Role of 5-HT₃ receptors in basal and K⁺-evoked dopamine release from rat olfactory tubercle and striatal slices

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1 The present study was aimed at examining the role of 5-HT₃ receptors in basal and depolarization-evoked dopamine release from rat olfactory tubercle and striatal slices. [³H]-dopamine ([³H]-DA) release was measured in both brain regions and endogenous dopamine release from striatal slices was also studied.

2 The selective 5-HT₃ receptor agonist 2-methyl-5-HT (0.5–10 µM) produced a concentration-dependent increase in [³H]-DA efflux evoked by K⁺ (20 mM) from slices of rat olfactory tubercle. 1-Phenylbiguanide (PBG) and 5-HT also increased K⁺-evoked [³H]-DA efflux.

3 5-HT (1–100 µM) increased in a concentration-dependent manner basal [³H]-DA release from olfactory tubercle and striatal slices as well as endogenous DA release from striatal slices. The selective 5-HT₃ receptor agonists 2-methyl-5-HT and 1-phenylbiguanide were weaker releasing agents. In all cases, the release was Ca²⁺ independent and tetrodotoxin insensitive.

4 5-HT₃ receptor antagonists such as ondansetron, granisetron and tropisetron (0.2 µM) significantly blocked the enhanced K⁺-evoked [³H]-DA efflux from rat olfactory tubercle slices induced by 2-methyl-5-HT. A ten fold higher concentration of the 5-HT₂ receptor antagonist ketanserin was ineffective.

5 Much higher concentrations, up to 50 µM, of the same 5-HT₃ receptor antagonists did not block the increase in basal [³H]-DA release from striatal or olfactory tubercle slices induced by 5-HT or the release of endogenous DA induced by 5-HT from striatal slices.

6 The DA uptake blocker nomifensine (10 µM) did not modify the enhancement in K⁺-evoked [³H]-DA efflux from rat olfactory tubercle slices induced by 2-methyl-5-HT. However, both nomifensine and cocaine significantly blocked the increase in basal dopamine release, either tritiated or endogenous, induced by 5-HT.

7 These results indicate that 5-HT₃ receptors only modulate DA release when the terminal fields of dopaminergic systems have been activated. In basal conditions, 5-HT₃ receptor agonists enhance DA release through a carrier-mediated mechanism. This may be of therapeutic relevance for the possible use of 5-HT₃ receptor antagonists under conditions of abnormal activation of the dopaminergic system.

Keywords: Dopamine release; 5-hydroxytryptamine; 5-HT₃ receptors; olfactory tubercle; striatum; 2-methyl-5-hydroxytryptamine; 1-phenylbiguanide; ondansetron; granisetron; tropisetron

Introduction

It has been suggested that antagonists at 5-hydroxytryptamine₃ (5-HT₃) receptors may be of value in the treatment of withdrawal reactions to drugs of abuse such as cocaine, morphine or ethanol (see reviews in Palfreyman *et al.*, 1993; Hagan *et al.*, 1993) and also in the treatment of schizophrenia (Hagan *et al.*, 1993). One of the mechanisms involved in these hypothetical therapeutic effects would be a blockade of the mesolimbic dopamine system activation induced by 5-HT₃ receptor stimulation (Costall *et al.*, 1987; Hagan *et al.*, 1987). It is known that agonists at this 5-HT receptor subtype are able to release dopamine from striatal and limbic regions both *in vitro* (Blandina *et al.*, 1989; Schmidt & Black, 1989; Yi *et al.*, 1991; Benuck & Reith, 1992; Jacocks & Cox, 1992) and *in vivo* (Jiang *et al.*, 1990; Chen *et al.*, 1991). Some *in vitro* experiments suggest that dopamine release by 5-HT₃ agonists is a receptor-mediated effect (Blandina *et al.*, 1989), while many other studies indicate that the release is carrier mediated (Schmidt & Black, 1989; Yi *et al.*, 1991; Benuck & Reith, 1992; Jacocks & Cox, 1992).

