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Species-selective binding of [^{3}H]-idazoxan to α_2-adrenoceptors and non-adrenoceptor, imidazoline binding sites in the central nervous system

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1 We have used the imidazoline derivative [^{3}H]-idazoxan to define α_2-adrenoceptors and non-adrenoceptor, imidazoline binding sites in cerebral cortex membranes of calf, mouse, rat, guinea-pig and man.

2 Competition experiments using the selective α-adrenoceptor drugs, rauwolscine and corynanthine, indicated that [^{3}H]-idazoxan bound to a single population of sites in the calf and mouse membranes. However, [^{3}H]-idazoxan also labelled non-adrenoceptor, imidazoline binding sites in the rat (15%), guinea-pig (30%) and human (40%) cerebral cortex membranes.

3 Competition experiments with adrenaline and cirazoline in the guinea-pig cortex, verified [^{3}H]-idazoxan binding to both α_2-adrenoceptors and to non-adrenoceptor, imidazoline binding sites.

4 It has been postulated by several groups that [^{3}H]-idazoxan may possess partial agonist activity. To investigate this further, saturation experiments were performed in the cerebral cortex membranes of all five species in the absence and presence of 300 μM guanosine triphosphate (GTP). GTP had no effect on [^{3}H]-idazoxan binding in guinea-pig cerebral cortex; in both rat and mouse membranes 300 μM GTP increased the dissociation constant for [^{3}H]-idazoxan by 2–3 fold without significantly affecting the B_{max}. GTP reduced the B_{max} by approximately 30% and 60% in calf and human cerebral cortex membranes, respectively, without significantly altering the K_d.

5 Saturation experiments were performed in the calf cerebral cortex membranes in the absence and presence of 300 μM GTP with the selective α_2-adrenoceptor agonist [^{3}H]-clonidine and the selective muscarinic antagonist [^{3}H]-quinuclidinyl benzilate (QNB). GTP reduced the B_{max} for [^{3}H]-clonidine without altering the K_d, but failed to affect either the B_{max} or the K_d for [^{3}H]-QNB.

6 Saturation experiments were performed in human cerebral cortex membranes in the presence of α_2-adrenoceptor blockade with and without GTP. GTP 300 μM reduced the B_{max} for [^{3}H]-idazoxan at the non-adrenoceptor, imidazoline binding sites, without affecting the K_d. GTP did not affect [^{3}H]-QNB binding to muscarinic sites.

7 Thus, there is a need to investigate further the pharmacological actions of [^{3}H]-idazoxan in view of its ability to recognise both α_2-adrenoceptors and non-adrenoceptor, imidazoline binding sites and because it might possess agonist activity at some of these sites.

Keywords: [^{3}H]-idazoxan; α_2-adrenoceptors; guanosine triphosphate; non-adrenoceptor; imidazoline binding sites

Introduction

The imidazoline derivative, idazoxan, was originally developed as a selective antagonist for α_2-adrenoceptors (Doxey *et al.*, 1983), and preliminary radioligand binding results supported this view. Hannah *et al.* (1983) showed that in rabbit cerebral cortex membranes, idazoxan displayed over 1000 fold selectivity for α_2-adrenoceptors binding sites labelled by [^{3}H]-clonidine, compared to α_1-adrenoceptor binding sites labelled by [^{3}H]-prazosin. Similarly, Pimoule *et al.* (1983) found that [^{3}H]-idazoxan binding in rat cerebral cortical membranes exhibited the characteristics of an α_2-adrenoceptor. However, while idazoxan selectively inhibited α_2-adrenoceptor-mediated inhibition of twitch responses in the rat isolated vas deferens, α_2-adrenoceptor-mediated mydriasis in the rat and α_2-adrenoceptor pressor responses in the pithed rat at doses or concentrations that failed to affect α_1-adrenoceptor-mediated responses, the degree of selectivity in these functional models was much less than that predicted by the radioligand experiments (Berridge *et al.*, 1983; Doxey *et al.*, 1983).

In a few studies idazoxan has also been reported to display partial agonism at both α_1- and α_2-adrenoceptors. Paciorek & Shepperson (1983) noted that idazoxan produced prazosin-sensitive pressor responses in the pithed rat. Significantly this 'agonist' activity of idazoxan was not shared by various 2-alkyl analogues of idazoxan (Doxey *et al.*, 1984). In the anaesthetized rabbit, Hannah and coworkers (1983) observed transient pressor responses to a bolus injection of idazoxan which were partially reduced by either the selective α_1-adrenoceptor antagonist, prazosin, or the selective α_2-adrenoceptor antagonist, yohimbine, but abolished by the non-selective α-adrenoceptor antagonist, phentolamine, suggesting the involvement of both α-adrenoceptor subtypes. Finally, Limberger & Starke (1983) reported that idazoxan inhibited [^{3}H]-noradrenaline release from pre-junctional nerve endings in the rabbit isolated ear artery. Since this effect was sensitive to yohimbine, but resistant to prazosin, idazoxan was assumed to be acting as a partial agonist at pre-junctional α_2-adrenoceptors.

More recently it has been recognized that [^{3}H]-idazoxan also binds to non-adrenoceptor, imidazoline binding sites, which have been identified in the rat cerebral cortex (Brown *et al.*, 1990), guinea-pig cerebral cortex (Wikberg & Uhlen, 1990), rabbit kidney (Lachaurd *et al.*, 1986), hamster adipocytes (MacKinnon *et al.*, 1989) and rabbit urethral smooth muscle (Yablonsky *et al.*, 1988). Pharmacologically these sites are characterized by a high affinity for idazoxan and the imidazoline derivative cirazoline, but low affinity for the

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endogenous catecholamines, noradrenaline and adrenaline. Although there is limited information concerning the functional response associated with these binding sites, it is noteworthy that idazoxan has been reported to inhibit [^{3}H]-noradrenaline release in the pulmonary artery (Gothert & Molderings, 1991) and to reduce prolactin release in rats (Krulich *et al.*, 1989) by a non-α-adrenoceptor mechanism.

