Br. J. Pharmacol. (1992), **106**, 139–142

Opposing roles for 5-HT_{1B} and 5-HT_3 receptors in the control of 5-HT release in rat hippocampus *in vivo*

[1]Keith F. Martin, Serina Hannon, Ian Phillips & David J. Heal

Boots Pharmaceuticals Research Department, Nottingham NG2 3AA

1 Intracerebral microdialysis was used to determine whether 5-hydroxytryptamine (5-HT) release in the ventral hippocampus of rats anaesthetized with chloral hydrate was modulated by 5-HT_3 receptors.

2 It was confirmed that 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole (RU 24969), a selective 5-HT_{1B} receptor agonist, decreased 5-HT release in a dose- and concentration-related manner when administered i.p. (1 and 5 mg kg^{-1}) or via the dialysis probe (0.1 and 1 μM) respectively. The effect of RU 24969 infusion (1 μM) was attenuated by concurrent infusion of metitepine (10 μM) into the hippocampus.

3 When infused into the hippocampus for 15 min, the selective 5-HT_3 receptor agonist, 2-methyl-5-hydroxytryptamine (2-methyl-5-HT; 0.1– 10 μM) increased dialysate 5-HT levels in a concentration-related manner; an effect which was abolished by concurrent infusion of 3-tropanyl-3,5-dichlorobenzoate (1 μM, MDL 72222), a selective 5-HT_3 antagonist.

4 MDL 72222 had no effects on hippocampal 5-HT release when administered via the dialysis probe (1 or 10 μM).

5 The data show that 5-HT_3 and 5-HT_{1B} receptors have opposing roles in the control of 5-HT release in the hippocampus, with 5-HT_3 receptors facilitating and 5-HT_{1B} receptors inhibiting 5-HT efflux, respectively. They also indicate that the facilitatory 5-HT_3 receptors are not tonically activated.

Keywords: 5-HT release; 5-HT_3 receptors; hippocampus; intracerebral microdialysis

Introduction

Radioligand binding and pharmacological studies have revealed several subtypes of 5-hydroxytryptamine (5-HT) receptor in the mammalian CNS. These receptors have been classified as 5-HT_1-like, 5-HT_2 and 5-HT_3 (Bradley *et al.*, 1986). Recently, 5-HT_4 receptors have also been identified (Bockaert *et al.*, 1990). The 5-HT_1-like receptor has been further subdivided into at least four subtypes: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} (Pazos *et al.*, 1985; Pazos & Palacios, 1985). It is now widely accepted that in the rat, 5-HT_{1B} receptors subserve the role of nerve terminal autoreceptors (Middlemiss, 1984; Engel *et al.*, 1986; Martin & Marsden, 1988). Furthermore, the 5-HT_{1A} receptor has also been shown to be located on 5-HT neurone cell bodies where it exerts an inhibitory action on 5-HT neurone firing (De Montigny *et al.*, 1984; Verge *et al.*, 1985; Sprouse & Aghajanian, 1987).

Although 5-HT_3 receptors are present in the CNS in fairly low concentrations, their relative distribution is high in the frontal cortex and the hippocampus (Kilpatrick *et al.*, 1987). Furthermore, the physiological role of 5-HT_3 receptors in the CNS is not clearly defined. It is known, however, that these receptors are linked to a monovalent cation channel (Derkach *et al.*, 1989) and that stimulation leads to depolarization of the membrane. It has also been suggested that 5-HT_3 receptors are involved in the control of cortical acetylcholine release (Barnes *et al.*, 1989) and, more recently, Galzin *et al.* (1990) have shown that the release of [^{3}H]-5-HT from guinea-pig frontal cortex slices is enhanced by 5-HT_3 receptor agonists. These findings suggest that 5-HT_3 receptors may play an important role in controlling the release of neurotransmitters, and in particular, may act as facilitatory autoreceptors in the 5-hydroxytryptaminergic system.

The purpose of the experiments described here, therefore, was to determine whether 5-HT_3 receptors are involved in the control of 5-HT release in rat hippocampus *in vivo*.

[1]Author for correspondence.

Methods

Animals

Male Wistar rats (Charles River) weighing 280–300 g were used. They were housed in pairs on a 12:12 h light:dark cycle (lights on: 07 h 00 min) in an ambient temperature of 21°C and had free access to food and water.

