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The profiles of interaction of yohimbine with anxiolytic and putative anxiolytic agents to modify 5-HT release in the frontal cortex of freely-moving rats

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1 The interaction of yohimbine with anxiolytic and putative anxiolytic agents to modify 5-hydroxytryptamine (5-HT) release in the frontal cortex of the freely-moving rat was assessed using the microdialysis technique.

2 The α_2-adrenoceptor antagonist, yohimbine (5.0 mg kg^{-1}, i.p.) increased maximally the extracellular levels of 5-HT in the rat frontal cortex by approximately 230% of the basal levels.

3 The α_2-adrenoceptor agonist, clonidine (30–100 μg kg^{-1}, i.p.) decreased dose-dependently the extracellular levels of 5-HT in the rat frontal cortex by approximately 0–60% of the basal levels. A 5 min pretreatment with clonidine (50 μg kg^{-1}, i.p.) prevented the yohimbine-induced increase in the extracellular 5-HT levels.

4 The benzodiazepine receptor agonist, diazepam (2.5 mg kg^{-1}, i.p.) and the 5-HT$_3$ receptor antagonist, ondansetron (100 μg kg^{-1}, i.p.) (5 min pretreatment) completely prevented the yohimbine (5.0 mg kg^{-1}, i.p.)-induced increases in the extracellular levels of 5-HT. The 5-HT$_{1A}$ receptor agonist, 8-OH-DPAT (0.32 mg kg^{-1}, s.c.) partially antagonized the yohimbine response.

5 A 5 min pretreatment with the 5-HT$_3$/5-HT$_4$ receptor ligand **R**(+)-zacopride (10 μg kg^{-1}, i.p.) reversed the yohimbine (5.0 mg kg^{-1}, i.p.)-induced increase in the extracellular levels of 5-HT to approximately 30% below the basal levels. A 5 min pretreatment with **S**(−)-zacopride (100 μg kg^{-1}, i.p.) failed to modify the response to yohimbine.

6 The present study provides evidence of the ability of the anxiogenic agent, yohimbine, to increase the activity of the central 5-hydroxytryptaminergic system and the ability of clonidine and various anxiolytic and putative anxiolytic agents to prevent the yohimbine response.

Keywords: 5-Hydroxytryptamine; *in vivo* microdialysis; α_2-adrenoceptor agonist and antagonist; diazepam; 5-HT$_{1A}$ receptor agonist; 5-HT$_3$ receptor antagonists; rat frontal cortex

Introduction

It is generally accepted that a reduction in the neuronal function of 5-hydroxytryptamine (5-HT) leads to an anxiolytic effect and that an increase in 5-HT function results in an anxiogenic profile (for reviews see Iversen, 1984; Chopin & Briley, 1987; Stein *et al.*, 1975; Thiebot, 1986). Thus, *p*-chlorophenylalanine (*p*-CPA), which reduces 5-HT neurotransmission by inhibition of tryptophan hydroxylase, showed anxiolytic-like effects in the rat social interaction test (File & Hyde, 1977; Barnes *et al.*, 1992b) and conflict procedures (Engel, 1986). These effects are reversed by the administration of 5-hydroxytryptophan (5-HTP) (Rochichaud & Sledge, 1969; Geller & Blum, 1970). Lesions produced by intraventricular or local stereotaxic administration of the 5-hydroxytryptaminergic neurotoxins, 5,6-dihydroxytryptamine (5,6-DHT) or 5,7-dihydroxytryptamine (5,7-DHT) result in anxiolytic-like effects in conflict tests (Stein *et al.*, 1975; Tye *et al.*, 1977) and in the rat social interaction tests (File *et al.*, 1979).

