Pharmacology Biochemistry and Behavior, Vol. 46, pp. 141–143, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

BRIEF COMMUNICATION

5-HT_{1A}-Mediated Lower Lip Retraction: Effects of 5-HT_{1A} Agonists and Antagonists

NICHOLAS A. MOORE,[1] GAYNOR REES, GRAHAM SANGER AND LISA PERRETT

Lilly Research Centre, Eli Lilly & Co., Erl Wood Manor, Windlesham, Surrey GU20 6PH, UK

Received 16 November 1992

MOORE, N. A., G. REES, G. SANGER AND L. PERRETT. *5-HT_{1A}-mediated lower lip retraction: Effects of 5-HT_{1A} agonists and antagonists.* PHARMACOL BIOCHEM BEHAV **46**(1) 141–143, 1993.—This study investigated the production of lower lip retraction (LLR) in the rat by the 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}) agonist 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT) and the effect of the putative 5-HT_{1A} antagonists pindolol and (1-(2-methoxy-phenyl)-4-[4-(2-phthalimido)-butyl]-piperazine (NAN190). 8-OH-DPAT (0.125–1.0 mg/kg, IP) caused a dose-related increase in LLR. Pindolol (10–40 mg/kg, IP) and NAN190 (2.5–10 mg/kg, IP) produced a dose-related block of 8-OH-DPAT-induced LLR. Pindolol (10–40 mg/kg, IP) when administered alone was also found to cause LLR, suggesting that pindolol behaves as a partial agonist in this model. This was not the case with NAN190 (2.5–10 mg/kg, IP), which failed to produce LLR; however, NAN190 (2.5–10 mg/kg, IP) produced a dose-related block of the pindolol-induced LLR. These results clearly demonstrate that the LLR model can be used to detect 5-HT_{1A} agonists, partial agonists, and antagonists.

8-OH-DPAT 5-HT_{1A} receptors Pindolol NAN190 Lower lip retraction Rat

ACTIVATION of 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}) receptors produces a number of behavioural and physiological changes; for example, the selective agonist 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT) produces a fall in body temperature and changes in ingestive behaviour and endocrine function mediated by 5-HT_{1A} receptor activation (9,10). The compound also produces a characteristic behavioural syndrome in rats, comprising of flat body posture, forepaw treading, head weaving, and hyperlocomotion (8). Recent studies have shown that as part of this syndrome rats exhibit a characteristic change in the musculature of the lower lip, which Berendsen et al. (2) designated "lower lip retraction" (LLR). Berendsen et al. (2) demonstrated that LLR was produced by the 5-HT_{1A} agonists 8-OH-DPAT, buspirone, ipsapirone, and RU24969. Agonists with selectivity for 5-$HT_{1B/1C}$ and 5-HT_2 receptors failed to produce the response. These authors also demonstrated that the putative 5-HT_{1A} antagonists pindolol and (1-(2-methoxy-phenyl)-4-[4-(2-phthalimido)-butyl]-piperazine (NAN190) antagonised 8-OH-DPAT-induced LLR (1).

Although some reports suggest that pindolol and NAN190 are 5-HT_{1A} antagonists, other studies suggested that these compounds may be weak partial agonists. Hjorth and Carlsson (5) reported that pindolol caused a selective reduction in brain 5-HT synthesis rate, similar to that seen with 8-OH-DPAT. These authors therefore concluded that this effect was due to a direct agonist-like effect at central 5-HT sites. Recently, Zhang and Barrett (11) reported that following chronic administration of 8-OH-DPAT pindolol substituted for 8-OH-DPAT in 8-OH-DPAT-trained pigeons, suggesting that under certain conditions pindolol can produce agonist-like actions at the 5-HT_{1A} receptor. It was initially claimed that NAN190 was a 5-HT_{1A} antagonist with no intrinsic activity (4). In in vitro studies, Rydelek-Fitzgerald et al. (6) reported that although NAN190 behaved as an antagonist in a 5-HT_{1A}-mediated adenylate cyclase assay the compound displayed a partial agonist profile in radioligand binding studies. In an operant paradigm, NAN190 failed to antagonise the effects of ipsapirone but produced an additive effect (3). Recently, Williams and Dourish (9) reported that NAN190 induced a feeding response similar to that observed with 5-HT_{1A} agonists.

In view of these reports, we looked at these agents in the LLR model in the rat.