Implantation of dialysis probes

Rats were anaesthetized with chloral hydrate (600 mg kg^{-1}, i.p.) and then held in a stereotaxic frame (David Kopf). A concentric microdialysis probe (500 μm o.d., CMA/Microdialysis) with a membrane 3 mm long was then implanted into the ventral hippocampus (co-ordinates for probe tip: anterior-posterior (AP) −4.4 mm, medio-lateral (ML) −4.2 mm, from bregma and dorso-ventral (DV) −8.6 mm from skull surface according to Paxinos & Watson, 1982). The probe was continuously perfused at a flow rate of 1 μl min^{-1} with an artificial CSF solution containing NaCl 147 mM, KCl 4 mM, $CaCl_2$ 4 mM and the selective 5-HT reuptake inhibitor, citalopram 1 μM. Anaesthesia was maintained throughout the experiment with supplementary doses of chloral hydrate (30 mg i.p.) when required. At the end of each experiment, the brain was removed and dialysis probe placement visually confirmed.

Perfusate analysis

Perfusates were collected every 30 min for the first 2 h after probe implantation and then every 15 min throughout the experiment. They were analysed for their 5-HT content by high performance liquid chromatography (h.p.l.c.) with electrochemical detection. Separation was achieved by reversed-phase liquid chromatography using a 10 cm × 2 mm column with Hypersil ODS2 3 μm packing (Technicol) and a mobile phase of 0.1 M phosphate, 0.93 M sodium octane sulphonic acid, 0.07% v/v dibutylamine and 12% v/v methanol

(pH 3.0). A Coulochem electrochemical detector with a Coulochem 5011 dual electrode analytical cell (ESA) was used. Electrode 1 was set at +0.1 V and electrode 2 at +0.28 V. The signal from electrode 2 was used to quantify the amount of 5-HT present. The detection limit for 5-HT was approximately 2 fmol on column.

Experimental protocol

Following a 2 h stabilization period, two 15 min baseline samples were obtained before any intervention. Drugs were then administered peripherally by the i.p. route. Alternatively, they were infused directly into the ventral hippocampus via the dialysis probe for one 15 min collection period.

Statistical analysis

Data were analysed by Dunnett's test or Student's *t* test. Where more than one experimental group was compared with a single control group the null hypothesis was rejected where $P<0.01$.

Drugs

All chemicals used were analytical or h.p.l.c. grade. The following drugs were used: 2-methyl-5-hydroxytryptamine (2-methyl-5-HT), 3-tropanyl-3,5-dichlorobenzoate (MDL 72222) (Research Biochemicals Inc.), 5-methoxy-3(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole (RU 24969) (Roussel Uclaf), citalopram (Lundbeck), metitepine and chloral hydrate (Sigma).

Results

Basal levels of 5-HT in the dialysate before any intervention were 21.94 ± 0.72 fmol per 15 min sample ($n = 58$).

Effects of RU 24969 on dialysate 5-hydroxytryptamine

Peripheral administration of the 5-HT_1-like receptor agonist, RU 24969, decreased the concentration of 5-HT in hippocampal dialysates in a dose-related manner (Figure 1a). The decrease was maximal 75 min after administration.

When RU 24969 was infused directly into the hippocampus via the dialysis probe for 15 min, there was a rapid, concentration-related decrease in 5-HT output (Figure 1b). For example, following infusion of RU 24969 at a concentration of 1 μM, 5-HT concentrations decreased by 47% in the 15 min period following the infusion. Extracellular 5-HT returned to basal values 60 min after cessation of the infusion. Concurrent infusion of the 5-HT receptor antagonist, metetipine (10 μM), with RU 24969 (1 μM) abolished the decrease in 5-HT release induced by RU 24969 alone (Figure 1c).