Anxiolytic benzodiazepine receptor agonists such as diazepam and chlordiazepoxide have also been demonstrated to inhibit 5-HT synthesis and metabolism in the rat or cat brain using *ex vivo* or *in vivo* measurements (Saner & Pletscher, 1979; Balfour, 1980; Collinge & Pycock, 1982). More recently, using the *in vivo* microdialysis technique, it has been reported that both the systemic and local administration of diazepam and flurazepam decreased the extracellular levels of 5-HT from the ventral hippocampus (Pei *et al.*, 1989) and frontal cortex (Barnes *et al.*, 1992a). It has also been demonstrated that exposure of rats to the elevated plus maze results in an increase in the levels of extracellular levels of 5-HT in the hippocampus, this effect being antagonized by the administration of diazepam and the 5-HT$_{1A}$ receptor agonist, ipsapirone (Wright *et al.*, 1992).

Some compounds which interact with 5-HT receptors such as the 5-HT$_{1A}$ receptor agonists buspirone, gepirone and ipsapirone, the 5-HT$_2$ receptor antagonists, ritanserin and ketanserin and the 5-HT$_3$ receptor antagonists, ondansetron, granisetron, tropisetron, MDL 72222 and zacopride, have been shown to have anxiolytic profiles in animal models of anxiety or in man (Cuelemans *et al.*, 1985; Goa & Ward, 1986; Newton *et al.*, 1986; Taylor, 1988; Lecrubier *et al.*, 1990; Briley & Chopin, 1991; Lader, 1991).

Yohimbine is a potent α_2-adrenoceptor antagonist (Goldberg & Robertson, 1983) and preclinical studies have indicated that yohimbine possesses anxiogenic-like effects in animal models of anxiety (Pellow *et al.*, 1985; 1987). In the clinical studies, it has been demonstrated that administration of yohimbine produces significant increases in subjective anxiety in healthy subjects (Charney *et al.*, 1983; 1992) and in patients with panic disorders (Albus *et al.*, 1992). The anxiogenic effects were antagonized by pretreatment with diazepam and clonidine (Charney *et al.*, 1983). The possible mechanism of such an effect may be the interaction of yohimbine at α_2-adrenoceptors resulting in an overactivity of the brain noradrenergic system which has been related to the production of some forms of anxiety (Charney *et al.*, 1983). There is also some evidence to indicate an interaction between α_2-adrenoceptors and 5-HT systems in the central nervous system (Fuxe, 1986; Maura *et al.*, 1982; Raiteri *et al.*, 1990) and the question arises as to whether 5-HT systems may play some role in the yohimbine-induced effects.

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The major aim of the present study was to investigate, by us of the *in vivo* microdialysis technique, the effect of systemic administration of yohimbine on the extracellular levels of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in the frontal cortex of freely-moving rats. A further objective was to determine if the effect of pretreatment with anxiolytic and putative anxiolytic agents could influence the effect of yohimbine on the extracellular levels of 5-HT.

Methods

Animals

Male hooded-Lister rats (400–450 g) were housed in groups of 5 in a controlled environment (temperature 21 ± 1°C) under a 12 h light/dark cycle (lights on 07 h 00 min) and were given free access to food and water.

Stereotaxic implantation of chronic indwelling guide cannulae

Rats were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.) and chloral hydrate (150 mg kg^{-1}, s.c.) before the insertion of 5 mm chronically indwelling guide cannulae (19 gauge stainless steel tubing; Coopers Needle Work Ltd) which were positioned above the frontal cortex (final probe tip location; right hemisphere, A +0.7, V −7.0, L −1.5, relative to bregma at an angle of 45°) and secured to the skull with screws and dental cement. The guide cannulae were kept patent with stylets.

Microdialysis probe construction, implantation and collection of dialysates

Microdialysis probes were constructed 'in house'. Briefly, stainless steel 'bodies' (11 mm, 23 gauge, Coopers Needle Work Ltd) and 'collars' (5 mm, 26 gauge, Coopers Needle Work Ltd) were fixed into a perspex holder. Dialysis membrane (external/internal diameter 310/220 μm, molecular weight cut off 40,000; AN69, Hospal Medical) was glued to the tip of the stainless steel 'body' and the end sealed with epoxy resin (Araldite) leaving 4 mm in total length exposed to the brain area. A silica glass fibre (external/internal diameter 140/74 μm, Scientific Glass Engineering PTY LTd) guided artificial cerebrospinal fluid (aCSF; mM: NaCl 126, $NaHCO_3$ 27.4, KCl 2.4, KH_2PO_4 0.5, $CaCl_2$ 1.1, $MgCl_2$ 1.3, Na_2HPO_4 0.49 and glucose 7.0; pH 7.4) to the tip of the microdialysis probe, which subsequently eluted from the probe (via coiled polypropylene tubing) following passage between the outer surface of the silica glass fibre and the inner surface of the dialysis membrane.