There is much behavioural, electrophysiological and neurochemical evidence suggesting that the 5-HT system may modulate the dopaminergic system, especially when the latter has been activated. In this regard, particular attention has been paid to 5-HT₃ receptors found in moderately high levels in terminal regions of the mesolimbic dopaminergic system,

such as the nucleus accumbens, olfactory tubercle and amygdala (Kilpatrick *et al.*, 1988; Waeber *et al.*, 1989; Barnes *et al.*, 1990). Ondansetron, a typical 5-HT₃ receptor antagonist, is able to block the behavioural effects of amphetamine, a dopamine-releasing drug, when amphetamine is injected into the nucleus accumbens but not when given systemically (Costall *et al.*, 1987). Ondansetron also attenuates the increase in dopamine turnover induced by injection of the neurokinin agonist DiMe-C7 into the ventral tegmental area (Hagan *et al.*, 1987). Chronic administration of another 5-HT₃ antagonist, MDL-73147EF, decreases the number of spontaneously active dopaminergic neurones in the ventral tegmental area (A10) (Sorensen *et al.*, 1989), which have been associated with the rewarding effect of drugs of abuse (Koob & Bloom, 1988). Tropisetron reverses the increase in dopamine release resulting from stress (Imperato *et al.*, 1990) or from the administration of morphine, nicotine and ethanol (Carboni *et al.*, 1989).

The present study was aimed at examining the role of 5-HT₃ receptors in basal and depolarization-evoked dopamine release from slices of rat olfactory tubercle, a terminal field of the mesolimbic dopaminergic system. Tritium efflux was measured after loading the slices with [³H]-dopamine both under basal conditions and after stimulation with KCl. Slices from rat striatum were comparatively studied. In this region, endogenous dopamine release was also studied. A preliminary report of this study has been presented elsewhere (Del Río & Zazpe, 1993).

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Methods

Preparation of brain slices

Olfactory tubercles or corpora striata from male Wistar rats (200–250 g) were dissected and cut sagittally into 350 μ m sections with a McIlwain tissue slicer. Each superfusion chamber contained tissue from two rats. The cut tissue was washed three times in gassed (95% O₂/5% CO₂) Krebs solution (NaCl 113; NaHCO₃ 25; KCl 4.75; NaH₂PO₄ 1.18; CaCl₂ 2.52; MgSO₄ 1.19; glucose 10 and pargyline 0.01 mM).

Release of endogenous dopamine

The slices were placed in 1 ml chambers and superfused in an overflow manner at a rate of 0.5 ml min⁻¹ with gassed Krebs solution at 37°C. After a 75 min equilibration period, 1.5 ml samples were collected into tubes containing 166 μ l of a solution of 1 N HClO₄, 0.1% ascorbic acid and 20.8 ng of dihydroxybenzylamine as an internal standard. At selected times, slices were exposed to the 5-HT agonist for 12 min by transferring the chamber inflow tubing to reservoirs containing medium of appropriate composition. When the interaction of a given compound with the 5-HT agonist was studied, slices were perfused with the compound 30 min before adding the 5-HT agonist. Dopamine (DA) release was calculated as percentage increase over basal release. The concentration of DA was determined by high-pressure liquid chromatography with electrochemical detection as previously described (Pérez-Otaño *et al.*, 1991).

Release of [³H]-dopamine

Slices were incubated with agitation in 5 ml of gassed Krebs solution with 0.1 μ M [³H]-dopamine (35 Ci mmol⁻¹, Amersham). After this time the tissue was washed three times with normal Krebs and the superfusion procedure was initiated as above. The equilibration period was 90 min. Samples were collected and 80 μ l aliquots were added to 4 ml of scintillation fluid (Biogreen no. 3) for radioactivity counting. Under the present experimental conditions, it is generally accepted that the radioactivity released from slices is predominantly [³H]-DA (e.g. Werling *et al.*, 1988).

