Increase of noradrenaline release in the hypothalamus of freely moving rat by postsynaptic 5-hydroxytryptamine_{1A} receptor activation

M. Suzuki, T. Matsuda, S. Asano, P. Somboonthum, K. Takuma & ¹A. Baba

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Osaka University, 1-6 Yamada-oka, Suita, Osaka, 565 Japan

1 5-Hydroxytryptamine (5-HT) plays a role in the regulation of noradrenergic neurones in the brain, but the precise mechanism of regulation of noradrenaline (NA) release by 5-HT_{1A} receptors has not been defined. The present study describes the effect of a highly potent and selective 5-HT_{1A} receptor agonist, 5-{3-[[[(2S)-1,4-benzodioxan-2-ylmethyl]amino]propoxy}-1,3-benzodioxole HCl (MKC-242), on NA release in the hypothalamus using microdialysis in the freely moving rat.

2 Subcutaneous injection of MKC-242 (0.5 mg kg⁻¹) increased extracellular levels of NA and its metabolite, 3-methoxy-4-hydroxyphenylglycol, in the hypothalamus and hippocampus.

3 The 5-HT_{1A} receptor agonists, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) (0.2 mg kg⁻¹) and buspirone (3 mg kg⁻¹) mimicked the effect of MKC-242 in increasing NA release in the hypothalamus.

4 The effects of MKC-242 and 8-OH-DPAT in the hypothalamus were antagonized by pretreatment with WAY100135 (10 mg kg⁻¹), a silent 5-HT_{1A} receptor antagonist.

5 Local administration of 8-OH-DPAT (10–100 µM), citalopram (1 µM), a 5-HT reuptake inhibitor, and MDL72222 (10 µM), a 5-HT₃ receptor antagonist, into the hypothalamus, had no effect on NA release.

6 Intracerebroventricular injection with 5,7-dihydroxytryptamine caused a marked reduction in brain 5-HT content, but the treatment affected neither basal NA levels nor the MKC-242-induced increase in NA release.

7 The effect of MKC-242 in increasing NA release was not attenuated by repeated treatment with the drug (0.5 mg kg⁻¹, once a day for 2 weeks).

8 The present results suggest that activation of postsynaptic 5-HT_{1A} receptors increases NA release in the hypothalamus.

Keywords: 5-Hydroxytryptamine (5-HT); 5-HT_{1A} receptors; postsynaptic; MKC-242; noradrenaline (NA) release; microdialysis

Introduction

Neuroanatomical (Pickel *et al.*, 1977; Steinbusch, 1981) and biochemical (Crespi *et al.*, 1980; McRae-Degueurce *et al.*, 1985; Reader *et al.*, 1986; Tian *et al.*, 1993) studies indicate that noradrenergic neurones are functionally connected with 5-hydroxytryptaminergic neurones in the brain. The regulation of noradrenergic neurones by 5-hydroxytryptamine (5-HT) may be mediated by an activation of specific 5-HT receptor subtypes such as 5-HT_{1A} (Fuller & Perry, 1989; Done & Sharp, 1994), 5-HT_{1B} (Clement *et al.*, 1992), 5-HT_{1C} (Blandina *et al.*, 1991), 5-HT₂ (Rasmussen & Aghajanian, 1986; Gorea & Ardrien, 1988; Done & Sharp, 1992) and 5-HT₃ (Blandina *et al.*, 1991). The involvement of 5-HT_{1A} receptors in this regulation is considered to be responsible for an antidepressant-like effect of 5-HT_{1A} receptor agonists, in view of the previous attempts to explain the effects of antidepressant treatments in terms of changes in function of brain NA. However, the possibility remains that 5-HT_{1A} agonists as indicated in previous studies (Fuller & Perry, 1989; Clement *et al.*, 1992; Tian *et al.*, 1993; Done & Sharp, 1994) activate noradrenergic neurones by directly blocking α_2 -adrenoceptors, since 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) (Crist & Superanant, 1987) and the metabolite of azapirone, 1-(2-pyrimidinyl)-piperazine, (Bianchi *et al.*, 1988; Gobbi *et al.*, 1990) have α_2 -adrenoceptor antagonist properties. Furthermore, it is not known whether the regulation of noradrenergic neurones is mediated by presynaptic or postsynaptic 5-HT_{1A} receptors. In

this paper, we examined the effect of 5-{3-[[[(2S)-1,4-benzodioxan-2-ylmethyl]amino]propoxy}-1,3-benzodioxole HCl (MKC-242), a novel 5-HT_{1A} receptor agonist with antidepressant-like effects (Egawa *et al.*, 1993), on NA release in the hypothalamus of the freely moving rat. A preliminary account of these findings was presented to the Japanese Pharmacological Society (Suzuki *et al.*, 1994).

