[³H]-idazoxan binds with high affinity to two sites on hamster adipocytes: an α_2 -adrenoceptor and a non-adrenoceptor site

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1 [³H]-idazoxan labels a single population of high affinity sites (K_d 2.26 ± 0.02 nM; B_{max} 372 ± 25 fmol mg⁻¹ protein) in hamster adipocyte membranes. In the presence of 1μ M yohimbine to preclude binding to α_2 -adrenoceptors, the density of [³H]-idazoxan binding sites was reduced (287 ± 18 fmol mg⁻¹ protein) without an apparent decrease in the affinity (K_d 2.19 ± 0.24 nM) of the radioligand.

2 Displacement studies indicate that α -adrenoceptor ligands with an imidazoline side chain completely inhibit [³H]-idazoxan binding to hamster adipocyte membranes; in contrast, the α_2 -adrenoceptor antagonists yohimbine, rauwolscine, BDF 6143 and phentolamine inhibited only 20–30% of the specific binding with affinity values consistent with an interaction at α_2 -adrenoceptors.

3 The low potency of noradrenaline and adrenaline in displacing [³H]-idazoxan binding to the second site on hamster adipocyte membranes indicates that it is unlikely that this site is a type of adrenoceptor.

4 These results suggests that [³H]-idazoxan binds with high affinity to two sites in hamster adipocytes: an α_2 -adrenoceptor and a non-adrenoceptor imidazoline site.

Introduction

Catecholamine-induced lipolysis from adipocytes isolated from white fat in man (Burns *et al.*, 1971), dog (Berlan & Dang Tran, 1978) and hamster (Hittelman *et al.*, 1973; Giudicelli *et al.*, 1977) is mediated through a stimulatory β -adrenoceptor and an inhibitory α -adrenoceptor. Studies indicate that α_1 - and α_2 -adrenoceptors exist in hamster (Garcia-Sainz *et al.*, 1980; Pecquery & Giudicelli, 1980) and human (Fain & Garcia-Sainz, 1983) fat cells. The antilipolytic effects of α -adrenoceptor agonists in hamster (Curtis-Prior & Tan, 1984; Tan & Curtis-Prior, 1984) and man (Berlan & Lafontan, 1980; Lafontan & Berlan, 1980; Curtis-Prior & Tan, 1984) are through the predominant α_2 -adrenoceptor.

The adipocyte is considered a suitable tissue to study the potency and selectivity of drugs that inter-

act with α_2 -adrenoceptors (Huerta-Bahena *et al.*, 1983). The α_2 -adrenoceptor present on human adipocytes is labelled with high affinity by [³H]-clonidine, [³H]-yohimbine and [³H]-rauwolscine (Tharp *et al.*, 1981; Berlan & Lafontan, 1982; Lafontan *et al.*, 1983; Richelsen & Pedersen, 1985). [³H]-clonidine binds with high affinity to hamster adipocyte membranes (Villeneuve *et al.*, 1985; Taouis *et al.*, 1986) whereas the labelling of α_2 -adrenoceptors by [³H]-yohimbine has proved difficult to demonstrate (Villeneuve *et al.*, 1985; Lafontan *et al.*, 1985). Yohimbine, rauwolscine and idazoxan reverse clonidine-induced inhibition of lipolysis in hamster fat cells (Tan & Curtis-Prior, 1983). The α_2 -adrenoceptor nature of the reversal is based upon rank order potency and not on receptor affinity.

In this study, we have examined the binding of [³H]-idazoxan to hamster fat cells and compared its

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displacement by adrenaline, noradrenaline and a number of imidazoline and non-imidazoline α -adrenoceptor ligands. We show that [^3H]-idazoxan, in addition to being a potent α_2 -adrenoceptor antagonist (Chapleo *et al.*, 1981), labels an imidazoline binding site on hamster adipocytes, with high affinity.