K⁺-evoked release of [³H]-dopamine

Slices were placed in a Krebs solution containing 39.75 mM KCl (tonicity maintained by reducing the NaCl concentration) and the flasks were shaken at 37°C for 20 min. After being washed the slices were incubated at 37°C for 30 min in normal Krebs buffer containing 0.1 μ M [³H]-dopamine

(35 Ci mmol⁻¹). Washed slices were placed into 1 ml superfusion chambers and superfused with Krebs solution (0.5 ml min⁻¹) at 37°C. After a 35 min washout period, 3 min samples of perfusate were collected. At 12 min (S₁) and 42 min (S₂) the slices were depolarized by changing the superfusion fluid for 6 min to a Krebs solution containing 20 mM KCl. All test drugs were added 15 min before S₂. The superfusate samples were collected over a 60 min period. S₂ and S₁ were calculated as K⁺-stimulated tritium increase on basal efflux.

Drugs and chemicals

The following drugs were used: [³H]-dopamine (Amersham), 5-hydroxytryptamine, creatinine sulphate, cocaine and pargyline (Sigma), 2-methyl-5-HT, 1-phenylbiguanide and nomifensine (RBI), tropisetron (Sandoz), granisetron (SmithKline Beecham) and ondansetron (Glaxo). Inorganic salts and all other reagents were from Merck.

Results

Effect of 5-HT agonists on K⁺-evoked [³H]-DA release from olfactory tubercle slices

Tritium efflux was practically the same after the two K⁺ pulses (S₂/S₁ ratio = 1.07 $\pm$ 0.07; *n* = 7). The selective 5-HT₃ receptor agonist 2-methyl-5-HT (2-Me-5-HT) produced a concentration-dependent increase in K⁺-evoked [³H]-DA efflux (S₂/S₁ ratio) from slices of rat olfactory tubercle preloaded with [³H]-DA (Figure 1). Phenylbiguanide (PBG) produced substantially the same effect and 5-HT was not significantly more potent than the selective 5-HT₃ agonists (Figure 1). The K⁺-evoked [³H]-DA efflux was virtually absent in the absence of Ca²⁺ in the superfusion medium (not shown). Tetrodotoxin (TTX, 1 μ M) partially blocked the K⁺-evoked tritium overflow. S₂/S₁ for control and TTX was 1.07 $\pm$ 0.07 and 0.83 $\pm$ 0.07 respectively (*n* = 5; *P* < 0.05). A similar percentage blockade was observed in slices stimulated with K⁺ and 2-Me-5-HT (2-Me-5-HT, 1.69 $\pm$ 0.07; TTX + 2-Me-5-HT, 1.35 $\pm$ 0.09).

Effect of 5-HT agonists on basal DA release from olfactory tubercle or striatal slices

The percentage release from striatal slices produced by 5-HT on either tritiated or endogenous DA was practically identical. The percentage release of [³H]-DA obtained from olfactory tubercle slices was slightly higher (Figure 2). Selective 5-HT₃ receptor agonists produced a weaker effect on DA

Figure 1 Concentration-dependent increase induced by 2-methyl-5-HT (●), 1-phenylbiguanide (Δ) and 5-HT (□) in K⁺-evoked [³H]-dopamine efflux from rat olfactory tubercle slices preloaded with [³H]-dopamine. Data are means ($\pm$ s.e.mean) of 4–10 experiments.

Figure 2 Effect of 5-HT on dopamine release from rat brain slices (per cent increase over basal values). Data are means ($\pm$ s.e.mean) of at least five separate experiments (Δ---Δ) [³H]-dopamine, olfactory tubercle; (●---●) [³H]-dopamine, striatum (□---□) endogenous dopamine, striatum.