Thus, there is preliminary evidence that, in addition to being an antagonist at some α_2-adrenoceptors, idazoxan may also act as a weak agonist in a number of other receptor systems. In the present study we have utilized the property of the guanine nucleotide, guanosine triphosphate (GTP), to reduce agonist binding, but not antagonist binding, to G-protein-coupled receptors (Hoffman *et al.*, 1980) to assess whether idazoxan might be a partial agonist at α_2-adrenoceptors and at non-adrenoceptor, imidazoline binding sites in the cerebral cortex from several species. Preliminary observations in calf cerebral cortex membranes have shown that GTP reduces [^{3}H]-idazoxan binding, although the precise nature of the receptor(s) involved was not examined in detail (Hussain *et al.*, 1991). An inhibitory effect of GTP on idazoxan binding to non-adrenoceptor, imidazoline binding sites would also indicate that these sites represent a G-protein-coupled receptor system.

Methods

Membrane preparation

Calf, rat, guinea-pig, mouse and human cerebral cortex membranes were prepared as described below. The calf brain was obtained from the local abattoir immediately after the slaughter of the animal. The human brain was obtained not more than 8 h *post-mortem* from the Department of Pathology, Queen's Medical Centre. The rat, guinea-pig and mouse cerebral cortices were obtained after killing the animals by decapitation and rapidly removing the brains and dissecting on ice. Cerebral cortices were homogenized in 20 volumes of ice cold Tris buffer (50 mM Tris HCl; pH 7.4) in a Polytron PT disrupter (setting 6; 20–30 s). The homogenate was then centrifuged at 20,000 r.p.m. for 10 min at 4°C. The supernatant was discarded and the pellet resuspended in 20 volumes of Tris buffer and re-centrifuged. The final pellet was resuspended in 4.9 vol of 50 mM Tris buffer for direct use in binding assays or stored at −20°C. Human and calf cerebral cortex membranes were prepared in a similar manner, except that homogenization of the cortices was achieved with an OMNI-GEN Macro homogenizer to minimize potential hazards associated with aerosol formation. Also, resuspension of the centrifuged pellet was with an Ultra turrax homogenizer sited in a laminar airflow hood.

Binding assays

The affinity of various imidazoline and non-imidazoline compounds for binding sites labelled by [^{3}H]-idazoxan was examined. Competition binding assays were performed by incubating the washed membranes: (200–480 μg protein for the different species) with [^{3}H]-idazoxan (1–2 nM) in the presence or absence of a range of 12–18 concentrations of the competing ligand in a total volume of 500 μl of Tris assay buffer (50 mM Tris HCl; pH 7.4 at 25°C). Non-specific binding was defined as the concentration of bound ligand in the presence of 10 μM cirazoline. After an incubation period of 90 min at 25°C, bound radioactivity was separated from free by vacuum filtration over Whatman GF/B glass fibre filters using a Brandel cell harvester (or Skatron cell harvester for bovine cerebral cortex membranes) and quantified by liquid scintillation spectrometry. Saturation experiments were performed under similar conditions with increasing concentrations of [^{3}H]-idazoxan (0.1 to 10–15 nM). For saturation experiments with [^{3}H]-clonidine and [^{3}H]-quinuclidinyl benzilate (QNB), 100 μM noradrenaline and 10 μM atropine, respectively, were used to determine non-specific binding. The protein content of the tissues was measured by the method of Bradford (1976) with bovine serum albumin as the standard and results expressed as fmol mg^{-1} protein. Kinetic analysis dissociation experiments with [^{3}H]-idazoxan were performed by adding the membranes at time 0 and terminating the incubation at the specific time points by the addition of 10 μM cirazoline.

Data analysis

Competition and saturation data analysis The inhibition of the radioligand by competing ligands was analysed to estimate the IC_{50} (concentration of competitor displacing 50% of specifically bound radioligand), with a non-linear least squares programme GraphPad (ISI). The IC_{50} value was converted to an affinity constant (K_i) using the expression derived by Cheng & Prusoff (1973):

$$K_i = IC_{50}/(1 + [L]/K_d)$$

In this expression, [L] and K_d represent the radioligand concentration and dissociation constant respectively. All data were initially analysed assuming a one site model of radioligand binding. The data, with Hill coefficients of less than unity were then analysed assuming a two site model. The model which gave a coefficient of correlation closest to one was accepted. GraphPad was also used to perform non-linear regression analysis for fitting data from saturation experiments. The expression used to derive B_{max} and K_d values was:

$$Y = AX/B + X \text{ where } A = B_{max} \text{ and } B = K_d$$

The function fits the data to a rectangular hyperbola graph. GraphPad was also used to analyse the kinetic data to determine the half-lives of the radioligand association and dissociation.

Drugs

Drugs and chemicals were obtained from Sigma or Fisons plc except: [^{3}H]-idazoxan (43 Ci mmol^{-1}) Amersham, UK; [^{3}H]-QNB (quinuclidinyl benzilate) (50 Ci mmol^{-1}) Amersham, UK; [^{3}H]-clonidine (30 Ci mmol^{-1}) Amersham, UK; idazoxan (Reckitt and Colman); cirazoline (Synthelabo Recherche); guanosine-5′ triphosphate disodium salt (Boehringer Mannheim); rauwolscine hydrochloride (Carl Roth, Karlsruhe, Germany); atropine sulphate (BDH Chemicals Ltd).

