Evidence that 5-HT₂ receptor activation decreases noradrenaline release in rat hippocampus *in vivo*

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1 Recent electrophysiological studies have shown that 5-HT₂/5-HT_{1C} receptor agonists inhibit the electrical activity of noradrenergic neurones in the rat locus coeruleus. Here we examine the effect of various agonists and antagonists of 5-HT₂/5-HT_{1C} receptors on noradrenaline release in hippocampus of anaesthetized rats using microdialysis.

2 Subcutaneous administration of the 5-HT₂/5-HT_{1C} receptor agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI: 0.2 and 0.5 mg kg⁻¹), caused a marked decrease (50% of pre-drug levels 60 min after injection) of noradrenaline in hippocampal dialysates which was long-lasting (> 120 min). Noradrenaline output also decreased in response to administration of the structural analogue of DOI, 1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane (DOB: 1 mg kg⁻¹, s.c.).

3 The effect of DOI on noradrenaline output was prevented by pretreatment with the 5-HT₂/5-HT_{1C} receptor antagonist, ritanserin (0.4 mg kg⁻¹, s.c.). Spiperone (0.2 and 1 mg kg⁻¹, s.c.), a 5-HT₂/dopamine D₂ receptor antagonist which has low affinity for 5-HT_{1C} receptors, also antagonized the effect of DOI (0.5 mg kg⁻¹, s.c.). Sulpiride (50 mg kg⁻¹, s.c.), a dopamine D₂ receptor antagonist did not alter the response to DOI (0.5 mg kg⁻¹, s.c.).

4 Both the non-selective 5-HT receptor agonist, quipazine (1 mg kg⁻¹, s.c.), and the 5-HT-releasing agent, *p*-chloroamphetamine (2 mg kg⁻¹, s.c.), decreased noradrenaline release in hippocampus and these effects were antagonized by pretreatment with ritanserin (0.4 mg kg⁻¹, s.c.).

5 Our data suggest that *in vivo*, noradrenaline release in hippocampus is inhibited by 5-HT₂ receptor activation. This effect is probably associated with a decrease in noradrenergic neuronal activity.

Keywords: Microdialysis; noradrenaline; 5-HT₂ receptors; 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI); ritanserin; quipazine; *p*-chloroamphetamine

Introduction

Several lines of evidence suggest that the activity of central noradrenergic neurones is regulated by the neurotransmitter 5-hydroxytryptamine (5-HT). In particular, the major source of noradrenergic innervation to the forebrain, the locus coeruleus (LC), receives a dense input of 5-HT-containing neurones (Pickel *et al.*, 1977; Leger & Descarries, 1978) and contains high levels of both 5-HT reuptake sites (Savaki *et al.*, 1985; De Souza & Kuyatt, 1987) and 5-HT binding sites (Weissmann-Nanopoulos *et al.*, 1985). In addition, both 5-HT denervation (Pujol *et al.*, 1978) and 5-HT synthesis inhibition cause an increase in noradrenaline synthesis in the LC (Crespi *et al.*, 1980; McRae-Degueurce *et al.*, 1985). Inhibition of 5-HT synthesis also results in an increase in basal firing rate of noradrenergic neurones in the LC (Ferron *et al.*, 1988). Taken together these findings support the view that central noradrenergic neurones may be under an inhibitory influence of 5-HT.

Evidence from *in vivo* electrophysiological studies suggests that the putative inhibitory influence of 5-HT on central noradrenergic neurones may be mediated by the 5-HT₂ or 5-HT_{1C} receptor. Thus, it has been shown that systemic injection of a range of 5-HT₂/5-HT_{1C} receptor agonists, including the phenylalkylamines 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) and 1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane (DOB), inhibits the firing rate of spontaneously active neurones in the rat LC (Rasmussen & Aghajanian, 1986; Gorea & Adrien, 1988). No studies to date, however, have examined whether noradrenaline release is decreased following 5-HT₂ receptor activation, as would be predicted from the latter work.

