ELSEVIER

European Journal of Pharmacology 276 (1995) 281–284

ejp

Short communication

Differential effects of WAY-100135 on the decrease in 5-hydroxytryptamine release induced by buspirone and NAN-190

Carol Routledge *, Jane Gurling, Mark A. Ashworth-Preece, Colin T. Dourish

Department of Neuropharmacology, Wyeth Research (UK), Huntercombe Lane South, Taplow, Maidenhead, Berkshire, SL6 OPH, UK

Received 30 January 1995; accepted 3 February 1995

Abstract

1-(2-Methoxyphenyl)-4-[(phthalimido)butyl] piperazine (NAN-190) and 8-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-8-azaspiro[4.5]decane-7,9-dione (buspirone) are 5-HT_{1A} receptor partial agonists which decrease 5-hydroxytryptamine (5-HT) release in vivo. In order to assess whether these ligands decrease 5-HT release by stimulating 5-HT_{1A} receptors we examined the ability of the selective 5-HT_{1A} receptor antagonist *N-tert*-butyl 3-4-(2-methoxyphenyl) piperazin-1-yl-2-phenylpropanamide dihydrochloride (WAY-100135) to block their inhibitory effects on 5-HT. NAN-190 (0.1 mg/kg s.c.) and buspirone (1.0 mg/kg s.c.) significantly decreased extracellular levels of 5-HT in hippocampal dialysates. WAY-100135 (10.0 mg/kg s.c.) attenuated the effect of buspirone but had no significant effect on the NAN-190-induced decrease in 5-HT release. These data demonstrate that buspirone is an agonist at the somatodendritic 5-HT_{1A} receptor but that the inhibitory effects of NAN-190 on 5-HT release may be mediated via a mechanism other than, or in addition to, 5-HT_{1A} receptor agonism.

Keywords: 5-HT_{1A} receptor antagonist; WAY-100135; NAN-190; Microdialysis

1. Introduction

The piperazine derivative 1-(2-methoxyphenyl)-4-[(phthalimido)butyl] piperazine (NAN-190) was one of the first putative 5-HT_{1A} receptor antagonists to be developed (Glennon et al., 1988). This compound was demonstrated to have high affinity for the 5-HT_{1A} receptor and to block some of the behavioural effects (5-hydroxytryptamine (5-HT) syndrome, stimulus properties) of the selective 5-HT_{1A} receptor agonist, 8-dihydroxy-2-(propylamino)tetralin (8-OH-DPAT) (Glennon et al., 1988; Hjorth and Sharp, 1990). However, it was later demonstrated that NAN-190 decreased hippocampal 5-HT release (Hjorth and Sharp, 1990) and raphe neuronal cell firing (Lum et al., 1990). These inhibitory effects were proposed to be a result of an agonist action of NAN-190 at somatodendritic 5-HT_{1A} receptors, suggesting that the compound behaves as a 5-HT_{1A} receptor partial agonist. However, it still remains uncertain whether the inhibitory effects of NAN-190 on 5-HT neuronal activity are due to an agonist action of this compound at somatodendritic 5-HT_{1A} receptors. In the present study, we have evaluated the effects of *N-tert*-butyl 3-4-(2-methoxyphenyl) piperazin-1-yl-2-phenylpropanamide dihydrochloride (WAY-100135) (Cliffe et al., 1993; Fletcher et al., 1993; Routledge et al., 1993) on the decrease in extracellular levels of hippocampal 5-HT induced by NAN-190. In addition, we have evaluated the effects of WAY-100135 on 8-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-8-azaspiro[4.5]decane-7,9-dione (buspirone), a ligand which was first demonstrated to decrease 5-HT neuronal activity in 1987 (Sprouse and Aghajanian, 1987), but which has awaited the development of selective antagonists to demonstrate unequivocally that its inhibitory effects are mediated via 5-HT_{1A} receptors.

