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Antagonism of the Stimulus Effects of Yohimbine and 8-Hydroxydipropylaminotetralin

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WINTER, J. C. AND R. A. RABIN. *Antagonism of the stimulus effects of yohimbine and 8-hydroxydipropylaminotetralin.* PHARMACOL BIOCHEM BEHAV 44(4) 851–855, 1993.—Stimulus control was established in rats using either 8-hydroxy-2-[di-*n*-propylamino]tetralin (DPAT) (0.2 mg/kg) or yohimbine (3 mg/kg). Tests were then conducted with purported antagonists at 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) receptors. Drugs studied were NAN-190, [+/−]-pindolol, and [−]-alprenolol. In addition, each drug was characterized in terms of its affinity for 5-HT$_{1A}$ and α_2-adrenoceptors by means of radioligand binding techniques. None of the antagonists tested provided complete blockade of the stimulus effects of either DPAT or yohimbine. However, [+/−]-pindolol produced a statistically significant intermediate degree of antagonism of both DPAT and yohimbine. The affinities of DPAT, yohimbine, and NAN-190 for the 5-HT$_{1A}$ and α_2-adrenergic receptors, respectively, were sufficiently high to lead to some ambiguity of interpretation of the behavioral data. However, the results with [+/−]-pindolol, which has high affinity for the 5-HT$_{1A}$ receptor (34 nM) and negligible affinity for the α_2-adrenoceptor (24,600 nM), indicate that a significant component of yohimbine-induced stimulus control is mediated by the 5-HT$_{1A}$ receptor.

8-OH-DPAT Yohimbine NAN-190 Alprenolol Pindolol Drug discrimination

IN previous studies, we observed significant generalization between the stimulus properties of 8-hydroxy-2-(di-*n*-propylamino)tetralin (DPAT) and yohimbine (20,22). Because of the relative specificity of DPAT for the 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) receptor [for review, see (17)] and of yohimbine as an antagonist at the α_2-adrenoceptor [for review, see (15)], these behavioral observations were initially puzzling. However, we recently presented evidence that, in addition to DPAT, yohimbine and related drugs have high affinity for, and behaviorally significant activity at, the 5-HT$_{1A}$ receptor (23).

A classic pharmacological method for the categorization of drugs is the use of specific antagonists (21). Thus, if two agonists act on the same receptor they can be expected to be similarly antagonized by competitive antagonists. In the present study, we examined the ability of selected serotonergic drugs to antagonize the stimulus effects of yohimbine and DPAT, respectively. In addition, each drug was characterized in terms of its affinity for 5-HT$_{1A}$ and α_2-adrenoceptors by means of radioligand binding techniques.

METHOD

Animals

Animals used in these studies were maintained in accordance with the Guide for Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. Male Fischer 344 rats were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, MA). They were housed in pairs under a natural light–dark cycle and allowed free access to water in the home cage. Rats were maintained at a body weight of 260–300 g by limiting access to food to 1 h per day.

Apparatus

Four small-animal test chambers (Coulbourn Instruments Model E10-10) housed in larger lightproof, sound-insulated boxes were used for all experiments. Each box had a houselight and exhaust fan. The chamber contained two levers mounted at opposite ends of one wall. Centered between the levers was a dipper that delivered 0.1 ml sweetened condensed milk diluted 2 : 1 with tapwater.

Procedure

At approximately 3 months of age, rats were assigned to one of two groups. After learning to drink from the dipper, subjects were trained to depress first one and then the other of the two levers. The number of responses for each reinforcement was gradually increased from 1 to 10 and all subsequent training and testing employed a fixed ratio (FR 10) schedule

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of reinforcement. Discrimination training was then begun. Fifteen minutes before each 10-min session, subjects were injected IP with either saline or drug. Following the administration of drug, every 10th response on the drug-appropriate lever was reinforced. Similarly, responses on the saline-appropriate lever were reinforced following injection of saline. For one half of the subjects, the left lever was designated as the drug-appropriate lever. During discrimination training, drug and saline were alternated on a daily basis. Drug-induced stimulus control was assumed to be present when, in five consecutive sessions, 83% or more of all responses prior to delivery of the first reinforcer were on the appropriate lever. Groups 1 and 2 were trained with DPAT (0.2 mg/kg) and yohimbine (3 mg/kg), respectively. A total of 36 rats were trained, equally divided between groups 1 and 2.