Methods

Preparation of adipocyte membranes

Golden male hamsters (2–3 months old; weight range, 100–150 g) were killed by cervical dislocation and the white adipose tissue carefully removed. Hamster adipocytes from epididymal fat were isolated essentially as described by Rodbell (1964) with minor modification with regard to the level of collagenase used in the digestion process. Adipocyte tissue (8–10 g per animal) was minced finely and digested with crude collagenase (1 mg ml^{-1}) in Krebs-Ringer bicarbonate buffer (5% w/v) containing 3.5% (w/v) fatty acid deficient bovine serum albumin and 1 mM CaCl_2 for 20 min at 37°C . During the digestion, the minced tissue was shaken vigorously approximately every 5 min by hand. The suspension containing the adipocytes was filtered through three layers of muslin and centrifuged at $400g$ for 1 min at room temperature. The infranatant was removed by aspiration and the cells washed three times with Krebs-Ringer bicarbonate buffer to remove the collagenase. Isolated adipocytes were suspended in 10 vol. lysing medium (5 mM Tris HCl, pH 7.4 containing 0.5 mM EDTA) and centrifuged at $400g$ for 1 min at room temperature. The membrane fraction was collected and the unbroken cells lysed by a further two washes in the 5 mM Tris HCl buffer. The membrane fractions were pooled and centrifuged at $30,000g$ for 15 min at 4°C . The final pellet was suspended in 50 mM Tris HCl buffer pH 7.4 containing 10 mM MgCl_2 and used immediately for ligand binding or stored for not more than 7 days under liquid nitrogen.

Male Sprague-Dawley rats (180–200 g) were stunned, decapitated and the cerebral cortex dissected from the other brain regions over ice. Tissues were homogenized in 20 volumes of 50 mM Tris HCl, 5 mM EDTA, pH 7.4 with a polytron PT 10 tissue disruptor (setting 10; $2 \times 10\text{ s}$ bursts). The homogenate was centrifuged at $48,000g$ for 15 min at 4°C . The supernatant was discarded and the pellet resuspended in the original volume of assay buffer (50 mM Tris HCl, 0.5 mM EDTA, pH 7.4). Membranes were washed twice by repeated centrifugation at $48,000g$ for 15 min at 4°C . The final pellet was

resuspended in assay buffer at an approximate protein level of 1 mg ml^{-1} . Membranes were stored under liquid nitrogen until used in the binding assay.

[^3H]-idazoxan binding

Saturation experiments Adipocyte membranes (250–300 μg protein) and nine concentrations of [^3H]-idazoxan over the range 0.1–12 nM were incubated to equilibrium in 50 mM Tris HCl buffer, pH 7.4 containing 10 mM MgCl_2 . Saturation studies were performed in the absence of yohimbine to determine the total density of binding sites or in the presence of $1\text{ }\mu\text{M}$ yohimbine to preclude [^3H]-idazoxan binding to α_2 -adrenoceptors. Incubations were carried out for 30 min at 25°C in a final volume of 0.5 ml. Bound ligand was separated from free by vacuum filtration over GF/B filters on a Brandell cell harvester. The filters were washed twice for 10 s with incubation buffer and the bound radioactivity determined by liquid scintillation counting. Non-specific binding at each free ligand concentration was determined in the presence of 1 mM phentolamine. Preliminary studies indicated that the imidazolines, cirazoline and naphazoline displaced non-specific [^3H]-idazoxan binding when defined by $10\text{ }\mu\text{M}$ phentolamine, a concentration often used in α_2 -adrenoceptor binding studies. The level of non-specific binding obtained with cirazoline and naphazoline was similar to the level obtained with 1 mM phentolamine.

Equilibrium binding parameters (K_d and B_{max}) were obtained by the iterative non-linear least square curve fitting programme 'ligand' (Munson & Rodbard, 1980).

