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Potential serotonergic and noradrenergic involvement in the discriminative stimulus effects of the selective imidazoline I_2-site ligand 2-BFI

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Abstract

The functional significance of imidazoline I_2 binding sites is unknown but microdialysis studies have indicated that the administration of I_2-site ligands leads to an increase in extracellular levels of monoamines. The specific I_2-site ligand 2-(-2-benzofuranyl)-2-imidazoline (2-BFI) generates a cue in drug discrimination, thereby indicating functional consequences of I_2-site ligand binding. In the present work, we explored the ability of selective noradrenergic and serotonergic ligands to substitute for 2-BFI. Hooded Lister rats were trained in two-lever operant chambers with condensed milk reward to distinguish 2-BFI (7 mg/kg) from saline vehicle, by pressing the correct lever to a predetermined success criterion. Training sessions were then interspersed with sessions in which animals were administered test substances and the proportion of lever presses on the 2-BFI-associated lever (substitution) recorded. Several agents exhibited significant partial substitution for 2-BFI: The monoamine-releasing agents D-amphetamine and fenfluramine dose-dependently substituted for 2-BFI, while norepinephrine (desipramine, reboxetine) and serotonin (clomipramine, citalopram) reuptake inhibitors substituted at one or more doses. Further investigation using specific receptor agonists and antagonists indicated a possible role for activation of α_1-adrenoceptors but failed to support involvement of α_2-adenoceptor, β-adrenoceptor or 5-HT_{1A} receptor activation. These results support the concept that the 2-BFI cue may contain both noradrenergic and serotonergic components.

Keywords: In vivo; Drug discrimination; Imidazoline I_2-site; Serotonin; Norepinephrine; Rat

1. Introduction

Imidazoline binding sites were originally identified as the nonadrenergic component of imidazoline binding (Michel and Ernsberger, 1992) and have been separated into at least three sites, I_1, I_2 and I_3 (Eglen et al., 1998). Imidazoline I_2-sites exhibit a discrete regional distribution in rat brain (Lione et al., 1998; MacInnes and Handley, 2001) but their functional significance is unknown, since neither their molecular structure(s) nor their second-messenger system has been elucidated. However, an imidazoline I_2-site has been localised to the enzyme monoamine oxidase (MAO; Raddatz et al., 1997; Escriba et al., 1999; Remaury et al., 2000). There have been relatively few functional studies of I_2-site ligands in vivo: I_2-site ligands have been found to increase food intake (Jackson et al., 1991; Brown et al., 1995; Menargues et al., 1995; Prasad and Prasad, 1996; Polidori et al., 2000), decrease immobility in the forced-swim test (Nutt et al., 1995) and potentiate morphine analgesia (Kolesnikov et al., 1996; Li et al., 1999; Sánchez-Blázquez et al., 2000).

The highly specific I_2-site ligand 2-(-2-benzofuranyl)-2-imidazoline (2-BFI) generates a cue in a two-lever rat drug discrimination paradigm (Jordan et al., 1996) and this cue generalises to other selective I_2-ligands (MacInnes and Handley, 2002). Microdialysis studies have shown that I_2-site ligands can increase extracellular levels of brain norepinephrine (Nutt et al., 1995; Hudson et al., 1999), dopamine (Hudson et al., 1999; Sastre-Coll et al., 2001) and serotonin (Adell et al., 1996; Ugedo et al., 1999). The pattern of substitution by other substances for 2-BFI suggests the possibility that such increases may be important in generating its discriminable stimulus. Thus, reversible inhibitors of MAO-A, but not of MAO-B, substituted for 2-BFI irrespective of whether or not they also bind to I_2-sites (MacInnes

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and Handley, 2002). The putative antiaddictive substance, ibogaine, which causes large increases in extracellular serotonin (Wei et al., 1998), also substituted (MacInnes and Handley, 2002). In addition, the α_2-adrenoceptor antagonist, ethoxy idazoxan, substituted partially for 2-BFI despite its negligible affinity for I_2-sites (Jordan et al., 1996).