Recent studies have shown that noradrenaline release measured by *in vivo* microdialysis is neurally derived and changes according to the level of LC neuronal activity (Abercrombie *et al.*, 1988; Kalen *et al.*, 1988). Here we have used the brain microdialysis technique to investigate further the nature of the interaction between 5-HT₂/5-HT_{1C} receptors and noradrenergic neurones. Noradrenaline release in hippocampus, a major efferent projection of the LC (Foote *et al.*, 1983), was measured following systemic administration of the 5-HT₂/5-HT_{1C} receptor agonists, DOI and DOB (Glennon *et al.*, 1988; Appel *et al.*, 1990), the non-selective 5-HT agonist, quipazine (Hoyer, 1988), and the 5-HT-releasing agent, *p*-chloroamphetamine (Pettibone & Williams, 1984). A preliminary account of these findings was presented to the British Pharmacological Society (Done & Sharp, 1991).

Methods

Microdialysis procedure

Male Sprague-Dawley rats (250–350 g; Harlan Olac, Bicester, U.K.) were maintained on a 12 h light/dark cycle with free access to food and water. Rats were anaesthetized with chloral hydrate (400 mg kg⁻¹, i.p. with supplementary doses of 60 mg kg⁻¹ as required) and a short loop microdialysis probe was stereotactically implanted into the ventral hippocampus (coordinates: AP – 4.0 mm, L – 4.6 mm, DV – 9.5 mm, relative to bregma and skull surface; Paxinos & Watson, 1982) and cemented in place. The probe was continuously perfused (1.2 µl min⁻¹) with artificial CSF (composition mM: NaCl 140, KCl 3, CaCl₂ 1.2, MgCl₂ 1.0, Na₂HPO₄ 1.2, NaH₂PO₄ 0.27 and glucose 7.2, pH 7.4) containing the noradrenaline uptake inhibitor, desipramine (5 µM). Desi-

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pramine was used to increase the basal level of noradrenaline in hippocampal dialysates to readily detectable amounts.

H.p.l.c.-e.c. analysis of noradrenaline

Noradrenaline in the perfusates was analysed as described previously (Done *et al.*, 1992; Sharp & Zetterstrom, 1992). The high performance liquid chromatography (h.p.l.c.) system utilised an h.p.l.c. column (4.6 × 150 mm containing spherical Microsorb C18, 5 µm particles, Dynamax/Rainin Inc.) and a mobile phase comprising 0.1 M NaH₂PO₄, 2 mM sodium octane sulphonate, 0.5 mM EDTA, and 12% (vol/vol) methanol (final pH 4.6, flow rate 1.1 ml min⁻¹). Electrochemical detection was by a glassy carbon electrode (BAS MF 1000, held at +0.7 V with respect to a Ag/AgCl reference electrode) connected to a BAS LC-4 potentiometer. Following collection, dialysate samples (20 µl) were injected immediately onto the analytical column using a Rheodyne 7125 injector. The approximate limit of detection was 10 fmol/sample.

Experimental design

After implantation of the dialysis probe a baseline period of 2–3 h was allowed for stabilization of noradrenaline output. At this time, injection of the α₂-adrenoceptor agonist, clonidine (1 mg kg⁻¹), decreases hippocampal noradrenaline output to 90% of pre-drug levels, within 40 min (Done *et al.*, 1992; Sharp & Zetterstrom, 1992), suggesting that a significant proportion of noradrenaline in the dialysates is of neuronal origin.

After the baseline period, drugs were administered subcutaneously and their effects were followed for 2 h. In some experiments 5-HT antagonists were injected subcutaneously 40 min before 5-HT agonists. The dose of quipazine used (1 mg kg⁻¹) induces 5-HT₂-mediated behaviour in rats (e.g. Wing *et al.*, 1990). The dose of *p*-chloroamphetamine used (2 mg kg⁻¹) has been shown to increase 5-HT release *in vivo* (Sharp *et al.*, 1986). Doses of ritanserin (0.4 mg kg⁻¹) and spiperone (0.2 mg kg⁻¹ and 1 mg kg⁻¹) were chosen on the basis of their ability to antagonize 5-HT₂ receptor-mediated head shakes in rats (Peroutka *et al.*, 1981; Backus *et al.*, 1990).

