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Short communication

Region-dependent effects of acute and chronic tranylcypromine in vivo on [^{3}H]2-BFI binding to brain imidazoline I_2 sites

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Abstract

An imidazoline I_2 site has been localised to monoamine oxidase. However, in vitro studies of the effect of monoamine oxidase inhibitors on imidazoline I_2-site radioligand binding have produced conflicting findings. Using the technique of autoradiography, we examined the effect of in vivo administration of the irreversible monoamine oxidase inhibitor tranylcypromine on binding of the imidazoline I_2 site-specific ligand [^{3}H]2-(-2-benzofuranyl)-2-imidazoline ([^{3}H]2-BFI) in four rat brain nuclei which are known to possess a high density of imidazoline I_2 sites, together with cerebral cortex and cerebellum which show weaker binding. A single acute pre-treatment with tranylcypromine significantly increased imidazoline I_2 site-specific binding in four regions: arcuate nucleus, interpeduncular nucleus, pineal gland and area postrema, but effects in cortical areas and cerebellum were not significant. The extent of the increase was proportional to the control binding in each region. In contrast, five daily treatments with the same dose of tranylcypromine significantly reduced [^{3}H]2-BFI binding in these same areas. The potential role of monoamine oxidase isoforms in these changes is discussed.

Keywords: Imidazoline I_2 site; [^{3}H]2-BFI([^{3}H]-2-(-2-benzofuranyl)-2-imidazoline; Autoradiography; Tranylcypromine; Brain; (Rat)

1. Introduction

Over 20 years of research has indicated that α_2-adrenoceptor ligands which contain an imidazoline moiety bind to sites other than α_2-adrenoceptors (Eglen et al., 1998). The imidazoline site has been separated into three subtypes, I_1, I_2 and I_3 (Michel and Ernsberger, 1992; Chan et al., 1994). The clonidine preferring imidazoline I_1 and idazoxan preferring imidazoline I_2 sites are located throughout the body and brain (Tesson et al., 1992; Diamant et al., 1992; Hudson et al., 1996; Lione et al., 1998), whilst the methoxy-idazoxan-preferring imidazoline I_3 site is located exclusively within the pancreatic β cells of the Islets of Langerhans (Chan et al., 1994). Molecular, pharmacological and immunocytochemical techniques have suggested that an imidazoline I_2 site may be localised to the enzyme monoamine oxidase (Alemany et al., 1995; Raddatz et al., 1997; Lalies et al., 1999; Escriba et al., 1999; Remaury et al., 2000). Monoamine oxidase catalyses the oxidative deamination of 5-hydroxytryptamine, dopamine, noradrenaline, and dietary amines (Von Korff, 1979; Kalgutkar et al., 1995).

In vitro studies with human brain homogenates have indicated that co-incubation with the irreversible monoamine oxidase-B inhibitor, tranylcypromine, concentration-dependently increases imidazoline I_2 site density measured by the binding of [^{3}H]2-(-2-benzofuranyl)-2-imidazoline ([^{3}H]2-BFI) while leaving affinity unchanged; however, under identical conditions, no potentiation of [^{3}H]2-BFI binding occurred in the cortex of the rat (Wiest and Steinberg, 1997). The effect is particular to tranylcypromine as co-incubation with pargyline or clorgyline failed to alter [^{3}H]2-BFI binding (Wiest and Steinberg, 1997). Indeed, cyclopropylamine monoamine oxidase inhibitors such as tranylcypromine inhibit monoamine oxidase in a manner different from that of the acetylenic monoamine oxidase inhibitors pargyline and deprenyl (see Kalgutkar et al., 1995 for a review). Co-incubation of tranylcypromine with human recombinant monoamine oxidase-B, but not human recombinant monoamine oxidase-A or rat recombinant monoamine oxidase-B also gave rise to potentiation of [^{3}H]2-BFI binding (Steinberg et al., 1999).

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Chronic in vivo administration of tranylcypromine (10 mg/kg twice daily for 7 days) had the opposite effect, significantly reducing the density of [^{3}H]idazoxan radiolabelled imidazoline I_2 sites in rat cortical homogenates (Alemany et al., 1995), an effect that is consistent with a loss of imidazoline I_2 binding sites associated with monoamine oxidase (Richards et al., 1998).