In the present work, we have explored the potential role of monoamines, particularly norepinephrine and serotonin, in contributing to the 2-BFI cue, by investigating the ability of monoamine-releasing agents, selective norepinephrine and serotonin reuptake inhibitors and several noradrenergic direct agonists to substitute for 2-BFI in drug discrimination. In all cases, the doses used were within the range previously shown active in behavioural experiments (Handley and Singh, 1986; McElroy and O'Donnell, 1988; Hughes et al., 1996; Jordan et al., 1995) and the risk of toxic effects was borne in mind in establishing the maximum dose administered.

2. Materials and methods

2.1. Subjects

Four groups of eight pair-housed male Hooded Lister rats (Charles River, UK), with a starting weight 100 g, were housed at an ambient temperature of 21 °C, humidity 45%, on a 12-h light/dark cycle (lights on at 0800 h) with free access to food and water. All work was performed in conformity with the Animals (Scientific Procedures) Act, 1986. Animals were transferred to an adjacent room for testing daily between 1000 and 1400 h (weekdays only).

2.2. Apparatus

Two-lever operant conditioning units (Campden Instruments, UK) were controlled by a remote Apple IIe computer using 'Operant Program for the Neurosciences' (Emmett-Oglesby et al., 1982). Each of the eight conditioning units contained a delivery hatch, equidistant from each of the two levers. Upon completion of the various fixed ratio (FR) schedules, a dipper presented a reward of sweetened condensed milk (diluted 1 part milk to 2 parts water; Nestle, UK). Food deprivation was not required as nondeprived rats show a high level of lever pressing for a condensed milk reward (Jordan et al., 1995, 1996).

2.3. Procedure

Following preliminary training to ensure rats consistently pressed either lever without bias to obtain one reward for every 10 lever presses (FR10), rats were admitted to daily 15-min training sessions with either 2-BFI (7 mg/kg^{-1}) or saline vehicle administered intraperitoneally 20 min before each session. No maximum was set to the number of reinforcers available during each training session. Discrimination training occurred in a repeated sequence, SDDSSDSSDD (S = saline day, D = drug day) and rats were rewarded on the FR10 schedule for pressing the 'correct' lever for that training day, i.e. either drug or saline. For 50% of rats, the left lever was set to deliver reward (i.e. correct) if the rats had received 2-BFI and the right lever if saline had been administered, with levers reversed for the remaining animals (Sanger, 1989).

Criteria for entry into test sessions were 10 consecutive training sessions where (i) $\geq$ 90% of all responses during the session were on the correct lever and (ii) at least 7 of the first 10 lever presses of the session were on the correct lever. Test days were added into the training cycle: STDTSDTSTD (D = drug day). Test sessions ended after 10 responses on one lever or 30 min, whichever was sooner; no reward was administered. Data from rats failing to complete a session, i.e. failing to make 10 responses on any one lever within 30 min, were excluded. The number of responses to the 2-BFI-associated lever was expressed as percentage of total responses. The group mean of these percentages represented the ability of a drug to substitute for 2-BFI. Rates of responding (responses per minute, RPM) were recorded for all rats completing the session. All doses of a single drug and associated saline control were given in a balanced pseudorandom order.

2.4. Statistics

Effects of treatment were analysed using repeated-measures analysis of variance, with degrees of freedom adjusted according to Mauchly's test of sphericity, and individual doses compared with vehicle by a priori contrasts (Statistical Package for the Social Sciences, Version 10).

2.5. Drugs

Drug treatments were distributed between groups as follows: Group 1, salbutamol, dobutamine, phenylephrine, clonidine; Group 2, WB4101 prazosin; Group 3, ST587, methoxamine, fenfluramine; Group 4, D-amphetamine, desipramine, WAY 100635, reboxetine, clomipramine, paroxetine, citalopram.