After stimulus control was well established, cross tests (tests of generalization) were conducted with a range of doses of pindolol, alprenolol, and NAN-190 in rats trained with either DPAT or yohimbine. These tests established any agonistic effects of the test drugs. In tests of antagonism, pindolol, alprenolol, and NAN-190 were administered in combination with DPAT in subjects trained with DPAT. Putative antagonists were injected 60 min before testing, that is, 45 min before either yohimbine or DPAT.

Cross tests were conducted once per week in each animal so long as performance during the remainder of the week did not fall below a criterion of 83% correct responding. In general, tests were equally divided between Thursday and Friday sessions. During cross tests, no responses were reinforced and the session was terminated after the emission of 10 responses on either lever. The distribution of responses between the two levers was expressed as the percentage of total responses emitted on the drug-appropriate lever. Comparisons of data were by means of individual applications of Wilcoxon's signed ranks test. Differences were considered significant if they would be expected to arise by random sampling alone with a probability <0.05.

Binding Assays

Rats were sacrificed by decapitation and either hippocampus or cerebral cortex removed. Tissues were homogenized (Dounce tissue grinder) in 50 mM Tris buffer (pH 7.4), and the homogenates were centrifuged at $40,000 \times g$ for 15 min at 4°C. The resulting pellets from the cortical homogenates were resuspended in the Tris buffer and again centrifuged at $40,000 \times g$ for 15 min. This procedure was repeated and the final pellet of cortical tissue was resuspended (50 mg wet weight/ml) in 50 mM Na phosphate buffer (pH 7.4) containing 4 mM $MgCl_2$ and 0.1% ascorbate. For the hippocampal tissue, the pellet after the first centrifugation was resuspended (40 vol/100 mg wet weight) in 50 mM Tris buffer (pH 7.4), and the samples were incubated at 37°C for 15 min to remove endogenous 5-HT (13). The samples were then centrifuged at $40,000 \times g$ for 15 min. The resulting hippocampal pellet was resuspended in the Tris buffer and again centrifuged at $40,000 \times g$ for 15 min. The final pellet was resuspended (6.25 mg wet weight/ml) in 50 mM Tris (pH 7.4) containing 4 mM $MgCl_2$, 10 mM pargyline, and 0.1% ascorbate.

[^{3}H]DPAT binding to hippocampal membranes was carried out in a final volume of 0.5 ml consisting of 50 mM Tris (pH 7.4), 4 mM $MgCl_2$, 10 μM pargyline, 0.1% ascorbate, [^{3}H]DPAT (1 nM; 124.9 Ci/mmol; DuPont/New England Nuclear, Boston, MA), and various concentrations of unlabeled drug. [^{3}H]Rauwolscine binding to cerebral cortical membranes was carried out in a final volume of 0.3 ml consisting of 50 mM Na phosphate buffer (pH 7.4) 4 mM $MgCl_2$, 0.1% ascorbate, [^{3}H]rauwolscine (5 nM; 75 Ci/mmol; DuPont/New England Nuclear), and various concentrations of unlabeled drugs. Incubations were initiated by the addition of either 0.4 ml hippocampal tissue or 0.2 ml cortical tissue and were carried out for either 25 min at 37°C ([^{3}H]DPAT binding) or 30 min at 30°C ([^{3}H]rauwolscine binding). In preliminary studies, these incubation times resulted in equilibrium binding under all conditions. All incubations were terminated using a Brandel cell harvester and the filters washed twice with cold 50 mM Tris buffer (pH 7.4). Radioactivity was measured by liquid scintillation spectrometry after incubating the filters in Liquiscint (National Diagnostics, Manville, NJ) scintillation cocktail overnight. Specific binding of [^{3}H]DPAT was defined as the difference in the amount of radioactivity bound in the absence and presence of 100 mM 5-HT. Specific binding of [^{3}H]rauwolscine was defined with 100 mM phentolamine. Data were analyzed by nonlinear regression using the program EBDA/LIGAND (Elsevier BIOSOFT).