2. Materials and methods

2.1. Experimental procedure

Male Sprague-Dawley rats were anaesthetised with a mixture of ketamine and xylazine (66.6:6.66 mg/kg i.m. respectively) and a guide cannula (CMA Microdialysis, Stockholm, Sweden) stereotaxically implanted directly above the dorsal hippocampus and cemented

* Corresponding author. SmithKline Beecham, New Frontiers Science Park North, Third Avenue, Harlow, Essex CM19 5AD, UK. Tel. (0279) 622457, fax (0279) 622230.

Elsevier Science B.V.
SSDI 0014-2999(95)00082-8

to the skull using dental acrylic. At the same time a cannula was implanted in the back of the neck under the skin and between the shoulder blades for subcutaneous administration of all drugs and vehicle. The purpose of the indwelling cannula was to alleviate injection stress. The animals were allowed a 24 h recovery period following which a microdialysis probe (dimensions: o.d. 0.5 mm, length 4.0 mm, CMA Microdialysis, Stockholm, Sweden) was lowered into the hippocampus via the guide cannula (tip co-ordinates P 4.8, L 4.7, V 8.0 ref. point bregma according to Paxinos and Watson, 1986). The probe was perfused with artificial cerebrospinal fluid (CSF; for composition see Routledge et al., 1993) at a flow rate of 1.0 μl/min. Following a 3.0 h stabilisation period 20 min microdialysis samples were taken and immediately injected onto a high performance liquid chromatography (HPLC) column for subsequent assay of 5-HT as described by Routledge et al. (1993). Three baseline control samples were taken followed by administration of WAY-100135 or vehicle and then 30 min later, by administration of 8-OH-DPAT, buspirone or vehicle.

At the end of the experiment placement of microdialysis probes was verified histologically.

2.2. Drugs

N-tert-Butyl 3-4-(2-methoxyphenyl) piperazin-1-yl-2-phenylpropanamide dihydrochloride (WAY-100135; racemate) (Wyeth Research, UK, 87% active moiety) was dissolved in 0.3% methyl cellulose and administered in a dose of 2.5 ml/kg s.c. 8-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-8-azaspiro[4.5]decane-7,9-dione hydrochloride (buspirone · HCl) (Research Biochemicals, St Albans, UK, 91% active moiety) and 1-(2-methoxyphenyl)-4-[(phthalimido)butyl] piperazine (NAN-190) (Wyeth Research, UK, 84% active moiety) were dissolved in 25% PEG : saline and administered in a volume of 1 ml/kg s.c. Controls received the appropriate volume of the appropriate vehicle.

2.3. Data analysis and statistics

Perfusate levels of 5-HT are expressed as a percent of the mean of absolute neurotransmitter collected in three pre-injection control samples. Data were analysed by two-way or three-way analysis of variance (ANOVA) with repeated measures and post-hoc testing carried out using the Tukey-Kramer test. A probability level of $P < 0.05$ was regarded as significant.

3. Results

Baseline extracellular levels of 5-HT in the rat hippocampus were 37.21 ± 7.98 fmol/20 μl dialysate (n = 36); levels remained relatively stable for the 5 h experimental period though there was a significant decrease in extracellular 5-HT levels at time point 240 min in vehicle/WAY-100135 groups. Vehicle injection had no significant effect on extracellular levels of 5-HT; in contrast, buspirone (1.0 mg/kg) and NAN-190 (0.1