All drugs were dissolved in 0.9% physiological saline except for reboxetine and WB4101, which were dissolved in deionised water. All drugs were administered intraperitoneally using a dose volume of 1 ml/kg 20 min before testing. Drugs that were administered as antagonists, i.e. prazosin, WB4101, WAY 100635, were administered 20 min before 2-BFI administration and testing commenced 20 min after this. Control data for these conditions were derived by replacing the antagonist with saline.

The following drugs were used: 2-BFI, gift from Phillipe Ladure, Pierre Fabre, (France); ST587, gift from Boehringer Ingelheim (Germany); reboxetine, gift from Pharmacia and Upjohn (UK); citalopram, gift from Lundbeck Limited (UK); paroxetine, gift from SmithKline Beecham (UK); desipra-

mine, clonidine, clomipramine, fenfluramine, WAY 100635, phenylephrine, methoxamine, D-amphetamine, prazosin, WB4101, salbutamol, dobutamine were purchased from Sigma (UK).

3. Results

All groups of rats reached criterion, i.e. 7 out of the first 10 lever presses and 90% of the total training session

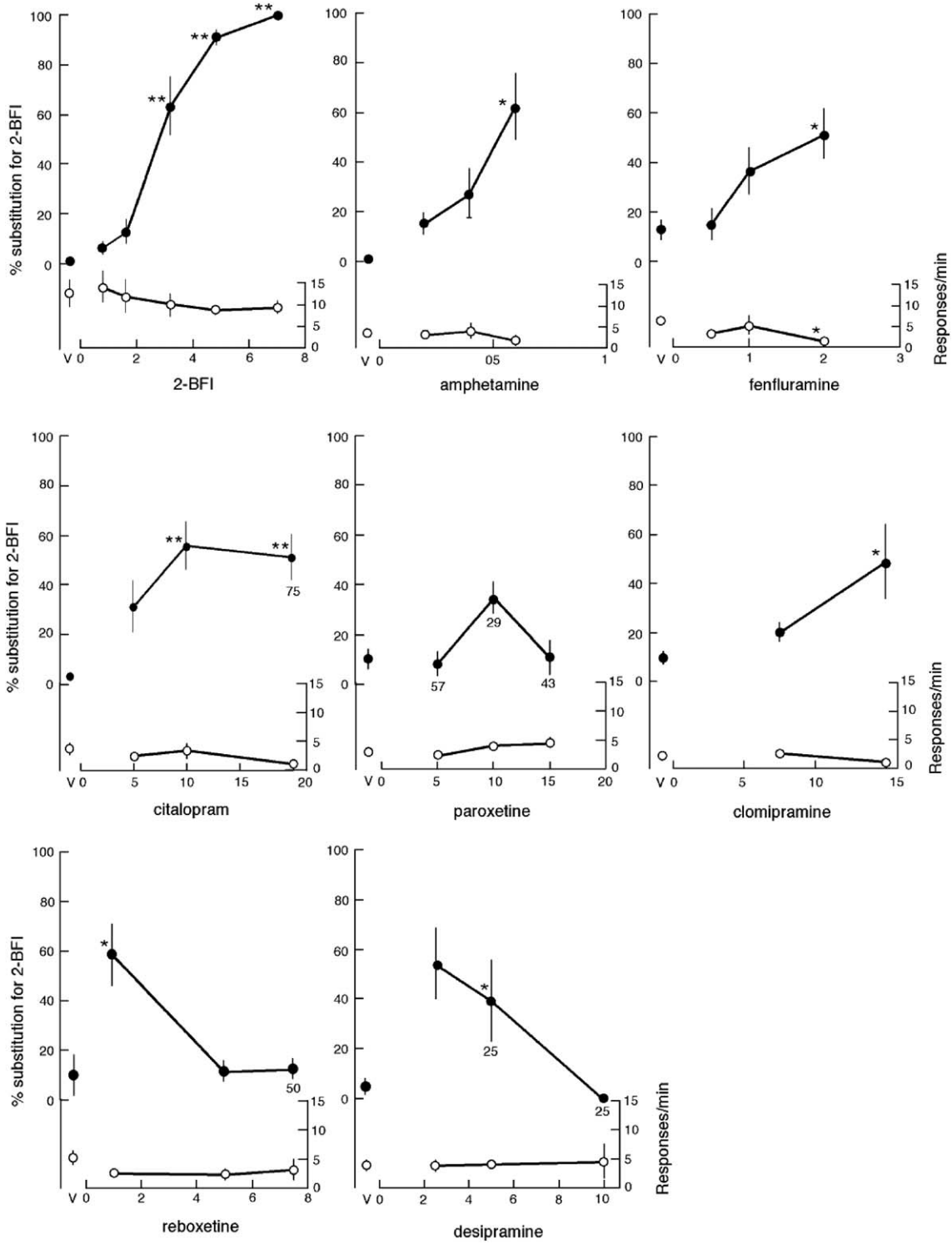

Fig. 1. The ability of 2-BFI itself, monoamine-releasing agents and reuptake inhibitors to substitute for 2-BFI. Closed circles: percentage of substitution for 2-BFI, 7 mg/kg (left-hand scale); open circles: RPM (right-hand scale). *X*-axis shows dose in milligrams per kilogram. Data are means (± S.E.M.) from eight rats, except 2-BFI ($n = 10$), paroxetine and amphetamine ($n = 7$). All animals completed the 30-min session except where percentage completion is shown beneath a data point. V: vehicle control. * $P < .05$, * * $P < .01$, relative to vehicle control.

