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A re-evaluation of the role of α_2-adrenoceptors in the anxiogenic effects of yohimbine, using the selective antagonist delequamine in the rat

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1 The acute behavioural effects of the α_2-adrenoceptor antagonists, yohimbine, idazoxan and delequamine (RS-15385-197) were compared in two tests of exploratory behaviour in the rat, operated in tandem. These were the elevated X-maze test (5 min) and a modified holeboard test (12 min), which comprised a holeboard arena with a small roof in one corner as a 'refuge'. Rats were first placed into this corner, thus enabling measurements of initial emergence latency and the number of forays. The experiments were always done with a concomitant vehicle control group, with 10–12 rats per group, and with the treatment blinded.

2 In order to validate the tests, the effects of representatives of four classes of psychoactive agents were examined, *viz.* picrotoxin (anxiogenic), chlordiazepoxide (anxiolytic), (+)-amphetamine (stimulant) and diphenhydramine (sedative). The modified holeboard tended to be more sensitive than the measurement of total arm entries in the elevated X-maze at detecting drug effects on exploratory behaviour, but unlike the X-maze it could not clearly identify each class of agent. Thus, picrotoxin (5 mg kg^{-1}, i.p.) reduced total arm entries and open arm exploration in the X-maze ($P<0.02$) and suppressed most measures of activity in the holeboard ($P<0.05$); chlordiazepoxide (7.5 mg kg^{-1}, i.p) increased total arm entries and open arm exploration ($P<0.02$) in the X-maze, without clear-cut effects in the holeboard; (+)-amphetamine (1 mg kg^{-1}, i.p.) had no significant effects in the X-maze, but increased most holeboard activities ($P<0.05$), and diphenhydramine (30 mg kg^{-1}, i.p.) reduced total arm entries in the X-maze ($P<0.002$) and hole exploration in the holeboard ($P<0.05$).

3 The actions of yohimbine most closely resembled those of picrotoxin. In the elevated X-maze, yohimbine (3 mg kg^{-1}, i.p.) decreased the total number of arm entries ($P<0.02$); a larger dose (10 mg kg^{-1}, i.p.) also reduced time spent on the open arms ($P<0.02$). In contrast, delequamine (3 mg kg^{-1}, i.p.) and idazoxan (3 mg kg^{-1}, i.p.) had no effect.

4 In the partially-shaded holeboard, yohimbine (3 mg kg^{-1}, i.p.) suppressed hole exploration ($P<0.05$); a higher dose (10 mg kg^{-1}, i.p.) increased emergence latency ($P<0.002$) and virtually abolished all activity. Delequamine (3 mg kg^{-1}, i.p.) and idazoxan (3 mg kg^{-1}, i.p.) did not influence emergence latency or holeboard activities.

5 The extent of the blockade of central α_2-adrenoceptors achieved during the tests was assessed by the ability of the doses used to reverse mydriasis induced by clonidine (300 $\mu g\ kg^{-1}$, s.c.) in anaesthetized rats. At a dose of 3 mg kg^{-1}, i.p., delequamine and idazoxan produced a rapid, sustained reversal of the clonidine response (by 87 ± 2 and $86\pm2\%$ respectively, 30 min after injection) whereas yohimbine produced a partial reversal of only $43\pm13\%$. The higher dose of yohimbine used in the exploratory tests (10 mg kg^{-1}, i.p.) was required in order to achieve $77\pm4\%$ reversal of clonidine-induced mydriasis.

6 We therefore conclude that blockade of central α_2-adrenoceptors *per se* does not have an anxiogenic effect, at least in the rat. Thus, yohimbine is not an ideal tool for studying α_2-adrenoceptor function in animals and some of the anxiogenic effects of yohimbine previously ascribed to α_2-adrenoceptor antagonism may be secondary to other effects of this poorly selective compound.

Keywords: Delequamine; RS-15385-197; yohimbine; idazoxan; α_2-adrenoceptors; anxiety; anxiogenic; X-maze; plus-maze; holeboard

Introduction

Since the original report by Holmberg & Gershon (1961), several groups have confirmed that the indole alkaloid, yohimbine, can induce feelings of anxiousness, fear or panic in humans. In normal subjects the effect tends to be mild anxiety (Uhde *et al.*, 1984; Mattila *et al.*, 1988; Krystal *et al.*, 1992); in patients with agoraphobia or panic disorder the agent induces an enhanced anxiety response or panic (Charney *et al.*, 1987), and in patients with post-traumatic stress disorder, yohimbine can induce panic and flashbacks (Southwick *et al.*, 1993). In contrast, patients with generalized anxiety disorder (Charney *et al.*, 1989) or depression (Heninger *et al.*, 1988) show mild anxiogenic responses to yohimbine similar to those of normal subjects. Yohimbine has therefore proved useful in teasing out sub-groups of responders, but this is only of value from an aetiological point of view if its precise pharmacological mechanism of action is understood.

Following the discovery of presynaptic α_2-adrenoceptors (see Langer, 1974) located both terminally and somato-dentrically on central noradrenergic neurones, and the actions of yohimbine at these sites, increasing noradrenaline release at synapses (Dietl *et al.*, 1981) and the firing rate of neurones in the locus coeruleus (Rasmussen & Jacobs, 1986) respectively, it has largely been assumed that yohimbine's anxiogenic effect in humans was due to these actions. In support of this view,

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synthetic α_2-adrenoceptor antagonists including idazoxan (Krystal *et al.*, 1992) and MK-912 (Gertz *et al.*, 1989) have been shown to induce mild anxiety in humans.