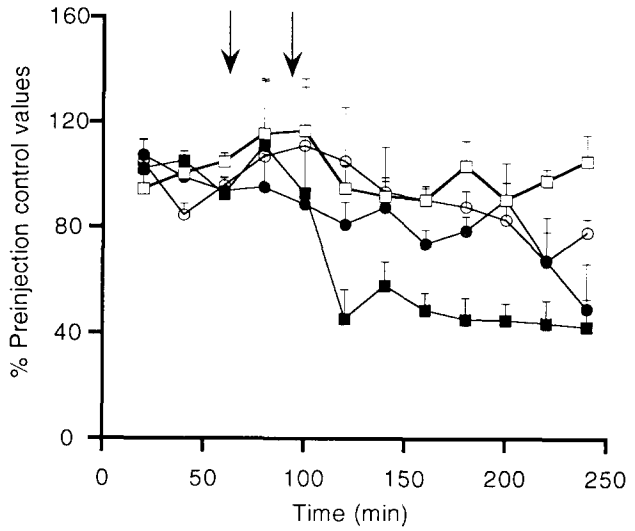

Fig. 1. Effects of WAY-100135 (10.0 mg/kg s.c.) on the buspirone (1.0 mg/kg s.c.) induced decrease in extracellular levels of 5-HT in the rat hippocampus. Each point represents mean ± S.E.M. for six animals per group. First arrow denotes administration of WAY-100135 or 0.3% methyl cellulose, the second denotes administration of buspirone and vehicle. See Results for statistical analysis of data. 0.3% methyl cellulose/vehicle (○), 0.3% methyl cellulose/buspirone (■), WAY-100135/vehicle (●), WAY-100135/buspirone (□).

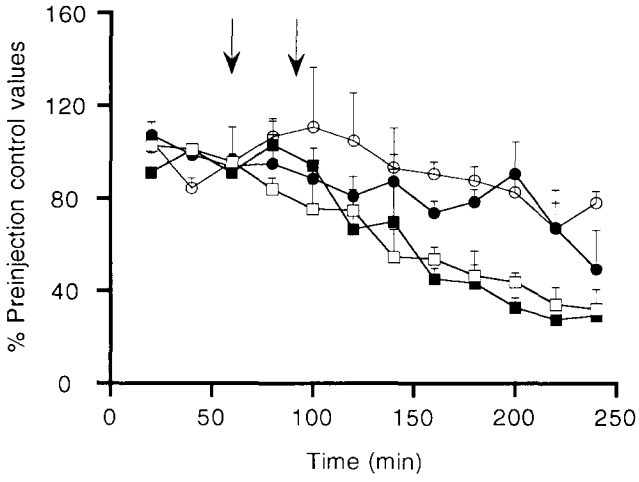

Fig. 2. Effects of WAY-100135 (10.0 mg/kg s.c.) on the NAN-190 (0.1 mg/kg s.c.) induced decrease in extracellular levels of 5-HT in the rat hippocampus. Each point represents mean ± S.E.M. for six animals per group. First arrow denotes administration of WAY-100135 or 0.3% methyl cellulose, the second arrow denotes administration of NAN-190 or vehicle. See Results for statistical analysis of data. 0.3% methyl cellulose/vehicle (○), 0.3% methyl cellulose/NAN-190 (■), WAY-100135/vehicle (●), WAY-100135/NAN-190 (□).

mg/kg) significantly ($P < 0.05$) decreased 5-HT release to 40 ± 3.1 and $30 \pm 1.2\%$ of preinjection control values respectively (Figs. 1 and 2). WAY-100135 at a dose of 10.0 mg/kg s.c. had no significant effect on extracellular levels of 5-HT, but completely blocked the buspirone-induced decrease in 5-HT release (Fig. 1). Statistical analysis using three-way ANOVA with repeated measures (followed by the Tukey-Kramer test) revealed a significant ($P < 0.05$) main effect of buspirone (1.0 mg/kg) to decrease 5-HT release and a significant interaction between WAY-100135 and buspirone ($F(1,20) = 2.354$, $P < 0.01$). In contrast, WAY-100135 at a dose of 10.0 mg/kg had no effect on the decrease in 5-HT release induced by 0.1 mg/kg NAN-190 (Fig. 2). Statistical analysis using three-way ANOVA with repeated measures (followed by the Tukey-Kramer test) revealed a significant ($P < 0.05$) main effect of NAN-190 (0.1 mg/kg) to decrease 5-HT release but no interaction between WAY-100135 and NAN-190 ($F(1,20) = 0.398$, $P = 0.867$).