At least 14 days after stereotaxic location of the guide cannulae, rats were immobilized by a soft-cloth wrapping technique and the microdialysis probe was gently implanted into the frontal cortex and secured with cyanoacrylate adhesive (Permabond C2). The rat was placed in a single animal cage (with free access to food and water) for 12 h before being transferred to the test cage (42 × 24 × 26 cm; length, width, height) where the animal also had free access to food and water. Following a 30 min period for the rat to habituate to the test box, the microdialysis probe was connected, via polypropylene tubing, to a microdialysis pump (CMA 100, Carnegie) and was perfused at a constant rate of 2.0 μl min^{-1} with aCSF. The dialysate collected over the first 60 min was discarded and subsequent samples were collected every 20 min for a period of up to 6 h. All samples were analysed immediately for levels of 5-HT and 5-HIAA by high performance liquid chromatography (h.p.l.c.) with electrochemical detection (e.c.d.).

H.p.l.c.-e.c.d. system for the quantification of 5-HT and 5-HIAA

For the simultaneous determination of 5-HT and 5-HIAA levels in dialysates, the h.p.l.c.-e.c.d. system comprised an isocratic pump (Waters 510 Solvent Delivery System) which was connected to an analytical column (Hypersil 5ODS; 150 × 4.6 mm; HPLC Technology) via an automatic injector (Waters Wisp). The analytical column was protected by a C_{18} guard column (Guard-Pak, Waters). The effluent from the