METHOD

Animals

Male Lister Hooded rats (Olac, Bicester, UK) were used in all studies. Animals were housed in groups of up to five in conventional ventilated metal cages, in a room maintained at

[1] To whom requests for reprints should be addressed.

21 ± 1°C, relative humidity 50 ± 5%, on a 12 L : 12 D cycle (light on 7:00 a.m.–7:00 p.m.). All rats had free access to food and water.

Procedure

Experiments were performed between 9:00 a.m. and 4:00 p.m. Animals were placed singly in Perspex cages. Following 60-min habituation, animals received either vehicle or agonist. In some studies, putative antagonists were administered 30 or 60 min before the agent inducing the LLR. Animals were observed before agonist administration and then at 5-min intervals for 60 min, starting 10 min after dosing using the following scoring system: 0, normal (lower incisors hardly visible); 1, lower lip slightly pulled back; 2, lower lip completely retracted with lower teeth clearly visible. Animals were also observed for any other behavioural changes (e.g., flat body posture), which were recorded but not quantified. All scoring was carried out by an observer who was unaware of the specific drug treatments.

Materials

The following drugs were dissolved or suspended in water: 8-OH-DPAT HBr (Research Biochemicals, Inc., Natick, MA), pindolol (Sigma Chemical Co., St. Louis, MO), NAN190 HBr (Research Biochemicals, Cookson Chemicals Ltd.). All drug solutions were freshly prepared and dosed by the IP route in a volume of approximately 1 ml/200 g.

Data Analysis

Comparisons between groups were made using an analysis of variance (ANOVA) procedure followed by the posthoc least square means multiple-comparison test within SAS Proc GLM (7). A probability of $p < 0.05$ was considered significant.

RESULTS

8-OH-DPAT-Induced LLR

8-OH-DPAT (0.25–2 mg/kg) produced a dose-related increase in LLR (Fig. 1a). The maximum response was observed following 1 mg/kg. The response had a rapid onset within 5 min and peaked between 10 and 20 min. The 8-OH-DPAT-induced LLR lasted about 30 min. 8-OH-DPAT also induced flat body posture over the same dose range as the LLR. The flat body posture had a similar time course to the LLR.

Antagonism of 8-OH-DPAT-Induced LLR

Pindolol (10–40 mg/kg) administered 1 h before 8-OH-DPAT significantly reduced the LLR produced by the 8-OH-DPAT (Fig. 1b). Pindolol (20 and 40 mg/kg) also reduced the flat body posture observed in 8-OH-DPAT-treated animals. NAN190 (1.25–10 mg/kg) administered 30 min before 8-OH-DPAT significantly reduced the LLR (Fig. 1c) and flat body posture. NAN190 alone failed to produce LLR or any other behavioural changes.

Pindolol-Induced LLR

In the pindolol/8-OH-DPAT studies, it was observed that pindolol alone (10–40 mg/kg) induced LLR (Fig. 2a). The LLR produced by pindolol was relatively short-lived, appearing within 5 min of dosing, and had a time course similar to that seen with 8-OH-DPAT. Some animals also exhibited