Results

Pharmacological characterization of [^{3}H]-idazoxan binding in the cerebral cortex from five species

Figure 1 shows the effect of idazoxan and the yohimbine diastereoisomers rauwolscine and corynanthine at sites labelled by 1–2 nM [^{3}H]-idazoxan in calf, mouse, rat, guinea-pig and human cerebral cortex membranes. Idazoxan was similarly effective in all five species, there being less than a 3 fold difference in the pK_i value between species (Table 1). In each tissue, rauwolscine possessed a high affinity for the sites labelled by [^{3}H]-idazoxan (pK_i around 8) and was between 30–100 fold more potent than corynanthine (Table 1), a finding consistent with labelling of α_2-adrenoceptors by [^{3}H]-idazoxan (McGrath, 1982; Daly *et al.*, 1988). However, in the rat, guinea-pig and human cerebral cortex, rauwolscine was clearly able to discriminate between two sites labelled by [^{3}H]-idazoxan (Figure 1c, d, e). The displacement curve for rauwolscine was characterized by a resistant component of [^{3}H]-idazoxan binding between 1 μM and 30 μM rauwolscine. The magnitude of the rauwolscine-resistant component was approximately 15%, 30% and 40% of the total [^{3}H]-idazoxan

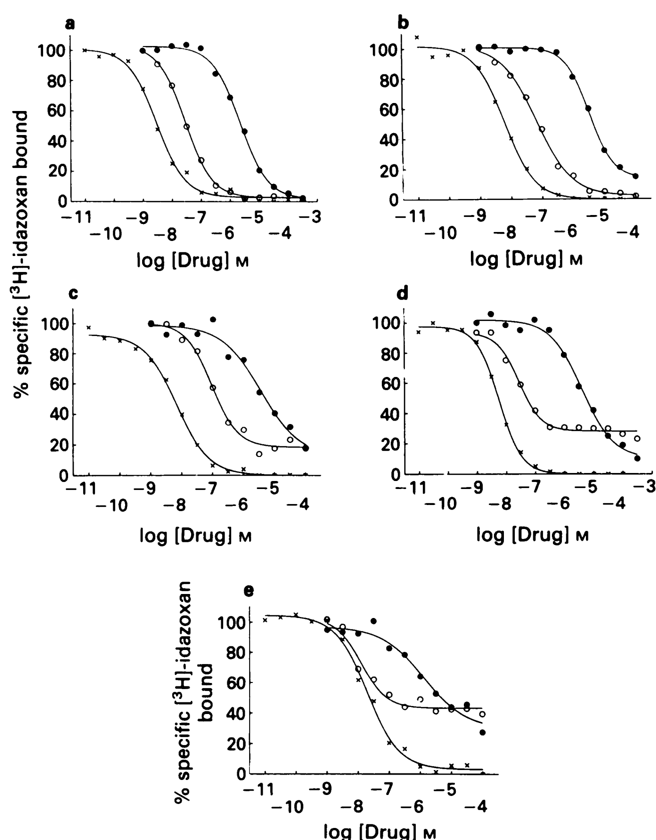

Figure 1 Inhibition of [^{3}H]-idazoxan binding in the cerebral cortex membranes of the (a) calf, (b) mouse, (c) rat, (d) guinea-pig, and (e) man by rauwolscine (○); corynanthine (●) and idazoxan (×). Non-specific binding was determined in the presence of 10 μM cirazoline and the assay was performed in 50 mM Tris buffer pH 7.4 (final volume 500 μl) for 90 min at 25°C. Non-specific binding was in the range of 10–15% of total binding. The figures are representative of 3 separate experiments.

binding in the rat, guinea-pig and human cerebral cortex membranes, respectively.

Figure 2 shows the effect of adrenaline and cirazoline against [^{3}H]-idazoxan binding in guinea-pig cerebral cortex membranes. Cirazoline completely displaced [^{3}H]-idazoxan binding with a pK_i of 7.29 and Hill slope (nH) of − 0.7 ($n = 2$). The displacement of [^{3}H]-idazoxan by adrenaline (nH of − 0.48 ± 0.07, $n = 5$), indicates a low affinity population of sites resistant to adrenaline binding, the IC_{50} of the low affinity component is > 100 μM adrenaline; the high affinity component (presumably binding to an adrenoceptor) represents 70% of the specific binding and has a pK_i of 7.62 ± 0.24 ($n = 5$). This is essentially in agreement with the observation made with rauwolscine and indicates that the rauwolscine-resistant component of [^{3}H]-idazoxan binding in the guinea-pig cerebral cortex is a non-adrenoceptor, imidazoline binding site (Figure 1d).

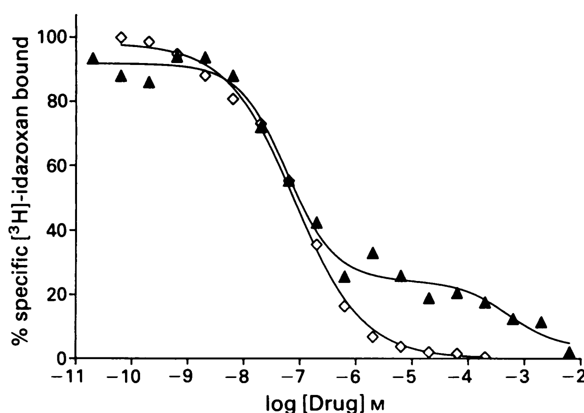

Figure 2 Inhibition of [^{3}H]-idazoxan binding in the guinea-pig cerebral cortex membranes by adrenaline (▲) and cirazoline (◇). Non-specific binding was determined in the presence of 10 μM cirazoline and the assay was performed in 50 mM Tris buffer pH 7.4 (final volume 500 μl) for 90 min at 25°C. Non-specific binding was in the range of 10–15% of total binding. The figure is representative of 3–5 separate experiments.

The effect of 300 μM GTP on saturation binding of [^{3}H]-idazoxan

Saturation curves were performed with increasing concentrations of [^{3}H]-idazoxan (0.1 to 10–15 nM) in calf, mouse, rat, guinea-pig and human cerebral cortex membranes in the presence and absence of 300 μM GTP (Figure 3). In the absence of 300 μM GTP, the K_d values for [^{3}H]-idazoxan varied from 0.81 ± 0.17 nM ($n = 7$) in calf cerebral cortex membranes to 6.67 ± 0.54 nM ($n = 4$) in human cerebral cortex membranes. The maximum number of binding sites varied from 119 ± 5 fmol mg^{-1} protein ($n = 7$) in calf cerebral cortex membranes to 499 ± 28.2 fmol mg^{-1} protein in mouse cerebral cortex membranes ($n = 5$). The dissociation constants and maximum number of binding sites for the other preparations are shown in Table 2.