Table 1 Effect of 5-HT and 5-HT₃ receptor agonists on release of tritiated or endogenous dopamine from rat striatal (str.) and olfactory tubercle (o.t.) slices

5-HT agonist (μM)	[³ H]-DA (o.t.)	[³ H]-DA (str.)	DA (str.)
5-HT (10)	405 $\pm$ 28	242 $\pm$ 12	188 $\pm$ 10
PBG (10)	241 $\pm$ 23	166 $\pm$ 14	118 $\pm$ 18
PBG (30)	438 $\pm$ 32	256 $\pm$ 20	238 $\pm$ 52
2-Me-5-HT (100)	122 $\pm$ 18	53 $\pm$ 7	n.d.

Data (means $\pm$ s.e.; $n = 4-6$) are expressed as per cent increase over basal release. PBG, 1 phenylbiguanide; n.d., not determined.

release (Table 1). The releasing effect of 2-Me-5-HT on endogenous dopamine was not determined because of interference of the chromatographic peak of this drug with dopamine. In all cases, the release was not modified by omission of Ca^{2+} in the superfusion medium and was tetrodotoxin insensitive (not shown).

Basal [³H]-DA release was also studied in olfactory tubercle slices pre-exposed for 20 min to a high- K^+ solution, as in the above experiments on K^+ -evoked [³H]-DA efflux, and no difference in percentage release was apparent after this previous exposure. Using these experimental conditions, the percentage increase in basal [³H]-DA release induced by 5 μM 5-HT was 188.7 ± 19.3 (mean $\pm$ s.e.mean of five experiments), and 2-Me-5-HT (10–50 μM) did not show any significant effect on basal [³H]-DA release. Endogenous DA release was not studied in striatal slices pre-exposed to a high level of K^+ since the rise in DA induced by 5-HT in the subsequent experiments was not high enough for accurate chromatographic quantification.

Effect of 5-HT₃ receptor antagonists on K^+ -evoked [³H]-DA release from olfactory tubercle slices

All of the studied 5-HT₃ receptor antagonists lacked any intrinsic effect on K^+ -stimulated release. For example, S_2/S_1 ratio after 2 μM ondansetron was 1.12 ± 0.10 ($n = 4$). Ondansetron (0.05–2 μM) produced a concentration-dependent blockade of the increased K^+ -evoked [³H]-DA efflux induced by 2-Me-5-HT in rat olfactory tubercle slices (Figure 3). Other 5-HT₃ receptor antagonists such as granisetron and tropisetron (0.2 μM) also blocked the effect of 2-Me-5-HT (Figure 3). A ten fold higher concentration (2 μM) of ketanserin, a 5-HT₂ receptor antagonist, did not modify the effect of 2-Me-5-HT (Figure 4). The increased K^+ -evoked [³H]-DA efflux induced by 5-HT was again blocked by ondansetron and not by ketanserin, 2 μM each. S_2/S_1 ratio was 1.96 ± 0.15 , 1.31 ± 0.14 and 1.72 ± 0.24 for 5-HT, 5-HT + ondansetron and 5-HT + ketanserin respectively ($n = 4-6$).

Effect of 5-HT₃ receptor antagonists on basal DA release

At variance with the above results, much higher concentrations (10 or 50 μM) of the 5-HT₃ receptor antagonists ondansetron, granisetron or tropisetron did not block the increase in basal [³H]-DA release from olfactory tubercle or striatal slices induced by 5–10 μM 5-HT or the release of endogenous DA induced by 10 μM 5-HT in striatal slices. Control release (per cent increase over basal) was essentially the same in all cases: [³H]-DA (olfactory tubercle), 213 ± 21 ; endogenous DA (striatum), 182 ± 23 ; [³H]-DA (striatum), 229 ± 25 . The results obtained in the presence of 5-HT₃ receptor antagonists were practically identical (not shown). The increase in [³H]-DA induced by the 5-HT₃ receptor agonist PBG was not blocked by either ondansetron or tropisetron, 50 μM each (not shown).