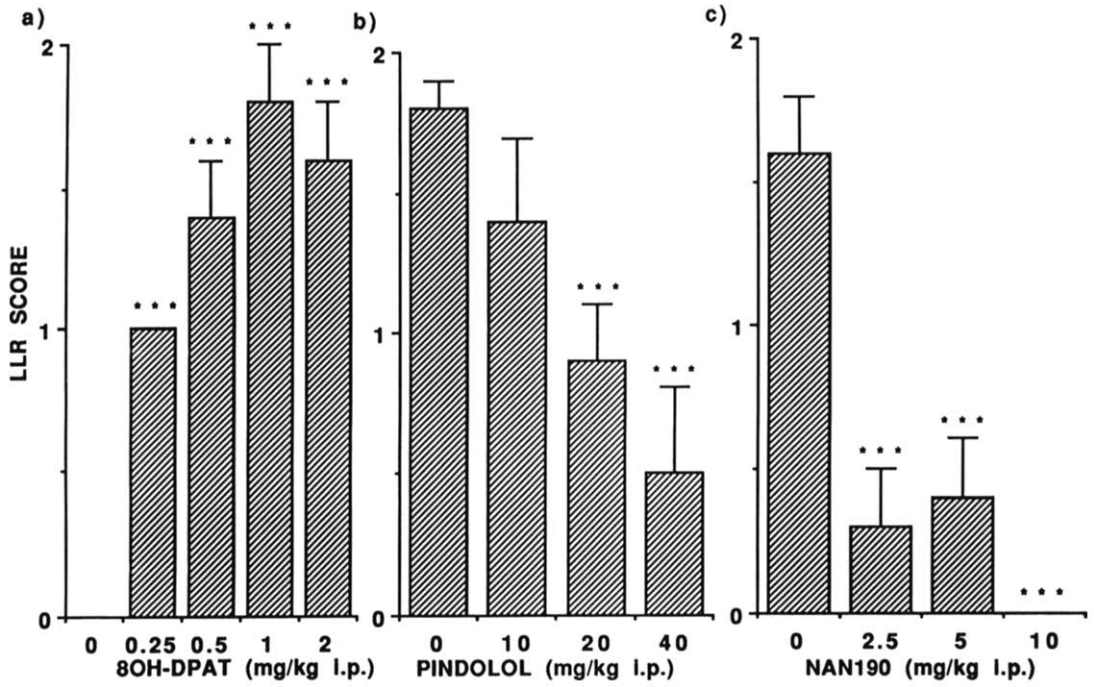

FIG. 1. (a). 8-Hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT)-induced lower lip retraction (LLR). (b). Effect of pindolol on 8-OH-DPAT (1 mg/kg)-induced LLR. Pindolol was administered 60 min before 8-OH-DPAT. (c). Effect of (1-(2-methoxy-phenyl)-4-[4-(2-phthalimido)-butyl]-piperazine (NAN190) on 8-OH-DPAT (1 mg/kg)-induced LLR. NAN190 was administered 30 min before 8-OH-DPAT. Each bar represents the mean (± SEM) for a group of eight rats. ***$p < 0.001$.

flat body posture following the highest dose of pindolol. NAN190 (2.5–10 mg/kg) administered 30 min before pindolol (40 mg/kg) significantly reduced the pindolol-induced LLR (Fig. 2b).

DISCUSSION

These results clearly show that the 5-HT$_{1A}$ agonist 8-OH-DPAT will induce a change in the musculature of the lower lip (LLR) and support the findings of Berendsen et al. (2). In our studies, approximately a 10-fold higher dose of 8-OH-DPAT was required to induce the response. This is presumably because 8-OH-DPAT was administered by the IP rather than the SC route. The LLR appeared to be one of the components of the 8-OH-DPAT-induced behavioural syndrome; animals showing LLR also exhibited flat body posture. However, it was found that LLR was easier to quantify than the other components of the syndrome.

Pindolol significantly reduced the LLR induced by 8-OH-DPAT and agrees with earlier reports that pindolol possesses 5-HT$_{1A}$ antagonist properties (1). High doses of pindolol were used in this study but this may be related to the fact that the pindolol was administered by the IP route and that pindolol was competing with a relatively high dose of 8-OH-DPAT. In agreement with other reports, NAN190 also significantly reduced the LLR induced by 8-OH-DPAT (1). During the pretreatment phase of the pindolol studies, it was noted that some animals showed a clear LLR response. Pindolol produced a dose-related increase in LLR that was prevented by pretreatment with NAN190. Previous studies failed to show pindolol-induced LLR; however, this may be due to the fact that lower doses of pindolol were used (1). Thus, in this model pindolol is able both to prevent the 8-OH-DPAT-mediated response and induce LLR itself, while NAN190 acts solely as an antagonist. The observation that pindolol behaves as a partial agonist supports data from a number of groups suggesting that pindolol displays both agonist and antagonist properties depending upon the model used (1,5,11).

In summary, these results show that the LLR response is an easily quantifiable component of the 5-HT$_{1A}$ behavioural syndrome and can be used to detect both 5-HT$_{1A}$ agonists, partial agonists, and antagonists. This study also demonstrates that caution should be exercised in interpreting the results from 5-HT$_{1A}$ behavioural studies; clearly, compounds that behave as antagonists in one study may appear to have agonistic properties in others.