Methods

Animals and drugs

Male Wistar rats were maintained under controlled environmental conditions (22 ± 1°C; 12–12 h light-dark cycle, lights on at 08 h 00 min food and water *ad libitum*) for at least 1 week before being used for the experiments during which time they were accustomed to being handled.

MKC-242, citalopram HBr and WAY100135 (N-tert-butyl-3-(4-(2-methoxyphenyl)piperazin-1-yl)-2-phenyl-propionamide) were gifts from Mitsubishi Chemical Co. (Japan), H. Lundbeck A/S (Denmark) and Wyeth Research (U.K.) Ltd., respectively. All other chemicals used were of the highest commercially available purity. All drugs were freshly prepared. MKC-242 and WAY100135 for s.c. injection (1 ml kg⁻¹) were suspended in 0.5% carboxymethylcellulose (CMC). 5,7-dihydroxytryptamine (5,7-DHT) was dissolved in 0.9% NaCl containing 0.2% ascorbic acid. Other drugs for peripheral (1 ml kg⁻¹) and the locus coeruleus (LC) injections were dissolved in 0.9% NaCl. For direct administration into the hy-

¹ Author for correspondence.

pothalamus via dialysis probe, drugs were dissolved in artificial cerebrospinal fluid (aCSF) (composition, mM: NaCl 140, KCl 3.35, MgCl₂ 1.15, CaCl₂ 1.26, Na₂HPO₄ 1.20 and NaH₂PO₄ 0.30, pH 7.4).

Microdialysis procedure

Rats (250–350 g) were anaesthetized with chloral hydrate (400 mg kg⁻¹, i.p.) and stereotactically implanted with guide cannulae for the dialysis probes (Eicom, Japan) at the hy-

pothalamus (A -1.8 and L 0.7, with the tip of the probe 10.5 mm deep from the top of the skull) or the hippocampus (A -5.3 and L 4.4, with the tip of the probe 6.5 mm deep from the top of the skull) (Paxinos & Watson, 1986). The active probe membranes were 2 mm (hypothalamus) and 3 mm (hippocampus) in length. The day after surgery the probes were perfused at a constant flow rate of 2 µl min⁻¹ with aCSF containing 1 µM desipramine (for NA and its metabolite, 3-methoxy-4-hydroxyphenylglycol (MHPG), assays) or 1 µM citalopram (for 5-HT assay). A 4 h stabilization period was

Figure 1 Effect of s.c. injection of MKC-242 on extracellular noradrenaline (NA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) levels in the hypothalamus (a) and hippocampus (b) of freely moving rats. Rats were treated with carboxymethylcellulose (CMC, ○) and MKC-242 at 0.5 (●) and 2.0 (▲) mg kg⁻¹ at zero time. Points are means ± s.e. mean of four to six determinations. **P* < 0.05, ^b*P* < 0.01, ^c*P* < 0.001, compared with the corresponding value of CMC-treatment (two-way ANOVA followed by Student's *t* test).

allowed before 20 min microdialysis samples (40 μ l) were taken and immediately injected onto an h.p.l.c. column for subsequent assay of amines. The probe recoveries (mean $\pm$ s.e.) for NA and MHPG were $19.0 \pm 0.9\%$ and $7.7 \pm 0.3\%$, respectively. For experiments of microinjection, guide cannulae were implanted in the locus coeruleus (A -9.8, L 1.2, and V 5.9 from the top of the skull) under pentobarbitone anaesthesia (40 mg kg⁻¹, i.p.) the day before the experiment.

Drugs were administered at 0 time. The LC injection (0.5 μ l per rat) and perfusion with drugs was carried out for a period of 2 min and 1 h (three fractions), respectively.

Analysis of dialysates

Dialysates were assayed by h.p.l.c. with electrochemical detection: Eicompak CA-ODS column (4.6 mm i.d. $\times$ 150 mm:

Figure 2 Effect of s.c. injection of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) (a) and buspirone (b) on extracellular noradrenaline (NA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) levels in the hypothalamus of freely moving rats. Rats were treated with saline (○), 8-OH-DPAT at 0.2 (▲) and 1.0 (△) mg kg⁻¹, and buspirone at 3.0 mg kg⁻¹ (●) at zero time. Points are means $\pm$ s.e. of four to five rats. ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$, compared with the corresponding value of saline treatment (two-way ANOVA followed by Student's *t* test).