4. Discussion

These data demonstrate that NAN-190 and buspirone decrease extracellular levels of 5-HT in the rat hippocampus, a response suggested to be indicative of activation of somatodendritic 5-HT_{1A} receptors on dorsal raphe neurones. This is in agreement with other data demonstrating NAN-190 and buspirone to be 5-HT_{1A} receptor partial agonists in vitro and in vivo (Sharp et al., 1989; Hjorth and Sharp, 1990; Lum et al., 1990; Rydelek-Fitzgerald et al., 1990; Greuel and Glaser, 1992). These data were supported by the demonstration that the non-selective 5-HT_{1A}/$_{1B}$/β-adrenoceptor antagonist (−)-pindolol blocked the inhibitory effects of these compounds (Hjorth and Sharp, 1990; Sharp et al., 1993). However, the poor selectivity of (−)-pindolol makes the results of the above experiments difficult to interpret. In the present study, the selective 5-HT_{1A} receptor antagonist WAY-100135 blocked the effects of buspirone on 5-HT release demonstrating that these inhibitory effects are mediated via 5-HT_{1A} receptor activation.

In contrast, WAY-100135 had no effect on the NAN-190-induced decrease in extracellular levels of 5-HT, suggesting that the inhibitory effects of NAN-190 may be due to an action other than 5-HT_{1A} receptor agonism. One possible explanation for the inhibitory effects of NAN-190 on 5-HT neuronal activity is blockade of facilitatory α_1-adrenoceptors located on raphe serotonergic cell bodies. NAN-190 has significant affinity for α_1-adrenoceptors (Glennon et al., 1988), and has been demonstrated to be 330-fold more potent at blocking α_1-adrenoceptor-mediated effects than 5-HT_{1A} receptor-mediated effects in vitro (Claustre et al., 1991). In addition, the α_1-adrenoceptor antagonist prazosin has been demonstrated to decrease extracellular levels of 5-HT in vivo (Claustre et al., 1991; Routledge et al., 1994) presumably via blockade of facilitatory α_1-adrenoceptors on raphe neurones. Further studies are required, however, to conclusively demonstrate this.

These data appear to contrast with those of Hjorth and Sharp (1990), who demonstrated that the inhibitory effects of NAN-190 were antagonised by (−)-pindolol. There are a number of methodological differences in these studies which may account for this discrepancy; these include anaesthetised versus unanaesthetised animals, the inclusion of citalopram in the perfusion fluid and the use of lower doses of NAN-190 in the study by Hjorth and Sharp. One additional explanation for this discrepancy may be the use of a non-selective 5-HT_{1A} receptor antagonist in this study. In addition to 5-HT_{1A} affinity, (−)-pindolol has significant affinity for β-adrenoceptors and 5-HT_{1B} receptors. In view of evidence that 5-HT_{1D} receptor antagonists can increase 5-HT release in the guinea-pig (Starkey and Skingle, 1994), it has been suggested that an antagonist at 5-HT_{1B} receptors may have a similar effect in the rodent. It is therefore possible that (−)-pindolol may reverse the inhibitory effects of NAN-190 by such a mechanism, i.e. physiological rather than pharmacological antagonism. It still remains to be determined if a 5-HT_{1B} receptor antagonist will increase 5-HT release in vivo.

An additional explanation which cannot be excluded at this stage is that NAN-190 has both α_1-adrenoceptor antagonist and 5-HT_{1A} receptor partial agonist activity; however, at the dose used in the present study (0.1 mg/kg s.c.) the α_1-adrenoceptor effects appear to predominate, hence the lack of effect of WAY-100135 on the NAN-190 response. Alternatively, NAN-190 may exert its inhibitory effect via an action unrelated to its effects at either α_1 or 5-HT_{1A} receptors, e.g. 5-HT_7.

In conclusion, these data demonstrate that buspirone behaves as a 5-HT_{1A} receptor agonist at the somatodendritic 5-HT_{1A} receptor in that the buspirone-induced decrease in 5-HT release is attenuated by the selective 5-HT_{1A} receptor antagonist WAY-100135. However, they also demonstrate that NAN-190 decreases 5-HT release in the rat hippocampus via a mechanism other than, or in addition to, 5-HT_{1A} receptor agonism as the NAN-190 response is not blocked by WAY-100135. Taken together with the results of previous studies from our laboratory (Routledge et al., 1994), these data suggest that NAN-190 may decrease 5-HT neuronal activity and 5-HT release via blockade of α_1-adrenoceptors. In addition, these data suggest that caution should be taken when using NAN-190 as some of the effects of this ligand may not be mediated via 5-HT_{1A} receptors.