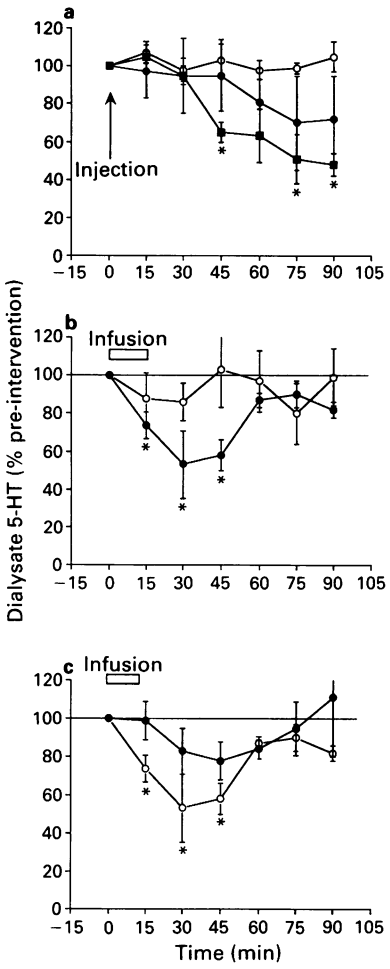

Figure 1 The effects of RU 24969 on rat hippocampal dialysate 5-hydroxytryptamine (5-HT) content. Concentric microdialysis probes were perfused with a solution containing NaCl 147 mM, KCl 4 mM, $CaCl_2$ 4 mM and citalopram 1 μM. Data are expressed as a percentage of the amount of 5-HT in the dialysate collected in the 15 min period immediately before the intervention. Each point represents the mean value with s.e. mean shown by vertical bars. *$P<0.01$ compared to control (Student's *t* test). (a) Saline (○, $n = 6$) or RU 24969 at dose 1(●, $n = 5$) and 5 mg kg^{-1} (■, $n = 5$) were injected i.p. at time zero. (b) RU 24969 at concentrations of 0.1 μM (○, $n = 5$) or 1 μM (●, $n = 5$) was infused into the hippocampus via the dialysis probe for the 15 min period indicated by the horizontal bar. (c) RU 24969 1 μM (○, $n = 5$) or metitepine, 10 μM, plus, RU 24969, 1 μM (●, $n = 4$) was infused directly into the hippocampus via the dialysis probe for the period indicated by the horizontal bar.

Effect of 2-methyl-5-hydroxytryptamine and MDL 72222

The dialysate level of 5-HT was significantly increased, in a concentration-related manner, by local infusion of the 5-HT_3 receptor agonist, 2-methyl-5-HT (Richardson *et al.*, 1985; Figure 2a). Levels of 5-HT were increased 40% and 50% during the infusion of 1 μM and 10 μM, respectively (Figure 2a). Following the infusion of the higher concentration, the levels of 5-HT continued to rise to a maximum increase of 100% in the next 15 min period. Following infusion of 2-methyl-5-HT at 1 μM, levels remained elevated for the 45 min post-infusion. By contrast, the levels of 5-HT rapidly returned to baseline following infusion of 2-methyl-5-HT at 10 μM. Infusion of 2-methyl-5-HT at a concentration of 0.1 μM was without effect (Figure 2a).

Local infusion of the 5-HT_3 receptor antagonist, MDL 72222 (0.1 and 1 μM), via the dialysis probe was without effect on the level of 5-HT measured in the dialysate (Figure 2b).

Infusion of MDL 72222 (1 μM) with 2-methyl-5-HT (1 μM) into the hippocampus via the probe completely prevented the increase in dialysate 5-HT concentrations observed following an infusion of 2-methyl-5-HT alone (Figure 2c).

Discussion

This study has used the technique of intracerebral microdialysis to determine whether 5-HT_3 receptors modulate the release of 5-HT in the hippocampus. The major finding of the experiments is that 5-HT_3 receptors facilitate 5-HT release in the ventral hippocampus of the anaesthetized rat.