Autoradiographic studies of imidazoline I_2 sites, as defined by [^{3}H]idazoxan or the imidazoline I_2 site-specific ligands [^{3}H]RS-45041-190 ([^{3}H]4-chlo-2-(imidazolin-2-yl) isoindoline) and [^{3}H]2-BFI, show these sites exhibit a discrete regional distribution in rat brain: all three ligands label the interpeduncular nucleus, area postrema and arcuate nucleus with high levels of tritiated ligand (>60 fmol/mg), whilst [^{3}H]2-BFI also strongly labels the pineal gland; in contrast the cerebral cortex exhibits much lower levels of binding (Mallard et al., 1992; MacKinnon et al., 1995; Lione et al., 1998). Thus, an autoradiographic approach is particularly well-suited to determining whether the potentiation of imidazoline I_2 site ligand binding by tranylcypromine that has been observed in some preparations in vitro (Wiest and Steinberg, 1997; Steinberg et al., 1999) also occurs after its in vivo administration. It may also be able to resolve whether this effect is regionally specific in the rat, potentially explaining the lack of potentiation in rat cortical homogenates (Wiest and Steinberg, 1997).

In view of the inconsistent findings that have been obtained in vitro, the major aim of the present study was to determine the effect of acute in vivo administration of tranylcypromine, in the rat, on imidazoline I_2 site ligand binding. By adopting an autoradiographic approach, we were able to target specific brain regions rich in imidazoline I_2 sites and compare them with the cerebral cortex. Effects of chronic tranylcypromine were also examined, both for comparison with acute effects and to determine whether the reduction in binding previously reported in cortical homogenates (Alemany et al., 1995) can be detected autoradiographically.

2. Materials and methods

2.1. Animals and drug treatment

Male Wistar rats (250–300 g), four per treatment group, were used. In the acute experiment rats received tranylcypromine (2.5 mg/kg) 45 min before intra-cardiac perfusion, whilst those used for the chronic experiment received tranylcypromine (2.5 mg/kg) once a day for 5 days. Tranylcypromine (*trans*-2-phenylcyclopropylamine hydrochloride, R.B.I.) was dissolved in 0.9% saline and administered intra-peritoneally (i.p.) in a final volume of 1 ml/kg. Control groups were dosed with 0.9% saline vehicle (1 ml/kg) following an identical protocol.

2.2. Autoradiography

Forty-five minutes after the last injection animals were anaesthetised with (halothane/nitrous oxide) and underwent intra-cardiac perfusion with 100 ml ice-cold phosphate (0.01 M) buffered saline (pH 7.4). Brains were removed and frozen in isopentane at -30 °C. Transverse sections (12 μM) were cut at -16 °C on a cryostat (Bright OTF, Bright Instruments, UK), thaw mounted onto Chrom-alum gelatin coated slides and stored at -70 °C until used. Nuclei of interest were identified by comparison with adjacent 30 μM cresyl violet stained sections according the rat brain atlas of Paxinos and Watson (1998). Cortical areas were examined on the same sections as the nuclei of interest.

Autoradiographic assays followed the method of Lione et al. (1998); 0.5 nM [^{3}H]2-BFI ([5,7-(*n*)-^{3}H]2-(2-benzofuranyl)-2-imidazoline; specific activity 70 Ci/mmol; radioactive concentration 200 μCi/ml, Amersham, UK) was used for the acute experiment, while for the chronic experiment an increase to 1 nM was required following pilot experiments that confirmed a marked reduction in imidazoline I_2 site ligand binding by tranylcypromine as previously reported (Alemany et al., 1995). 10 μM BU224 HCl (2-(4,5-dihydroimidaz-2-yl)-quinoline hydrochloride, R.B.I) was used to determine non-specific binding. Slides were apposed to [^{3}H]Hyperfilm (Amersham) for 6 weeks. The resulting autoradiograms were quantified by computer-assisted densitometry (MCID version 4, Microcomputer Image Device, Imaging Research, St Catherines, OT, Canada) and values converted to fmol ^{3}H-ligand/mg wet tissue with ^{3}H-microscale standards (Amersham). For each region of interest examined, one data point was obtained for each rat by collapsing the results from three sections per nucleus and three determinations per section. Statistical analysis was by one- or two-way analysis of variance (ANOVA) with Tukey-*B* post-hoc tests. Correlation coefficients (r) were calculated from the raw data.