In olfactory tubercle slices pre-exposed to a high level of K^+ , 5-HT₃ receptor antagonists did not block the increase in basal [³H]-DA release induced by 5-HT. For example, 2 μM

Figure 3 Effect of 5-HT₃ and 5-HT₂ receptor antagonists on the enhanced K^+ -evoked [³H]-dopamine efflux from rat olfactory tubercle slices induced by 2-methyl-5-HT. Data are means ($\pm$ s.e.mean) of at least five separate experiments. * $P < 0.01$ vs 2-Me-5-HT (Student's t test).

Figure 4 Lack of effect of nomifensine (nomif.) on the enhanced K^+ -evoked [³H]-dopamine efflux from rat olfactory tubercle slices induced by 2-methyl-5-HT. Data are means ($\pm$ s.e.mean) of five separate experiments. * $P < 0.01$ vs control (Student's t test).

5-HT produced a percentage increase over basal values of 77.3 ± 11.7 and 70.3 ± 9.4 ($n = 5$) when tested alone and in the presence of 2 μM ondansetron respectively. A higher ondansetron concentration (10 μM) was also unable to block the enhanced release after 10 μM 5-HT; percentage increases were 378 ± 29 and 358 ± 22 for 5-HT and 5-HT + ondansetron respectively.

Effect of DA uptake blockers on K^+ -evoked and basal DA release from olfactory tubercle and striatal slices

Nomifensine (10 μM) did not show an intrinsic effect on K^+ -evoked [³H]-DA efflux from rat olfactory tubercle slices. The same concentration of nomifensine did not modify the enhancing effect of 2-Me-5-HT on K^+ -evoked tritium efflux (Figure 4).

Figure 5 Blockade by nomifensine (nomif.) and cocaine of dopamine release from rat brain slices (per cent increase over basal values) induced by 5-HT or 1-phenylbiguanide (PBG). Data are means ($\pm$ s.e.mean); $n = 4-7$. (a) [3 H]-dopamine, striatum; (b) [3 H]-dopamine, olfactory tubercle; (c) endogenous dopamine, striatum. * $P < 0.01$ vs 5-HT (Student's t test).

Basal [3 H]-DA release was not modified by 2 μ M nomifensine or 10 μ M cocaine and was enhanced by five fold higher concentrations of the DA uptake blockers (Figure 5a and b). Both nomifensine (10 μ M) and cocaine (50 μ M) were able to significantly block the much larger increase in basal [3 H]-DA release from striatal or olfactory tubercle slices induced by 5-HT (Figure 5a and b). The lower concentrations of nomifensine and cocaine, 2 and 10 μ M respectively, also produced a small but significant decrease in 5-HT-induced [3 H]-DA release (not shown). Nomifensine also blocked the increase in endogenous DA release from striatal slices produced by 5-HT or PBG (Figure 5c).

Discussion

The present results provide evidence for the modulation by 5-HT₃ receptors of K⁺-evoked dopamine release from slices of rat olfactory tubercle. Conversely, when terminal fields of the mesolimbic or nigrostriatal dopaminergic system are not activated with K⁺, 5-HT and 5-HT₃ receptor stimulants enhance dopamine release through a carrier-mediated mechanism.

Previous studies have addressed the role of 5-HT₃ receptors in dopamine release by using either slices (Blandina *et al.*, 1989; Benuck & Reith, 1992; Jacocks & Cox, 1992) or synaptosome preparations (Yi *et al.*, 1991) from rat brain regions and measuring either endogenous dopamine release or tritium efflux after preloading the preparation with [3 H]-DA. In the present study, all experiments were carried out in brain slices, which allow the detection of effects mediated by receptors located not only on nerve terminals but also on interneurons. As to the interest in measuring tritium efflux or endogenous dopamine release, previous studies indicate that tritium efflux closely mimics endogenous dopamine release (Herdon *et al.*, 1985; Werling *et al.*, 1988). The almost identical results obtained with striatal slices in the present study (Figure 3) corroborate this assumption.