Competition experiments

[^3H]-idazoxan binding to hamster adipocytes Competition experiments in hamster adipocytes, were carried out in 50 mM Tris HCl buffer, pH 7.4 containing 10 mM MgCl_2 , 1.5 nM [^3H]-idazoxan and various drugs over the concentration range 10^{-3} to 10^{-12} M in a final volume of 0.5 ml. Conditions for the competition assays were identical to those described for the saturation experiments. Non-specific binding was defined by 1 mM phentolamine and represented approximately 25% of total binding. Each determination was carried out in duplicate. Inhibition of specific binding of [^3H]-idazoxan by the drug was analysed to estimate the IC_{50} (concentration of drug displacing 50% of specific binding). The inhibitory constant (K_i) was calculated from the IC_{50} by the equation of Cheng & Prusoff (1973).

[^3H]-idazoxan binding to rat cortical α_2 -adrenoceptors The displacement of [^3H]-idazoxan

from α_2 -adrenoceptors in rat cortical membranes (100–200 μg protein) was carried out in 50 mM Tris HCl buffer, pH 7.4 containing 0.5 mM EDTA. Binding conditions were as previously described for hamster adipocyte membranes. Under these conditions, no evidence was obtained for [^3H]-idazoxan at a free ligand concentration of 1.5 nM, binding to an imidazoline site in rat cortex. Non-specific binding was defined by 10 μM phentolamine and represented approximately 35% of total binding.

Protein level was determined by the method of Lowry *et al.* (1951) with bovine serum albumin used as the protein standard.

Computer analysis of displacement curves

Binding isotherms from displacement studies were analysed by a non-linear least square parametric curve fitting programme capable of fitting to a single or two site model. The programme provided a sum of square error for a single or two site model. The single site and two site models for each isotherm were compared using the differential *F* value defined by the following equation:

$$F = \frac{(SS_1 - SS_2)/(df_1 - df_2)}{SS_2/df_2}$$

where SS_1 is the sum of squares error for the single site, SS_2 is the sum of squares error for the two site model, df_1 is the degrees of freedom for the single site model and df_2 the degrees of freedom for the two site model (Munson & Rodbard, 1980; Petrash & Bylund, 1986). A two site fit was assumed to be significantly better than a single site fit if the determined *F* value had a $P < 0.05$.

Chemicals and drugs

[^3H]-idazoxan (40 Ci mmol $^{-1}$) was purchased from Amersham, U.K. Drugs were obtained from the following sources; noradrenaline, naphazoline, phenylephrine, clonidine and adrenaline from Sigma; cirazoline from Synthelabo; rauwolscine from Inverbeff; idazoxan from Reckitt & Colman; UK 14304 (5-bromo-6(2-imidazolin-2-ylamino)-quinaxoline tartrate) from Pfizer; phentolamine from Ciba-Geigy; BHT 920 (6-allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo[4,5-d]azepine dihydrochloride) from Boehringer Ingelheim; WB 4101 (2-(2,6-dimethoxyphenoxyethyl)-aminomethyl-1,4-benzodioxan) from Ward Blenkinsop; cirazoline and BDF 6143 (4-chloro-2-[2-imidazoline-2-ylamino]-isindoline hydrochloride) were synthesized by Syntex. All other chemicals were of the highest purity commercially available.

Figure 1 [^3H]-idazoxan saturation binding was carried out under control conditions (□) K_d 1.7 nM, B_{max} 396 fmol mg $^{-1}$ protein, or in the presence of 1 μM yohimbine (●), K_d 2.2 nM, B_{max} 260 fmol mg $^{-1}$ protein. The results presented are of a single experiment.