Drugs

The following drugs were purchased from commercial sources: 8-hydroxy-dipropylaminotetralin HBr and NAN-190 HBr (1-(2-methoxyphenyl)-4-[4-(2-thalimido)butyl]piperazine HBr) (RBI, Natick, MA), [+/−]pindolol and [−]alprenolol *d*-tartrate hydrate (Sigma Chemical Co., St. Louis, MO), and yohimbine HCl (Aldrich, Milwaukee, WI).

RESULTS

Figure 1 shows the effects of [+/−]-pindolol, [−]-alprenolol, and NAN-190 alone and in combination with DPAT in rats trained with DPAT as a discriminative stimulus. Neither [−]alprenolol nor NAN-190 displayed agonistic effects, that is, when given alone (open symbols), responding was appropriate for the nondrug condition. In contrast, [+/−]pindolol at a dose of 10 mg/kg resulted in an intermediate degree of DPAT-appropriate responding, that is, the degree of drug-lever appropriate responding was statistically significantly different from either training condition. When tested for possible antagonistic activity, none of the three drugs blocked completely the stimulus effects of DPAT (closed symbols) but each drug at one or more doses caused partial antagonism, that is, the degree of drug-appropriate lever selection was significantly reduced but did not reach that observed in the no-treatment control condition. When a combination of [+/−]pindolol (2 mg/kg) and NAN-190 (0.75 mg/kg) was given, additive antagonism was not observed but only half the animals tested completed the session (point indicated by "+" in Fig. 1).

When [+/−]pindolol, [−]alprenolol, and NAN-190 were examined in rats trained with yohimbine as a discriminative stimulus, the results were as shown in Fig. 2. Neither dose of NAN-190 produced significant antagonism and responding was severely depressed at a dose of 1.5 mg/kg. A statistically significant degree of antagonism was produced only at the highest dose of [−]alprenolol (20 mg/kg) but the pattern of responding was intermediate in nature, that is, complete antagonism was not observed. The most effective of the three drugs was [+/−]-pindolol, but again the degree of antagonism was intermediate. At doses of 2 and 5 mg/kg of [+/−]pindolol, responding was significantly different from both training conditions.

The data of Table 1 indicate a high degree of selectivity by

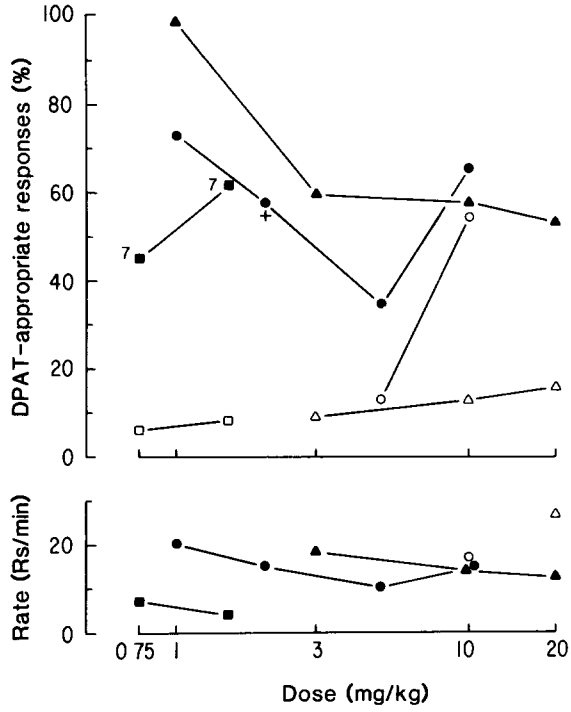

FIG. 1. Effects of [−]alprenolol (triangles), [+/−]pindolol (circles), and NAN-190 (squares) alone (open symbols) and in combination with DPAT (closed symbols) in rats trained with DPAT (0.2 mg/kg) as a discriminative stimulus. Each point is the mean of one determination in each of 10 subjects. The number of subjects that completed the session, if other than 10, is indicated adjacent to the data point. The point indicated by "+" is for [+/−]pindolol (2 mg/kg) plus NAN-190 (0.75 mg/kg) given prior to DPAT. Ordinate: upper panel, mean percentage of responses on the DPAT-appropriate lever; lower panel, response rate. Abscissa: Dose plotted on a log scale.

