain dose curves. Therefore, we also included the ED_{50}s from active sigma drugs tested previously (DTG, haloperidol, BD614) (9,31) to yield a correlation based on six compounds. Although (+)-3-PPP and PCP have some affinity for sigma receptors [the latter was tested behaviorally in a previous study (31)], they were not included in the correlation because 1) their failure to produce dystonic effects precluded the calculation of an ED_{50} and 2) they have known actions through other receptor systems (e.g., PCP, NMDA, acetylcholine, dopamine) that may account for their lack of potency in the head torque assay (7,8). As shown in Fig. 3, least squares regression of the log ED_{50}s from the other six drugs revealed a highly significant linear trend ($r = .94$, $T = 5.60$, $df = 4$, $p = 0.005$).

DISCUSSION

Unilateral rubral microinjections of (+)-pentazocine, dextrallorphan, and (+)-SKF 10,047 in the rat produced dose-dependent torsional movements of the head. These findings are consistent with previously reported effects of sigma ligands in the rat red nucleus and the high concentration of sigma receptors in this area of the brain (13,31). Although correlations do not establish causation and other possible explanations of the effects cannot be entirely excluded, the high correlation between sigma binding affinity and potency found in this and a previous experiment (31) suggests that sigma receptors mediate the dystonic effects of these drugs.

The lack of effect of various drugs that possess very low or no affinity for sigma receptors also supports this conclusion. The failure of intrarubral injections of selective dopaminergic and

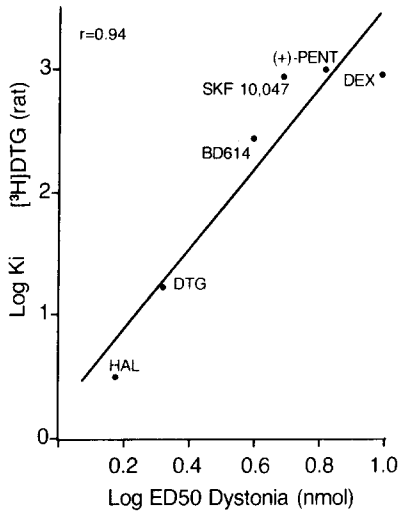

FIG. 3. Relationship between potency in displacing [^{3}H]DTG from rat brain membranes and ED_{50} in producing head torque. The significant correlation between binding affinity and potency in this test suggests that sigma receptors mediate the actions of these compounds.

serotonergic ($5HT_{1A}$) compounds to produce postural asymmetries is consistent with the relative absence of dopamine and serotonin receptors in the rat red nucleus. The lack of effect of the selective serotonin ($5HT_{1A}$) agonist, 8-OHDPAT, is noteworthy since some of the motor dysfunctions associated with intracerebroventricular administration of certain sigma ligands are qualitatively similar to those associated with the serotonin syndrome (unpublished observations). A similar lack of effect of selective dopaminergic compounds is significant because nonselective sigma ligands such as haloperidol bind to dopamine receptors (26). Since selective dopamine D1 and D2 antagonists failed to produce postural changes in this study, it is unlikely that rubral dopamine receptors were responsible for the effects observed in this study or the previously reported dystonic actions of haloperidol (31).

The only compound with high affinity for sigma receptors in this study that had no effect in the behavioral assay was (+)-3PPP, a ligand that also acts through dopamine receptors (7, 11, 16). The lack of efficacy of this substance cannot be attributed to antagonism of sigma receptors because (+)-3PPP failed to attenuate the effects of DTG. The discrepancy between the in vitro and in vivo potency of (+)-3PPP is unusual, but it is not a finding that is unique to this study. (+)-3PPP exhibits weaker sigma-like actions in several other systems than would be predicted by its high binding affinity. These effects include the unusually weak ability of (+)-3PPP to inhibit carbachol-stimulated phosphoinositide turnover (3,4), electrically induced contractions of the guinea pig ileum (5), firing of rubral neurons (19), and [^{3}H](+)-SKF 10,047 binding in in vivo binding experiments (30). Since (+)-3PPP appears to have dopaminergic activity (7, 11, 16), it is possible that under physiological conditions, the ligand acts primarily through dopamine receptors or alternatively, that unusual interactions arise when (+)-3PPP concurrently activates dopamine and sigma receptors.

An alternative explanation for the lack of effect of (+)-3PPP in this behavioral assay is that it interacts with a different type of sigma binding site than the one mediating the postural effects reported here. This possibility is supported by a number of studies which suggest the existence of multiple sigma or sigma-like binding sites. For example, the binding affinities of (+)-opiates are higher in both rat and guinea pig brain when [^{3}H](+)-3PPP or [^{3}H](+)-pentazocine is used as the sigma receptor probe than when [^{3}H]DTG is used [(10, 15, 17), unpublished observation]. We have also reported that benzomorphan and nonbenzomorphan ligands bind to rat brain sigma sites with differential sensitivity to ultraviolet light and compete by a mechanism which is uncompetitive, suggesting distinct, but allosterically coupled binding sites (2). In addition, pharmacological profiles at sigma binding sites have been shown to differ between species and tissue types (1, 15, 23). In particular, a sigma-like site has been identified on PC12 cells which exhibits lower affinity for (+)-opiates and lower molecular weight compared to sigma sites of guinea pig brain (1,15).

Consequently, the strong correlation between behavioral potency and sigma affinity in this study suggests that the dystonic effects of sigma ligands are mediated by a sigma receptor type which exhibits relatively low affinity for (+)-opiate compounds or which is activated by (+)-opiates with low efficacy. This is important because the potency of sigma ligands in another functional assay, inhibition of carbachol or oxotremorine-M-stimulated phosphoinositide turnover in rat brain, correlated very well with sigma binding affinities determined in guinea pig brain using [^{3}H](+)-3-PPP ($r = .92$) (3,4). Since (+)-opiates were potent in the phosphoinositide assay, the phosphoinositide effect may be mediated through interactions with a different sigma receptor type. Clearly, more studies will be required in order to clarify the issue of sigma site heterogeneity and relationship to function.

In summary, the results of this study suggest that sigma receptors in the rat red nucleus are involved in the regulation of posture. Compounds that bind to sigma receptors produce marked postural changes when unilaterally microinjected into the red nucleus, and their potencies correlate well with their affinity for rat brain sigma receptors labelled with [^{3}H]DTG. These effects appear to be mediated through a sigma receptor type with low affinity for (+)-opiates compared to nonopiate sigma ligands perhaps a site related to that found on PC12 cells (15). The observed postural changes cannot be attributed to dopamine or serotonin ($5HT_{1A}$) receptors since selective compounds at these receptors were without effect. A previous study also demonstrated the lack of effect of PCP (31), suggesting that PCP receptors are also not involved. These findings thus strengthen the idea that sigma receptors are involved in the control of posture and that some of the motor effects of typical neuroleptics may result from interactions with sigma, rather than dopamine receptors (31).

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