Results

The binding of [^3H]-idazoxan to membranes of hamster adipocytes was saturable and of high affinity (Figure 1). Iterative non-linear analysis of the binding isotherms demonstrated that [^3H]-idazoxan labelled a single population (K_d 2.26 $\pm$ 0.02 nM, B_{max} 373 $\pm$ 25 fmol mg $^{-1}$ protein) of binding sites (Table 1). The saturation data was also fitted to a two-site model (double rectangular hyperbola). The two-site fit was not significantly better than the one-site fit. Saturation studies were repeated in the presence of 1 μM yohimbine to preclude [^3H]-idazoxan binding to α_2 -adrenoceptors (Figure 1). The number of [^3H]-idazoxan binding sites (B_{max} 287 $\pm$ 18 fmol mg $^{-1}$ protein) was reduced, in the presence of 1 μM yohimbine, without an apparent decrease in the affinity (K_d 2.19 $\pm$ 0.24 nM) of the radioligand (Table 1). Thus α_2 -adrenoceptors only

Table 1 [^3H]-idazoxan binding to hamster adipocyte membranes

	K_d (nM)	B_{max} (fmol mg $^{-1}$ protein)
Control	2.26 $\pm$ 0.02	373 $\pm$ 25
Yohimbine (1 μM)	2.19 $\pm$ 0.24	287 $\pm$ 18*

Saturation experiments were carried out with 9 concentrations of [^3H]-idazoxan over the range 0.1–12 nM. In addition, saturation studies were repeated in the presence of 1 μM yohimbine to preclude [^3H]-idazoxan binding to α_2 -adrenoceptors. Each value represents the mean $\pm$ s.e. mean of three separate determinations. Statistical significance relative to control density. * $P < 0.05$.

Figure 2 Displacement of [^3H]-idazoxan binding to hamster adipocyte membranes by phentolamine. Membranes were incubated with 1.5 nM [^3H]-idazoxan and increasing concentrations of phentolamine for 30 min at 25°C. The result shown is a single experiment performed in duplicate. Computer-assisted curve fitting demonstrated that a two site fit was significantly better than a one site fit, $P < 0.001$. (one site, ssmin 334010, d.f. 9; two site, ssmin 1473, d.f. 7, F value 76).

contribute approximately 23% of the total [^3H]-idazoxan binding to hamster adipocytes. The failure of 1 μM yohimbine to alter the K_d of [^3H]-idazoxan for the remaining sites implies that [^3H]-idazoxan has similar affinity for both the α -adrenoceptor and the non α_2 -adrenoceptor site.

The displacement of [^3H]-idazoxan binding to adipocyte membranes by phentolamine was complex with Hill slopes considerably less than unity. Computer analysis of the displacement curves indicated that phentolamine displaced [^3H]-idazoxan binding with high (Site_1 , pKi 7.92) and low (Site_2 , pKi 5.21) affinity (Figure 2). Complex displacement curves for [^3H]-idazoxan binding to adipocytes were not confined to phentolamine. Noradrenaline and the α_2 -adrenoceptor antagonists, yohimbine, rauwolscline and BDF 6143 displaced [^3H]-idazoxan with low Hill slopes that could be resolved into two sites (Table 2).

We therefore compared the ability of a number of drugs with affinity for α_2 -adrenoceptors to displace [^3H]-idazoxan from Site_1 in hamster adipocytes and from α_2 -adrenoceptors in rat cerebral cortex (Table 2). The displacement of [^3H]-idazoxan binding by the α_2 -adrenoceptor antagonists was monophasic in rat cerebral cortex, with Hill slopes close to unity. The antagonist affinity rank order of BDF 6143 > phentolamine > rauwolscline > yohimbine > imiloxan, is consistent with [^3H]-idazoxan labelling an α_2 -adrenoceptor in rat cortex. The affinity values at the α_2 -adrenoceptor in rat cortex were similar to the values obtained for the displacement of [^3H]-idaz-

Table 2 Displacement of [^3H]-idazoxan binding to hamster adipocyte and rat cerebral cortical membranes