Several parallel studies have been carried out using various measures of 'anxiety' in the rat. Yohimbine has been shown to have anxiogenic-like effects in the elevated X-maze (Handley & Mithani, 1984; Pellow *et al.*, 1985a; Ferrari *et al.*, 1989; Baldwin *et al.*, 1989; Wada & Fukuda, 1991), social interaction (Guy & Gardner, 1985; Pellow *et al.*, 1985b), holeboard exploration (Chopin *et al.*, 1986), light-dark box (Merlo Pich & Samanin, 1989), conditioned place aversion (File, 1986) and fear-potentiated startle tests (Davis *et al.*, 1979). Fewer studies have been conducted with other α_2-adrenoceptor antagonists, but the evidence is by no means clear-cut. For example, imiloxan and piperoxan are apparently anxiogenic in the elevated X-maze (Handley & Mithani, 1984) and this latter agent is anxiogenic in the fear-potentiated startle test (Davis *et al.*, 1979). On the other hand, whereas idazoxan has been reported by some authors (Handley & Mithani, 1984; Soderpalm & Engel, 1989) to be anxiogenic in the elevated X-maze test, others have found it to be without effect in this test (File, 1987; Moser, 1989). Similarly, 1-pyrimidyl piperazine, a metabolite of buspirone with α_2-adrenoceptor antagonist properties, is not anxiogenic in this test (Moser, 1989), nor is the selective α_2-adrenoceptor antagonist, atipamezole (Kauppila *et al.*, 1991).

The main problem in the interpretation of these findings is that yohimbine and, to a lesser extent, these other drugs are relatively promiscuous agents, with affinities for receptors associated with other neurotransmitter systems. Recently, we have described the pharmacology of a highly potent and selective α_2-adrenoceptor antagonist, delequamine (RS-15385-197; Clark *et al.*, 1989; Brown *et al.*, 1993), an agent which readily penetrates into the CNS and has actions at central α_2-adrenoceptors with the expected augmentation of noradrenergic neurotransmission (Redfern *et al.*, 1993). In order to clarify the involvement of α_2-adrenoceptors in the anxiogenic effects of yohimbine, we have compared its effects in ethological tests of 'neophobia' sensitive to psychoactive agents with those of delequamine and idazoxan at doses equieffective at blocking central α_2-adrenoceptors. We tested rats in an elevated X-maze and then immediately exposed the same rats to a modified holeboard, in order to increase the information from the experiment.

A preliminary account of part of this work has been presented to the British Pharmacological Society (Redfern & Williams, 1989; 1990).

Methods

Animals

Male Sprague-Dawley rats (Charles River, U.K.) weighing 120–200 g arrived in the animal unit 4–8 days before testing and were immediately housed singly, on a 12 h light-dark cycle (on: 08 h 00 min; off: 20 h 00 min) until testing. The same experimenter handled the rats at each stage up to and including testing. The rats were transferred to the testing room on the afternoon before the tests, and exposed to a standard handling procedure (lifted out of the cage onto the experimenter's arm three times at 4 min intervals). The following morning they were handled again in the same way before starting the tests. The tests were conducted between 09 h 00 min and 12 h 30 min over three consecutive days, with equal numbers of drug- and vehicle-treated rats in each test session. The observer was blind to the treatment. Drug or vehicle was injected intraperitoneally (i.p.) 30 min before the tests.

Elevated X-maze

The apparatus was constructed of plywood, and comprised 2 open arms (length 50 cm, width 10 cm) facing each other and 2 enclosed arms (length 50 cm, width 10 cm) with walls along the sides and at the end of the arms (height 20 cm). The X-maze was supported firmly on a metal base so that it was 50 cm above the floor. Lines were drawn across the entrance to each arm, thus forming a centre square 10×10 cm. The light intensities were 23 lux in the centre square, 8 lux at the extremities of the enclosed arms and 36 lux at the extremities of the open arms.

A rat was placed on an open arm with its forepaws inside the centre square, and observed from an adjacent room for 5 min by means of a closed circuit TV camera mounted vertically above the X-maze. An arm entry was deemed to have occurred when both forepaws crossed an entry line, even if the rat advanced no further; the duration on the arm was defined as the period between consecutive arm entries. The number of entries onto each arm and the time spent on each arm were noted. From these observations were derived the total number of arm entries, the number of open arm entries expressed as a percentage of the total, and the time spent on the open arms as a percentage of the total time. After testing in the X-maze each rat was transferred immediately to the holeboard.

Partially-shaded holeboard

The apparatus (height 30 cm, width 46 cm, length 75 cm) was constructed of plywood, covered with thick polythene to facilitate cleaning, and fixed 70 cm above the ground. A small shelf (12×15 cm) was fixed 10 cm above the floor in one corner, with a steep roof on top of the shelf to prevent rats from climbing onto it. The floor contained 8 holes of diameter 4 cm. The holes were spaced 13–15 cm apart, and 13 cm from the edges of the box (23 cm from the end containing the shaded corner), so that a rat could walk around the edge of the holeboard without difficulty, and also so that the holes could not readily be reached by a rat in the shaded corner. Sheets of black polythene were suspended beneath the floor such that there was a different field of view through each hole. The floor was marked with a line which bisected the floor longitudinally, and with three lines across the width of the box, so forming 8 equally-sized sectors. The light intensities were 14 lux in the shaded corner, 95–130 lux in the other 3 corners, 170 lux in the centre of the open arena, and 7 lux at the ground level 70 cm beneath the holes.

A rat was placed head first in the shaded corner, and its behaviour was observed from an adjacent room by means of a closed-circuit TV camera mounted vertically above the holeboard. Observations were made on the initial emergence latency (a foray from the shaded corner was defined as occurring when all 4 paws were visible), the duration of the first foray, the number of forays, time spent in the open arena, number of line crossings (with all four paws), number of rears and the number of head dips (nose below level of floor), over a 12 min observation period, dividing the counts into three 4 min periods. After removing the rat from the holeboard, both sets of apparatus were wiped clean.

Mydriasis tests

The doses of the α_2-adrenoceptor antagonists used in the X-maze and holeboard were tested in a separate group of rats for reversal of mydriasis induced by clonidine, which is a centrally-mediated response (Berridge *et al.*, 1983). Rats (250–440 g; 6–10 per group) were anaesthetized with pentobarbitone sodium (60 mg kg^{-1}, i.p.), and their body core temperature maintained at 37°C with a heating lamp. Pupil diameter was measured with an illuminated inspection glass with $\times 7$ magnification (RS Components Ltd). Mydriasis was induced by injection of clonidine (300 μg kg^{-1}, s.c.) and pupil diameter was measured 20 min later; 5 min later, the α_2-adrenoceptor antagonist was administered intraperitoneally, and pupil diameter measured at 5 min intervals for up to 30 min after injection of the antagonist.