Eicom, Japan) and graphite electrode (Eicom) set at +550 mV (for NA and MHPG) or +450 mV (for 5-HT) against an Ag/AgCl reference electrode were used as previously described (Matsuda *et al.*, 1989). The mobile phase for NA and MHPG assays contained 95 mM sodium phosphate buffer (pH 6.0), 1.85 mM sodium octanesulphonic acid, 134 μ M EDTA and 5% (v/v) methanol, and that for 5-HT assay contained 80 mM sodium phosphate buffer (pH 6.0), 1.39 mM sodium octanesulphonic acid, 134 μ M EDTA and 20% v/v methanol.

Lesions of 5-HT neurones with 5,7-DHT

Rats (220–250 g) were anaesthetized with pentobarbitone (40 mg kg⁻¹, i.p.). Lesions of 5-HT neurones were carried out by injection of 5,7-DHT (150 μ g as free base, 20 μ l per rat, over a period of 2 min) into the lateral ventricles (A -0.8, L -1.5 and V 4.5 from skull surface). Desipramine at 25 mg kg⁻¹, i.p. was injected 30 min before 5,7-DHT to pro-

tect noradrenergic neurones. The animals were used 2 weeks after the i.c.v. injection.

Data analysis

The average of three fractions before drug administration was defined as 100% (control), and the subsequent perfusate levels were expressed as a percentage of the control. Statistical analyses were conducted by two-way ANOVA followed by Student's *t* test. *P* values of 5% or less were considered statistically significant.

Results

Basal levels of NA and MHPG, corrected with probe recoveries, in the rat hypothalamus, were 200 $\pm$ 12 and 287 $\pm$ 13 pg per fraction, respectively (means $\pm$ s.e., *n* = 56). The

Figure 3 Effects of WAY100135 on MKC-242- and 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT)-induced increases in extracellular noradrenaline (NA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) levels in the hypothalamus. MKC-242 at 0.5 mg kg⁻¹ (a) and 8-OH-DPAT at 0.2 mg kg⁻¹ (b) were injected s.c. at zero time. WAY100135 (10 mg kg⁻¹) was administered s.c. 30 min before the agonists: (○) the agonists; (●) WAY100135/the agonists; (△) vehicle control. Points are means $\pm$ s.e. of four to six rats. ^a*P* < 0.05, ^b*P* < 0.01, compared with the corresponding value of CMC treatment (two-way ANOVA followed by Student's *t* test). Two-way ANOVA analysis showed that the effects of WAY100135 on MKC-242- and 8-OH-DPAT-induced NA release were significant (MKC-242 vs. WAY100135/MKC-242, *P* = 0.042; 8-OH-DPAT vs. WAY100135/8-OH-DPAT, *P* = 0.001).

Figure 4 Effect of perfusion with 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), citalopram or MDL72222 on extracellular noradrenaline (NA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) levels in the hypothalamus. 8-OH-DPAT (a), citalopram (b) and MDL72222 (c) were infused into the hypothalamus via the dialysis probe for 1 h period indicated by the horizontal bar. The concentrations of drugs were: 0 (○), 1 (●), 10 (△) and 100 (▲) μM. Points are means ± s.e. of three to five. Two-way ANOVA analysis revealed no significant effect of either 8-OH-DPAT, citalopram or MDL72222 vs aCSF controls.

Table 1 Effect of 5-hydroxytryptamine (5-HT) neuronal lesions with 5,7-dihydroxytryptamine (5,7-DHT) on noradrenaline (NA), dopamine (DA), 5-HT and their metabolite levels in rat whole brain (except for cerebellum)

	NA	MHPG	DA	DOPAC	HVA	5-HT	5-HIAA
Vehicle	474 ± 14	141 ± 2	1505 ± 47	145 ± 6	189 ± 17	748 ± 28	492 ± 48
5,7-DHT	484 ± 22	130 ± 2	1237 ± 54*	138 ± 7	165 ± 5	48 ± 5***	28 ± 3***

Rats were injected i.c.v. with 20 µl of 5,7-DHT creatinine sulphate (314.8 µg) and vehicle 14 days before the experiment. Results (ng g⁻¹ tissue) are mean ± s.e. mean from three to five rats. **P* < 0.05, ****P* < 0.01, compared with vehicle (Student's *t* test).

Figure 5 Effect of pretreatment with 5,7-dihydroxytryptamine (5,7-DHT) on MKC-242-induced increase in extracellular noradrenaline (NA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) levels in the

basal level of NA in the present study was similar to that reported previously in the hypothalamus (Qadri *et al.*, 1991).