	pKi		Rat cortex
	Hamster adipocytes Site_1	Site_2	
Yohimbine	7.28 $\pm$ 0.02	4.48 $\pm$ 0.16	7.25 $\pm$ 0.08
Rauwolscline	7.23 $\pm$ 0.04	<4.00	7.10 $\pm$ 0.06
BDF 6143	9.10 $\pm$ 0.06	5.92 $\pm$ 0.04	8.66 $\pm$ 0.22
Phentolamine	7.92 $\pm$ 0.14	5.21 $\pm$ 0.02	8.33 $\pm$ 0.21
Noradrenaline	6.21 $\pm$ 0.10	<4.00	6.70 $\pm$ 0.13*
Adrenaline	6.92 $\pm$ 0.12	<4.00	7.49 $\pm$ 0.13*
Imiloxan	5.67 $\pm$ 0.08		6.68 $\pm$ 0.09
Cimetidine	4.30 $\pm$ 0.05		4.53 $\pm$ 0.07
Histamine	4.78 $\pm$ 0.07		5.13 $\pm$ 0.07

The affinity values were obtained for the displacement of specific binding of 1.5 nM [^3H]-idazoxan from hamster adipocyte and rat cortical membranes. Site_1 represents the site that displays high affinity towards the α_2 -adrenoceptor antagonists but only represents 20–30% of specific binding. Site_2 , comprising the remaining specific binding, displays low affinity towards the α_2 -adrenoceptors and adrenaline and noradrenaline. Each value represents the mean $\pm$ s.e.mean of three separate determinations. *denotes Hill slope less than unity.

oxan binding to Site_1 in adipocytes, suggesting that Site_1 represents the α_2 -adrenoceptor. Inhibition of [^3H]-idazoxan binding to hamster adipocyte membranes by the α_2 -adrenoceptor antagonist imiloxan, the α_1 -adrenoceptor antagonist prazosin and the H_2 -histamine receptor antagonist cimetidine were monophasic, with Hill slopes close to unity. Each drug displaced [^3H]-idazoxan binding with low affinity. All drugs, however were capable of displacing 100% specific binding.

The nature of the second [^3H]-idazoxan binding site on hamster adipocytes was examined by use of a number of α -adrenoceptor ligands. In the presence of 1 μM yohimbine to block [^3H]-idazoxan binding to the α_2 -adrenoceptor, the α -adrenoceptor ligands with the imidazoline side chain displaced [^3H]-idazoxan to the remaining sites with a potency order of cirazoline > naphazoline > UK 14304 > clonidine (Table 3). Cirazoline displaced [^3H]-idazoxan with a Hill slope of unity. In contrast, naphazoline, UK 14304 and clonidine displaced [^3H]-idazoxan binding with Hill slopes significantly different from unity. Noradrenaline and the non-imidazoline α_2 -adrenoceptor agonist BHT 920 failed to displace [^3H]-idazoxan binding from this site on hamster adipocytes. The low affinity of the α_1 -adrenoceptor antagonist WB 4101 and agonist phenylephrine

Table 3 Displacement of [³H]-idazoxan binding to hamster adipocytes in the presence of yohimbine (1 μ M)

	<i>pIC</i> ₅₀	<i>nH</i>
Cirazoline	9.02 $\pm$ 0.13	1.00
Naphazoline	7.90 $\pm$ 0.32	0.50 $\pm$ 0.08
UK 14304	7.52 $\pm$ 0.22	0.70 $\pm$ 0.05
Clonidine	5.71 $\pm$ 0.21	0.72 $\pm$ 0.06
BHT 920	<4.00	—
Phenylephrine	<4.00	—
WB 4101	<5.00	—
Noradrenaline	<4.00	—

Inhibition of [³H]-idazoxan (1.5 nM) binding to hamster adipocyte membranes in the presence of yohimbine (1 μ M) to preclude binding to α_2 -adrenoceptors. Each value represents the mean $\pm$ s.e.mean of four separate determinations.

against [³H]-idazoxan binding suggests that the potency of cirazoline and naphazoline is due to their imidazoline side chain and not to an interaction with α_1 -adrenoceptors.