GTP, 300 μM, had no significant effect on [^{3}H]-idazoxan binding in guinea-pig cerebral cortex (Figure 3d, Table 2). In both rat and mouse cerebral cortex membranes, 300 μM GTP increased the dissociation constant for [^{3}H]-idazoxan by 2–3 fold without significantly affecting B_{max} (Figure 3b,c and Table 2). In marked contrast, 300 μM GTP reduced B_{max} by approximately 30% and 60% in calf and human cerebral cortex membranes, respectively, without significantly altering K_d (Figure 3a,e and Table 2).

Association of [^{3}H]-idazoxan to calf cortex membranes was rapid, equilibrium was reached within 2 min. Figure 4 shows that the half-life for the dissociation of specific [^{3}H]-idazoxan binding from calf cerebral cortex membranes was unaffected by the presence of 300 μM GTP, thereby supporting the complementary saturation experiments which indicate that GTP does not affect the affinity of idazoxan.

Table 1 Displacement of [^{3}H]-idazoxan (1–2 nM) from calf, human, guinea-pig, rat and mouse cerebral cortex membranes

Species	*Rauwolscine* pK_i (nH)	*Corynanthine* pK_i (nH)	*Idazoxan* pK_i (nH)
Calf	7.91 ± 0.08; (− 1.09 ± 0.15)	6.24 ± 0.08; (− 0.79 ± 0.02)	8.57 ± 0.25; (− 0.97 ± 0.08)
Man	8.26 ± 0.09; (− 0.59 ± 0.07)	6.55 ± 0.08; (− 0.63 ± 0.07)	8.19 ± 0.12; (− 0.80 ± 0.10)
Guinea-pig	7.94 ± 0.02; (− 0.77 ± 0.15)	5.93 ± 0.02; (− 0.97 ± 0.31)	8.56 ± 0.03; (− 0.92 ± 0.07)
Rat	7.36 ± 0.02; (− 0.72 ± 0.09)	6.02 ± 0.16; (− 0.78 ± 0.16)	8.66 ± 0.22; (− 0.79 ± 0.08)
Mouse	7.59 ± 0.07; (− 0.66 ± 0.08)	5.84 ± 0.04; (− 1.02 ± 0.10)	8.47 ± 0.09; (− 0.95 ± 0.11)

The pK_i values were calculated from the displacement of 1–2 nM [^{3}H]-idazoxan from calf, human, guinea-pig, rat and mouse cerebral cortex membranes as described in Methods. Each value represents the mean ± s.e.mean of three separate experiments.

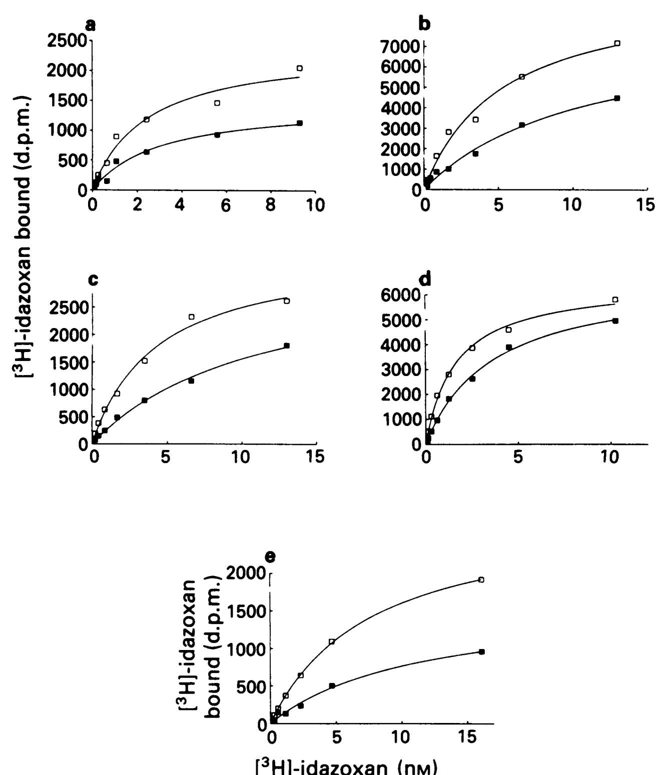

Figure 3 Saturation curves of [^{3}H]-idazoxan binding to (a) calf, (b) human, (c) guinea-pig, (d) mouse and (e) rat cerebral cortex membranes. The preparations were incubated with increasing concentrations of [^{3}H]-idazoxan in the absence (□) or presence of 300 μM GTP (■). Non-specific binding was determined in the presence of 10 μM cirazoline and the assay was performed in 50 mM Tris buffer pH 7.4 (final volume 500 μl) for 90 min at 25°C. Non-specific binding was in the range of 10–15% of total binding. B_{max} and K_d values were calculated with the computer curve fitting programme GraphPad. The data shown are representative of one experiment with each point determined in duplicate. Similar results were obtained in 3–7 other experiments.

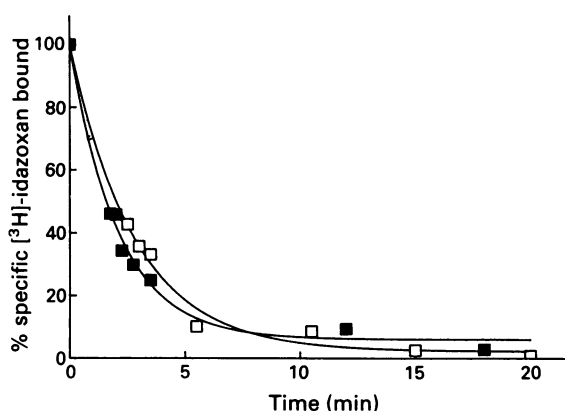

Figure 4 The effect of 300 μM GTP on the dissociation of [^{3}H]-idazoxan from calf cerebral cortex membranes. Membranes were preincubated for 30 min at 25°C in the presence of 1–2 nM [^{3}H]-idazoxan. Specific binding was determined at various time intervals with the addition of 10 μM cirazoline to the samples starting at time 0 with (■) or without (□) 300 μM GTP. Dissociation of [^{3}H]-idazoxan followed first order kinetics with half-lives of 1.40 ± 0.42 and 1.48 ± 0.48 min, with and without GTP respectively. Half-lives were not significantly different ($P > 0.05$ paired t test).