Drugs used

Delequamine HCl (RS-15385-197) was synthesized by Dr R. Clark, Syntex, Palo Alto, Ca, U.S.A. The following drugs were purchased: yohimbine HCl (Sigma), idazoxan HCl (Sigma), (+)-amphetamine sulphate (SKF), diphenhydramine HCl (Sigma), picrotoxin (Sigma) and chlordiazepoxide HCl (Sigma). All drugs were dissolved in saline (0.9% w/v NaCl, aq.), and injected intraperitoneally in a volume of 1 ml kg^{-1}. Doses refer to the base.

Statistics

All data are expressed as mean ± standard error of the mean (s.e.mean). Each drug-treated group was compared with its own vehicle-treated group by a 2-tailed Mann-Whitney U-test; differences between groups were considered to be significant if $P<0.05$. An arbitrary 100 s cut-off was imposed on the emergence latency data.

Results

General features of the behavioural tests used

Examination of the data from vehicle-treated rats for both the X-maze and holeboard tests (Figures 1–6) reveals some variability between batches of rats; this justifies our use of concomitant vehicle control groups. Using these data from vehicle-treated rats ($n=84$), there were no correlations between any parameters in the elevated X-maze and any in the holeboard (e.g. total arm entries in X-maze *vs.* line crossings in holeboard: $r=0.079$; % time on open arms in X-maze *vs.* time in open arena of holeboard: $r=0.003$), which indicates that the two tests are complementary rather than equivalent. In the modified holeboard, we also examined the data in three 4 min 'bins'; there were never any significant drug effects during the first 4 min which were not significant after 12 min, although the converse was often the case. Therefore, the data shown in Figures 3 and 6 are after 12 min in the holeboard. One parameter which we recorded, the duration of the first foray, was unaffected by any of the drugs tested, and the data have therefore been omitted from the figures.

Validation of the tests with centrally-acting agents

(+)-Amphetamine In the elevated X-maze, (+)-amphetamine (1 mg kg^{-1}, i.p.) increased the total number of arm entries, but not significantly. There was no effect on the percentage of entries that were onto the open arms, nor on the duration of open arm entries (Figure 1). In the partially-shaded holeboard, (+)-amphetamine did not affect emergence latency (Figure 2) but significantly increased forays from the 'refuge corner', time spent out in the open arena, line crossings, rears and head dips (Figure 3).

Diphenhydramine Diphenhydramine (30 mg kg^{-1}, i.p.) significantly reduced the total number of arm entries in the X-maze without affecting the proportion of open: enclosed arm entries, or the duration on the open arms (Figure 1). There was a general trend for a reduction in all activities in the holeboard, but only head dips and the number of holes explored were significantly reduced (Figure 3). A lower dose of diphenhydramine (10 mg kg^{-1}, i.p.) had no significant effect in either test (Figures 1–3).

Picrotoxin Picrotoxin (2 mg kg^{-1}, i.p.) had no significant effects in the X-maze, but significantly reduced head dips and line crossings in the holeboard (Figure 3). A higher dose (5 mg kg^{-1}, i.p.) significantly reduced total arm entries, the proportion of open arm entries, and the time spent on the open arms in the X-maze (Figure 1). In the holeboard, emergence

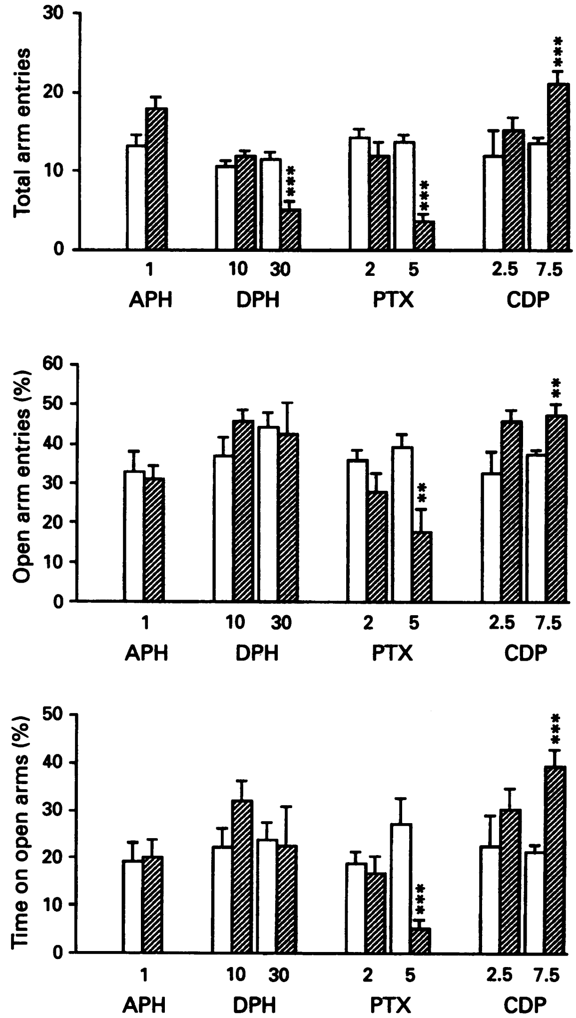

Figure 1 Effects of representatives of four classes of psychoactive agents on the activity of male Sprague-Dawley rats, housed singly for 4–8 days before testing, in the elevated X-maze (5 min). Open columns, vehicle-treated; hatched columns, drug-treated; 11–12 rats per group. Abbreviations: APH, (+)-amphetamine; DPH, diphenhydramine; PTX, picrotoxin; CDP, chlordiazepoxide. The treatment was administered on a blind basis 30 min before testing. Doses in mg kg^{-1}, i.p. are given below the column pairs. $^{**}P<0.02$; $^{***}P<0.002$ between drug-treated and corresponding vehicle control group (Mann-Whitney U-test, 2-tailed).