Discussion

This study indicates that [³H]-idazoxan labels with high affinity two sites in hamster adipocytes; an α_2 -adrenoceptor and a non-adrenoceptor site. Saturation studies indicate that [³H]-idazoxan has similar affinity towards the two sites and that α_2 -adrenoceptors only represent a relatively small proportion of the binding of [³H]-idazoxan. The results of displacement studies provide additional evidence for [³H]-idazoxan binding to α_2 -adrenoceptors and a non-adrenoceptor site. The first binding site (Site₁) representing approximately 20–30% of specific binding has a pharmacological profile consistent with binding to an α_2 -adrenoceptor. In contrast, the other site (Site₂) representing 70–80% specific binding, is unlikely to be an α_2 -adrenoceptor since the antagonists yohimbine, rauwolscine, and BDF 6143 failed to displace [³H]-idazoxan binding at pharmacologically relevant concentrations. Furthermore, the low affinity of noradrenaline and adrenaline towards this binding site makes it unlikely that it is an adrenoceptor.

Competition experiments indicate that compounds containing the imidazoline side chain are potent displacers of [³H]-idazoxan binding to Site₂ in adipocytes. The rank order potency of: α_1 -adrenoceptor agonists, (cirazoline and naphazoline) > the imidazoline α_2 -adrenoceptor agonists, UK 14304 > clonidine

> the non-imidazoline α_2 -adrenoceptor agonist, BHT 920 is consistent with [³H]-idazoxan labelling an imidazoline site. The failure of the α_1 -adrenoceptor agonist phenylephrine and the antagonist WB 4101 to displace [³H]-idazoxan from Site₂ in adipocytes, suggests that the high affinity of cirazoline and naphazoline is due to the imidazoline side chain and not to an interaction at α_1 -adrenoceptors. Cirazoline has been reported to display higher affinity towards [³H]-clonidine binding than [³H]-prazosin binding to rat brain (Van Meel *et al.*, 1981). In field stimulated guinea-pig ileum, cirazoline competitively antagonized the α_2 -adrenoceptor mediated inhibition of twitch height with a *pA*₂ of 7.56 (Ruffolo & Waddell, 1982). Thus cirazoline can act as an α_1 -adrenoceptor agonist and an α_2 -adrenoceptor antagonist. The finding of this study that cirazoline displaces [³H]-idazoxan binding, in the presence of yohimbine, with high affinity indicates that this compound also binds to the imidazoline site in hamster adipocytes.

Imidazoline binding sites, labelled by [³H]-idazoxan, have been described previously. The imidazoline related compounds, cirazoline and UK 14304 displace [³H]-idazoxan from rabbit urethral membranes with high affinity, whereas yohimbine, rauwolscine and the phenylethanolamines, adrenaline, noradrenaline and α -methyl-($\pm$)-noradrenaline are essentially devoid of affinity (Yablonsky *et al.*, 1988). Imidazoline binding sites have been identified in rabbit cerebral cortex and kidney (Couprie *et al.*, 1987; Hamilton *et al.*, 1988) and rabbit adipocytes (Langin & Lafontan, 1989). The affinity rank order of cirazoline (*pKi* 8.50) > naphazoline (*pKi* 8.3) UK 14304 (*pKi* 6.52) > clonidine (*pKi* 5.90) for the displacement of [³H]-idazoxan from rabbit urethral membranes is in agreement, with the exception of UK 14304, with the values obtained in this study. The affinities reported by Langin & Lafontan (1989) for naphazoline (*pKi* 8.00) and clonidine (*pKi* 6.01) suggests that the imidazoline binding site in hamster adipocytes is likely to be similar to the one that exists in rabbit adipocytes. However, the affinity of UK 14304 in both rabbit urethral membranes and adipocytes (*pKi* 6.02) is less than its affinity (*pKi* 7.52) for the imidazoline site in hamster adipocytes. In both rabbit urethral and adipocyte membranes, the affinity of idazoxan was higher at the imidazoline binding site than at the α_2 -adrenoceptor in the corresponding tissue. This contrasts with the findings of this study in which [³H]-idazoxan has a similar affinity at the α_2 -adrenoceptor and the imidazoline binding site. Whether this represents a species difference between the imidazoline sites or a difference in sensitivity of [³H]-idazoxan and UK 14304 in the different buffer systems used has still to be resolved. The α_2 -adrenoceptor agonist, *p*-aminoclonidine