Subcutaneous injection of MKC-242 caused a significant dose-dependent increase in extracellular NA level in the hypothalamus of the freely moving rat (Figure 1a). The extracellular level of MHPG, a metabolite of NA, was also increased by MKC-242, but the increase in MHPG concentration was smaller than that of NA. At a dose of 0.1 mg kg⁻¹, MKC-242 was ineffective (data not shown). A similar effect of MKC-242 on NA release was also observed in the hippocampus (Figure 1b). The effect of MKC-242 was mimicked by the 5-HT_{1A} receptor agonists, 8-OH-DPAT and buspirone: 8-OH-DPAT at 0.2 and 1.0 mg kg⁻¹ and buspirone at 3.0 mg kg⁻¹ significantly increased extracellular levels of NA and MHPG in the hypothalamus (Figure 2). 8-OH-DPAT-induced increase in NA release in the hypothalamus was also observed even when the perfusion was carried out with aCSF lacking desipramine (data not shown). In rats pretreated with WAY100135 at 10 mg kg⁻¹, MKC-242 and 8-OH-DPAT did not increase extracellular levels of NA and MHPG (Figure 3). WAY100135 alone did not affect the basal release of NA in the hypothalamus (data not shown), in agreement with previous data (Routledge *et al.*, 1993). When 8-OH-DPAT (10, 100 µM), citalopram (1 µM) and MDL 72222 (10 µM) were administered locally via the dialysis probe, they had no effect on hypothalamic NA release (Figure 4).

Lesions of 5-HT neurones with 5,7-DHT caused a marked reduction in 5-HT (by 94%) and 5-hydroxyindoleacetic acid (5-HIAA) (by 95%) levels and a slight reduction in the dopamine level (by 18%), while it did not affect NA, MHPG, HVA and DOPAC levels in the brain (Table 1). In 5,7-DHT-treated rats, MKC-242 (0.5 mg kg⁻¹) significantly increased extracellular NA and MHPG levels (Figure 5). There was no difference in basal NA release between control and 5,7-DHT-treated groups: the NA levels (means ± s.e., *n* = 3–5) in the hypothalamus of vehicle- and 5,7-DHT-treated rats were 165 ± 31 and 168 ± 26 pg per fraction, respectively. The LC injection of 8-OH-DPAT at 2 µg did not cause a significant increase in NA release in the hypothalamus, although it showed a tendency to increase (Figure 6).

Repeated treatment with MKC-242 (once a day for 2 weeks at 0.5 mg kg⁻¹) did not affect the increase in NA release in the hypothalamus caused by MKC-242. While the chronic treatment showed a tendency to attenuate the effect of MKC-242 on 5-HT release, the decrease in 5-HT release was not significant in rats chronically treated with the agonist (Figure 7).

hypothalamus. MKC-242 at 0.5 mg kg⁻¹ was injected s.c. 14 days after rats were injected i.c.v. with saline (●) and 5,7-DHT at 150 µg (□). Only saline/CMC controls (○) are shown, since there was no difference in the effect of CMC between saline- and 5,7-DHT-treated rats. Points are means ± s.e. of three to five rats. **P* < 0.05, ^b*P* < 0.01, ^c*P* < 0.001, compared with the corresponding value of CMC treatment (two-way ANOVA followed by Student's *t* test). Two-way ANOVA analysis revealed no significant difference in the effects of MKC-242 on NA and MHPG release between 5,7-DHT- and saline-treated groups.

Discussion

We have recently developed a novel compound, MKC-242, which is a potent and selective 5-HT_{1A} receptor agonist with a

K_i value in the subnanomolar range in [³H]-8-OH-DPAT binding and with a low or negligible affinity for 5-HT transporter and other neurotransmitter receptors (Yoshikawa *et al.*, 1994). In the present study, we examined, by *in vivo* micro-

Figure 6 Effect of microinjection of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) into the LC on noradrenaline (NA) release in the hypothalamus. Saline (○) and 8-OH-DPAT at 2 μ g (●) were injected into the LC, and the perfusion was carried out in the hypothalamus. Points are means $\pm$ s.e. of three rats. Two-way ANOVA analysis revealed no significant differences in the effects of MKC-242 on NA and 3-methoxy-4-hydroxyphenylglycol (MHPG) release between saline and 8-OH-DPAT.