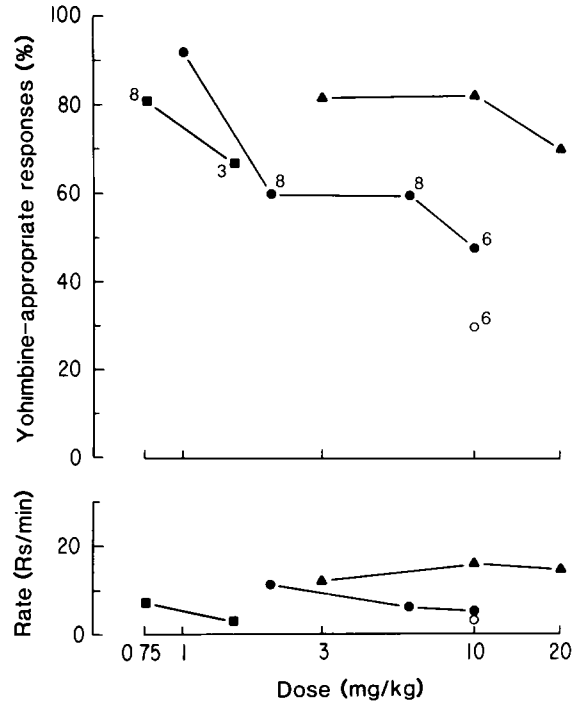

FIG. 2. Effects of [−]alprenolol (triangles), [+/−]pindolol (circles), and NAN-190 (squares) alone (open symbols) and in combination with yohimbine (closed symbols) in rats trained with yohimbine (3 mg/kg) as a discriminative stimulus. All other details are as in Fig. 1.

[+/−]pindolol and [−]-alprenolol for the 5-HT_{1A} receptor. In contrast, the data for NAN-190 suggest possible activity at both serotonergic and adrenergic receptors.

DISCUSSION

Complete blockade of the stimulus properties of DPAT by NAN-190 has been reported in rats (8,9) and pigeons (1). In the present investigation (Fig. 1), stimulus control by DPAT was significantly attenuated at doses of NAN-190 of 0.75 and 1.5 mg/kg. However, at both doses of the antagonist responses following the training dose of DPAT were intermediate in nature, that is, significantly different from both training conditions. [For a discussion of the interpretation of intermediate results, see (18)]. Differences between rats and pigeons, especially with respect to the presence or absence of the 5-HT_{1B} receptor, offer an explanation for the difference between the present results and those of Barrett and Gleeson (1). A reconciliation of the present data with those of Glennon and colleagues (8,9) is more problematic. In their studies, NAN-190 at a dose of 3.2 mg/kg was maximally effective. Our failure to achieve complete blockade of DPAT is plausibly explained by the inability of our subjects to complete test sessions at doses of NAN-190 significantly in excess of 1.5 mg/kg. Reasons for this differential rate-suppressant effect are not apparent. The only procedural difference between the present experiments and those of Glennon et al. (8,9) of which we are aware is the use by the latter of a 2.5-min extinction session for purposes of testing. In contrast, our test sessions

TABLE 1

DISSOCIATION CONSTANTS AT THE 5-HT_{1A} AND α_2-ADRENOCEPTOR BINDING SITES

5-HT_{1A} Ligands	K_d (nM)*	
	HT_{1A}	α_2
DPAT	1.3 (0.04)	611 (144)
Yohimbine	83 (19.3)	30 (8.3)
NAN-190	1.9 (0.24)	90 (28)
[+/−]-Pindolol	34 (11)	24,600 (5,500)
[−]-Alprenolol	52 (10.6)	26,000 (15,000)
[−]-Propranolol	106 (19.2)	23,200 (2,250)

The affinities of unlabeled drugs for the 5-HT_{1A} and α_2-adrenoceptor binding sites were determined from competition experiments as described in the Method Section using 1 nM [^{3}H]DPAT or 5 nM [^{3}H]rauwolscine, respectively.