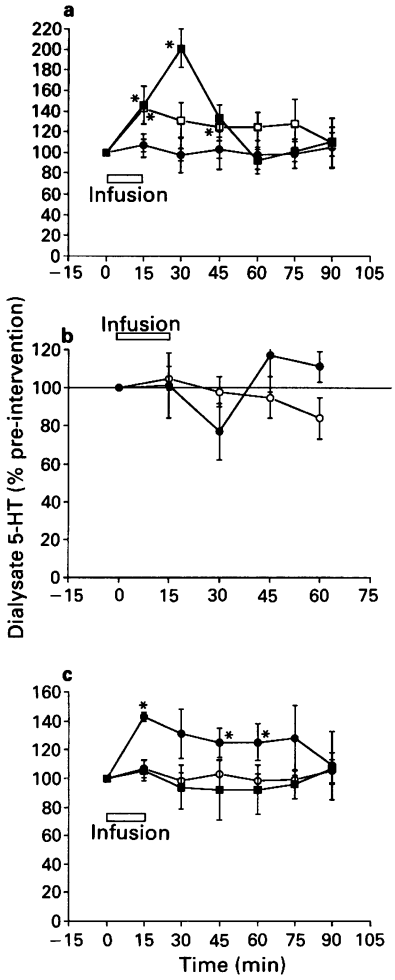

Figure 2 Effects of the 5-HT_3 receptor agonist, 2-methyl-5-HT, and antagonist, MDL 72222, on rat hippocampal dialysate 5-hydroxytryptamine (5-HT) content. (a) 2-Methyl-5-HT, 0.1 μM (●, $n = 5$), 1 μM (□, $n = 4$) or 10 μM (■, $n = 4$) was infused directly into the hippocampus as described in (b); control values (○, $n = 5$). *$P<0.01$ compared to control (Student's t test). (b) MDL 72222, 1 μM (○, $n = 4$) or 10 μM (●, $n = 4$), was infused directly into the hippocampus via the dialysis probe for the 15 min period indicated by the horizontal bar. (c) Either 2-methyl-5-HT, 1 μM (●, $n = 4$) or MDL 72222, 1 μM, plus 2-methyl-5-HT, 1 μM (■, $n = 4$) was infused into the hippocampus via the dialysis probe for 15 min as indicated by the horizontal bar; control values (○, $n = 6$). *$P<0.05$ compared to control (Dunnett's test). Each point represents the mean with s.e. mean indicated by vertical bars. See legend to Figure 1 for further details.

Furthermore, we have shown that these receptors do not appear to be tonically activated.

In the experiments described here, we have used rats anaesthetized with chloral hydrate and have included the specific 5-HT re-uptake inhibitor, citalopram, in the dialysate. Previous studies have clearly shown that under these experimental conditions, a significant proportion of the 5-HT measured results from depolarization-evoked, calcium-dependent release from 5-HT-containing neurones (Sharp *et al.*, 1991).

The observations that peripheral and local administration of RU 24969 inhibited 5-HT release are in close agreement with previous findings (Sharp *et al.*, 1989a; Auerbach *et al.*, 1991). We have also shown that the effects of locally applied RU 24969 were attenuated by metitepine, a 5-HT receptor antagonist which has some selectivity for 5-HT_1-like sites (Hibert & Middlemiss, 1986). Similar findings have also been reported using *in vivo* voltammetry; Martin & Marsden (1986) found that the effects of RU 24969 on 5-HT release and metabolism in the rat suprachiasmatic nucleus were specifically mediated by 5-HT_1-like receptors. These authors also showed that this effect involved 5-HT_{1B}, but not 5-HT_{1A}, receptors located in the nerve terminal region (Martin & Marsden, 1988). Furthermore, using *in vitro* techniques, Middlemiss (1984) has demonstrated that the nerve terminal autoreceptor in the rat is of the 5-HT_{1B} subtype. In view of the above, and because RU 24969 has marginally more affinity for 5-HT_{1B} receptors than 5-HT_{1A} receptors (Hoyer *et al.*, 1985), it is probable that the inhibition described here, is also due to its agonist effect at terminal 5-HT_{1B} receptors.

The physiological role of 5-HT_3 receptors is still not fully understood. However, it has been claimed that these sites are involved in the control of cortical acetylcholine release (Barnes *et al.*, 1989; Bianchi *et al.*, 1990), where they exert an inhibitory effect. In addition, it has also been reported that the *in vitro* release of cortical [^{3}H]-5-HT is increased by 5-HT_3 receptor activation (Galzin *et al.*, 1990). The present data are in agreement with these latter findings. The 5-HT_3 receptor agonist, 2-methyl-5-HT (Richardson *et al.*, 1985), concentration-dependently increased dialysate 5-HT levels. This effect was abolished by an equimolar concentration of MDL 72222, a highly selective 5-HT_3 receptor antagonist (Fozard, 1984). Although these data clearly indicate that 5-HT_3 receptors located in a 5-HT neurone terminal field facilitate 5-HT release, they do not allow firm conclusions to be drawn concerning the anatomical location of these sites. However, we postulate that they may be located on 5-HT nerve terminals, but further experimentation is required to confirm this hypothesis. The 5-HT_3 receptor antagonist, MDL 72222, failed to alter 5-HT release when given directly into the hippocampus. This finding indicates that the 5-HT_3 receptors responsible for mediating the effects of 2-methyl-5-HT are not tonically activated by endogenous 5-HT, under which circumstance, MDL 72222 would have been predicted to decrease 5-HT release.