lever presses were to the correct lever after a similar number of training sessions (Group 1, 52.2 ± 15.1; Group 2, 60.3 ± 21.4; Group 3, 63.5 ± 21.7; Group 4, 54.3 ± 13.1), and levels of discrimination remained stable throughout testing. 2-BFI given as a test substance dose-dependently substituted for the training dose with no significant changes in response rate (Fig. 1). The training dose itself produced 100% substitution. The monoamine-releasing agents D-amphetamine [$F(3,18)=11.51$, $P<.001$] and fenfluramine [$F(3,15)=5.85$, $P<.01$] dose-dependently substituted for 2-BFI over the dose range tested, maximum substitution reaching 63% for D-amphetamine and 52% for fenfluramine (Fig. 1). Response rates decreased at the highest doses tested.

The serotonin reuptake inhibitors citalopram [$F(3,15)=8.7$, $P<.01$] and clomipramine [$F(2,20)=7.5$, $P<.01$] induced significant substitution for 2-BFI (Fig. 1), although two rats failed to complete the session with the highest dose of citalopram. In the case of citalopram, substitution appeared to plateau at 10 mg/kg (56%) and did not increase as the dose was raised further. Clomipramine produced significant substitution at 15 mg/kg. Paroxetine decreased session completion at all doses tested (see Fig. 1 legend) and could not be analysed statistically.

The norepinephrine reuptake inhibitors reboxetine and desipramine proved capable of inducing significant substitution for 2-BFI but substitution declined as doses were increased, despite lack of change in response rates (Fig. 1). Reboxetine produced 50% completion failure at 7.5 mg/kg and this dose was excluded from the analysis. All rats completed the remaining sessions and there was a significant effect of treatment [$F(2,14)=12.61$, $P<.01$] with 59% substitution at 1 mg/kg ($P=.001$). However, substitution declined when the dose was increased to 5 mg/kg. In the case of desipramine, 54% substitution ($P<.05$, paired t test) was seen at 2.5 mg/kg but only two rats completed the sessions at higher doses.