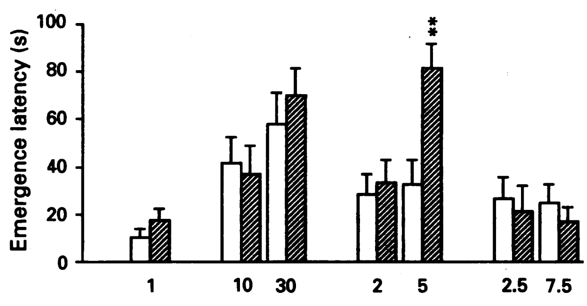

Figure 2 Effects of representatives of four classes of psychoactive agents on emergence latency from the covered corner of the modified holeboard. The rats had been transferred directly from their 5 min test in the elevated X-maze. Column identity and abbreviations as in Figure 1. An arbitrary 100s cut-off has been imposed on these data. Note that only picrotoxin (5 mg kg^{-1}, i.p.) significantly prolonged the emergence latency ($^{**}P<0.002$; Mann-Whitney U-test, 2-tailed).

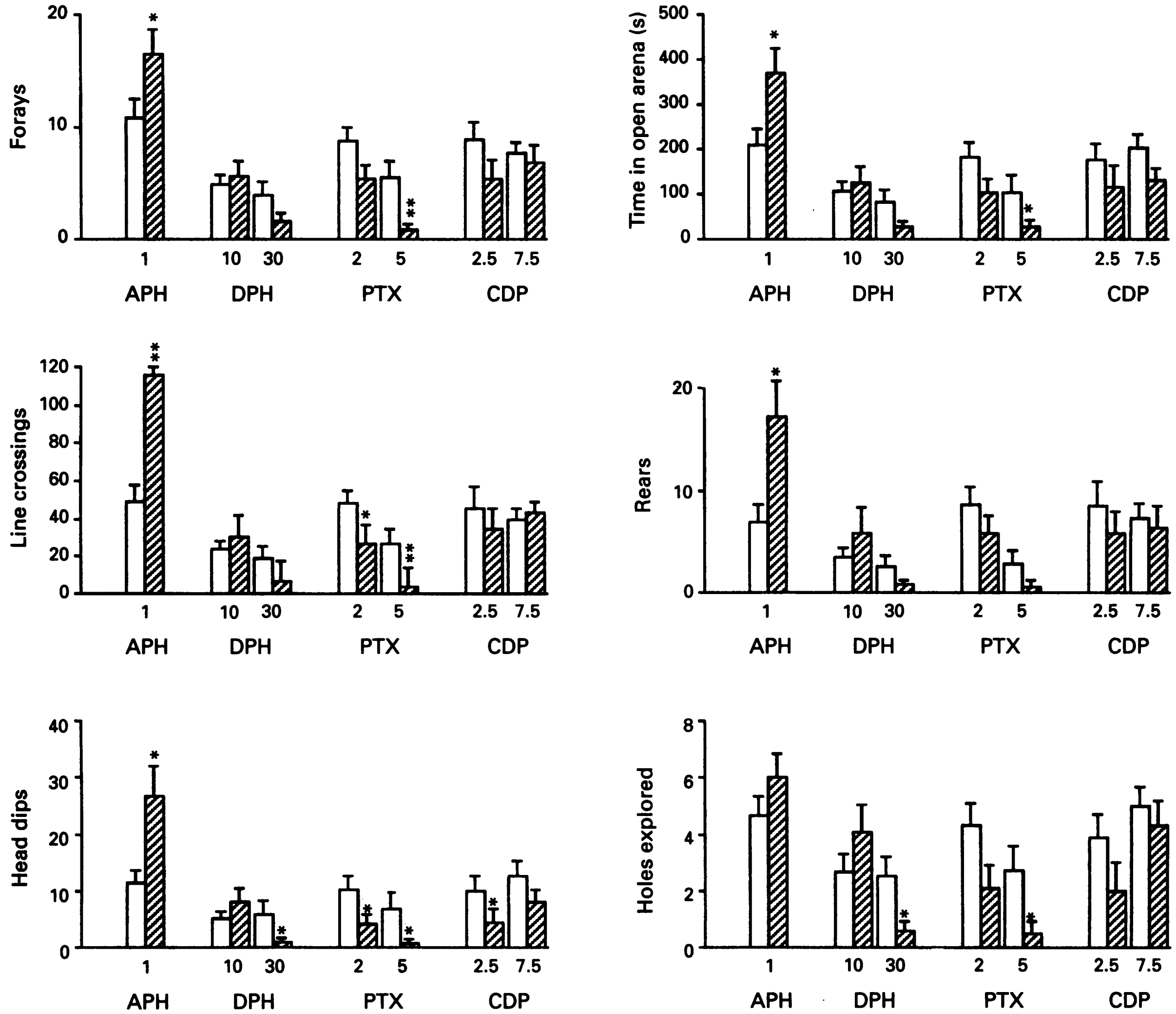

Figure 3 Effects of representatives of four classes of psychoactive agents on activities in the modified holeboard over 12 min. Column identity and abbreviations as in Figure 1. *$P<0.05$; **$P<0.02$ between drug-treated and corresponding vehicle control group (Mann-Whitney U-test, 2-tailed).

latency was significantly elevated (Figure 2), and most measures of activity were significantly reduced (Figure 3).

Chlordiazepoxide Chlordiazepoxide (2.5 mg kg^{-1}, i.p.) had no significant effects in the elevated X-maze (Figure 1). In the holeboard, the activity scores tended to be lower in the drug-treated group, but this attained significance only for head dips (Figure 3). At a higher dose (7.5 mg kg^{-1}, i.p.) there was a significant increase in total arm entries, in the percentage of entries made onto the open arms, and in the percentage of time spent on the open arms (Figure 1). In the holeboard, the time spent in the open arena was reduced, but this just failed to reach statistical significance ($P=0.05$; Figure 3).