Figure 7 Effects of chronic treatment with MKC-242 on noradrenaline (NA) release (a) and 5-hydroxytryptamine (5-HT) release (b) in the hypothalamus. MKC-242 at 0.5 mg kg⁻¹ was injected s.c. in rats treated once a day for 14 days with carboxymethyl cellulose (CMC) (○) and MKC-242 at 0.5 mg kg⁻¹ (▲). Only acute CMC controls (○) are shown, since the effect of chronic CMC was similar to that of acute CMC. Points are means $\pm$ s.e. of three to five rats. ^a P < 0.05, ^b P < 0.01, ^c P < 0.001, compared with the corresponding value of CMC control (two-way ANOVA followed by Student's *t* test). Two-way ANOVA analysis revealed no significant differences in the effects of MKC-242 on NA and 5-HT release between acute and chronic groups.

dialysis, the effect of MKC-242 on rat hypothalamic NA release, in order to clarify the 5-HT_{1A} receptor-mediated regulation of noradrenergic neurones. Systemic administration of MKC-242, as well as other 5-HT_{1A} receptor agonists such as 8-OH-DPAT and buspirone, increased extracellular NA and its metabolite, MHPG, in the hypothalamus and hippocampus. It is unlikely that the increase in NA release by the drugs is due to an inhibition of the reuptake, since desipramine, an inhibitor of NA reuptake, was included in the perfusion fluid in this study. We also observed that the effects of MKC-242 and 8-OH-DPAT on the extracellular NA level were almost completely blocked by pretreatment with WAY100135, a selective antagonist at presynaptic and postsynaptic 5-HT_{1A} receptors (Fletcher *et al.*, 1993). These results suggest that activation of 5-HT_{1A} receptors increases NA release. This was also supported by the previous observation that systemic administration of 8-OH-DPAT and buspirone increased NA turnover in the hypothalamus (Fuller & Perry, 1989). In contrast, Broderick & Piercey (1991) reported that buspirone and ipsapirone depressed NA release in the hippocampus, although they found that the 5-HT_{1A} receptor agonists excited NA neuronal cell firing rates in the LC. The discrepancy might be due to a difference in the method used to detect NA release: Broderick & Piercey (1991) used *in vivo* voltammetric recording of monoamine release.

The results of the present study are consistent with the findings of Done & Sharp (1994) who have shown that 8-OH-DPAT increased the release of NA in the hippocampus as measured by microdialysis. They further suggested that the effect of 8-OH-DPAT might be due to activation of presynaptic 5-HT_{1A} autoreceptors, since the effect was mimicked by NAN-190 and MDL 73005EF, 5-HT_{1A} receptor antagonists with agonist properties at presynaptic 5-HT_{1A} receptors (Gartside *et al.*, 1990; Hjorth & Sharp, 1990; Sprouse, 1991; Greuel & Glaser, 1992). In contrast with this proposal, the present study demonstrates that treatment with 5,7-DHT which destroys presynaptic 5-hydroxytryptaminergic nerve fibres does not alter the effect of MKC-242 in increasing NA release. This finding suggests that NA release is modulated in part by postsynaptic 5-HT_{1A} receptors, although it does not rule out the possibility that the presynaptic 5-HT_{1A} receptors play a role in NA release as previously suggested by Done & Sharp (1994). In view of the earlier finding that NA release is under inhibitory control by 5-HT (Done & Sharp, 1992), it may be considered that 5,7-DHT lesions eliminate the inhibitory tone on noradrenergic neurones, and increase NA release. However, the present study showed that there was no difference in basal NA release between vehicle- and 5,7-DHT-treated rats. This may be explained by 5,7-DHT-induced supersensitivity of 5-HT₂ receptors as previously reported (Butler *et al.*, 1990; Heal *et al.*, 1990), since 5-HT₂ receptors can exert an inhibitory influence on NA release (Done & Sharp, 1992).

Feuerstein & Hertting (1986) showed that the 5-HT re-

ceptor agonists, 5-HT, 2-methyl-5-HT and 5-carboxamido-tryptamine, increased [³H]-NA release in hippocampal slices. Furthermore, Blandina *et al.* (1991) reported that 5-HT₃ receptors mediated an inhibition of NA release from hypothalamic slices in the presence of ritanserin, a 5-HT₂/5-HT_{1C} receptor antagonist. However the present *in vivo* study showed that local administration of 8-OH-DPAT, citalopram and MDL72222 did not affect NA release in the hypothalamus. The lack of effect of 8-OH-DPAT suggests that the 5-HT_{1A} receptors controlling NA release are not present in noradrenergic nerve terminals in the hypothalamus. Earlier studies (Renaud *et al.*, 1975; Crespi *et al.*, 1980; Reader *et al.*, 1986) suggest that the LC which receives a rich 5-HT innervation (Pickel *et al.*, 1977; Steinbusch, 1981) may be a possible site for the action of the 5-HT_{1A} agonist. In this connection, Broderick & Piercey (1991) found that azapirones increased NA neuronal firing rate in the LC. In contrast, Gorea *et al.* (1991) reported that microiontophoretic application of 8-OH-DPAT did not modify the spontaneous firing rate of neurones in the LC. The present study showed that microinjection of 8-OH-DPAT into the LC did not increase NA release in the hypothalamus. It remains to be determined where the postsynaptic 5-HT_{1A} receptors controlling NA release are localized in the brain.