The facilitation of 5-HT release by 2-methyl-5-HT is not unexpected because several authors have previously shown that 5-HT_3 receptors have stimulatory effects on target neurones where they facilitate the release of neurotransmitters (Gaddum & Piccarelli, 1957; Fozard & Mobarak Ali, 1978; Hagen *et al.*, 1987; Carboni *et al.*, 1989). However, this is the first demonstration that endogenous 5-HT release is facilitated by 5-HT_3 receptor stimulation. In conclusion, the data presented in this paper, demonstrate that *in vivo*, 5-HT_{1B} and 5-HT_3 receptors have opposing roles in the control of rat hippocampal 5-HT release.

References

AUERBACH, S.B., RUTTER, J.J. & JULIANO, P.J. (1991). Substituted piperazine and indole compounds increase extracellular serotonin in rat diencephalon as determined by in vivo microdialysis. *Neuropharmacol.*, **30**, 307–311.

BARNES, J.M., BARNES, N.M., COSTALL, B., NAYLOR, R.J. & TYERS, M.B. (1989). 5-HT_3 receptors mediate inhibition of acetylcholine release in cortical tissue. *Nature*, **338**, 762–763.

BIANCHI, C., SINISCALCHI, A. & BEANI, L. (1990). 5-HT_{1A} agonists increase and 5-HT_3 agonists decrease acetylcholine efflux from the cerebral cortex of freely moving guinea-pigs. *Br. J. Pharmacol.*, **101**, 448–452.

BOCKAERT, J., SEBBEN, M. & DUMUIS, A. (1990). Pharmacological characterization of 5-hydroxytryptamine$_4$ (5-HT_4) receptors positively coupled to adenylate cyclase in adult guinea pig hippocampal membranes: effects of substituted benzamide derivatives. *Molec. Pharmacol.*, **37**, 408–411.

BRADLEY, P.B., ENGEL, G., FENIUK, W., FOZARD, J.R., HUMPHREY, P.P.A., MIDDLEMISS, D.N., MYLECHARANE, E.J., RICHARDSON, B.P. & SAXENA, P.R. (1986). Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. *Neuropharmacol.*, **25**, 563–576.

CARBONI, E., ACQUAS, E., FRAU, R. & DI CHIARA, G. (1989). Differential inhibitory effects of a 5-HT_3 antagonist on drug-induced stimulation of dopamine release. *Eur. J. Pharmacol.*, **164,** 515–519.

DE MONTIGNY, C., BLIER, P. & CHAPUT, Y. (1984). Electrophysiologically-identified serotonin receptors in rat CNS: effect of antidepressant treatment. *Neuropharmacol.*, **23,** 1511–1520.

DERKACH, V., SURPRENANT, A. & NORTH, R.A. (1989). 5-HT_3 receptors are membrane ion channels. *Nature*, **339,** 706–709.

ENGEL, G., GÖTHERT, M., HOYER, D., SCHLICKER, E. & HILLENBRAND, K. (1986). Identity of inhibitory presynaptic 5-hydroxytryptamine (5-HT) autoreceptors in the rat brain cortex with 5-HT_{1B} binding sites. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **332,** 1–7.

FOZARD, J.R. (1984). MDL 72222: a potent and highly selective antagonist at neuronal 5-hydroxytryptamine receptors. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **326,** 36–44.

FOZARD, J.R. & MOBARAK ALI, A.T.M. (1978). Receptors for 5-hydroxytryptamine on the sympathetic nerves of rabbit heart. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **301,** 223–235.

GADDUM, J.H. & PICARELLI, Z.P. (1957). Two kinds of tryptamine receptor. *Br. J. Pharmacol. Chemother.*, **12,** 323–328.

GALZIN, A.M., PONCET, V. & LANGER, S.Z. (1990). 5-HT_3 receptor agonists enhance the electrically-evoked release of [^{3}H]5-HT in guinea pig frontal cortex slices. *Br. J. Pharmacol.*, **100,** 307P.

HAGEN, R.M., BUTLER, A., HILL, J.M., JORDAN, C.C., IRELAND, S.J. & TYERS, M.B. (1987). Effect of the 5-HT_3 receptor antagonist, GR 38032F, on the response to injection of a neurokinin agonist into the ventral tegmental area of the rat brain. *Eur. J. Pharmacol.*, **138,** 303–305.