binds to two sites in bovine ventrolateral medulla: an α_2 -adrenoceptor and a non-adrenoceptor site, insensitive to catecholamines (Meeley *et al.*, 1986; Ernsberger *et al.*, 1987). This second site has also been identified in human brainstem (Bricca *et al.*, 1988) where it is labelled by [3 H]-clonidine (Bricca *et al.*, 1989). In displacement studies, cirazoline displaced [3 H]-clonidine with high affinity whereas μ molar concentrations of yohimbine were required to induce the same effect. At present, insufficient evidence is available to determine whether the non-adrenoceptor [3 H]-clonidine binding site in human brainstem is identical with the non-adrenoceptor [3 H]-idazoxan binding site on hamster adipocytes. The low affinity of clonidine towards the non-adrenoceptor site on hamster adipocytes would argue against these sites being similar.

The displacement of [3 H]-idazoxan binding to Site₂ in adipocyte membranes is apparently selective for the imidazoline structure. Imiloxan, an imidazole α_2 -adrenoceptor antagonist was considerably less potent in displacing [3 H]-idazoxan from this site in hamster adipocytes than at the α_2 -adrenoceptor in rat brain. Imidazole binding sites have also been described. Displacement of the binding of the imidazole H_2 -histamine receptor antagonist [3 H]-cimetidine to membranes of cerebral cortical and medulla pons indicated that this ligand was not binding to an H_2 -receptor but to an imidazole site (Rising *et al.*, 1980; Subramanian & Slotkin, 1981). Clonidine and phentolamine potently inhibited [3 H]-cimetidine binding to the imidazole site in rat brain (Burkard, 1978; Subramanian & Slotkin, 1981). In this study, the imidazoles histamine and cimetidine although displacing [3 H]-idazoxan binding completely, were only of low potency.

[3 H]-idazoxan is used frequently as a radioligand to label α_2 -adrenoceptors in both central and peripheral tissue. A recent autoradiographical and binding study carried out in a sodium phosphate buffer, suggests that [3 H]-idazoxan labels a hetero-

ogeneous population of binding sites in rat brain and that one population of these sites is selectively labelled by [3 H]-rauwolscine (Boyajian *et al.*, 1987; Boyajian & Leslie, 1987). There were approximately 4 times the number of 'idazoxan' sites as for rauwolscine. A possible explanation for the apparent discrepancy between the density of sites identified by the two radioligands is that [3 H]-idazoxan labels an imidazoline binding site in addition to α_2 -adrenoceptors. [3 H]-idazoxan binding to rat cortical membranes was carried out in this study under conditions that define α_2 -adrenoceptors. The existence of imidazoline binding sites in rat cortex, must await specifically designed studies.

Evidence from studies on isolated adipocytes suggest that it is the α_2 -adrenoceptor that is responsible for modifying α -adrenergic stimulation of lipolysis in human (Berlan & Lafontan, 1982), rat (Sundin & Fain, 1983) and hamster adipocytes (Carpene *et al.*, 1980). Cirazoline, a compound shown in this study to possess high affinity for the imidazoline binding site on hamster adipocytes, had no effect on noradrenaline- or isoprenaline-stimulated lipolysis, at concentrations up to 1×10^{-5} M (Curtis-Prior & Tan, 1983). Under identical conditions, a concentration-dependent inhibition of lipolysis was observed with clonidine. Thus at present the functional significance of the imidazoline binding site in hamster adipocytes remains uncertain.

These results provide additional evidence that [3 H]-idazoxan labels in addition to α_2 -adrenoceptors, a non-adrenoceptor imidazoline site in hamster adipocytes that is similar to the site recently described in rabbit adipocytes (Langin & Lafontan, 1989). The hamster adipocyte may provide a useful test system for exploring the relationship between α_2 -adrenoceptors and imidazoline binding sites.

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