3. Results

3.1. Acute administration of tranylcypromine

In vehicle-treated animals, levels of [^{3}H]2-BFI (0.5 nM) specific binding (Table 1) differed significantly between the areas examined ($F(7,22) = 99.721$, $P < 0.0001$). The groups of brain regions defined by post-hoc analysis as significantly different from one another were: arcuate nucleus > area postrema > interpeduncular nucleus and pineal gland > cortical areas and cerebellum. Acute administration of tranylcypromine (one dose 2.5 mg/kg 45 min before perfusion) increased [^{3}H]2-BFI specific binding in a number of regions (Table 1). Comparison of vehicle and tranylcypromine-treated groups demonstrated significant

Table 1
Effects of acute tranylcypromine administration on specific binding of [^{3}H]2-BFI in rat brain nuclei

Region	Control	Tranylcypromine	%Change	Sig
	fmol/mg tissue			
Arcuate nucleus	56.5 ± 3.6	106.1 ± 6.5	+88	$P < 0.001$
Area postrema	38.6 ± 2.9	69.2 ± 4.2	+79	$P < 0.005$
Pineal gland	26.1 ± 1.6	44.9 ± 1.4	+72	$P < 0.001$
Interpeduncular nucleus	28.4 ± 2.3	45.5 ± 0.6	+60	$P < 0.001$
Cortex (parietal/occiptal border)	8.5 ± 1.0	14.2 ± 1.0	+67	ns
Cortex (occiptal)	7.5 ± 0.5	11.1 ± 0.7	+48	ns
Cortex (parietal)	6.9 ± 0.4	10.8 ± 0.8	+57	ns
Cerebellum	4.6 ± 0.3	7.7 ± 0.1	+67	ns

Each value represents mean ± standard error of the mean from a minimum of four rats.
%Change: percent change from vehicle control. Sig: significance of difference from vehicle control value for the corresponding region.

main effects of treatment ($F(1,48) = 230.3$, $P < 0.0001$) and region ($F(7,48) = 280.8$, $P < 0.0001$). The significant treatment × region interaction ($F(7,48) = 26.2$, $P < 0.0001$) indicated that the extent of the increase differed significantly between regions. Indeed, the percentage increases in [^{3}H]2-BFI binding after tranylcypromine were significantly correlated with the amount of control binding in each area ($r = 0.47$, $P < 0.01$). Tranylcypromine significantly increased [^{3}H]2-BFI binding in the arcuate nucleus, pineal gland, interpeduncular nucleus and area postrema when compared to the same nuclei in vehicle-treated animals (Table 1). The increases observed in cortical areas and cerebellum were not statistically significant. Non-specific binding defined in the presence of saturating concentrations (10 μM) of BU224 in adjacent 12 μM brain sections was low (< 10%).

3.2. Chronic administration of tranylcypromine

In the control group chronically treated with vehicle, binding varied across areas as for the acute experiment ($F(7,31) = 79.5$, $P < 0.0001$). Chronic administration of tranylcypromine (Table 2) significantly reduced [^{3}H]2-BFI specific binding within the arcuate nucleus, pineal gland, interpeduncular nucleus and area postrema when compared to the same nuclei in vehicle-treated animals (Table 2). The reductions observed in cortical areas and cerebellum were not statistically significant. There was a significant main effect of treatment ($F(1,48) = 32.8$, $P < 0.0001$) and region ($F(7,48) = 115.3$, $P < 0.0001$). The extent of the decrease caused by chronic treatment with tranylcypromine varied across regions (treatment x region interaction $F(7,48) = 5.8$, $P < 0.0001$) but there was no significant relationship between the amount of control binding and the decreases observed in different regions $r = -0.46$, $P > 0.8$).

4. Discussion

As previously reported, [^{3}H]2-BFI specific binding exhibited a distinct regional variation (Mallard et al., 1992; MacKinnon et al., 1995; Lione et al., 1998). In contrast to previous binding studies with rat cortex homogenates (Wiest and Steinberg, 1997), the autoradiographical approach was able to demonstrate that acute administration of the irreversible monoamine oxidase inhibitor tranylcypromine significantly increased [^{3}H]2-BFI specific bin-

Table 2
Effects of chronic tranylcypromine administration on specific binding of [^{3}H]2-BFI in rat brain nuclei

Region	Control	Tranylcypromine	%Change	Sig
	fmol/mg tissue			
Interpeduncular nucleus	52.0 ± 2.1	28.9 ± 4.9	−44	iP < 0.01
Arcuate nucleus	169.8 ± 6.9	122.2 ± 13.5	−28	$P < 0.05$
Pineal gland	41.6 ± 4.5	23.7 ± 2.4	−42	$P < 0.05$
Area postrema	104.9 ± 5.7	43.0 ± 9.6	−59	$P < 0.005$
Cortex (parietal)	8.3 ± 0.5	5.0 ± 0.7	−40	ns
Cortex (parietal/occiptal border)	8.4 ± 0.8	5.2 ± 0.6	−38	ns
Cortex (occiptal)	7.8 ± 0.4	5.6 ± 0.4	−28	ns
Cerebellum	5.0 ± 0.5	2.8 ± 0.7	−44	ns