It is known that the effects of 5-HT_{1A} agonists on body temperature and corticosterone secretion are attenuated by repeated treatment with the agonists (Nash *et al.*, 1989; Larson *et al.*, 1990; Nakano *et al.*, 1992). In contrast to these postsynaptic 5-HT_{1A} receptor-mediated responses, the desensitization of 5-HT_{1A} autoreceptors is not confirmed. Electrophysiological studies (Blair & Montigny, 1987; Godbout *et al.*, 1991) suggest that 5-HT_{1A} autoreceptors are desensitized by chronic 5-HT_{1A} receptor agonists, while neurochemical observations using *in vivo* microdialysis (Beer *et al.*, 1990; Kreiss & Lucki, 1992; Sharp *et al.*, 1993; Soderpalm *et al.*, 1993; Hjorth & Auerback, 1994) are controversial. The present study suggests that the postsynaptic 5-HT_{1A} receptors controlling NA release are not desensitized following the repeated treatment. As regards the desensitization of 5-HT_{1A} autoreceptors, we could not reach any conclusion: the significant effect of MKC-242 in decreasing 5-HT release was not observed in rats chronically treated with the drug, while there was no difference in the effect of MKC-242 on 5-HT release between the acute and chronic treatment.

In conclusion, the present study indicates that activation of postsynaptic 5-HT_{1A} receptors causes NA release. The 5-HT_{1A} receptors controlling NA release are possibly localized on the NA cell body rather than the nerve terminals, and the regulation may be involved in the pharmacology of 5-HT_{1A} receptor agonists.

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References

- BEER, M., KENNETT, G.A. & CURZON, G. (1990). A single dose of 8-OH-DPAT reduces raphe binding of [³H]8-OH-DPAT and increases the effect of raphe stimulation of 5-HT metabolism. *Eur. J. Pharmacol.*, **178**, 179–187.
- BIANCHI, G., CACCIA, S., DELLA VEDOVA, F. & GARATTINI, S. (1988). The α_2 -adrenoceptor antagonist activity of ipsapirone and gepirone is mediated by their common metabolite 1-(2-pyrimidinyl)-piperazine (PmP). *Eur. J. Pharmacol.*, **151**, 365–371.
- BLANDINA, P., GOLDFARB, J., WALCOTT, J. & GREEN, J.P. (1991). Serotonergic modulation of the release of endogenous norepinephrine from rat hypothalamic slices. *J. Pharmacol. Exp. Ther.*, **256**, 341–347.
- BLIER, P. & DE MONTIGNY, C. (1987). Modification of 5-HT neuron properties by sustained administration of the 5-HT_{1A} agonist gepirone: Electrophysiological studies in the rat brain. *Synapse*, **1**, 470–480.
- BRODERICK, P.A. & PIERCEY, M.F. (1991). 5-HT_{1A} agonists uncouple noradrenergic somatodendritic impulse flow and terminal release. *Brain Res. Bull.*, **27**, 693–696.
- BUTLER, P.D., PRANZATELLI, M.R. & BARKAI, A.I. (1990). Regional central serotonin-2 receptor binding and phosphoinositide turnover in rats with 5,7-dihydroxytryptamine lesions. *Brain Res. Bull.*, **24**, 125–129.
- CLEMENT, H.W., GEMSA, D. & WESEMANN, W. (1992). Serotonin-norepinephrine interactions: a voltammetric study on the effect of serotonin receptor stimulation followed in the N. raphe dorsalis and the Locus coeruleus of the rat. *J. Neural Transm.*, **88**, 11–23.
- CRESPI, F., BUDA, M., MCRAE-DEGUEURCE, A. & PUJOL, J.F. (1980). Alteration in tyrosine hydroxylase activity in the LC after administration of p-chlorophenylalanine. *Brain Res.*, **19**, 501–509.