Investigation of noradrenergic α_1-adrenoceptor agonists (Fig. 2) showed that methoxamine was able to substitute for 2-BFI [$F(4,28)=3.69$, $P<.05$] and that this substitution reached a plateau of 64% at 0.75 mg/kg with a higher dose failing to substitute significantly. Response rates appeared to decline as doses were increased. In the case of phenylephrine, individual doses of 1.0 and 1.5 mg/kg substituted

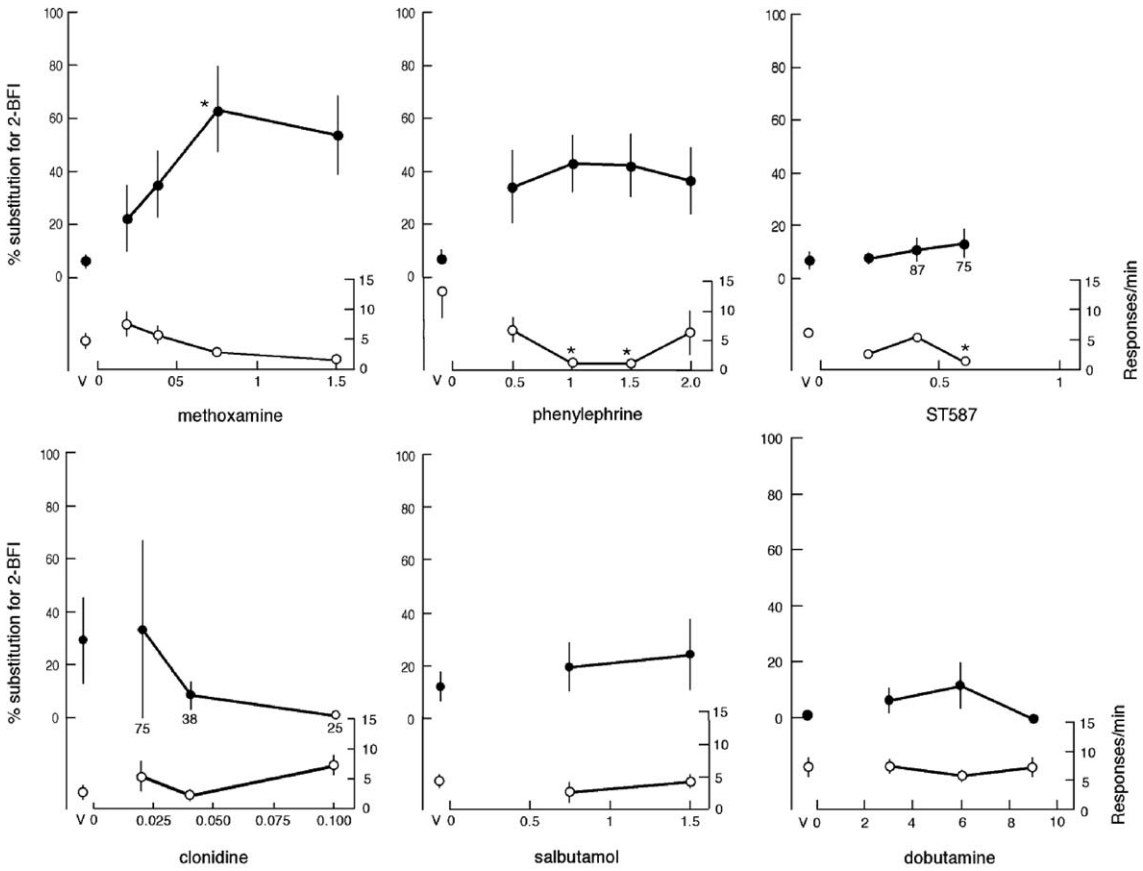

Fig. 2. The ability of some noradrenergic agents to substitute for 2-BFI. Closed circles: percentage of substitution for 2-BFI, 7 mg/kg (left-hand scale); open circles: RPM (right-hand scale). X-axis shows dose in milligrams per kilogram. Data are means (± S.E.M.) from eight rats. All animals completed the 30-min session except where percentage completion is shown beneath a data point. V: vehicle control. * $P<.05$, relative to vehicle control.