*The K_d values for the radioligands were determined from nonlinear regression analysis of the equilibrium saturation experiments using 0.5–20 nM [^{3}H]DPAT or [^{3}H]rauwolscine. Data are expressed as the mean of 3 to 5 separate experiments with the SEM in parentheses.

ended as soon as 10 responses had been emitted on either lever. The relevance to the present data of the reported inability of NAN-190 to block the hypothermic, hyperglycemic, and cortisol-releasing effects of DPAT (14,24) and NAN-190's properties as a mixed agonist/antagonist at 5-HT_{1A} receptors in some tissues (5,10) is uncertain at this time.

A group of drugs first characterized as β-adrenoceptor antagonists was subsequently shown to have high affinity for serotonergic receptors, especially those of the 5-HT_{1A} and 5-HT_{1B} subtypes (6,11,12). In drug discrimination studies, attempts to block DPAT-induced stimulus control in rats with β-adrenoceptor antagonists have yielded mixed results. Thus, two groups failed to observe antagonism of the DPAT cue by [+/−]-propranolol (4,7) while others reported complete blockade of stimulus control by [−]-pindolol and [−]-alprenolol (16). In the present investigation, a significant but intermediate level of antagonism of DPAT was seen following pretreatment with [+/−]-pindolol and [−]-alprenolol (Fig. 1). A plausible explanation of these discrepant results regarding antagonism of DPAT-induced stimulus control by β-adrenoceptor antagonists is suggested by the training conditions of the respective studies. The intermediate degree of antagonism seen in Fig. 1 following [+/−]-pindolol and [−]-alprenolol and the absence of antagonism by [+/−]-propranolol reported previously (4,7) were observed in animals trained with a dose of DPAT of 0.2 mg/kg or greater administered IP with a pretreatment time of 15 min. In contrast, the complete blockade of the DPAT-induced cue by [−]-pindolol and [−]-alprenolol (16) occurred in rats trained following SC administration of a dose of DPAT of 0.05 mg/kg with a pretreatment time of 30 min. However, it should be noted that when the present group of rats trained with 0.2 mg/kg DPAT IP and a pretreatment time of 15 min (n = 13) were cross-tested with 0.05 mg/kg DPAT SC 30 min before testing 83% DPAT-appropriate responding was observed, with 10 of 13 subjects choosing the DPAT-appropriate lever. This result suggests, at least for the present subjects, that the stimulus effects of DPAT under the two distinct testing conditions are nonetheless similar.

In Fig. 1, the biphasic antagonism of DPAT by [+/−]pindolol, that is, a greater degree of antagonism at a dose of 3 mg/kg than at 10 mg/kg, is readily explained in terms of the emergence of agonistic effects at the higher dose. The present observation that a combination of [+/−]pindolol (2 mg/kg) and NAN-190 (0.75 mg/kg) did not result in additive antagonism is compatible with competition by the two antagonists at a common receptor.

Since the initial report of the stimulus properties of yohimbine (19), no studies of interactions of β-adrenoceptor antagonists with the yohimbine cue have been reported. The pattern of interaction of the β-adrenoceptor antagonists and NAN-190 with the stimulus properties of yohimbine seen in Fig. 2 is similar to that shown in Fig. 1 for DPAT. Thus, complete antagonism was not observed with any combination and [+/−]-pindolol was the most effective of the antagonists tested. However, doses of NAN-190 that resulted in an intermediate degree of antagonism of the DPAT-induced cue could not be shown to be effective vs. yohimbine. The data of Table 1 show a high affinity for the 5-HT_{1A} receptor by [−]-propranolol, [+/−]-pindolol, and [−]-alprenolol but negligible affinity for the α_2-adrenoceptor. Thus, the partial antagonism of yohimbine by [+/−]-pindolol and [−]-alprenolol in conjunction with the binding data of Table 1 are suggestive of an action by yohimbine at 5-HT_{1A} receptors.

The present data suggest that NAN-190 and selected β-adrenoceptor antagonists with high affinity for 5-HT_{1A} receptors are less than fully effective antagonists of the stimulus properties of DPAT. However, pindolol, with negligible affinity for α_2-adrenoceptors, was nearly as effective against yohimbine as it was against DPAT. This finding is consistent with our earlier conclusion that yohimbine-induced stimulus control is mediated to a significant degree by actions at 5-HT_{1A} receptors (23). It remains to be established whether the recently described DPAT antagonist, [S]-5-fluoro-DPAT (2,3), will provide more complete blockade of the stimulus properties of yohimbine and DPAT.

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