Effects of α_2-adrenoceptor antagonists

Elevated X-maze Although delequamine (3 mg kg^{-1}, i.p.), as well as idazoxan (3 mg kg^{-1}, i.p.) appeared to increase the time spent on the open arms, this failed to reach statistical significance (Figure 4). Yohimbine (3 mg kg^{-1}, i.p.) decreased the total number of arm entries ($P<0.02$) without affecting the ratio of open: enclosed arm entries or the time spent exploring the open arms (Figure 4). A larger dose (10 mg kg^{-1}, i.p.) reduced the % time spent on the open arms ($P<0.02$) and total arm entries ($P<0.002$; Figure 4).

Partially-shaded holeboard Yohimbine (3 mg kg^{-1}, i.p.) suppressed head dips ($P<0.02$) and the number of holes explored ($P<0.05$) without significantly inhibiting other activities (Figure 6). A higher dose (10 mg kg^{-1}, i.p.) increased emergence latency ($P<0.002$; Figure 5) and virtually abolished all activity (Figure 6). Delequamine and idazoxan (both at 3 mg kg^{-1}, i.p.) did not affect emergence latency or holeboard activities (Figures 5, 6).

Mydriasis tests The degree of central α_2-adrenoceptor blockade achieved during the exploratory tests was determined by comparing the ability of the drugs to reverse mydriasis induced by clonidine (300 μg kg^{-1}, s.c.) in anaesthetized rats. At a dose of 3 mg kg^{-1}, i.p., delequamine and idazoxan produced a rapid, sustained reversal of the clonidine response (by 87 ± 2 and 86 ± 2% respectively, 30 min after injection; Figure 7) whereas yohimbine produced a partial reversal of only 43 ± 13%. The higher dose of yohimbine used in the exploratory tests (10 mg kg^{-1}, i.p.) was required in order to achieve 77 ± 4% reversal of clonidine-induced mydriasis (Figure 7).

Discussion

The present study was undertaken to re-examine the apparent involvement of central α_2-adrenoceptors in the anxiogenic actions of yohimbine. We compared the effects of this agent with those of the more selective α_2-adrenoceptor antagonist, delequamine (RS-15385-197), in ethological tests of anxiety in the

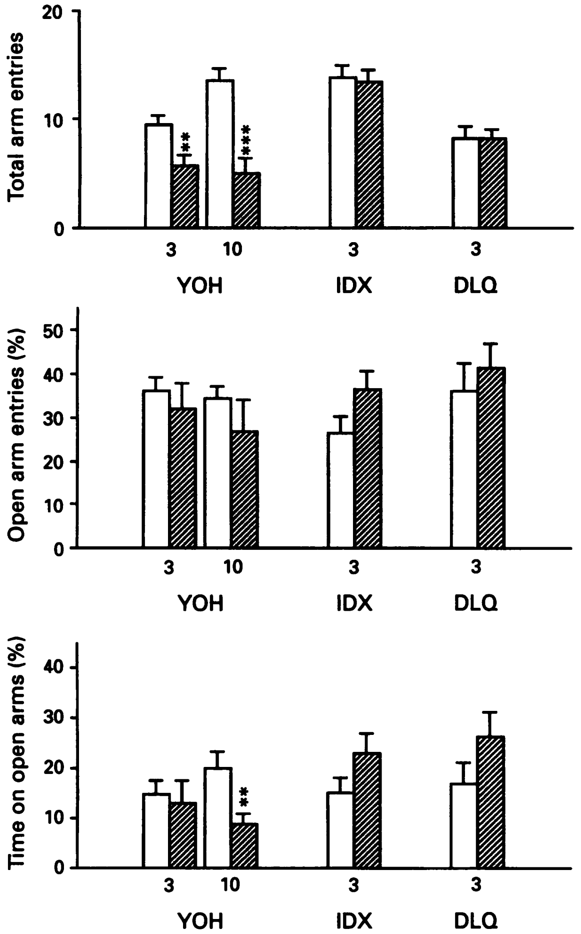

Figure 4 Effects of the three α_2-adrenoceptor antagonists on activities in the elevated X-maze over 5 min. Column identity and *n* numbers as in Figure 1. Abbreviations: YOH, yohimbine; IDX, idazoxan; DLQ, delequamine. Doses in mg kg^{-1}, i.p. are given below the column pairs. Note that only yohimbine had any significant effects. $^{**}P<0.02$; $^{***}P<0.002$ between drug-treated and corresponding vehicle control group (Mann-Whitney U-test, 2-tailed).

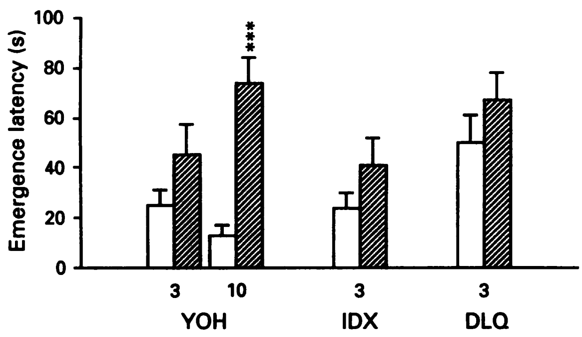

Figure 5 Effects of the three α_2-adrenoceptor antagonists on emergence latency from the covered corner of the modified holeboard. Column identity and abbreviations as in Figure 4. An arbitrary 100s cut-off has been imposed on these data. Note that only yohimbine (10 mg kg^{-1}, i.p.) significantly prolonged the emergence latency ($^{***}P<0.002$; Mann-Whitney U-test, 2-tailed).

rat. Delequamine is a highly potent and selective α_2-adrenoceptor antagonist *in vitro* and *in vivo* which readily penetrates into the CNS (Clark *et al.*, 1989; MacKinnon *et al.*, 1992; Brown *et al.*, 1993; Redfern *et al.*, 1993). In binding studies it is >100 fold more selective for α_2-adrenoceptors versus other receptors tested (Brown *et al.*, 1993), which is far superior to either yohimbine or idazoxan.