significantly ($P < .05$) but this effect could not be considered reliable since the overall effect of treatment failed to reach significance [$F(4,28) = 2.49$, $P = .066$]. ST587 [$F(2,12) = 1.19$, $P > .3$] failed to substitute up to doses significantly reducing response rates. The β_1-adrenoceptor agonist dobutamine [$F(2,14) = 0.84$, $P > .05$] and the β_2-adrenoceptor agonist salbutamol [$F(2,14) = 0.65$, $P > .05$] failed to substitute at any dose tested. In the case of clonidine, too few animals completed the sessions for statistical analysis, little 2-BFI-appropriate responding was seen among those that did complete.

Pretreatment with the α_1-adrenoceptor antagonist WB4101 (1.5 and 3.0 mg/kg) reduced the ability of 2-BFI (7 mg/kg) to cause responding on the drug-appropriate lever (Fig. 3). However, prazosin (0.5 and 1.0 mg/kg) failed to alter the effect of 2-BFI (Fig. 3). Neither WB4101 nor prazosin substituted for 2-BFI when given alone. The 5-HT$_{1A}$ antagonist WAY 100635 (0.8 mg/kg) did not induce 2-BFI-apropriate responding, neither did it reduce that of 2-BFI when administered 20 min previously (Table 1).

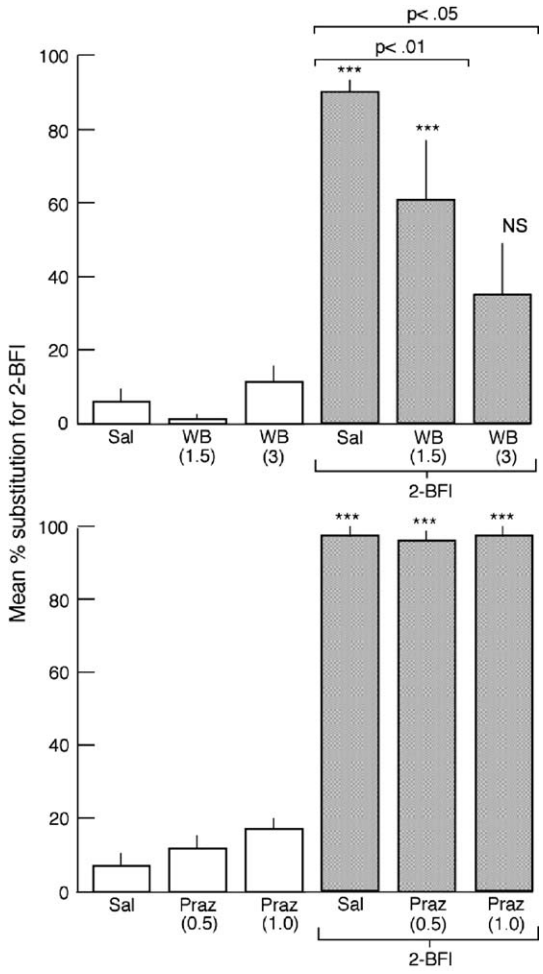

Fig. 3. Antagonism of 2-BFI—appropriate responding following pretreatment with WB4101 or prazosin. Sal: saline control; WB (1.5): WB4101, 1.5 mg/kg; WB (3): WB4101, 3 mg/kg; Praz (0.5): prazosin, 0.5 mg/kg; Praz (1): prazosin, 1 mg/kg; 2-BFI dose was 7 mg/kg. *** $P < .001$ compared to the corresponding control in the absence of 2-BFI. NS $P > .05$ compared to the corresponding control in the absence of 2-BFI.