In keeping with several previous studies (Nurse *et al.*, 1988; Schmidt & Black, 1989; Yi *et al.*, 1991; Benuck & Reith, 1992; Jacocks & Cox, 1992), 5-HT-enhanced [3 H]-DA release was prevented by DA uptake blockers and not by 5-HT₃ receptor antagonists. Partial depletion of endogenous DA stores by pre-exposure of the slices to a high level of K⁺ did not modify the effect of 5-HT or 2-Me-5-HT on basal [3 H]-DA release and, likewise, 5-HT₃ receptor antagonists were unable to block the enhancement in basal [3 H]-DA efflux induced by 5-HT. This is consonant with the ability of PBG to promote [3 H]-DA efflux in striatal slices from rats pretreated with reserpine (Benuck & Reith, 1992): not only are endogenous DA stores markedly reduced but DA storage mechanisms are also affected. Release of either endogenous or tritiated dopamine by 5-HT or 5-HT₃ receptor agonists was not dependent on Ca²⁺ and was also tetrodotoxin insensitive, thus supporting the notion of a carrier-mediated release of the neurotransmitter (see Levi & Raiteri, 1993). However, our results are not in accordance with those reported by Blandina *et al.* (1989), who found that 5-HT₃ receptor antagonists are able to prevent endogenous DA release from striatal slices. Since in their study all experiments were carried out in the presence of nomifensine, it is possible that other mechanisms may still be operative in such an experimental condition. The comparatively low density of 5-HT₃ receptors in the striatum compared with limbic and cortical areas (Kilpatrick *et al.*, 1988) raises some doubt about their physiological significance in this brain region.

K⁺-stimulated dopamine release from the olfactory tubercle showed a strong Ca²⁺ dependence, consistent with an exocytotic mechanism. At a 20 mM K⁺ concentration, the neuronal firing would be activated and this would activate in turn Ca²⁺ influx (Illes, 1986). However, K⁺-evoked dopamine release was only partially tetrodotoxin sensitive, indicating that only a part of the K⁺-stimulated release is dependent on excitatory input from sites distal to the terminal (Jacocks & Cox, 1992). Tetrodotoxin blocked to approximately the same extent the enhancement by 2-Me-5-HT of the K⁺-stimulated release, suggesting that 5-HT₃ receptor activation potentiates the K⁺ effect, probably by contributing to a greater ionic influx through opening of the associated 5-HT-gated channel (Derkach *et al.*, 1989).

Forebrain 5-HT₃ receptors are concentrated in terminal fields of the mesolimbic dopaminergic system, such as the olfactory tubercle (Kilpatrick *et al.*, 1988) so their role at this level should be more relevant than at the striatum. Interestingly, it seems that these receptors are only operative in the control of dopamine release when dopaminergic terminals have been depolarized by a high potassium concentration. Under these experimental conditions, there is not a marked difference in potency between the specific 5-HT₃ receptor agonists 2-Me-5-HT or PBG and 5-HT, as is the case when their effects on basal dopamine release are compared. It is possible that under conditions of K⁺-stimulated exocytotic release 5-HT may act also on presynaptic receptors inhibiting dopamine release (see Levi & Raiteri, 1993). The enhancement of K⁺-evoked dopamine release by 5-HT seems to be dependent on 5-HT₃ receptor stimulation and not on activa-

tion of any other 5-HT receptor subtype. The lack of blockade by ketanserin of the 5-HT effect argues against the involvement of 5-HT₂ receptors, which rather appear to control carrier-mediated release of dopamine (Nash, 1990; Huang & Nichols, 1993).

The results of the present study showing that 5-HT₃ receptor antagonists are only able to prevent the effect of serotonergic stimulation on terminal fields of the mesolimbic dopaminergic system when this system has been previously

activated by K⁺ offer parallelism with many *in vivo* studies and stress the potential psychiatric interest of a class of drugs that would only be affecting the dopaminergic system under conditions of abnormal activation.