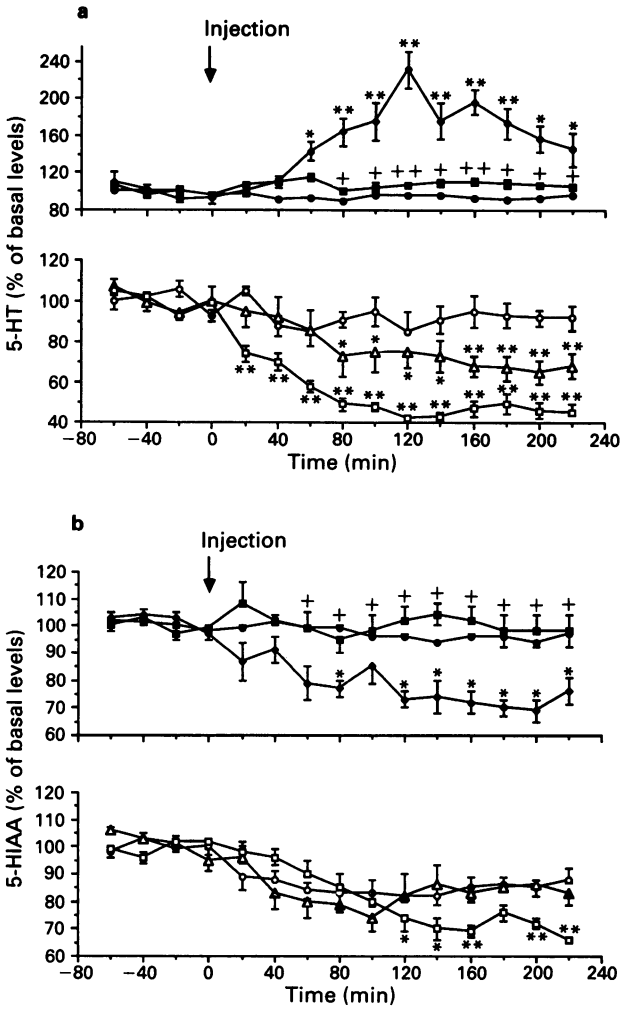

Figure 1 Effect of yohimbine (5.0 mg kg^{-1}, i.p.) (◆), clonidine (30 μg kg^{-1}, i.p. (○), 50 μg kg^{-1}, i.p. (△) and 100 μg kg^{-1}, i.p.) (□) and vehicle (saline, 1.0 ml kg^{-1}, i.p.) (●) and the ability of clonidine (50 μg kg^{-1}, i.p.) (■) to modify the yohimbine (5.0 mg kg^{-1}, i.p.)-induced increase in (a) 5-hydroxytryptamine (5-HT) or decrease in (b) 5-hydroxyindoleacetic acid (5-HIAA) in the rat frontal cortex dialysates with measurements commencing 12 h after the probe implantation. Clonidine was administered 5 min prior to the injection of yohimbine when co-administered. 5-HT and 5-HIAA levels are expressed as the percentage of the meaned absolute amount in the 4 collections preceding the drug treatment. Data represent the mean ± s.e.mean of four to eight determinations. Significant increases or decreases compared to the basal levels and differences between the yohimbine-treated and clonidine + yohimbine-treated groups are indicated as $^{*}P<0.05$, $^{**}P<0.01$ (single factor ANOVA followed by Dunnett's *t* test). $^{+}P<0.05$, $^{++}P<0.01$ (two factor ANOVA followed by *t* test) respectively. Basal levels of 5-HT and 5-HIAA were – yohimbine: 54 ± 3.30 fmol/40 μl, 2.66 ± 0.05 pmol/40 μl; clonidine 30 μg kg^{-1}: 67 ± 4.90 fmol/40 μl, 4.6 ± 0.28 pmol/40 μl; clonidine 50 μg kg^{-1}: 69 ± 3.7 fmol/40 μl, 5.54 ± 0.11 pmol/40 μl; clonidine 100 μg kg^{-1}: 110 ± 5.60 fmol/40 μl, 5.82 ± 0.07 pmol/40 μl; yohimbine + clonidine: 95 ± 2.38 fmol/40 μl, 5.30 ± 0.45 pmol/40 μl; vehicle: 54 ± 2.50 fmol/40 μl, 2.41 ± 0.05 pmol/40 μl respectively.

analytical column was passed into an electrochemical detector (ESA Coulochem with model 5011 analytical cell, working electrode potential + 0.45 V versus a solid state reference electrode incorporated within the analytical cell). The output from the electrode was monitored with a recording/plotting integrator (Hewlett-Packard 3392A). A guard cell (ESA model 5020) was placed between the pump and injector and was set at + 0.50 V (relative to a solid state reference electrode incorporated within the cell) to reduce the background current originating from the mobile phase. The h.p.l.c.-e.c.d. system, with the exception of the integrator, was maintained at a constant temperature of 4°C inside a glass-fronted cool cabinet. The optimized mobile phase (methanol 11% v/v, disodium hydrogen orthophosphate 106 mM, citric acid 36 mM, tetraethylammonium bromide 2 mM, pH 6.2–6.3; slight adjustments to the pH and/or methanol concentration were made to overcome column to column variation) was delivered to the analytical column at a rate of 1.4 ml min^{-1}. Injections of external standards were made in order to identify and calibrate the peaks resulting from the injection of the dialysates.

Drugs

Yohimbine hydrochloride (Sigma), 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino) tetralin hydrobromide, Research Biochemicals Inc.), ondansetron hydrochloride dihydrate (Glaxo), **R**(+)-zacopride and **S**(−)-zacopride hydrochloride ((±)4-amino-*N*-(1-azabicyclo[2,2,2]oct-3yl)-5-chloro-2-methoxy benzamide, A.H. Robins) and clonidine hydrochloride (Research Biochemicals Inc.) were dissolved in distilled water and diluted to volume in saline (0.9% w/v NaCl). Diazepam (Sigma) was prepared as a suspension in 10% polyethylene glycol 400 in saline. All drugs were used as received and were freshly prepared immediately before use.