Table 1
Effect of WAY 100635 on 2-BFI responding

	Dose (mg/kg)	Mean percentage of substitution for 2-BFI ± S.E.M.	No. entering/ No. completing session	Mean rate of responding ± S.E.M.
Saline		10.7 ± 3.3	7/7	2.4 ± 0.5
Saline + 2-BFI	7.0	97.1 ± 2.1 ***	7/7	4.38 ± 0.97
WAY 100635	0.8	9.0 ± 4.3**	7/7	2.1 ± 0.5
WAY 100635 + 2-BFI	0.8	97.1 ± 2.9 ***	7/7	4.34 ± 1.16
	7.0	*		

* Not significantly different from saline + 2-BFI, $P > .05$.
** Not significantly different from saline control.
*** Significantly different from saline control, $P < .001$.

4. Discussion

2-BFI itself, when administered as a test drug, produced dose-dependent substitution, reaching 100% at the training dose of 7.0 mg/kg. Its discriminative stimulus appears to be pharmacologically specific, in that other I_2-site ligands, reversible MAO-A inhibitors and ibogaine substitute fully for 2-BFI, while reversible MAO-B inhibitors, SKF 10,047, amiloride and diazepam neither substitute nor disrupt responding (Jordan et al., 1996; MacInnes and Handley, 2002). This specificity was further confirmed here by the inability of, for instance, salbutamol and dobutamine to produce any 2-BFI-appropriate responding.

In the present study, the monoamine-releasing agents D-amphetamine and fenfluramine substituted partially and dose-dependently over the dose range tested. Although a tendency to reduce rates of responding precluded extension of the dose range, this finding indicates a potential role of elevated synaptic concentrations of one or more monoamines in generating the 2-BFI cue. Consistent with this suggestion, both noradrenergic and serotonergic reuptake inhibitors exhibited significant substitution at one or more doses. Again, interference with responding prevented extension of the dose range but significant levels of substitution were observed with one or more doses of citalopram, clomipramine, reboxetine and desipramine that did not significantly affect rates of responding. However, it is not clear whether the apparent inverted-U-shaped dose–response relationship seen with reboxetine and desipramine was a real effect or an artefact of response disruption. Response disruption has been observed previously with desipramine and methoxamine in methamphetamine-trained rats (Munzar and Goldberg, 1999) and with D-amphetamine, desipramine and fenflur-

amine in nicotine-trained rats (Mansbach et al., 1998) but, while serotonin reuptake inhibitors had marked effects on responding rates in the present experiments, other studies have shown it possible to train rats to discriminate citalopram 2.5 mg/kg from saline, and paroxetine did not reduce response rates in these rats (Millan et al., 1999). Similarly, we have previously trained rats to discriminate clonidine 0.2 mg/kg from saline (Jordan et al., 1993) but 0.1 mg/kg caused responding to cease in 75% of the present 2-BFI-trained rats. Thus, the ability of a drug to disrupt responding may be related to the training drug used.

The potential contribution of norepinephrine to the 2-BFI cue was investigated by administration of several direct agonists. No evidence was found for β-adrenoceptor involvement, in that neither the β_1-adrenoceptor agonist dobutamine nor the β_2-adrenoceptor agonist salbutamol produced 2-BFI-appropriate responding. The potential role of the α_2-adrenoceptor is complex: 2-BFI has negligible affinity for α_2-adrenoceptors (Nutt et al., 1995), yet α_2-adrenoceptor antagonists that do not bind to I_2-sites induce 2-BFI-appropriate responding (Jordan et al., 1996). It has been suggested that this could be due to an increase in synaptic norepinephrine, consequent on blocking presynaptic α_2-adrenoceptors, thus mimicking a noradrenergic component of the 2-BFI discriminative stimulus (Jordan et al., 1996). This explanation also suggests that postsynaptic α_2-adrenoceptor activation cannot be important in generating the 2-BFI cue, since these receptors would have been blocked by the α_2-adrenoceptor antagonist. This was supported in the present work by the failure of clonidine to produce any significant amount of substitution for 2-BFI in those rats that continued to respond.