Further examination of the effect of GTP in calf and human cerebral cortex membranes

The finding that 300 μM GTP significantly reduced [^{3}H]-idazoxan binding in calf and human cerebral cortex membranes, without affecting the dissociation constant, prompted further experiments to examine this phenomenon in greater detail. Figure 5 shows the effect of 300 μM GTP on saturation curves for the binding of the selective α_2-adrenoceptor agonist, [^{3}H]-clonidine and the selective muscarinic antagonist, [^{3}H]-QNB to calf cerebral cortex membrane. GTP (300 μM) significantly reduced the B_{max} for [^{3}H]-clonidine binding from 51.7 ± 14.7 to 29.0 ± 5.5 fmol mg^{-1} protein ($n = 4$), but the dissociation constant in the presence (4.35 ± 1.39 nM) and absence (3.56 ± 1.27 nM) of 300 μM GTP were not significantly different (Figure 5a). In marked contrast, 300 μM GTP failed to affect either the dissociation constant or the B_{max} value for [^{3}H]-QNB binding (Figure 5b).

Figure 6 shows the effect of 300 μM GTP on [^{3}H]-idazoxan binding to human cerebral cortex membranes in the presence of 1 μM rauwolscine (to mask α_2-adrenoceptor binding sites). GTP (300 μM) significantly reduced the B_{max} for [^{3}H]-idazoxan at the non-adrenoceptor, imidazoline sites from 86.6 ± 11.7 to 55.7 ± 11.7 ($n = 3$) fmol mg^{-1} protein, without affecting the dissociation constant (9.71 ± 0.86 nM and 9.94 ± 2.29 nM in the absence and presence, respectively, of 300 μM GTP). In contrast, 300 μM GTP failed to affect either the dissociation constant (1.00 ± 0.58 nM and 1.29 ± 0.78 nM ($n = 3$) in the absence and presence, respectively, of GTP) or the B_{max} (567 ± 53 and 562 ± 51 fmol mg^{-1} protein ($n = 3$) in the absence and presence, respectively, of GTP) of [^{3}H]-QNB binding to human cerebral cortex.

Discussion

In the present study we have shown that the imidazoline derivative [^{3}H]-idazoxan binds to both α_2-adrenoceptor and non-adrenoceptor, imidazoline binding sites in cerebral cortex membranes from several species. The α_2-adrenoceptor binding sites have been identified by the 100 fold greater potency of rauwolscine compared to its diastereoisomer, corynanthine (see: McGrath, 1982), while the non-adrenoceptor, imidazoline component of [^{3}H]-idazoxan binding has been identified as that portion of the cirazoline-sensitive [^{3}H]-idazoxan binding which is resistant to either adrenaline or

Table 2 Effect of GTP on the specific binding of [^{3}H]-idazoxan to calf, human, guinea-pig, rat and mouse cerebral cortex membranes

	K_d (nM)		B_{max} (fmol mg^{-1})	
Species	*Control*	*GTP* (300 μM)	*Control*	*GTP* (300 μM)
Calf	0.81 ± 0.17	1.03 ± 0.07	119.4 ± 4.5	82.2 ± 4.2**
Man	6.67 ± 0.54	5.39 ± 0.69	148.7 ± 9.7	56.8 ± 3.0**
Guinea-pig	3.25 ± 0.81	4.86 ± 0.87	209.7 ± 5.0	232.3 ± 7.6
Rat	5.07 ± 0.74	11.17 ± 1.51*	128.8 ± 10.4	127.0 ± 13.1
Mouse	4.44 ± 0.34	13.99 ± 2.20*	499.0 ± 28.2	423.9 ± 34.9

Saturation experiments were performed in the absence and presence of 300 μM GTP. Values shown are the mean ± s.e.mean of 3–7 experiments.
*$P < 0.05$; **$P < 0.01$; unpaired t test.

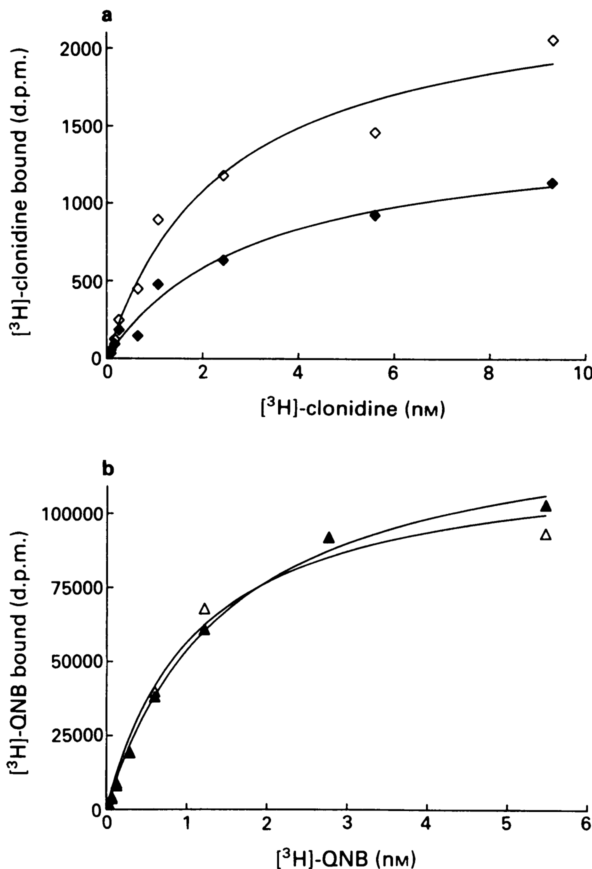

Figure 5 Saturation curves of (a) [³H]-clonidine and (b) [³H]-quinuclidinylbenzoate ([³H]-QNB) binding to calf cerebral cortex membranes. (a) The preparation was incubated with increasing concentrations of [³H]-clonidine; in the absence (◇) and presence (◆) of 300 μM GTP; non-specific binding was defined in the presence of 10 μM adrenaline. (b) The preparation was incubated with increasing concentrations of [³H]-QNB in the absence (△) and presence (▲) of 300 μM GTP; non-specific binding was defined in the presence of 10 μM atropine. The assay was performed in 50 mM Tris buffer pH 7.4 (final volume 500 μl) for 90 min at 25°C. Non-specific binding was in the range of 10–15% of total binding. B_{max} and K_d values were calculated with the computer curve fitting programme GraphPad. The data shown are representative of one experiment with each point determined in duplicate. Similar results were obtained in 2 other experiments.