Results

Effect of yohimbine and clonidine and their interaction to modify the extracellular levels of 5-HT and 5-HIAA

The administration of yohimbine (5.0 mg kg^{-1}, i.p.), caused marked increases in the extracellular levels of 5-HT in the rat frontal cortex (by approximately 230% of the basal levels), the maximal increases in the dialysate 5-HT levels were detected approximately 120 min following the injection, and then gradually decreased. At the end of the experiment (220 min), the levels of 5-HT were still significantly 40% higher than the basal levels (Figure 1). Yohimbine (5.0 mg kg^{-1}, i.p.) also caused a slight (25%) but significant decrease in the extracellular levels of 5-HIAA in the rat frontal cortex (Figure 1).

The administration of clonidine (30–100 μg kg^{-1}, i.p.) reduced in a dose-dependent manner the extracellular levels of 5-HT by some 0–60% of the basal levels in the rat frontal cortex. Such reductions began 20 min after the injection and reached a maximal effect in 120 min and remained at this level for the duration of the experiment (Figure 1). The high dose of clonidine (100 μg kg^{-1}, i.p.) (but not 30 and 50 μg kg^{-1}, i.p.) also slightly but significantly reduced the extracellular 5-HIAA levels from 98% ($t = 0$) to 70% (maximum reduction, Figure 1).

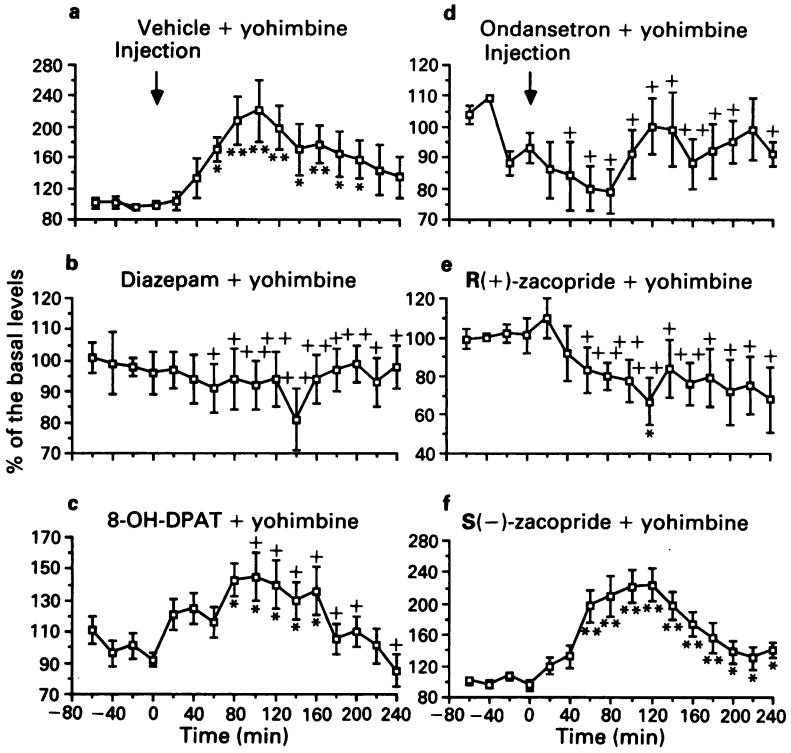

Figure 2 The ability of (a) vehicle (saline, 1.0 ml kg^{-1}, i.p.), (b) diazepam (2.5 mg kg^{-1}, i.p.), (c) 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT, 0.32 mg kg^{-1}, s.c.), (d) ondansetron (100 μg kg^{-1}, i.p.), (e) **R**(+)-zacopride (10 μg kg^{-1}, i.p.) and (f) **S**(−)-zacopride (100 μg kg^{-1}, i.p.) to modify the yohimbine (5.0 mg kg^{-1}, i.p.)-induced increases or decreases in the extracellular levels of 5-HT in the rat frontal cortex dialysates with measurement commencing 12 h after the probe implantation. Diazepam, 8-OH-DPAT, ondansetron, **R**(+)-zacopride, **S**(−)-zacopride or vehicle were administered 5 min prior to the injection of yohimbine. 5-HT levels are expressed as the percentage of the meaned absolute amount in the 4 collections preceding the drug treatment. Data represent the mean ± s.e.mean of four to six determinations. Significant increases or decreases compared to the basal levels and differences between the vehicle + yohimbine treated and drugs + yohimbine treated groups are indicated as $^{*}P<0.05$, $^{**}P<0.01$ (single factor ANOVA followed by Dunnett's t test); $^{+}P<0.05$ and $^{++}P<0.01$ (two factor ANOVA followed by t test) respectively. Basal levels of 5-HT were – diazepam: 56 ± 2.10 fmol/40 μl, 8-OH-DPAT: 54 ± 2.39 fmol/40 μl; ondansetron: 38 ± 0.98 fmol/40 μl; **R**(+)-zacopride: 44 ± 2.83 fmol/40 μl; **S**(−)-zacopride: 47 ± 2.69 fmol/40 μl.