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References

- BARNES, J.M., BARNES, N.M., CHAMPANERIA, S., COSTALL, B. & NAYLOR, R.J. (1990). Characterization and autoradiographic localization of 5-HT₃ receptor recognition sites identified with [³H]-(S)-zacopride. *Neuropharmacology*, **29**, 1037–1045.
- BENUCK, M. & REITH, M.E.A. (1992). Dopamine releasing effect of phenylbiguanide in rat striatal slices. *Naunyn-Schmied. Arch. Pharmacol.*, **345**, 666–672.
- BLANDINA, P., GOLDFARB, J., CRADDOCK-ROYAL, B. & GREEN, J.P. (1989). Release of endogenous dopamine by stimulation of 5-hydroxytryptamine₃ receptors in rat striatum. *J. Pharmacol. Exp. Ther.*, **251**, 803–809.
- CARBONI, E., ACQUAS, E., FRAU, R. & DI CHIARA, G. (1989). Differential inhibitory effects of a 5-HT₃ antagonist on drug-induced stimulation of dopamine release. *Eur. J. Pharmacol.*, **164**, 515–519.
- COSTALL, B., DOMENEY, A.M., NAYLOR, R.J. & TYERS, M.B. (1987). Effects of the 5-HT₃ receptor antagonist, GR 38032F, on raised dopaminergic activity in the mesolimbic system of rat and marmoset brain. *Br. J. Pharmacol.*, **92**, 881–894.
- CHEN, J., VAN PRAAG, H.M. & GARDNER, E.L. (1991). Activation of 5-HT₃ receptor by 1-phenylbiguanide increases dopamine release in the rat nucleus accumbens. *Brain Res.*, **543**, 354–357.
- DEL RIO, J. & ZAZPE, A. (1993). Release of dopamine from rat olfactory tubercle is mediated by activation of 5-HT₃ receptors. *Soc. Neurosci. Abstr.*, **23**, 1380.
- DERKACH, V., SUPRENT, A. & NORTH, R.A. (1989). 5-HT₃ receptors are membrane ion-channels. *Nature*, **339**, 706–709.
- HAGAN, R.M., BUTLER, A., HILL, J.M., JORDAN, C.C., IRELAND, S.J. & TYERS, M.B. (1987). Effect of the 5-HT₃ receptor antagonist, GR 38032F, on responses to injection of a neurokinin agonist into the ventral tegmental area of the brain. *Eur. J. Pharmacol.*, **138**, 303–305.
- HAGAN, R.M., KILPATRICK, G.J. & TYERS, M.B. (1993). Interactions between 5-HT₃ receptors and cerebral dopamine function: implications for the treatment of schizophrenia and psychoactive substance abuse. *Psychopharmacology*, **112**, S68–S75.
- HERDON, H., STRUPISH, J. & NAHORSKI, S.R. (1985). Differences between the release of radiolabelled and endogenous dopamine from superfused rat brain slices: effect of depolarizing stimuli, amphetamine and synthesis inhibition. *Brain Res.*, **348**, 309–320.
- HUANG, X. & NICHOLS, D.E. (1993). 5-HT₂ receptor-mediated potentiation of dopamine synthesis and central serotonergic deficits. *Eur. J. Pharmacol.*, **238**, 291–296.
- ILLES, P. (1986). Mechanisms of receptor-mediated modulation of transmitter release in noradrenergic, cholinergic and sensory neurons. *Neuroscience*, **17**, 909–928.
- IMPERATO, A., PUGLISI-ALLERGRA, S., ZOCCHI, A., SCROCCO, M.G., CASOLINI, P. & ANGELUCCI, L. (1990). Stress activation of limbic and cortical dopamine release is prevented by ICS205-930 but not diazepam. *Eur. J. Pharmacol.*, **175**, 211–214.