Drugs

The drugs used, together with their suppliers, were as follows; DOI hydrochloride, DOB hydrobromide, quipazine maleate (all from Research Biochemicals Inc., Semat, St. Albans, U.K.), *p*-chloroamphetamine hydrochloride, spiperone, sulpiride hydrochloride (all from Sigma Chemical Co., Poole, U.K.) and ritanserin tartrate (Janssen Pharmaceuticals, Breese, Belgium). DOI, DOB, quipazine and *p*-chloroamphetamine were dissolved in saline (0.9% NaCl, w/v). Ritanserin, spiperone and sulpiride were dissolved in a few drops of glacial acetic acid and then diluted to volume with 5% glucose solution.

Data analysis

Noradrenaline in each sample is expressed as a percentage of the absolute amount of noradrenaline in the sample collected immediately before drug administration. Time points are expressed as the mean ± s.e.mean of the results obtained from 4–7 rats. Values for individual time points over the 120 min postdrug period were accumulated to give area under the curve. Area under the curve data were analysed statistically by the Kruskal-Wallis ANOVA followed by Mann-Whitney U-test. *P* values of 5% or less were considered statistically significant.

Results

Effect of DOI and DOB on noradrenaline output in rat hippocampus

Figure 1 shows that the 5-HT_{2/1C} receptor agonist DOI, at doses of 0.2 and 0.5 mg kg⁻¹, caused a marked decrease of noradrenaline in hippocampal dialysates of anaesthetized rats. A dose of 0.5 mg kg⁻¹ DOI decreased noradrenaline to about 50% of pre-drug levels within 60 min of injection, and this effect was long-lasting (> 120 min). A higher dose of DOI (2 mg kg⁻¹) had a similar effect on noradrenaline output compared to 0.5 mg kg⁻¹ (*n* = 5, data not shown). At a dose of 0.02 mg kg⁻¹, DOI was ineffective compared to saline-injected controls. Thus, overall the slope of the dose-response curve looks steep (Figure 7).

DOB (1 mg kg⁻¹) also decreased hippocampal noradrenaline release by 50 ± 19% (60 min post-drug, *n* = 3) compared to pre-drug levels.

A dose of 0.5 mg kg⁻¹ DOI was chosen for antagonist experiments, since of the doses used, this produced the most consistent and reproducible decrease in hippocampal noradrenaline output.

Effect of pretreatment with ritanserin, spiperone or sulpiride on DOI response

Pretreatment with the 5-HT_{2/1C} receptor antagonist, ritanserin (0.4 mg kg⁻¹, s.c.) completely prevented the inhibitory effect of DOI (0.5 mg kg⁻¹, s.c.) on hippocampal noradrenaline output (Figure 2). Pretreatment with 0.2 mg kg⁻¹ of the 5-HT₂/dopamine D₂ antagonist, spiperone, also blocked the effect of DOI (0.5 mg kg⁻¹, s.c.) although only partially (Figure 3). A higher dose of spiperone (1 mg kg⁻¹) completely prevented the effect of DOI (Figure 3). When injected alone neither ritanserin nor spiperone altered noradrenaline output over the 2 h post-drug period. Figure 4 shows that pretreatment with the D₂ receptor antagonist, sulpiride (50 mg kg⁻¹, s.c.), 40 min before DOI (0.5 mg kg⁻¹, s.c.), did not alter the response to DOI. Since sulpiride was dissolved in acid, this experiment also confirms that the blockade of the DOI effect by ritanserin and spiperone (which were also dissolved in acid) cannot be accounted for by vehicle alone.

Figure 1 Effect of 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI, 0.02–0.5 mg kg⁻¹, s.c.) on noradrenaline in hippocampal microdialysates of the anaesthetized rat. Rats were injected with either saline (Δ) or DOI (0.02 mg kg⁻¹, ▲; 0.2 mg kg⁻¹, □; 0.5 mg kg⁻¹, ●) at the time point indicated. Each point represents the mean of data obtained from 5–7 rats; s.e.mean shown by vertical bars. The mean (± s.e.mean) basal output of noradrenaline for the above groups combined was 0.096 ± 0.006 pmol/20 min sample (*n* = 26). Statistical analysis of area under the curve data revealed that DOI (0.2 and 0.5 mg kg⁻¹)-injected groups and the saline-injected controls were significantly different (*P* < 0.01).