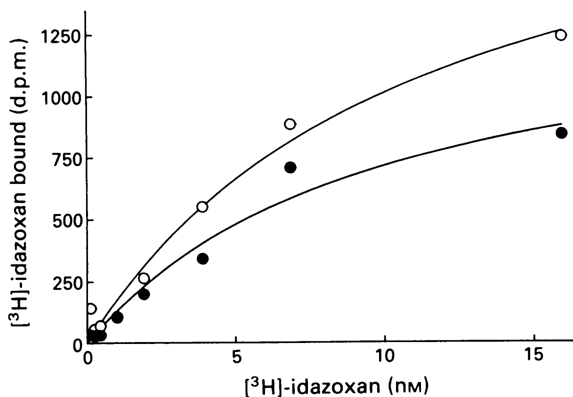

Figure 6 Saturation curve of [³H]-idazoxan binding to human cerebral cortex membranes. The preparation was incubated with increasing concentrations of [³H]-idazoxan in the presence of 1 μM rauwolscine (○), or in the presence of 1 μM rauwolscine + 300 μM GTP (●). Non-specific binding was determined in the presence of 10 μM cirazoline and the assay was performed in 50 mM Tris buffer pH 7.4 (final volume 500 μl) for 90 min at 25°C. Non-specific binding was in the range of 10–15% of total binding. B_{max} and K_d values were calculated with the computer curve-fitting programme GraphPad. The data shown are representative of one experiment with each point determined in duplicate. Similar results were obtained in 2 other experiments.

rauwolscine. In calf and mouse cerebral cortex membranes, idazoxan labels only α_2-adrenoceptors, while in the rat, guinea-pig and human cerebral cortex membranes there is an increasing contribution made by imidazoline binding sites to total [³H]-idazoxan binding. These results are in agreement with earlier reports suggesting the presence of non-adrenoceptor, imidazoline binding sites in the rat (Brown *et al.*, 1990), guinea-pig (Wikberg & Uhlen, 1990) and human cerebral cortex (De Vos *et al.*, 1991).

GTP had no effect on [³H]-idazoxan binding in the guinea-pig cerebral cortex membranes, the B_{max} and K_d values remaining unaltered in the presence of 300 μM GTP. These results suggest that idazoxan might be acting as an antagonist at both α_2-adrenoceptors and at non-adrenoceptor, imidazoline binding sites, in this preparation. GTP reduced the affinity of [³H]-idazoxan for α_2-adrenoceptors in the mouse cerebral cortex membranes; and also in the rat where 85% of the [³H]-idazoxan binding sites are α_2-adrenoceptors. This increase in K_d with no change in B_{max} would be expected of agonist modulation by guanine nucleotides (U'Prichard *et al.*, 1976; Hoffman *et al.*, 1980), suggesting that [³H]-idazoxan may have agonist activity at α_2-adrenoceptors.

A decrease in the B_{max} of [³H]-idazoxan binding at α_2-adrenoceptors was observed in the calf and human cerebral cortex membranes in the presence of GTP. It has been suggested that the affinity of certain receptors could be reduced by GTP to such a degree that the binding is no longer observable, therefore manifesting itself as a decrease in B_{max} (Ofri *et al.*, 1992). This action of GTP is consistent with its effects on agonist binding to a number of receptor systems e.g. the full α_2-adrenoceptor agonist [³H]-UK-14,304 in human platelet membranes (Schloos *et al.*, 1987); the partial α_2-adrenoceptor agonist [¹²⁵I]-*p*-iodoclonidine in human platelet membranes (Gerhardt *et al.*, 1990); [³H]-5-hydroxytryptamine (5-HT) in rat hippocampus (Mallat & Hamon, 1982); [³H]-8-OH-DPAT in rat cortical membranes (Harrington & Peroutka, 1990) and the endogenous agonist [¹²⁵I]-neuropeptide Y in human SK-N-MC neuroblastoma cells (Feth *et al.*, 1991). The difference in the effect of GTP on [³H]-idazoxan binding in rat and mouse, and calf and man could be a reflection of α_2-adrenoceptor heterogeneity, e.g. the low Hill slopes for rauwolscine in Table 1.

The effect of GTP does not appear to be non-specific because it had no effect on [³H]-QNB binding in the human and calf cortex membranes, and no effect was observed on [³H]-idazoxan binding in the guinea-pig cerebral cortex membranes. The effects of GTP observed in the calf and human cerebral cortex membranes are qualitatively similar to the effects observed with the α_2-adrenoceptor partial agonist radioligand [³H]-clonidine in the calf cortex (Figure 5a). The 'agonist' action of idazoxan at α_2-adrenoceptors is consistent with the observations of Limberger & Starke (1983); however, idazoxan should still be considered essentially as an antagonist at most α_2-adrenoceptors.

The data from the saturation studies in the human cerebral cortex membranes show a reduction in B_{max} by GTP suggesting that idazoxan might be an agonist at non-adrenoceptor binding sites and that these sites could be part of the G-protein receptor superfamily; in support of this there is a similar effect of GTP in the porcine renal cortex (Hussain *et al.*, 1992). A parallel observation has been made for rauwolscine, an α_2-adrenoceptor antagonist (De Vos *et al.*, 1991). Rauwolscine binding at 5-HT_{1A} receptors in the human frontal cortex membranes was decreased by GTP (500 μM), indicating an agonist role for rauwolscine at these receptors. Interestingly, GTP has also been reported to influence [³H]-aminoclonidine binding to non-adrenoceptor imidazoline binding sites in the calf ventral lateral medulla (Ernsberger, 1992). Further evidence suggesting agonist activity of idazoxan at non-adrenoceptor, imidazoline binding sites has been reported by Gothert & Molderings (1991) who observed that when α_2-adrenoceptors were blocked by either rauwolscine or by exposure to phenoxybenzamine, [³H]-adrenaline

overflow from the rabbit pulmonary artery was inhibited by idazoxan. In addition, a chronic infusion study with idazoxan ($1.1\ mg\ kg^{-1}\ h^{-1}$ for 5 days) in rabbits showed a down regulation of [^{3}H]-idazoxan binding sites in the kidney membranes, suggesting agonist activity of [^{3}H]-idazoxan at the non-adrenoceptor sites in this preparation (Yakubu *et al.*, 1990).

