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BRIEF COMMUNICATION

In Vivo Interactions of NAN-190, a Putative Selective 5-HT_{1A} Antagonist, With Ipsapirone

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DEANS, C., M. LEATHLEY AND A. GOUDIE. *In vivo interactions of NAN-190, a putative selective 5-HT_{1A} antagonist, with ipsapirone*. PHARMACOL BIOCHEM BEHAV **34**(4) 927–929, 1989.—The actions of NAN-190, a putative 5-HT_{1A} antagonist, were assessed in rats. The selective 5-HT_{1A} agent ipsapirone suppressed operant responding, but this effect was *not* antagonised by NAN-190, which suppressed responding itself in a dose-related manner, and had additive effects when administered with ipsapirone. These data do not support suggestions that NAN-190 is a 5-HT_{1A} antagonist. NAN-190 may be a 5-HT_{1A} partial agonist which can antagonise effects of full 5-HT_{1A} agonists.

Ipsapirone 5-HT_{1A} receptors NAN-190 Operant responding

5-HT_{1A} receptor systems are involved in a range of physiological/behavioural processes including: The control of temperature and blood pressure, mediation of aspects of the 5-HT behavioural syndrome, regulation of food and fluid intake, and of sexual activity, anxiety and depression (2). However, a major problem in analysing actions of 5-HT_{1A} systems has been the absence of selective 5-HT_{1A} antagonists (1,2). Agents such as pindolol and alprenolol interact in a stereospecific manner with 5-HT_{1A} agonists (14). However, these agents also have well known actions as β-adrenergic antagonists, and also possible agonist actions at the 5-HT_{1B} receptor (6).

Electrophysiological studies suggest that the buspirone analogue BMY 7378 may be an effective 5-HT_{1A} antagonist, although the use of this compound may be compromised by its lack of specificity for the 5-HT_{1A} receptor (1). Furthermore, the potential 5-HT_{1A} antagonist actions of BMY 7378 do not yet appear to have been studied in behavioural assays. It is, therefore, of interest that recently, an arylpiperazine, 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine (NAN-190), was described as binding selectively with high affinity at 5-HT_{1A} receptors (7), and antagonising the 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) discriminative stimulus (6). Since the 8-OH-DPAT discriminative stimulus is mediated by 5-HT_{1A} receptors (14), NAN-190 may be a selective 5-HT_{1A} antagonist that could be useful for the analysis in vivo of 5-HT_{1A} receptors.

The work reported here addressed this issue further by determining whether NAN-190 antagonises the effects on operant behaviour of ipsapirone, an agent with a relatively high degree of selectivity for the 5-HT_{1A} receptor (13), and which generalizes to the 8-OH-DPAT discriminative stimulus (14). Since ipsapirone generalizes to the 8-OH-DPAT discriminative stimulus and NAN-190 antagonises it, if NAN-190 is a specific antagonist at 5-HT_{1A} receptors, it should presumably antagonise actions of ipsapirone on operant responding.

METHOD

Animals

Twenty-four female Wistar rats (bred in the Psychology Department of Liverpool University) served as subjects. They were individually housed in a humidity and temperature controlled room. After daily operant sessions, they were fed supplementary food on a regime designed to maintain body weights fixed at 90% of free feeding weights.

Apparatus

Standard operant chambers (Coulbourn Instruments, USA) in

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sound-damped, ventilated housing were used. Reinforcements consisted of 45 mg pellets. A minicomputer was used to control experimental contingencies and to record behavioural data.

Procedure

Animals were initially shaped to lever press in daily operant sessions. Subsequently, animals were transferred to a schedule in which a single response on one lever was required to obtain reward, i.e., a Fixed Ratio (FR) 1 schedule. The number of responses required to obtain reward was increased gradually up to 20 over a number of days of training. All animals were therefore trained finally on an FR 20 schedule of reinforcement. After extensive FR 20 training, "test" sessions were introduced in which the effect of ipsapirone (at a constant dose), in the presence and absence of NAN-190 (at 4 doses between 0 and 2 mg/kg), on response rate were studied. "Test" sessions were always separated by at least two days, and on the day before a "test" session animals always received saline injections. This procedure was adopted in order to allow effects of drug treatments on individual rates of operant responding to be expressed as percentages of rates on the immediately preceding saline session. Before all "test" sessions, animals received two injections. The first (105 min pretreatment) was of either saline or NAN-190. The second (60 min pretreatment) was of either saline or ipsapirone. Drug combinations were studied in a nonsystematic pseudorandom order.