Figure 2 Antagonism of the 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) induced inhibition of hippocampal noradrenaline release by ritanserin (Rit). DOI (0.5 mg kg^{-1} , s.c.) was injected into control rats (●) or rats treated 40 min previously with 0.4 mg kg^{-1} , s.c. ritanserin (○). Results are means $\pm$ s.e.mean (vertical bars) of data obtained from 5–7 rats. The mean ($\pm$ s.e.mean) basal output of noradrenaline for the above groups combined was $0.106 \pm 0.008 \text{ pmol/20 min sample}$ ($n = 12$). Statistical analysis of area under the curve data revealed that the ritanserin/DOI and DOI alone groups were significantly different ($P < 0.01$).

Figure 3 Reversal of 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI)-induced inhibition of noradrenaline release in hippocampus by spiperone. DOI (0.5 mg kg^{-1} , s.c.) was injected into control rats (●) or rats treated 40 min previously with either 0.2 mg kg^{-1} (□) or 1 mg kg^{-1} , s.c. (○) spiperone. Results are means $\pm$ s.e.mean (vertical bars) of data obtained from 4–7 rats. The mean ($\pm$ s.e.mean) basal output of noradrenaline for the above groups combined was $0.118 \pm 0.007 \text{ pmol/20 min sample}$ ($n = 16$). Statistical analysis of area under the curve data revealed that the spiperone (0.2 mg kg^{-1})/DOI and DOI alone groups were significantly different ($P < 0.05$) and the spiperone (1 mg kg^{-1})/DOI and DOI alone groups were significantly different ($P < 0.01$).

The data for DOI in combination with ritanserin and spiperone are summarized in Figure 7.

Effect of quipazine on noradrenaline output in hippocampus

Figure 5 shows that the non-selective 5-HT receptor agonist quipazine (1 mg kg^{-1} , s.c.) induced an immediate and marked

Figure 4 Lack of effect of sulpiride (Sulp) in blocking 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI)-induced inhibition of noradrenaline release in hippocampus. DOI (0.5 mg kg^{-1} , s.c.) was injected into control rats (●) or rats treated 40 min previously with 50 mg kg^{-1} , s.c. sulpiride (○). Results are means $\pm$ s.e.mean (vertical bars) of data from 4–7 rats. The mean ($\pm$ s.e.mean) basal output of noradrenaline for the above groups combined was $0.104 \pm 0.007 \text{ pmol/20 min sample}$ ($n = 11$). Statistical analysis of area under the curve data revealed no significant difference between sulpiride/DOI and DOI alone groups.

Figure 5 Effect of quipazine (Quip) on noradrenaline release in hippocampus. Quipazine (1 mg kg^{-1} , s.c.) was injected into control rats (●) or rats treated 40 min previously with 0.4 mg kg^{-1} , s.c. ritanserin (Rit) (○). Results are means $\pm$ s.e.mean (vertical bars) of data from 5 rats. The mean ($\pm$ s.e.mean) basal output of noradrenaline for the above groups combined was $0.121 \pm 0.008 \text{ pmol/20 min sample}$ ($n = 10$). Statistical analysis of area under the curve data revealed that the quipazine-injected group and the saline-injected controls were significantly different ($P < 0.01$). In addition the ritanserin/quipazine and quipazine alone groups were significantly different ($P < 0.05$).

decrease of noradrenaline in hippocampal dialysates. The maximum effect was a reduction of hippocampal noradrenaline to 50% of pre-drug levels, 60 min after injection. Pretreatment with ritanserin (0.4 mg kg^{-1}) antagonized the effect of quipazine (Figure 5).

The data for quipazine in combination with ritanserin are summarized in Figure 7.