In studies on the effects of drugs on the level of anxiety in animals it is desirable to employ more than one appropriate behavioural test, as no one test is ideal. Some such tests are based on the conflict between the motivation to explore a novel environment, and an aversion to it. An example of this type of test suitable for use in rats is the elevated X-maze (plus-maze), consisting of two enclosed arms (with walls) and two open arms (without walls) elevated above the ground (Handley & Mithani, 1984; Pellow *et al.*, 1985a). Drugs which are anxiolytic in man increase the proportion of open arm activity, whereas anxiogenic agents decrease it (Pellow *et al.*, 1985a). As this test is capable of detecting an anxiogenic effect of yohimbine (Handley & Mithani, 1984; Pellow *et al.*, 1985a; Ferrari *et al.*, 1989; Baldwin *et al.*, 1989; Wada & Fukuda, 1991), it would be expected to be suitable for detecting any anxiogenic effects of agents in the same class as yohimbine, such as delequamine. Another test measures the emergence latency of a rat from a small, dark area into a larger, brightly lit arena (File, 1985): an increase in anxiety will prolong the emergence latency. A third potentially useful test is the holeboard, in which a rat is placed in a chamber with holes in the floor into which it can insert its head (head dipping): a greater level of anxiety suppresses head dipping and other aspects of exploratory locomotion (File, 1985). However, all of these tests are affected to varying degrees by changes in the level of arousal.

In the interests of efficiency, and in order to minimize the number of animals used, we felt it would be worthwhile to combine these three tests either physically or temporally, so as to maximize the information derived from each individual animal. We therefore combined the emergence test with the holeboard in a single apparatus, and conducted it in tandem with the elevated X-maze test. After first carrying out pilot studies to optimize our test conditions, representative drugs from four classes of centrally-acting agents (sedative, stimulant, anxiolytic and anxiogenic) were tested in order to determine firstly if the modified, partially-shaded holeboard could distinguish between them, and secondly to establish whether this test would provide worthwhile additional information to that obtained with the elevated X-maze relating to the effects of drugs on exploratory behaviour. Data accumulated from the control (saline-treated) rats were used to examine the relationships between activities in these two tests. In fact, none was found for any of the parameters, indicating that these two tests are not equivalent, and therefore vindicating our decision to operate both tests.

Thus we investigated whether the modified holeboard could be used to distinguish between drugs affecting the level of anxiety and drugs affecting the level of arousal. The drugs used were diphenhydramine, which is sedative in man (Carruthers *et al.*, 1978), (+)-amphetamine, which is stimulant in man (Ivy & Krasno, 1941), chlordiazepoxide, which is anxiolytic in man (Randall & Kappell, 1973) and picrotoxin, which has anxiogenic-like actions in rats (File & Lister, 1984). On the evidence obtained from the drugs tested, it would appear that our modified holeboard test cannot distinguish between sedative and anxiogenic drugs, as both diphenhydramine and picrotoxin were found to suppress most activities in the modified holeboard, i.e. unique profiles of activity were not found. However, in the elevated X-maze, in the same rats, the distinction between these two agents was clear: although both drugs reduced the total number of arm entries, picrotoxin reduced the proportion of open arm activity whereas diphenhydramine did not. The elevated X-maze also distinguished between a stimulant and an anxiolytic drug: whereas both (+)-amphetamine and chlordiazepoxide increased the total number of entries onto both types of arm, chlordiazepoxide increased open arm preference whereas (+)-amphetamine did not. In the partially-shaded holeboard, the sedative rather than the anxiolytic properties of chlordiazepoxide predominated; others have reported that chlordiazepoxide and other benzodiazepines have sedative effects when given acutely, which recede over several days of treatment to