However, in some systems idazoxan can act as an antagonist at brain non-adrenoceptor, imidazoline binding sites, for example Olmos *et al.* (1991, 1992) reported that chronic treatment with idazoxan ($10\ mg\ kg^{-1}$, i.p. every 12 h, 7 days) consistently increased the density of imidazoline receptors in the brain of Wistar-Kyoto and Sprague-Dawley rats. This up-regulation is consistent with the effect of a chronic treatment with an antagonist on receptor number. There are several reports in the literature of a lack of effect of Gpp(NH)p or GTP on UK-14,304 displacement of [^{3}H]-idazoxan binding at non-α_2-adrenoceptor sites (Michel *et al.*, 1989; Langin *et al.*, 1990; Zonnenschein *et al.*, 1990; Lachaud-Pettiti *et al.*, 1991). The apparent contradiction between these previous reports and the current study may derive from different species and tissues used or from methodological differences (e.g. [^{3}H]-idazoxan saturation isotherms were constructed in the present study while the previous studies employed inhibition curves, additionally, it is possible that UK-14,304 is not an agonist at the non-adrenoceptor, imidazoline binding sites).

Thus, there is a need to re-evaluate the actions of idazoxan in light of its ability to recognize both α_2-adrenoceptors and non-adrenoceptor, imidazoline binding sites, and because it may possess agonist activity at some of these sites.

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References

BERRIDGE, T.L., GADIE, B., ROACH, A.G. & TULLOCH, I.F. (1983). α_2-Adrenoceptor agonists induce mydriais in the rat by an action within the central nervous system. *Br. J. Pharmacol*, **78**, 507–515.

BRADFORD, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*, **72**, 248–254.

BROWN, C.M., MACKINNON, A.C., MCGRATH, J.C., SPEDDING, M. & KILPATRICK, A.T. (1990). α_2-Adrenoceptor subtypes and imidazoline-like binding sites in the rat brain. *Br. J. Pharmacol.*, **99**, 803–809.

CHENG, Y.-C. & PRUSOFF, W.H. (1973). Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (IC_{50}) of an enzymatic reaction. *Biochem. Pharmacol*, **22**, 3099–3108.

DALY, C.J., MCGRATH, J.C. & WILSON, V.G. (1988). An examination of the post-junctional α-adrenoceptors subtypes for (−)-noradrenaline in several isolated blood vessels form the rabbit. *Br. J. Pharmacol.*, **95**, 473–484.

DEVOS, H., CZERWIEC, E., DEBACKER, J.-P., DEPOTTER, W. & VAUQUELIN, G. (1991). [^{3}H]-Rauwolscine behaves as an agonist for the 5-HT_{1A} receptors in human frontal cortex membranes. *Eur. J. Pharm. (Mol. Pharmacol. Sec.)*, **207**, 1–8.

DOXEY, J.C., ROACH, A.G. & SMITH, C.F.C. (1983). Studies on RX-781094: a selective potent and specific antagonist of α_2-adrenoceptors. *Br. J. Pharmacol.*, **78**, 489–505.

DOXEY, J.C., ROACH, A.G., STRACHAN, D.A. & VIRDEE, N.K. (1984). Selectivity and potency of 2-alkyl analogues of the α_2-adrenoceptor antagonist (RX 781094) in peripheral systems. *Br. J. Pharmacol*,. **83**, 713–722.

ERNSBERGER, P. (1992). Heterogeneity of imidazoline binding sites: proposal I_1 and I_2 subtypes. *Fundam. Clin. Pharmacol.*, **6**, P18.

FETH, F., RASCHER, W. & MICHEL, M.C. (1991). G-Protein coupling and signalling of Y_1-like neuropeptide Y receptors in SK-N-MC cells. *Naunyn Schmieds Arch. Pharmacol*,. **344**, 1–7.

GERHARDT, M.A., WADE, S.M. & NEUBIG, R.R. (1990). *p*-[^{125}I]-Iodoclonidine is a partial agonist at the α_2-adrenergic receptor. *Mol. Pharmacol.*, **38**, 214–221.

GOTHERT, M. & MOLDERINGS, G.J. (1991). Involvement of presynaptic imidazoline receptors in the α_2-adrenoceptor-independent inhibition of noradrenaline release by imidazoline derivatives. *Naunyn-Schmieds Arch. Pharmacol.*, **343**, 271–282.

HANNAH, J.A.M., HAMILTON, C.A. & REID, J.L. (1983). RX781094, a new potent α_2 adrenoceptor antagonist. *In vivo* and *in vitro* studies in the rabbit. *Naunyn Schmieds Arch. Pharmacol.*, **322**, 221–227.

HARRINGTON, M.A. & PEROUTKA, S.J. (1990). Modulation of 5-hydroxytryptamine$_{1A}$ receptor density by nonhydrozyable GTP analogues. *J. Neurochem.*, **54**, 294–299.

HOFFMAN, B.B., MULLIKIN-KILPATRICK, D. & LEFKOWITZ, R.J. (1980). Heterogeneity of radioligand binding to α-adrenergic receptors. Analysis of guanine nucleotide regulation of agonist binding in relation to receptor subtypes. *J. Biol. Chem.*, **255**, 4645–4652.

HUSSAIN, J.F., KENDALL, D.A. & WILSON, V.G. (1992). A possible linkage of non-adrenoceptor, imidazoline binding sites labelled by [^{3}H]-idozoxan to K^+ channels and G-proteins. *Br. J. Pharmacol.*, **107**, 323P.