Effect of *p*-chloroamphetamine on noradrenaline output in hippocampus

Figure 6 shows that the 5-HT-releasing agent *p*-chloroamphetamine (2 mg kg⁻¹, s.c.), like quipazine and DOI, induced a long-lasting decrease in hippocampal noradrenaline output, the maximum effect being about 40% reduction of pre-drug control levels. The effect of *p*-chloroamphetamine was antagonized by pretreatment with 0.4 mg kg⁻¹ ritanserin (Figure 6).

The data for *p*-chloroamphetamine in combination with ritanserin are summarized in Figure 7.

Discussion

Our results show that systemic injection of the phenylalkylamine hallucinogen, DOI, which has high binding affinity for both 5-HT₂ and 5-HT_{1C} receptors (Glennon *et al.*, 1988; Appel *et al.*, 1990), decreases levels of noradrenaline in hippocampal dialysates of anaesthetized rats. Both ritanserin and spiperone prevented this inhibitory effect of DOI. Receptor binding studies show that ritanserin has high affinity for both 5-HT₂ and 5-HT_{1C} receptors, while spiperone has high affinity for 5-HT₂ but low affinity for 5-HT_{1C} receptors (Hoyer, 1988). These findings strongly suggest that the inhibitory effect of DOI on noradrenaline release is mediated by the 5-HT₂ receptor. Indeed the complete blockade by spiperone of the DOI effect suggests that the 5-HT_{1C} receptor does not influence noradrenaline release under the present experimental conditions. Although spiperone is a dopamine D₂ receptor antagonist (Stefanini *et al.*, 1980) pretreatment with the selective D₂ receptor antagonist, sulpiride, did not alter the response to DOI, suggesting that the D₂ receptor is not involved.

Two other drugs with 5-HT₂ agonist properties, quipazine and DOB (Vetulani *et al.*, 1980; Glennon *et al.*, 1983; Glennon *et al.*, 1986), like DOI, decreased hippocampal noradrenaline output. The effect of quipazine was also antagonized by ritanserin and given the above discussion, it would seem reasonable to assume that the effect of quipazine is mediated by 5-HT₂ rather than 5-HT_{1C} receptors.

Figure 6 Effect of *p*-chloroamphetamine (PCA) on noradrenaline release in hippocampus. *p*-Chloroamphetamine (2 mg kg⁻¹, s.c.) was injected into control rats (●) or rats treated 40 min previously with 0.4 mg kg⁻¹, s.c. ritanserin (Rit) (○). Results are means ± s.e.mean (vertical bars) of data from 4–5 rats. The mean (± s.e.mean) basal output of noradrenaline for the above groups combined was 0.113 ± 0.007 pmol/20 min sample (*n* = 9). Statistical analysis of area under the curve data revealed that the *p*-chloroamphetamine-injected group and the saline-injected controls were significantly different (*P* < 0.01). In addition the ritanserin/*p*-chloroamphetamine and *p*-chloroamphetamine alone groups were significantly different (*P* < 0.05).

Figure 7 Summary of data expressed as means (± s.e.mean, vertical bars) of areas under the curve for the 120 min post-drug period: 100% represents no change in noradrenaline output over the 120 min post-drug period. Doses (mg kg⁻¹) are shown in the figure. ^a*P* < 0.01 versus saline-injected controls; ^b*P* < 0.01 versus 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI, 0.5 mg kg⁻¹, s.c.) alone; ^c*P* < 0.05 versus DOI (0.5 mg kg⁻¹, s.c.) alone; ^d*P* < 0.05 versus quipazine (1 mg kg⁻¹, s.c.) alone; ^e*P* = 0.05 versus *p*-chloroamphetamine (2 mg kg⁻¹, s.c.) alone. Data analysis by Kruskal-Wallis ANOVA followed by Mann-Whitney U-test.