Each value represents mean ± standard error of the mean from a minimum of four rats.
%Change: percent change from vehicle control. Sig: significance of difference from vehicle control value for the corresponding region.

ding. This effect was seen in brain nuclei previously reported to exhibit high levels of imidazoline I_2 site binding (Mallard et al., 1992; MacKinnon et al., 1995; Lione et al., 1998) and increases in cortical and cerebellar [^{3}H]2-BFI binding were not significant. This regional variation may explain why the previous study did not detect any increase in binding in rat cortical homogenates (Wiest and Steinberg, 1997). At this stage, it is not possible to state whether the potentiation of [^{3}H]2-BFI specific binding was due to a change in B_{MAX} or K_D, but it is consistent with the ability of tranylcypromine to enhance the binding of [^{3}H]2-BFI in human brain homogenates through an increase in B_{MAX} (Wiest and Steinberg, 1997).

The extent of the increase in [^{3}H]2-BFI specific binding across brain regions was proportional to the amount of binding in each region in control animals. The reason for this proportionality is not known, but a potential explanation lies in the suggestion (Wiest and Steinberg, 1997) that tranylcypromine treatment causes a conformational change in monoamine oxidase, or a closely associated protein, exposing cryptic imidazoline I_2 binding sites (Wiest and Steinberg, 1997).

In contrast to acute administration, chronic in vivo administration of tranylcypromine reduced imidazoline I_2 site-specific binding in the same nuclei where enhancement of this binding occurred after its acute administration. A similar effect has previously been observed in rat cortical homogenates after chronic in vivo treatment with tranylcypromine, using [^{3}H]idazoxan as the radioligand (Alemany et al., 1995). Here we show that there is a pronounced regional variation in the size of this effect.

Ligand binding to imidazoline I_2 sites fits a two-site model for which 2-BFI exhibits K_I values of 1.71 and 242 nM, respectively (Lione et al., 1998). Studies with monoamine oxidase-A and monoamine oxidase-B knockout mice indicate that the high-affinity [^{3}H]idazoxan-labelled sites appear to be associated with monoamine oxidase-B and the low affinity-sites with monoamine oxidase-A (Remaury et al., 2000), but the autoradiographic distribution of [^{3}H]2-BFI specific binding in rat brain (Mallard et al., 1992; MacKinnon et al., 1995; Lione et al., 1998 and present findings) does not correspond with that of either monoamine oxidase-A or monoamine oxidase-B when individual nuclei are assessed for the amount of ligand bound (Saura et al., 1992). However, enzyme kinetic studies indicate that imidazoline I_2 site ligands show preference for inhibiting monoamine oxidase-A over monoamine oxidase-B (Lalies et al., 1999), and monoamine oxidase-A, but not monoamine oxidase-B, inhibitors substitute for 2-BFI in a rat drug discrimination paradigm (MacInnes and Handley, 2000, 2001). The autoradiographic distribution of [^{3}H]2-BFI specific binding, obtained with a concentration of [^{3}H]2-BFI close to its K_D for the high-affinity imidazoline I_2 site, may include binding to both isoenzymes, depending on their relative local abundance. Alternatively, the imidazoline I_2 site binding detected autoradiographically may represent a further subgroup of monoamine oxidase: autoradiographic data has suggested that imidazoline I_2 site ligands bind to a smaller number of sites than do either monoamine oxidase-A or monoamine oxidase-B inhibitors (Saura et al., 1992; Lione et al., 1998), and monoamine oxidase-A in particular appeared to be heterogenous in electron-spin resonance studies (Huang and Eiduson, 1977).

The data presented here demonstrates acute in vivo tranylcypromine treatment increased [^{3}H]2-BFI binding in a region-dependent manner, while chronic treatment significantly reduced it in the same regions. Further examination of these effects would assist understanding of the relationship between imidazoline imidazoline I_2 sites and the isoforms of monoamine oxidase. This has implications for the treatment of such disorders as depression and Parkinson's disease (The Parkinson Study Group, 1993; Sambunaris et al., 1997).

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