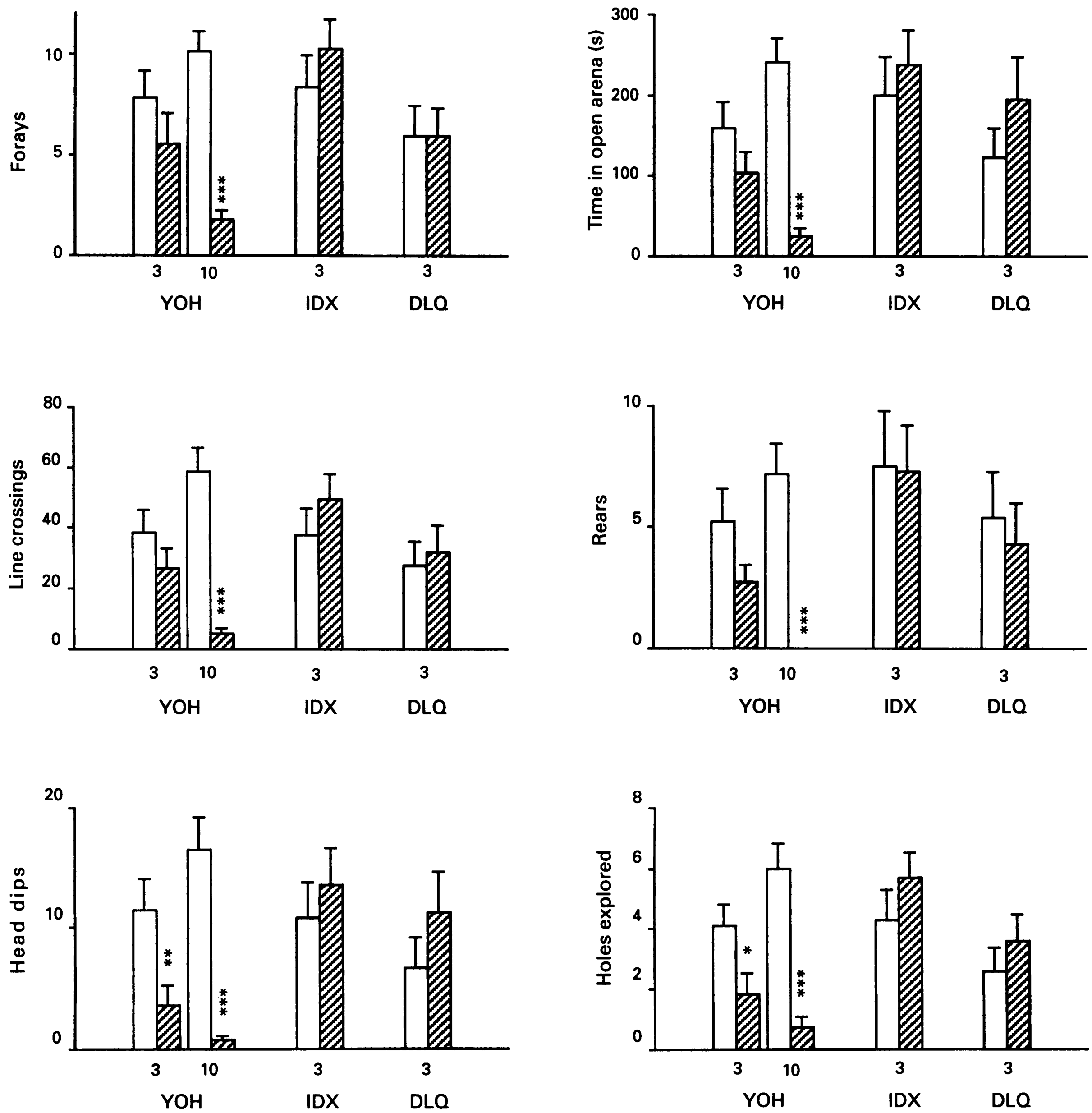

Figure 6 Effects of the three α_2-adrenoceptor antagonists on activities in the modified holeboard over 12 min. Column identity and abbreviations as in Figure 4. Note that only yohimbine had any effects in this test, with the higher dose (10 mg kg^{-1}, i.p.) virtually abolishing most activities. *$P<0.05$; **$P<0.02$; ***$P<0.002$ between drug-treated and corresponding vehicle control group (Mann-Whitney U-test, 2-tailed).

reveal their anxiolytic action (Pellow *et al.*, 1985a). In contrast to the suppression of hole exploration by chlordiazepoxide at a dose of 2.5 mg kg^{-1}, i.p. in our modified holeboard, this same dose has been reported to increase head dipping in rats placed in a conventional holeboard (File *et al.*, 1985). However, the two tests are subtly different; in a conventional holeboard the rat has no clear-cut 'refuge corner' and the anxiolytic properties of chlordiazepoxide may well be seen as an increase in head-dipping, whereas in our modified holeboard it is possible that the sedative effect of chlordiazepoxide will decrease exploration of the holes merely because of a reduction in the time spent in the area which contains them. The modified holeboard did detect the stimulant effects of (+)-amphetamine.

One problem with using the elevated X-maze alone is that changes in total arm entries are relied upon to indicate changes in the level of arousal. Because of the fact that the modified holeboard involves the observation of several distinct types of activity (locomotion, rears and head dips), whereas the measurement of total arm entries in the elevated X-maze is simply a crude indicator of locomotion, the holeboard test would be expected to be more sensitive to the effects of centrally-acting drugs than the X-maze. This was borne out by the data: the holeboard detected changes in activity when there was no significant effect on total arm entries in the X-maze, for amphetamine (1 mg kg^{-1}, i.p.), chlordiazepoxide (2.5 mg kg^{-1}, i.p.) and picrotoxin (2 mg kg^{-1}, i.p.). Also, it is not correct to assume that changes in total arm entries merely represent changes in the level of arousal; an increase in anxiety, if sufficiently intense, would be expected to suppress all activity in the elevated X-maze as well as reducing the proportion of open arm activity. This was the case with the higher dose of picrotoxin (5 mg kg^{-1}, i.p.). Conversely, a decrease in the level of anxiety would be expected to increase all activity in addition to increasing the proportion of open arm activity; this occurred with chlordiazepoxide (7.5 mg kg^{-1}, i.p.). To summarize, from this and previous studies (Handley & Mithani 1984; Pellow *et al.*, 1985a) the elevated X-maze can distinguish anxiolytic and anxiogenic agents from stimulant and sedative drugs respectively. Our modified holeboard did not discriminate in this way, but was more sensitive than the elevated X-maze test in

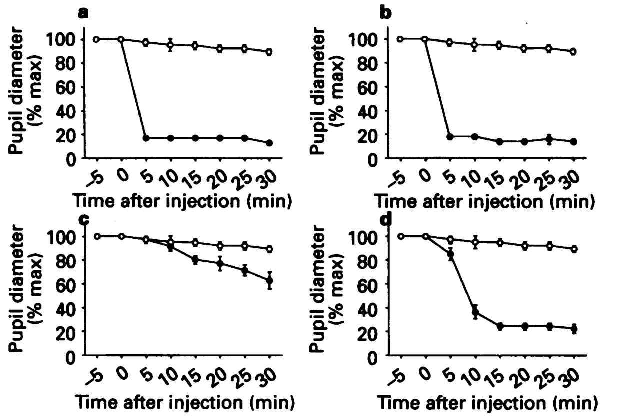

Figure 7 Confirmation of the extent of blockade of central α_2-adrenoceptors by the doses of antagonists used in the behavioural tests, by their ability to reverse mydriasis induced by clonidine ($300\,\mu g\,kg^{-1}$, s.c.) in anaesthetized rats: (○) clonidine controls ($n=5$); (●) clonidine followed by drug administration at time zero ($n=6-10$). (a) Delequamine, $3\,mg\,kg^{-1}$, i.p.; (b) idazoxan, $3\,mg\,kg^{-1}$, i.p.; (c) yohimbine $3\,mg\,kg^{-1}$, i.p.; (d) yohimbine $10\,mg\,kg^{-1}$, i.p. Note that 30 min post-treatment would be the time of exposure to the X-maze test in conscious rats.

detecting general behavioural effects of centrally-acting drugs; a measure of total arm entries in the elevated X-maze is not a reliable indicator of the effects of drugs on the level of arousal. Operation of the two tests in tandem permitted both sensitivity and selectivity in the detection of the effects of centrally-acting drugs.