- CRIST, J. & SUPRENANT, A. (1987). Evidence that 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) is a selective α_2 -adrenoceptor antagonist on guinea-pig submucous neurones. *Br. J. Pharmacol.*, **92**, 341–347.
- DONE, C.J.G. & SHARP, T. (1992). Evidence that 5-HT₂ receptor activation decreases noradrenergic release in rat hippocampus *in vivo*. *Br. J. Pharmacol.*, **107**, 240–245.
- DONE, C.J.G. & SHARP, T. (1994). Biochemical evidence for the regulation of central noradrenergic activity by 5-HT_{1A} and 5-HT₂ receptors: microdialysis studies in the awake and anaesthetized rat. *Neuropharmacology*, **33**, 411–421.
- EGAWA, M., ABE, M., TABATA, R., SAITO, K.-I., TOBE, A., MATSUDA, T. & BABA, A. (1993). Pharmacological effects of MKC-242: a novel potential anxiolytic and antidepressant compound with selective 5-HT_{1A} agonistic profile. *Soc. Neurosci. Abstr.*, **19**, 1243.
- FEUERSTEIN, T.J. & HERTTING, G. (1986). Serotonin (5-HT) enhances hippocampal noradrenaline (NA) release: Evidence for facilitatory 5-HT receptors within the CNS. *Naunyn-Schmied Arch Pharmacol.*, **333**, 191–197.
- FLETCHER, A., CLIFFE, I.A. & DOURISH, C.T. (1993). Silent 5-HT_{1A} receptor antagonists: utility as research tools and therapeutic agents. *Trends Pharmacol. Sci.*, **14**, 441–448.
- FULLER, R. & PERRY, K.W. (1989). Effects of buspirone and its metabolite, 1-(2-pyrimidinyl)piperazine, on brain monoamines and their metabolites in rats. *J. Pharmacol. Exp. Ther.*, **248**, 50–56.
- GARTSIDE, S.E., COWEN, P.J. & HJORTH, S. (1990). Effects of MDL 73005EF on central pre- and postsynaptic 5-HT_{1A} receptor function in the rat *in vivo*. *Eur. J. Pharmacol.*, **191**, 291–400.
- GOBBI, M., FRITTOLI, E. & MENNINI, T. (1990). Antagonist properties of 1-(2-pyrimidinyl)-piperazine at presynaptic α_2 -adrenoceptors in the rat brain. *Eur. J. Pharmacol.*, **180**, 183–186.
- GODBOUT, R., CHAPUT, Y., BLIER, P. & DE MONTIGNY, C. (1991). Tandospirone and its metabolite, 1-(2-pyrimidinyl)-piperazine. I. Effects of acute and long-term administration of tandospirone on serotonin transmission. *Neuropharmacology*, **30**, 679–690.
- GOREA, E. & ARDRIEN, J. (1988). Serotonergic regulation of noradrenergic coerulean neurons: electrophysiological evidence for the involvement of 5-HT₂ receptors. *Eur. J. Pharmacol.*, **154**, 285–291.
- GOREA, E., DAVENNE, D., LANFUMEY, L., CHASTANET, M. & ADREIN, J. (1991). Regulation of noradrenergic coerulean neuronal firing mediated by 5-HT₂ receptors: involvement of the prepositus hypoglossal nucleus. *Neuropharmacology*, **30**, 1309–1318.
- GREUEL, J.M. & GLASER, T. (1992). The putative 5-HT_{1A} receptor antagonists NAN-190 and BMY 7378 are partial agonists in the rat dorsal raphe nucleus *in vitro*. *Eur. J. Pharmacol.*, **211**, 211–219.
- HEAL, D.J., HURST, E.M., PROW, M.R. & BUCKETT, W.R. (1990). An investigation of the role of 5-hydroxytryptamine in the attenuation of presynaptic α_2 -adrenoceptor-mediated responses by antidepressant treatments. *Psychopharmacology*, **101**, 100–106.
- HJORTH, S. & AUERBACH, S.B. (1994). Lack of 5-HT_{1A} autoreceptor desensitization following chronic citalopram treatment, as determined by *in vivo* microdialysis. *Neuropharmacology*, **33**, 331–334.
- HJORTH, S. & SHARP, T. (1990). Mixed agonist/antagonist properties of NAN-190 at 5-HT_{1A} receptors: behavioural and *in vivo* brain microdialysis studies. *Life Sci.*, **46**, 955–963.
- KREISS, D.S. & LUCKI, I. (1992). Desensitization of 5-HT_{1A} auto receptors by chronic administration of 8-OH-DPAT. *Neuropharmacology*, **31**, 1073–1076.
- LARSSON, L.G., RENYI, L., ROSS, S.B., SVENSSON, B. & ANGEBY-MOLLER, K. (1990). Different effects on the responses of functional pre- and postsynaptic 5-HT_{1A} receptors by repeated treatment of rats with the 5-HT_{1A} receptor agonist 8-OH-DPAT. *Neuropharmacology*, **29**, 86–91.