HUSSAIN, J.F., WILSON, V.G. & KENDALL, D.A. (1991). The effect of guanine nucleotides on [^{3}H]-idazoxan binding in the bovine cerebral cortex. *Br. J. Pharmacol.*, **104**, 269P.

KRULICH, L., JURCOVICOVA, J. & LE, T. (1989). Prolactin (PRL) release-inhibiting properties of the α_2-adrenergic receptor antagonist idazoxan: comparison with yohimbine. *Life Sci.*, **44**, 809–818.

LACHAUD, V., COUPRY, I., PODEVIN, R.-A., DAUSSE, J.-P., KOENIG, E. & PARINI, A. (1986). Interaction of clonidine and rilmenidine with imidazoline-preferring receptors. *J. Hypertens*,. **6** (Suppl. 4), S511–S518.

LACHAUD-PETTITI, V., PODEVIN, R.-A., CHIETIEN, Y. & PARINI, A. (1991). Imidazoline-guanidinium and α_2-adrenergic binding sites in basolateral membranes from human kidney. *Eur. J. Pharmacol. (Mol. Pharmacol. Sec.)*, **206**, 23–31.

LANGIN, D., PARIS, H. & LAFONTAN, M. (1990). Binding of [^{3}H]-idazoxan and its methoxyderivative [^{3}H]-RX821002 in human fat cells: [^{3}H]-idazoxan but not [^{3}H]-RX821002 labels additional non-α_2-adrenergic binding sites. *Mol. Pharmacol.*, **37**, 876–885.

LIMBERGER, N. & STARKE, K. (1983). Partial agonist effect of 2-[2-(1,4-benzodioxanyl)]-2-imidazoline (RX781094) at presynaptic α_2-adrenoceptors in rabbit ear artery. *Naunyn-Schmieds Arch. Pharmacol.*, **324**, 75–78.

MACKINNON, A.C., BROWN, C.M., SPEDDING, M. & KILPATRICK, A.T. (1989). [^{3}H]-idazoxan binds with high affinity to two sites on hamster adipocytes: an α_2-adrenoceptor and a non-adrenoceptor sites. *Br. J. Pharmacol.*, **98**, 1143–1150.

MALLAT, M.C. & HAMON, M. (1982). Ca^{2+}-guanine interaction in brain membranes. I. Modulation of central 5-hydroxytryptamine receptors in the rat. *J. Neurochem.*, **38**, 151–161.

MCGRATH, J.C. (1982). Evidence for more than one type of post-junctional α-adrenoceptor. *Biochem. Pharmacol.*, **31**, 467–484.

MICHEL, M.C., BRODDE, O.-E., SCHNEPEL, B., BEHRENDT, J., TSCHAUDA, R., MOTULSKY, H.J. & INSEL, P.A. (1989). Nonadrenergic [^{3}H]-idazoxan binding sites are physically distinct from α_2-adrenergic receptors. *Mol. Pharmacol.*, **35**, 324–330.

OFRI, D., RITTER, A.M., LIU, Y., GIOANNINI, T.L., HILLER, J.M. & SIMON, E.J. (1992). Characterization of solubilized opioid receptors: reconstitution and uncoupling of guanine nucleotide-sensitive agonist binding. *J. Neurochem.*, **58**, 628–635.

OLMOS, G., MIRALLES, A., BARTUREN, F. & GARCIA-SEVILLA, J.A. (1991). Repeated idazoxan increases brain imidazoline receptors in normotensive (WKY) but not in hypertensive (SHR) rats. *J. Neurochem.*, **57**, 1811–1813.

OLMOS, G., MIRALLES, A., BARTUREN, F. & GARCIA-SEVILLA, J.A. (1992). Characterization of brain imidazoline receptors in normotensive and hypertensive rats: differential regulation by chronic imidazoline drug treatment. *J. Pharmacol. Exp. Ther.*, **260**, 1000–1007.

PACIOREK, P.M. & SHEPPERSON, N.B. (1983). α_1-Adrenoceptor agonist activity of α_2-adrenoceptor antagonist in the pithed rat preparation. *Br. J. Pharmacol.*, **79**, 12–14.

PIMOULE, C., SCATTON, B. & LANGER, S.Z. (1983). [^{3}H]-RX781094: a new antagonist ligand labels α_2-adrenoceptors in the rat brain cortex. *Eur. J. Pharmacol.*, **95**, 79–85.

SCHLOOS, J., WELLSTEIN, A. & PALM, D. (1987). Agonist binding at α_2-adrenoceptors of human platelets using [^{3}H]-UK14,304: regulation by Gpp(NH)p and cations. *Naunyn-Schmieds Arch. Pharmacol.*, **336**, 48–59.

U'PRICHARD, P.C., GREENBERG, D.A. & SNYDER, S.H. (1976). Binding characteristics of a radioligand agonist and antagonist at central nervous system α-noradrenergic receptors. *Mol. Pharmacol.*, **13**, 454–473.

WIKBERG, J.E.S. & UHLEN, S. (1990). Further characterization of the guinea-pig cerebral cortex idazoxan receptor: solubilization, distinction from the imidazoline site, and demonstration of cirazoline as an idazoxan receptor-selective drug. *J. Neurochem.*, **55**, 192–203.

YABLONSKY, F., RIFFAUD, J.P., LACOLLE, J.Y. & DAUSSE, J.P. (1988). Evidence for non-adrenergic binding sites for [^{3}H]-idazoxan in the smooth muscle of rabbit urethra. *Eur. J. Pharmacol.*, **154**, 209–212.

YAKUBU, M.A., DEIGHTON, N.M., HAMILTON, C.A. & REID, J.L. (1990). Differences in the regulation of [^{3}H]-idazoxan and [^{3}H]-yohimbine binding sites in the rabbit. *Eur. J. Pharmacol.*, **176**, 305–311.

ZONNENSCHEIN, R., DIAMANT, S. & ATLAS, D. (1990). Imidazoline receptors in the rat liver cells: a novel receptor or a subtype of α_2-adrenoceptors. *Eur. J. Pharmacol.*, **190**, 203–215.

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