α_1-Adrenoceptor agonists differed in their effects. Methoxamine produced up to 64% substitution for 2-BFI at a dose of 0.75 mg/kg, which did not significantly affect responding, and this may have been a maximum effect since a higher dose did not substitute significantly. The substitution produced by phenylephrine, however, did not reach statistical significance and ST587 did not produce 2-BFI-appropriate responding. ST587 is a partial agonist at α_2-adrenoceptors (Badia and Salles, 1989; Salles et al., 1994) and this may explain why, as with clonidine, its testable dose range was severely limited by response failure. Failure to penetrate the brain sufficiently does not appear to explain the effects of the α_1-adrenoceptor agonists. It has not yet been firmly established whether the 2-BFI cue is central in origin, although it clearly does have other central effects after its peripheral administration (Olmos et al., 1994; Nutt et al., 1995; Hudson et al., 1999). ST587 is highly lipophilic (Jonge et al., 1981). There is also good evidence that both methoxamine and phenylephrine have direct central effects, since both these agents were able to induce membrane translocation of protein kinase C in the cortex and the hippocampus after their peripheral administration, in doses similar to those used here (Szmigielski and Gorska, 1997). Methoxamine substitution for phenylethylamine in drug discrimination has been attributed to the central actions of these compounds (Schechter, 1991), although methoxamine's discriminative stimulus has also been attributed to an increase in blood pressure (Lal et al., 1990). However, I_2-site ligands do not appear to affect blood pressure or heart rate (Brown et al., 1995).

α_1-Adrenoceptor antagonists were investigated to throw further light on the potential role of these receptors. 2-BFI responding was substantially and dose-dependently reduced by WB4101. However, prazosin was ineffective, and interestingly, similar doses of prazosin were unable to antagonise the amphetamine, or methamphetamine, cues in drug discrimination (Arnt, 1996; Munzar and Goldberg, 1999) but were able to decrease discrimination in phenylephrine-trained rats (Schechter, 1991). Unlike prazosin, WB4101 is approximately 30-fold selective for the α_{1A}- over the α_{1B}-adrenoceptor subtype (Morrow and Creese, 1986), raising the possibility of a selective involvement of α_{1A}-adrenoceptors in the 2-BFI cue. An alternative explanation could lie in the affinity of WB4101 for 5-HT_{1A} receptors (Norman et al., 1985); however, the 5-HT_{1A} antagonist WAY 100635 was without effect, suggesting that 5-HT_{1A} receptor activation may not be relevant either to the putative serotonergic component of the 2-BFI cue or to the antagonistic actions of WB4101.

The mechanism by which imidazoline I_2-site ligands raise synaptic concentrations of monoamine remains to be established. These ligands bind to a site on MAO distinct from the catalytic site (Alemany et al., 1995) and the high-affinity component of this binding appears to be located to MAO-B (Remaury et al., 2000), yet the discriminative stimulus generated by 2-BFI generalises to reversible MAO-A but not MAO-B inhibitors (MacInnes and Handley, 2002), raising the possibility that this cue is related to inhibition of MAO-A through binding to the low-affinity I_2-site located on this isoform (Remaury et al., 2000). The results from the present study provide evidence that its ability to increase synaptic concentrations of both norepinephrine and serotonin (Nutt et al., 1995; Adell et al., 1996; Hudson et al., 1999; Ugedo et al., 1999) may contribute to its discriminative stimulus. The α_1-adrenoceptor remains a candidate among postsynaptic noradrenergic receptors and a serotonergic component could explain why ibogaine also substitutes for 2-BFI (MacInnes and Handley, 2002), since this compound is a potent elevator of synaptic serotonin (Wei et al., 1998).

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