Despite the high potency of delequamine as a centrally acting α_2-adrenoceptor antagonist, the compound was devoid of effects on behaviour in the tests used. Yohimbine reduced exploratory activity in these tests whereas idazoxan and delequamine did not, even though the doses of yohimbine used were equieffective (10 mg kg^{-1}) or less effective (3 mg kg^{-1}) at blocking central α_2-adrenoceptors, as assessed by effects on clonidine-induced mydriasis, a reliable measure of interaction at central α_2-adrenoceptors (Berridge *et al.*, 1983). Taking the reduced open arm activity in the elevated X-maze as an index of increased anxiety (Handley & Mithani, 1984; Pellow *et al.*, 1985a), it appears from the results of these tests that, unlike yohimbine, delequamine and idazoxan did not have anxiogenic effects at doses effective at blocking central α_2-adrenoceptors. The effects we observed with yohimbine in the elevated X-maze are in agreement with previously reported studies (Handley & Mithani, 1984; Pellow *et al.*, 1985a; Johnston & File, 1989). However, in contrast to the conclusions of Handley & Mithani (1984) our data suggest that acute blockade of central α_2-adrenoceptors *per se* is not associated with increased anxiety in the rat and that the anxiogenic effects of yohimbine therefore may be due to some other property of this rather non-selective drug.

Before considering these non-adrenoceptor interactions of yohimbine, an alternative explanation for its distinctive anxiogenic effect could arise from dissimilar binding profiles of yohimbine and delequamine at the subtypes of the α_2-adrenoceptor. Up to three subtypes have been proposed: α_{2A}, α_{2B} and α_{2C}; a further subtype, α_{2D}, may be the rat homologue of the human α_{2A}-adrenoceptor (Ruffolo *et al.*,1993; MacKinnon *et al.*, 1994). There is evidence for the existence of two of these subtypes in rat brain, namely $\alpha_{2A/D}$ and α_{2C} (Lorenz *et al.*, 1990; Uhlen *et al.*, 1992; MacKinnon *et al.*, 1992); they have different anatomical distributions (Wamsley *et al.*, 1992), which may suggest heterogeneous expression on neurones of different neurotransmitter identities. Indeed, as well as presynaptic autoreceptors on noradrenergic neurones, which are believed to be of the α_{2D} subtype in the rat cortex (Trendelenburg *et al.*, 1993), there are also α_2-adrenoceptors on 5-hydroxytryptamine (5-HT) cell bodies and terminals which mediate tonic inhibitory influences of noradrenaline on 5-HT neurotransmission (Garratt *et al.*, 1991; Tao & Hjorth, 1992; Rosin *et al.*, 1993; Feuerstein *et al.*, 1993). Although these are distinct from the $\alpha_{2A/D}$ subtype, their precise identity is still unclear (Maura *et al.*, 1992; Gobbi *et al.*, 1993). Yohimbine has a relatively higher affinity for α_{2B}- and α_{2C}- than $\alpha_{2A/D}$-adrenoceptors (see MacKinnon *et al.*, 1994), but delequamine has high, equivalent affinities for each of the subtypes (Brown *et al.*, 1993; J.W. Regan, personal communication). In order to explain our data in terms of subtype selectivity one could postulate that yohimbine is preferentially blocking the inhibitory α_2-adrenoceptors on 5-HT neurones, and thereby enhancing 5-HT neurotransmission, which would raise the level of anxiety (see Iversen, 1984; Chopin & Briley, 1987) whereas delequamine is simultaneously blocking the noradrenergic autoreceptors, with no net effect on 5-HT neurotransmission. Certainly, yohimbine, at a dose (5 mg kg^{-1}, i.p.) intermediate between those of the present study, more than doubles the release of 5-HT in the frontal cortex in conscious rats (Cheng *et al.*, 1993). In contrast, studies of the effects of delequamine on the levels of 5-HT and its metabolite, 5-hydroxyindole acetic acid, in rat brain indicated an absence of any effect, at a dose (0.5 mg kg^{-1}, p.o.) which doubled the concentration of the noradrenaline metabolite, 3-methoxy-4-hydroxy-phenylglycol (Redfern *et al.*, 1993). However, a more straightforward explanation for these effects of yohimbine on 5-HT neurotransmission involves non-adrenoceptor interactions. Yohimbine has a high affinity for $5\text{-}HT_{1A}$ receptors (Winter & Rabin, 1992), possessing partial agonist activity (Arthur *et al.*, 1993); such ligands are anxiogenic in rats tested in the elevated X-maze (Moser, 1989). Other possible explanations for the anxiogenic action of yohimbine include a dual action at adrenoceptors and dopamine receptors (Johnston & File, 1989); the interaction of yohimbine with the benzodiazepine modulatory site on the $GABA_A$ receptor (Lal *et al.*, 1983) has been discounted as an explanation of its anxiogenic effects (Pellow *et al.*, 1985b).

Are the results of our tests relevant to the anxiogenic effects of yohimbine in human subjects? The elevated X-maze has recently been both championed (Handley & McBlane, 1993) and criticised (Dawson & Tricklebank, 1995), on the grounds of its usefulness as a model of human anxiety, and for its predictive value with respect to clinically-useful anti-anxiety agents. However, this is beyond the scope of our application of this test; we merely employed it to detect a behavioural effect of yohimbine, consistent with its anxiogenic properties, and to show that this was not shared by two more selective agents of the same pharmacological class, at a dose which blocked central α_2-adrenoceptors. Furthermore, behavioural effects of yohimbine were even detected at a dose which only partially affected central α_2-adrenoceptors. We therefore conclude that blockade of central α_2-adrenoceptors *per se* does not have an anxiogenic effect, at least in the rat.

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