- JACOBS, H.M. & COX, B.M. (1992). Serotonin-stimulated release of [³H]dopamine via reversal of the dopamine transporter in rat striatum and nucleus accumbens: a comparison with release elicited by potassium, N-methyl-D-aspartic acid, glutamic acid and D-amphetamine. *J. Pharmacol. Exp. Ther.*, **262**, 356–364.
- JIANG, L.H., ASHBY, Jr. C.R., KASSER, R.J. & WANG, R.Y. (1990). The effect of intraventricular administration of the 5-HT₃ receptor agonist 2-methyl-serotonin on the release of dopamine in the nucleus accumbens: an *in vivo* chronocoulometric study. *Brain Res.*, **513**, 156–160.
- KILPATRICK, G.J., JONES, B.J. & TYERS, M.B. (1988). The distribution of specific binding of the 5-HT₃ receptor ligand [³H] GR 65630 in rat brain using quantitative autoradiography. *Neurosci. Lett.*, **94**, 156–160.
- KOOB, G.F. & BLOOM, F.E. (1988). Cellular and molecular mechanisms of drug dependence. *Science*, **242**, 715–722.
- LEVI, G. & RAITERI, M. (1993). Carrier-mediated release of neurotransmitters. *Trends Neurosci.*, **16**, 415–419.
- NASH, J.F. (1990). Ketanserin pretreatment attenuates MDMA-induced dopamine release in the striatum as measured by *in vivo* microdialysis. *Life Sci.*, **47**, 2401–2408.
- NURSE, B., RUSSELL, V.A. & TALJAARD, J.J.F. (1988). Characterization of the effects of serotonin on the release of [³H]dopamine from rat nucleus accumbens and striatal slices. *Neurochem. Res.*, **13**, 403–407.
- PALFREYMAN, M.G., SCHMIDT, C.J., SORENSEN, S.M., DUDLEY, M.W., KEHNE, J.H., MOSER, P., GITTO, M.W. & CARR, A.A. (1993). Electrophysiological, biochemical and behavioural evidence for 5-HT₂ and 5-HT₃ mediated control of dopaminergic function. *Psychopharmacology*, **112**, S60–S67.
- PEREZ-OTAÑO, I., HERRERO, M.T., OSET, C., DE CEBALLOS, M.L., LUQUIN, M.R., OBESO, J.A. & DEL RIO, J. (1991). Extensive loss of brain dopamine and serotonin induced by chronic administration of MPTP in the marmoset. *Brain Res.*, **567**, 127–132.
- SCHMIDT, C.J. & BLACK, C.K. (1989). The putative 5-HT₃ agonist phenylbiguanide induces carrier-mediated release of [³H]dopamine. *Eur. J. Pharmacol.*, **167**, 309–310.
- SOERSEN, S., HUMPHREYS, T.M. & PALFREYMAN, M.G. (1989). Effect of acute and chronic MDL 73,147EF, a 5-HT₃ receptor antagonist, on A9 and A10 dopamine neurones. *Eur. J. Pharmacol.*, **163**, 115–118.
- WAEBER, C., HOYER, D. & PALACIOS, J.M. (1989). 5-Hydroxytryptamine receptors in the human brain, autoradiographic visualization using [³H]ICS 205-930. *Neuroscience*, **31**, 393–400.
- WERLING, L.L., FRATTALI, P., PORTOGHESE, P.S., TAKEMORI, A.E. & COX, B.M. (1988). Kappa receptor regulation of dopamine release from striatum and cortex of rats and guinea-pigs. *J. Pharmacol. Exp. Ther.*, **246**, 282–286.
- YI, S.-J., GIFFORD, A.N. & JOHNSON, K.M. (1991). Effect of cocaine and 5-HT₃ receptor antagonists on 5-HT-induced [³H]dopamine release from rat striatal synaptosomes. *Eur. J. Pharmacol.*, **199**, 185–189.

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