Drugs

Ipsapirone HCl (Troponwerke, Köln, F.R.G.) and NAN-190 HBr (Dr. R. A. Glennon, Virginia Commonwealth University, USA) were made up as salts, dissolved in gently heated 0.9% saline at an injection volume of 2 ml/kg of rat. NAN-190 was administered at doses up to 2.0 mg/kg IP, this being a dose that antagonised the 8-OH-DPAT discriminative stimulus (6). Higher doses of NAN-190 were not studied due to solubility problems. The fixed dose of ipsapirone (7.5 mg/kg, IP) was the approximate ED_{50} for suppression of FR 20 responding (8). The rationale behind the choice of the ED_{50} dose of ipsapirone was that it should have been possible with such a dose to detect both potentiation and antagonism of the actions of ipsapirone by NAN-190.

RESULTS

The results are plotted in Fig. 1, which shows the effects on response rates of injections of various doses of NAN-190 alone (i.e., in conjunction with injections of saline), and in conjunction with the constant 7.5 mg/kg dose of ipsapirone. The point at S (above the 0 mg/kg dose of NAN-190) shows the effect of saline injections alone. The point at I (also above the 0 mg/kg dose of NAN-190) shows the effect of ipsapirone alone at 7.5 mg/kg.

These data were analysed with a two-factor (4 doses of NAN, presence/absence of ipsapirone) repeated measures ANOVA. This revealed a highly significant effect, $F(1,23)=277.6$, $p<0.001$, of the presence or absence of ipsapirone, showing that ipsapirone, at the 7.5 mg/kg dose studied, suppressed operant responding. In addition, there was a highly significant effect, $F(3,69)=54.5$, $p<0.001$, of the dose of NAN-190, indicating that, as shown in Fig. 1, NAN-190 produced dose-related suppression of operant responding. The most important finding from the ANOVA was, however, that there was *no* significant interaction between the two factors, $F(3,69)=1.97$, NS. Thus, the suppressant effect of ipsapirone on operant responding was neither potentiated nor antagonised by NAN-190. Instead, Fig. 1 suggests unequivocally that NAN-190 and ipsapirone simply had additive effects in suppressing responding. For example, the 2 mg/kg dose of NAN-190 suppressed responding by 29% (relative to the effect of saline alone). The same dose of NAN-190 increased by a similar amount (23%) the suppressant effect of ipsapirone (i.e., relative to the effect of ipsapirone alone). For each dose of NAN-190 studied, the degree of suppression of responding in conjunction with saline was similar to the degree of suppression of responding in conjunction with ipsapirone (see Fig. 1).

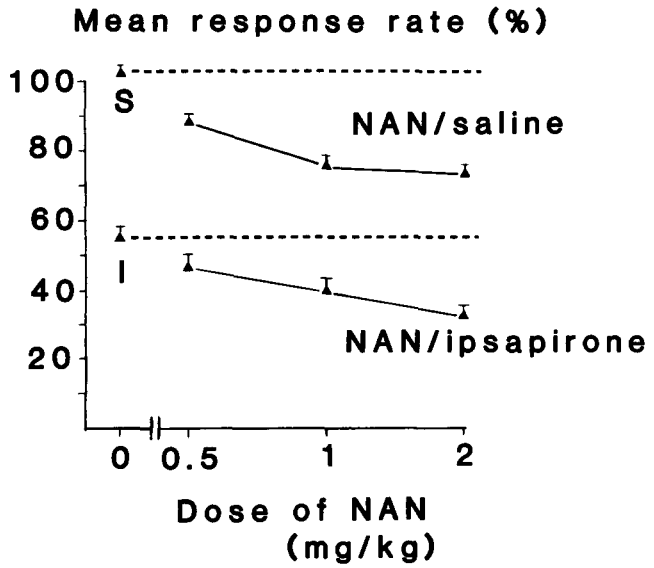

FIG. 1. Effects of NAN-190, at various doses (log scale), on operant responding (mean ± S.E. percent of previous saline baseline session). The effects of NAN-190 are shown separately when injected together with saline and with ipsapirone. The point S above the 0 mg/kg dose of NAN-190 shows the effect of saline injections alone. The dotted line along the x axis at this point can be used as a reference to assess the effects of NAN-190 alone on responding (i.e., relative to the relevant injection control). The point I above the 0 mg/kg dose of NAN-190 shows the effect of ipsapirone alone at 7.5 mg/kg. The dotted line along the x axis at this point can be used as a reference to assess the effects of various doses of NAN-190 on the actions of ipsapirone.

DISCUSSION

The finding that ipsapirone suppressed Fixed Ratio responding in rats accords with other studies from this laboratory (8). More generally, animal studies have shown that agents acting at the 5-HT_{1A} receptor, such as buspirone, gepirone and ipsapirone, typically have suppressant actions on unpunished operant responding [e.g., (10)], as reported here.

Despite the fact that ipsapirone had a clear rate-suppressant effect, this was not antagonised by NAN-190. These data do *not* support the hypothesis that NAN-190 is a selective 5-HT_{1A} antagonist. As such, they appear to disagree with the findings of Glennon *et al.* (6) in the 8-OH-DPAT drug discrimination bioassay. It is, therefore, necessary to consider possible causes of these apparently discrepant findings.