- MATSUDA, T., SEONG, Y.H., AONO, H., KANDA, T., BABA, A., SAITO, K., TOBE, A. & IWATA, H. (1989). Agonist activity of a novel compound, 1-[3[(3,4-methylenedioxypheyl)propyl]-4-phenyl piperazine (BP-554), at central 5-HT_{1A} receptors. *Eur. J. Pharmacol.*, **170**, 75–82.
- MCRAE-DEGUEURCE, A., DENNIS, T., LEGER, L. & SCATTON, B. (1985). Regulation of noradrenergic neuronal activity in the locus coeruleus by serotonergic afferents. *Physiol. Psychol.*, **13**, 188–196.
- NAKANO, Y., MATSUDA, T., TAKUMA, K., YOSHIKAWA, T. & BABA, A. (1992). Sex difference for tolerance of 5-HT_{1A} receptor-mediated temperature and corticosterone responses in mice. *Eur. J. Pharmacol.*, **219**, 339–341.
- NASH, J.F. JR., MELTZER, H.Y. & GUDELSKY, G.A. (1989). Selective cross-tolerance to 5-HT_{1A} and 5-HT₂ receptor-mediated temperature and corticosterone responses. *Pharmacol. Biochem. Behav.*, **33**, 781–785.
- PAXINOS, G. & WATSON, C. (1986). *The Rat Brain in Stereotaxic Coordinates*. Second edition. San Diego: Academic Press.
- PICKEL, V.M., JOH, T.H. & REIS, D. (1977). A serotonergic innervation of noradrenergic neurons in locus coeruleus. Demonstrations by immunocytochemical localization of the transmitter specific enzymes tyrosine and tryptophan hydroxylase. *Brain Res.*, **131**, 197–214.
- QADRI, F., BADPER, E., STADLER, T. & UNGER, T. (1991). Angiotensin II-induced noradrenaline release from anterior hypothalamus in conscious rats: a brain microdialysis study. *Brain Res.*, **563**, 137–141.
- RASMUSSEN, K. & AGHAJANIAN, G.K. (1986). Effect of hallucinogens on spontaneous and sensory-evoked locus coeruleus unit activity in the rat: reversal by selective 5-HT₂ antagonists. *Brain Res.*, **385**, 395–400.
- READER, T.A., BRIERE, R., GRONDIN, L. & FERRON, A. (1986). Effect of p-chlorophenylalanine on cortical monoamines and on electrical activity of noradrenergic neurons. *Neurochem. Res.*, **11**, 1025–1035.
- RENAUD, B., BUDA, M., LEWIS, D. & PUJOL, J.F. (1975). Effect of 5,6-dihydroxytryptamine on tyrosine hydroxylase activity in central catecholamine neurons of the rat. *Biochem. Pharmacol.*, **24**, 1739–1742.
- ROUTLEDGE, C., GURLING, J., WRIGHT, I.K. & DOURISH, C.T. (1993). Neurochemical profile of the selective and silent 5-HT_{1A} receptor antagonist WAY100135: an *in vivo* microdialysis study. *Eur. J. Pharmacol.*, **239**, 195–202.
- SHARP, T., MCQUADE, R., BRAMWELL, S. & HJORTH, S. (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**, 339–346.
- SODERPALM, B., LUNDIN, B. & HJORTH, S. (1993). Sustained 5-hydroxytryptamine release-inhibitory and anxiolytic-like action of the partial 5-HT_{1A} receptor agonist, buspirone, after prolonged chronic administration. *Eur. J. Pharmacol.*, **239**, 69–73.
- SPROUSE, J.S. (1991). Inhibition of dorsal raphe firing by MDL 73005EF, a novel 5-HT_{1A} receptor ligand. *Eur. J. Pharmacol.*, **201**, 163–169.
- STEINBUSCH, H.W.M. (1981). Distribution of serotonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals. *Neuroscience*, **6**, 557–618.
- SUZUKI, M., MATSUDA, T., YOSHIKAWA, T., SOMBOONTHUM, P. & BABA, A. (1994). Increase in norepinephrine release by serotonin_{1A} agonists in hypothalamus of freely moving rats. *Jpn. J. Pharmacol.*, **64** (Suppl. I), 156P.
- TIAN, Y., EATON, M.J., GOUDREAU, J.L., LOOKINGLAND, K.J. & MOORE, K.E. (1993). Neurochemical evidence that 5-hydroxytryptaminergic neurons tonically inhibit noradrenergic terminating in the hypothalamus. *Brain Res.*, **607**, 215–221.
- YOSHIKAWA, T., MATSUDA, T., TAKUMA, K., SUZUKI, M., EGAWA, M., TOBE, A. & BABA, A. (1994). Neurochemical profile of a novel serotonin_{1A} receptor agonist, MKC-242. (Abstr.) *Neurochem. Res.*, **19**, 904.

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