HIBERT, M. & MIDDLEMISS, D.N. (1986). Stereoselective blockade at the 5-HT autoreceptor and inhibition of radioligand binding to central 5-HT recognition sites by the optical isomers of methiothepin. *Neuropharmacol.*, **25,** 1–4.

HOYER, D., ENGEL, G. & KALKMAN, H.O. (1985). Molecular pharmacology of 5-HT_1 and 5-HT_2 recognition sites in rat and pig brain membranes: radioligand binding studies with [^{3}H]5-HT, [^{3}H]8-OH-DPAT, (−)[^{125}I]cyanopindolol, [^{3}H]mesulergine and [^{3}H]ketanserin. *Eur. J. Pharmacol.*, **118,** 13–23.

KILPATRICK, G.J., JONES, B.J. & TYERS, M.B. (1987). Identification and distribution of 5-HT_3 receptors in rat brain using radioligand binding. *Nature*, **330,** 746–748.

MARTIN, K.F. & MARSDEN, C.A. (1986). *In vivo* voltammetry in the suprachiasmatic nucleus of the rat: effects of RU 24969, methiothepin and ketanserin. *Eur. J. Pharmacol.*, **121,** 135–140.

MARTIN, K.F. & MARSDEN, C.A. (1988). *In vivo* identification of the serotonin (5-HT) autoreceptor in the rat suprachiasmatic nucleus (SCN). In *New Concepts in Depression* ed. Briley, M. & Fillion, G. pp. 66–68. Basingstoke: Macmillan Press.

MIDDLEMISS, D.N. (1984). The putative 5-HT_1 receptor agonist, RU 24969, inhibits the efflux of 5-hydroxytryptamine from rat frontal cortex slices by stimulation of the 5-HT autoreceptor. *J. Pharm. Pharmacol.*, **37,** 434–437.

PAXINOS, S. & WATSON, C. (1982). *The Rat Brain in Stereotaxic Co-ordinates.* Sydney: Academic Press.

PAZOS, A. & PALACIOS, J.M. (1985). Quantitative autoradiographic mapping of serotonin receptors in the rat brain. 1. Serotonin receptors. *Brain Res.*, **346,** 205–230.

PAZOS, A., HOYER, D. & PALACIOS, J.M. (1985). The binding of serotonergic ligands to the choroid plexus: characterization of a new type of serotonin recognition site. *Eur. J. Pharmacol.*, **106,** 539–546.

RICHARDSON, B.P., ENGEL, G., DONATSCH, P. & STADLER, P.A. (1985). Identification of serotonin M-receptor subtypes and their specific blockade by a new class of drugs. *Nature*, **316,** 126–131.

SHARP, T., BRAMWELL, S.R. & GRAHAME-SMITH, D.G. (1989a). 5-HT_1 agonists reduce 5-hydroxytryptamine release in rat hippocampus *in vivo* as determined by brain microdialysis. *Br. J. Pharmacol.*, **96,** 283–290.

SHARP, T., BRAMWELL, S.R., HJORTH, S. & GRAHAME-SMITH, D.G. (1989b). Pharmacological characterization of 8-OH-DPAT-induced inhibition of rat hippocampal 5-HT release *in vivo* as measured by microdialysis. *Br. J. Pharmacol.*, **98,** 989–997.

SHARP, T., BRAMWELL, S.R. & GRAHAME-SMITH, D.G. (1991). Release of endogenous 5-hydroxytryptamine in rat ventral hippocampus evoked by electrical stimulation of the dorsal raphe nucleus as detected by microdialysis: sensitivity to tetrodotoxin, calcium and calcium antagonists. *Neuroscience*, **39,** 629–637.

SPROUSE, J.S. & AGHAJANIAN, G.K. (1987). Electrophysiological responses of serotonergic dorsal raphe neurons to the 5-HT_{1A} and 5-HT_{1B} agonists. *Synapse*, **1,** 3–9.

VERGE, D., DAVAL, G., PATEY, A., GOZLAN, H., EL MESTIKAWY, S. & HAMON, M. (1985). Presynaptic 5-HT autoreceptors on serotonergic cell bodies and/or dendrites, but not terminals, are of the 5-HT_{1A} subtype. *Eur. J. Pharmacol.*, **113,** 463–464.

(Received January 15, 1992
Revised February 3, 1992
Accepted February 5, 1992)