The administration of clonidine (50 μg kg^{-1}, i.p.) 5 min prior to the injection of yohimbine (5.0 mg kg^{-1}, i.p.) completely prevented the yohimbine-induced increases in the extracellular levels of 5-HT (Figure 1). The reduction in 5-HIAA levels induced by yohimbine were returned by clonidine to the vehicle-treated control levels (respective values of 73% and 102% at 120 min after drug administration, Figure 1).

Effect of diazepam, 8-OH-DPAT, ondansetron, R(+)-zacopride and S(−)-zacopride on the yohimbine-induced increases in the extracellular levels of 5-HT in the rat frontal cortex

The administration of diazepam (2.5 mg kg^{-1}, i.p.) and ondansetron (100 μg kg^{-1}, i.p.) 5 min prior to the injection of yohimbine (5.0 mg kg^{-1}, i.p.) completely prevented the yohimbine (5.0 mg kg^{-1}, i.p.)-induced increases in the extracellular levels of 5-HT in the rat frontal cortex (Figure 2). The 5-HT$_{1A}$ receptor agonist 8-OH-DPAT (0.32 mg kg^{-1}, s.c.) partially prevented the effect of yohimbine, reducing the maximal yohimbine-induced increase in the 5-HT levels from 230% to 140% of the basal levels (t = 100 min, Figure 2). At the end of the experiment (220 min following the injection), the 5-HT levels had returned to the basal levels shown in the vehicle treated controls (Figure 2).

The administration of R(+)-zacopride (10 μg kg^{-1}, i.p.) 5 min before the injection of yohimbine (5.0 mg kg^{-1}, i.p.) reversed the yohimbine (5.0 mg kg^{-1}, i.p.)-induced increases in the extracellular 5-HT levels, the 5-HT levels significantly decreasing to approximately 30% below the basal levels (Figure 2). S(−)-zacopride (100 μg kg^{-1}, i.p.) administered 5 min before the injection of yohimbine (5.0 mg kg^{-1}, i.p.) failed to modify significantly the yohimbine-induced increases in the 5-HT levels (Figure 2).

Diazepam (2.5 mg kg^{-1}, i.p.), ondansetron (100 μg kg^{-1}, i.p.) R(+)-zacopride (10 μg kg^{-1}, i.p.), S(−)-zacopride (100 μg kg^{-1}, i.p.) and 8-OH-DPAT (0.32 mg kg, s.c.) failed to modify significantly ($P > 0.05$) yohimbine-induced decrease in 5-HIAA levels (data not shown).

Discussion

Using the *in vivo* microdialysis technique, the present study has demonstrated that the systemic administration of the α_2-adrenoceptor antagonist, yohimbine, having anxiogenic properties in animal models of anxiety and man (Charney *et al.*, 1983; 1992; Pellow *et al.*, 1987), significantly increased the extracellular levels of 5-HT in the frontal cortex of the freely-moving rat. Yohimbine also caused a slight but significant reduction in the frontal cortical dialysate 5-HIAA levels. Clonidine caused a dose-dependent reduction in the extracellular 5-HT levels, and the frontal cortical dialysate 5-HIAA levels were slightly but significantly reduced by the high dose of clonidine. Such findings might indicate that the anxiogenic-like effects induced by either an anxiogenic agent such as yohimbine or aversive situations (Wright *et al.*, 1992) result in increased 5-HT neuronal activity in the nerve terminal regions.