The 5-HT-releasing agent, *p*-chloroamphetamine, also decreased hippocampal noradrenaline output, an effect blocked by ritanserin. *p*-Chloroamphetamine, at a dose similar to that used in the present study, has previously been shown to release 5-HT *in vivo* (Sharp *et al.*, 1986), which supports the idea that the effect of this drug on noradrenaline output is mediated by endogenous 5-HT. However, *p*-chloroamphetamine also releases dopamine (Sharp *et al.*, 1986), and it is therefore possible that the drug normally releases noradrenaline. It is unlikely, however, that *p*-chloroamphetamine would release noradrenaline in the present experiments since a noradrenaline uptake inhibitor, desipramine, was present in the perfusion medium. Desipramine would prevent entry of *p*-chloroamphetamine into the noradrenergic nerve terminals in the region of the microdialysis probe and thereby circumvent any indirect noradrenaline releasing action.

The 5-HT₂ agonist-induced decrease in hippocampal noradrenaline release may well be directly related to a decrease in firing rate of noradrenergic neurones in the LC. Thus, it has been reported previously that systemic administration of 5-HT_{2/1C} agonists, including quipazine and phenylalkylamines such as DOB and DOM, inhibits the spontaneous electrical activity of noradrenergic neurones in the LC (Rasmussen & Aghajanian, 1986; Rasmussen *et al.*, 1986; Gorea & Adrien, 1988). Furthermore, the latter studies showed that the inhibitory actions of quipazine and the phenylalkylamines are antagonized by 5-HT_{2/1C} antagonists.

It should be noted that whereas the phenylalkylamines and quipazine cause a near complete inhibition of firing of neurones in the LC, noradrenaline output in hippocampus

was reduced to only 50% of control values. The latter effect compares with a 90% reduction in hippocampal noradrenaline output induced by 1 mg kg⁻¹ clonidine (Done *et al.*, 1992; Sharp & Zetterstrom, 1992), a drug which is well known to inhibit LC neuronal firing completely. The quantitative difference in the effects of the 5-HT_{2/1C} agonists on hippocampal noradrenaline output versus LC neuronal firing may suggest that, at least in the case of 5-HT_{2/1C} agonists (but presumably not clonidine), the inhibition of LC neuronal firing may be offset by compensatory changes in noradrenaline release at the nerve terminal. Alternatively, not all noradrenergic neurones innervating hippocampus may be under the inhibitory influence of 5-HT₂ receptors. In this respect it would be interesting to know the effects of 5-HT_{2/1C} agonists on noradrenaline output in other brain areas.

Our experiments show that neither ritanserin nor spiperone influence the basal output of noradrenaline in hippocampus. This finding suggests that there is no basal 5-hydroxytryptaminergic tone at the 5-HT₂ receptor inhibiting noradrenaline output under the present experimental conditions. An earlier report showed that the 5-HT_{2/1C} antagonist, ketanserin, has no consistent effect on LC firing (Gorea & Adrien, 1988). However, in another study both ritanserin and LY 53857 (also a 5-HT_{2/1C} antagonist) were observed to cause a slight increase in LC firing (Rasmussen & Aghajanian, 1986). The latter study suggests that under certain, as yet undefined, conditions central noradrenergic neurotransmission may be

under an inhibitory tone mediated by the 5-HT₂ receptor. We are investigating this issue at present.

Although we have no direct evidence to suggest that the 5-HT₂-induced reduction of noradrenaline release observed in the present study is mediated centrally, this seems likely since intracerebroventricular injection of quipazine induces a reduction of noradrenergic neuronal firing (Gorea & Adrien, 1988). Interestingly, recent electrophysiological studies indicate that the inhibitory effect of 5-HT_{2/1C} agonists on noradrenergic activity is mediated, not via a direct action in the LC but involves afferents to the LC from the medulla (Gorea *et al.*, 1991). It is unlikely that 5-HT₂ agonists inhibit noradrenaline release by a direct action at the nerve terminal since local perfusion of DOI into hippocampus has no effect on noradrenaline release (this laboratory, unpublished observations).

In summary, this study demonstrates that DOI, DOB, quipazine and *p*-chloroamphetamine decrease noradrenaline output in hippocampus of anaesthetized rats. Furthermore our results suggest that this effect is 5-HT₂-mediated. These findings are consistent with several lines of evidence indicating an inhibitory influence of 5-HT, mediated via the 5-HT₂ receptor, in the regulation of central noradrenergic transmission.

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