One possible "explanation" for our data may simply be that bioassays which involve rates of operant responding may be incapable of detecting actions of 5-HT_{1A} antagonists. It is not at present known whether the rate suppressant actions of ipsapirone can be antagonised by nonselective 5-HT_{1A} antagonists such as pindolol. It is, therefore, possible that the assay used was not appropriate for 5-HT_{1A}-mediated effects. In this context it should perhaps be noted that ipsapirone may have functional actions at dopamine receptors (12), and that it can also act as an $alpha_1$ antagonist, although only at high doses (9). Since NAN-190 has

high affinity for the $alpha_1$ binding site (6), it is possible that the additive effects of ipsapirone and NAN-190 reported here were mediated by common actions at this receptor site. However, activity studies (11) demonstrate unequivocally that "sedative" actions of ipsapirone and related compounds in rats are mediated by actions at the 5-HT_{1A} receptor, suggesting that the suppression of operant responding observed may well have been 5-HT_{1A} receptor mediated. Furthermore, although it is possible that we were simply using the "wrong" bioassay to detect antagonistic actions of NAN-190 at the 5-HT_{1A} receptor, it should be noted that other authors have successfully used assays involving rates of operant responding to demonstrate effects of other 5-HT antagonists on actions of 5-HT agonists [see, e.g., (15)]. Consequently, while we cannot prove unequivocally that the assay used involved actions at the 5-HT_{1A} receptor, this is certainly a plausible hypothesis. Even if the conclusion that antagonistic actions of NAN-190 can only be seen in specific bioassays is valid, it is of some importance, since it indicates that NAN-190 does not antagonise all of the behavioural effects of agents such as ipsapirone.

An alternative possible "explanation" for the apparent discrepancy between our data and the findings of Glennon *et al.* (6) relates to our use of ipsapirone, rather than 8-OH-DPAT, as the 5-HT_{1A} specific agent to assess possible antagonistic actions of NAN-190. Ipsapirone is generally regarded as partial 5-HT_{1A} agonist (13). However, there are conditions under which ipsapirone can antagonise actions of 8-OH-DPAT, suggesting that, in some assays ipsapirone may act as a 5-HT_{1A} antagonist [e.g., (9)]. If the rate suppressant actions of ipsapirone reported here were due to 5-HT_{1A} antagonistic actions of ipsapirone, one would not expect a putative 5-HT_{1A} antagonist such as NAN-190 to reverse such effects. In contrast, one would expect additive effects of the two agents, as reported. In support of this suggestion, it should be noted that there is evidence (4) that agents such as 8-OH-DPAT may stimulate food intake by acting as agonists at 5-HT_{1A} autoreceptors, counteracting tonic serotonergic inhibition of feeding. If ipsapirone, at the dose used in this study, acted as an antagonist at such autoreceptors, it would be expected to suppress appetitive food-maintained operant responding (as reported), and to have additive actions with NAN-190 if this agent is a 5-HT_{1A} antagonist. However, this interpretation of the data seems unlikely since other evidence shows that at 5 mg/kg (3) and higher doses (8) ipsapirone stimulates, rather than suppresses, food intake in rats, an effect due presumably to agonist actions at 5-HT_{1A} autoreceptors (4). Furthermore, it is not at all clear why agents such as ipsapirone appear to act as 5-HT_{1A} agonists in some bioassays and yet to antagonise actions of 8-OH-DPAT in other assays (5,13). It has consequently previously been suggested (5) that the ability of ipsapirone to antagonise some actions of the full agonist 8-OH-DPAT may be related to the fact that ipsapirone is a partial agonist at 5-HT_{1A} receptors.

The suggestion that a partial 5-HT_{1A} agonist may "antagonise" some actions of 8-OH-DPAT may be of relevance in reassessing the report by Glennon *et al.* (6) that NAN-190 antagonises the 8-OH-DPAT discriminative stimulus. It is possible that such "antagonism" was also actually due to NAN-190 acting as a partial agonist in the drug discrimination procedure. If NAN-190 is a 5-HT_{1A} partial agonist, its behavioural effects might be expected to summate with those of the partial 5-HT_{1A} agonist ipsapirone, as shown in Fig. 1. Although definitive conclusions about the putative 5-HT_{1A} antagonist actions of NAN-190 are clearly precluded at present, it is, nevertheless, apparent that the interpretation of the data proposed here, and the reassessment of the data reported by Glennon *et al.* (6), is a reasonably parsimonious interpretation of the data currently available. Thus, the data reported caution against the use of NAN-190 as a selective antagonist of ipsapirone in behavioural studies and show clearly that the drug has intrinsic actions on behaviour itself.

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