The influence of α_2-adrenoceptor ligands to modulate the activity of 5-HT neurones may occur either by an action on α_2-adrenoceptors on the 5-HT nerve terminals (Fuxe, 1965; Maura *et al.*, 1982; Raiteri *et al.*, 1990) or by an action on 5-HT neurones of the dorsal raphe nucleus (Fuxe, 1965). In the present study, therefore, the increases in the extracellular 5-HT levels induced by administration of yohimbine could be the result of blockade of an inhibitory noradrenergic innervation located on the 5-HT nerve terminals in the forebrain or 5-HT cells in the midbrain. This hypothesis is supported by data showing that the injection of the α_2-adrenoceptor antagonist, idazoxan into the dorsal raphe nucleus caused an elevation of the firing rate of 5-HT neurones in the dorsal raphe (Freeman, & Aghajanian, 1984), and systemic administration of idazoxan increased the extracellular levels of 5-HT and 5-HIAA in the rat frontal cortex (Garratt *et al.*, 1991). In the present study, the activation of α_2-adrenoceptors by clonidine, decreasing the extracellular levels of 5-HT when administered alone and preventing the yohimbine-induced increases in the 5-HT levels in the rat frontal cortex, are consistent with *in vitro* results showing that noradrenaline inhibits the depolarization-induced release of [^{3}H]-5-HT from rat hippocampal slices (Frankhuyzen & Mulder, 1980). In addition there are *in vivo* data indicating that clonidine inhibits depolarization-induced endogeneous 5-HT release in the hippocampus of freely-moving rats (Yoshioka *et al.*, 1992).

The present study also demonstrated that the yohimbine-induced increases of the extracellular levels of 5-HT were completely antagonized by pretreatment with diazepam, ondansetron and R(+)-zacopride, partially antagonized by 8-OH-DPAT and not affected by the administration of S(−)-zacopride. Diazepam, clonidine, ondansetron, R(+)-zacopride and 8-OH-DPAT have all been demonstrated to reduce, to different extents, the behavioural response to an aversive situation in the animal models of anxiety or possess antianxiety effects in man (Hoehn-Saric *et al.*, 1981; Engel *et al.*, 1984; Barnes *et al.*, 1990; Costall *et al.*, 1990; Young & Johnson, 1991). The doses chosen in the present study were based on the doses producing anxiolytic-like effects in the animal models of anxiety. The doses of diazepam, 8-OH-DPAT and R(+)-zacopride are also those we have previously demonstrated to decrease 5-HT release in the rat frontal cortex (Barnes *et al.*, 1992a) and for diazepam and 8-OH-DPAT in the rat ventral hippocampus (Pei *et al.*, 1989; Sharp *et al.*, 1989). However, this is not taken to imply that anxiolytic-like actions are necessarily related to a reduction in 5-HT release. Thus ondansetron failed to modify the extracellular levels of 5-HT when administered alone (Barnes *et al.*, 1992a) yet completely prevented the yohimbine-induced increase in the extracellular levels of 5-HT. But it remains possible that the actions of diazepam, 8-OH-DPAT and R(+)-zacopride in antagonizing the yohimbine-induced increases in the extracellular levels of 5-HT may reflect indirect and opposing actions. Yet there are additional possibilities of direct drug interactions on the 5-HT systems. Thus the ability of diazepam to decrease the depolarization-induced 5-HT release from slices of cortex (Collinge & Pycock, 1982) and hippocampus *in vitro* (Balfour, 1980), probably reflects the action of the benzodiazepine receptor agonists to enhance the action of GABA at GABA$_A$ receptors to reduce 5-hydroxytryptaminergic transmission in the brain (Stein *et al.*, 1977). Thus, in the present study, it is possible that diazepam potentiates the action of the GABA receptor to inhibit the synthesis (Saner & Pletscher, 1979) and metabolism of 5-HT (Koe, 1979) and to reduce 5-HT release in the terminal regions (Barnes *et al.*, 1992a) to attenuate the yohimbine-induced increases in the extracellular levels of 5-HT in the rat frontal cortex.