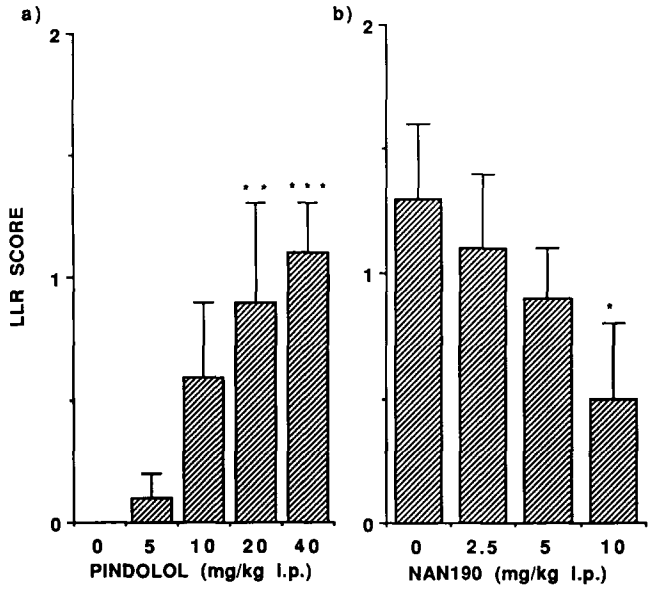

FIG. 2. (a). Pindolol-induced lower lip retraction. (b). Effect of (1-(2-methoxy-phenyl)-4-[4-(2-phthalimido)-butyl]-piperazine (NAN190) on pindolol (40 mg/kg)-induced lower lip retraction. NAN190 was administered 30 min before pindolol. Each bar represents the mean (± SEM) for a group of eight rats. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

REFERENCES

1. Berendsen, H. H. G.; Broekkamp, C. L. E.; Van Delft, A. M. L. Antagonism of 8-OH-DPAT-induced behaviour in rats. Eur. J.Pharmacol. 187:97–103; 1990.
2. Berendsen, H. H. G.; Jenk, F.; Broekkamp, C. L. E. Selective activation of 5-HT1a receptors induces lower lip retraction in the rat. Pharmacol. Biochem. Behav. 33:821–827; 1989.
3. Deans, C.; Leathley, M.; Goudie, A. J. In vivo interactions of NAN-190, a putative selective 5-HT$_{1A}$ antagonist, with ipsapirone. Pharmacol. Biochem. Behav. 34:927–929; 1989.
4. Glennon, R. A.; Naiman, N. A.; Pierson, M. E.; Teiteler, M.; Lyon, R. A.; Weisberg, E. NAN-190: An arylpiperazine analog that antagonizes the stimulus effects of the 5-HT$_{1A}$ agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT). Eur. J. Pharmacol. 154:339–341; 1988.
5. Hjorth, S.; Carlsson, A. Is pindolol a mixed agonist-antagonist at central serotonin (5-HT) receptors? Eur. J. Pharmacol. 129: 131–138; 1986.
6. Rydelek-Fitzgerald, L.; Teitler, M.; Fletcher, P. W.; Ismaiel, A. M.; Glennon, R. A. NAN-190: Agonist and antagonist interactions with brain 5-HT$_{1A}$ receptors. Brain Res. 532:191–196; 1990.
7. SAS user's guide: Statistics. ed. 5. Cary, NC: SAS Institute; 1985: 433–507.
8. Tricklebank, M. D.; Forler, C.; Fozard, J. R. The involvement of subtypes of the 5-HT$_1$ receptor and of catecholaminergic systems in the behavioural response to 8-hydroxy-2-(di-*n*-propylamino)tetralin in the rat. Eur. J. Pharmacol. 106:271–282; 1984.
9. Williams, A. R.; Dourish, C. T. Effects of the putative 5-HT$_1$a receptor antagonist NAN-190 on free feeding and on feeding induced by the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT in the rat. Eur. J. Pharmacol. 219:105–112; 1992.
10. Wozniak, K. M.; Durcan, M. J.; Linnoila, M. Is NAN-190 an effective antagonist of the hypothermia and hyperglycemia induced by the 5-HT$_{1A}$ receptor agonist, 8-OH-DPAT? Eur. J. Pharmacol. 193:253–256; 1991.
11. Zhang, L.; Barrett, J. E. Modification of the discriminative stimulus effects of 8-OH-DPAT, buspirone and the beta-adrenoreceptor antagonist pindolol after chronic administration of the 5-HT$_{1A}$ agonist 8-OH-DPAT in the pigeon. Behav. Pharmacol. 2:369–378; 1991.