References

Claustre, Y., L. Rouquier, A. Serrano, J. Benavides and B. Scatton, 1991, Effect of the putative 5-HT_{1A} receptor antagonist NAN-190 on rat brain serotonergic transmission, Eur. J. Pharmacol. 204, 71.

Cliffe, I.A., C.I. Brightwell, A. Fletcher, E.A. Forster, H.L. Mansell, Y. Reilly, C. Routledge and A.C. White, 1993, (*S*)-*N*-*tert*-Butyl-3-(4-(2-methoxyphenyl)-piperazin-1-yl)-2-phenylpropranamide [(*S*)-WAY-100135]: a selective antagonist at presynaptic and postsynaptic 5-HT_{1A} receptors, J. Med. Chem. 36, 1509.

Fletcher, A., D.J. Bill, S.J. Bill, I.A. Cliffe, G.M. Dover, E.A. Forster, J.T. Haskins, D. Jones, H.L. Mansell and Y. Reilly, 1993, WAY-100135: a novel, selective antagonist at presynaptic and postsynaptic 5-HT_{1A} receptors, Eur. J. Pharmacol. 237, 283.

Glennon, R.A., N.A. Naiman, M.E. Pierson, M. Titeler, R.A. Lyon and E. Weisberg, 1988, NAN-190: an arylpiperazine analog that antagonises the stimulus effects of the 5-HT_{1A} agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), Eur. J. Pharmacol. 154, 339.

Greuel, J.M. and T. Glaser, 1992, The putative 5-HT_{1A} receptor antagonists NAN-190 and BMY 7378 are partial agonists in the dorsal raphe nucleus in vitro, Eur. J. Pharmacol. 211, 211.

Hjorth, S. and T. Sharp, 1990, Mixed agonist/antagonist properties of NAN-190 at 5-HT_{1A} receptors: behavioural and in vivo brain microdialysis studies, Life Sci. 46, 955.

Lum, J.T., W.E. Hoffman and M.F. Piercey, 1990, 5-HT_{1A} agonist-like effects of NAN-190, Soc. Neurosci. Abstr. 427-1, P1034.

Paxinos, S. and C. Watson, 1986, The Rat Brain in Stereotaxic Coordinates (Academic Press, New York).

Routledge, C., J. Gurling, I.K. Wright and C.T. Dourish, 1993, Neurochemical profile of the selective and silent 5-HT_{1A} receptor antagonist WAY100135: an in vivo microdialysis study, Eur. J. Pharmacol. 239, 195.

Routledge, C., J. Hartley, J. Gurling, M. Ashworth-Preece, G. Brown and C.T. Dourish, 1994, In vivo characterisation of the putative 5-HT_{1A} receptor antagonist SDZ 216,525 using two in vivo models of somatodendritic 5-HT_{1A} receptor function, Neuropharmacology 33, 359.

Rydelek-Fitzgerald, L., M. Tietler, P.W. Fletcher, A.M. Ismaiel and R.A. Glennon, 1990, NAN-190: agonist and antagonist interactions with brain 5-HT_{1A} receptors, Brain Res. 502, 191.

Sharp, T., S.R. Bramwell and D.G. Graham-Smith, 1989, 5-HT_1 agonists reduce 5-hydroxtryptamine release in hippocampus in vivo as determined by brain microdialysis, Br. J. Pharmacol. 96, 283.

Sharp, T., R. McQuade, S. Bramwell and S. Hjorth, 1993, Effect of acute and repeated administration of 5-HT_{1A} receptor agonists on 5-HT release in rat brain in vivo, Naunyn-Schmied. Arch. Pharmacol. 348, 339.

Sprouse, J.S. and G.K. Aghajanian, 1987, Responses of hippocampal pyramidal cells to putative 5-HT_{1A} and 5-HT_{1B} agonists: a comparative study with dorsal raphe neurones, Neuropharmacology 27, 707.

Starkey, S.J. and M. Skingle, 1994, 5-HT_{1D} as well as 5-HT_{1A} autoreceptors modulate 5-HT release in the guinea-pig dorsal raphe nucleus, Neuropharmacology 33, 393.