The ability of 8-OH-DPAT to attenuate significantly the yohimbine-induced increase in the 5-HT release may reflect the actions of a 5-HT$_{1A}$ receptor ligand with intrinsic activity to reduce the release of 5-HT in terminal regions following interaction with somato-dendritic 5-HT autoreceptors (Hutson *et al.*, 1989; Sharp *et al.*, 1989; Barnes *et al.*, 1992a). The partial blockade by 8-OH-DPAT may reflect the use of a modest dose. Thus, it has previously been demonstrated that idazoxan completely blocked the effects of 8-OH-DPAT in moderating 5-HT release and metabolism in the suprachiasmatic nucleus (SCN) of the chloral hydrate anaesthetized rat (Marsden & Martin, 1986).

The present results showing that R(+)-zacopride but not S(−)-zacopride significantly antagonized the effects of yohimbine in increasing the extracellular 5-HT levels are in agreement with the behavioural finding that R(+)-zacopride

possesses anxiolytic-like activity at low ($\mu g\,kg^{-1}$) dose levels whereas effective doses of S(−)-zacopride are at least 4 orders of magnitude higher than in the animal models of anxiety (Barnes *et al.*, 1990; Young & Johnson, 1991). Both R(+)-zacopride and S(−)-zacopride have potent antagonist effects at the 5-HT_3 receptors but their different potentials in modifying the effects of yohimbine adds to the differential activities of R(+)- and S(−)-zacopride to inhibit emesis and dopamine-induced hyperactivity, to reduce anxiety-related behaviours, to improve cognitive performance and to modify the extracellular levels of 5-HT in the rat frontal cortex (Barnes *et al.*, 1990; Costall *et al.*, 1990; Barnes *et al.*, 1992a). However, in addition to their 5-HT_3 receptor antagonism, S(−)-zacopride has a 5-HT_3 agonist potential and both R(+)- and S(−)-zacopride have been demonstrated to have agonist/partial agonist effects at the 5-HT_4 receptors in many tissues (see review by Eglen *et al.*, 1990; King, 1990; Bockaert *et al.*, 1992). In addition, R(+)-zacopride binds with much greater affinity than S(−)-zacopride to an unspecified recognition site (Barnes *et al.*, 1990; Kidd *et al.*, 1992). However, the relevance of such actions to their ability to modify behaviour in an aversive situation and neurotransmitter release is not known. It is reasonable to presume that the differential activities of R(+)- and S(−)-zacopride to modify the yohimbine-induced increases in the 5-HT release may reflect their interaction at 5-HT_3, 5-HT_4 and/or the R(+)-zacopride recognition site.

The extracellular levels of 5-HIAA were also slightly but significantly reduced by the administration of yohimbine and reductions caused by the yohimbine interaction with anxiolytic and putative anxiolytic agents were also significant. These results, however, are not in agreement with the previous finding that the α_2-adrenoceptor antagonist, idazoxan, increased 5-HIAA levels in the hypothalamus and dorsal raphe nucleus (Garratt *et al.*, 1991). The difference could be the result of a discrepancy between the different methods used. 5-HT could be deaminated before or after release and it would be difficult to conclude whether 5-HIAA accurately reflects 5-HT utilization or simply provides an index of monoamine oxidase activity. Thus, the changes in the extracellular levels of 5-HIAA are not necessarily an indication of corresponding changes in 5-HT release.

In summary, the present studies have demonstrated that the α_2-adrenoceptor antagonist, yohimbine, caused significant increases in the extracellular levels of 5-HT in the frontal cortex of freely-moving rats which are antagonized by pretreatment with the anxiolytic agent, diazepam, and the putative anxiolytic agents, clonidine, ondansetron, R(+)-zacopride and 8-OH-DPAT and not affected by S(−)-zacopride. The neurochemical data are interpreted in terms of drug action on 5-hydroxytryptaminergic systems to support a role of 5-HT in the behavioural response to drugs modifying behaviour to aversive situations in the animal models of anxiety.

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