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EFFECTS OF IDAZOXAN ON CATECHOLAMINE SYSTEMS IN RAT BRAIN

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Abstract—Experiments have been performed to assess the potency of idazoxan (RX 781094) at α and β-adrenoceptors and dopamine receptors and on catecholamine uptake processes in rat brain. The effects of idazoxan on the turnover rates of noradrenaline and dopamine have been determined.

Radioligand binding studies with cerebral cortex membranes have demonstrated that idazoxan exhibits 46-fold selectivity for α_2- adrenoceptors labelled by (^{3}H)-idazoxan (Mean $K_i \pm$ S.E.M. = 3.1 ± 0.4 nM) compared with α_1-adrenoceptors labelled by (^{3}H)-prazosin (Mean $K_i \pm$ S.E.M. = 142 ± 27 nM). Under the same conditions, yohimbine showed 6-fold selectivity for α_2-adrenoceptors. Idazoxan had low affinity for β-adrenoceptors labelled by (^{3}H)-dihydroalprenolol (IC_{50} value >10 μM), for dopamine receptors labelled by (^{3}H)-domperidone (IC_{50} value >20 μM), for the (^{3}H)-noradrenaline uptake site in rat hypothalamus (IC_{50} = 31 μM) and for the (^{3}H)-dopamine uptake site in rat striatum (IC_{50} value ~ 800 μM).

In rats treated with α-methyl-*p*-tyrosine, idazoxan (10–80 mg/kg, po) produced a marked increase (63% at 10, 217% at 20 mg/kg, po) in the apparent rate of turnover of noradrenaline in rat cortex/striatum, without affecting the rate of turnover of dopamine. This was in contrast to yohimbine (5–20 mg/kg, po) which increased the turnover rates of both catecholamines.

In the absence of α-methyl-*p*-tyrosine, idazoxan (5–40 mg/kg, po) produced a dose related increase in the MHPG concentration and a small (20–30%) reduction in the steady state concentration of NA; the duration of the reduction was dose-related. DA steady state concentrations were unaffected.

Idazoxan is a new selective α_2-adrenoceptor antagonist which should prove a valuable investigative tool in neurochemical studies and which may be a useful clinical agent in the management of the affective disorders.

The release of noradrenaline from noradrenergic neurones has been shown to be regulated by compounds which act on α_2-adrenoceptors. *In vivo*, the rate of decline of the concentration of noradrenaline in rat brain following treatment with the tyrosine hydroxylase inhibitor α-methyl-*p*-tyrosine (α-MPT) has been shown to be reduced with concomitant administration of the α_2-adrenoceptor agonist clonidine, and increased with the α_2-antagonist yohimbine [1, 2]. Yohimbine also accelerated the α-MPT-induced decline of the rat brain dopamine concentration; these effects are thought to be secondary to effects on noradrenergic neurones [3]. Alternatively, Scatton *et al.* [4] showed that yohimbine blocked dopamine receptors and proposed that this gave rise to the increased rate of dopaminergic transmission.

Idazoxan (RX 781094, 2-(2-(1,4-benzodioxanyl)-2-imidazoline HCl) has been described as a potent and selective α_2-adrenoceptor antagonist in the rat from both *in vitro* and *in vivo* experiments; yohimbine was both less potent and less selective than idazoxan under the same conditions [5]. Similarly in the rat CNS the EEG synchrony and behavioural depression produced by α_2-agonists were reversed by idazoxan and yohimbine [6].

This paper describes the affinity of idazoxan for catecholamine receptors and uptake sites in rat brain and compares the effects of idazoxan and yohimbine on the apparent rates of turnover of noradrenaline and dopamine.

Preliminary findings have been presented to the British Pharmacological Society [7, 8].

MATERIALS AND METHODS

Materials. The physiological salt solution contained 118 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 24 mM $NaHCO_3$ and 1.3 mM $CaCl_2$. This was equilibrated at 25° with 95% O_2/5% CO_2 before use, final pH 7.4. (^{3}H)-Prazosin was a gift from Pfizer Ltd, specific activity 33 Ci/mmole; (^{3}H)-dihydroalprenolol ((^{3}H)-DHA), 49.4 Ci/mmole and (^{3}H)-domperidone ((^{3}H)-DOM), 43 Ci/mmole were obtained from New England Nuclear; 1-[7,8-^{3}H]-noradrenaline HCl, 8–10 Ci/mmole and [2,5,6-^{3}H]-dopamine, 6 Ci/mmole were obtained from Amersham International plc; (^{3}H)-idazoxan was prepared by custom synthesis (Amersham International plc) by catalytic tritium-bromine exchange and purified by preparative TLC by Dr. P. Taylor of Reckitt and Colman; the specific activity was 30 Ci/mmole. Drug concentrations are in terms of the salts. Saline was 154 mM NaCl. Activated alumina was prepared from Type WN3, Neutral (Sigma) which was refluxed for 1 hr in 2 M HCl, washed to neutrality and dried at

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200° until free-flowing. The following compounds were gifts: prazosin (Pfizer); WB 4101 (Ward Blenkinsop); mianserin (Organon); and phentolamine (Ciba–Geigy). Yohimbine was obtained from Sigma.

Binding studies. Cerebral cortex membranes were prepared and binding assays carried out as described previously [9, 10]. Rat striatal tissue was used for (^{3}H)-DOM binding, final protein concentration approximately 0.25 mg/ml. The incubation time was 15 min for (^{3}H)-idazoxan and (^{3}H)-DHA and 30 min for (^{3}H)-prazosin and (^{3}H)-DOM experiments. Specific binding of (^{3}H)-idazoxan (1 nM) was defined with L-adrenaline (300 μM), (^{3}H)-DHA (1 nM) with alprenolol (1μM), (^{3}H)-prazosin (0.3 nM) with WB 4101 (2 μM) and (^{3}H)-DOM (1.5 nM) with haloperidol (1 μM). The concentration of the compound which produced 50% displacement of the specific binding (IC_{50} value) was determined from the 'Hill' linearization method. The apparent affinity of the compound for the binding site (K_i-value) was determined from the IC_{50} value, using the Cheng and Prusoff equation [11].

Measurement of the inhibition of noradrenaline and dopamine uptake. Rat hypothalamus (NA) or striatum (DA) was homogenized in ice-cold 0.25 M sucrose (1.3 ml per hypothalamus or striatum) and centrifuged at 1000 *g* for 15 min at 2°. The supernatant was diluted 16 times with ice-cold 0.2 M sodium phosphate buffer pH 7.4 containing 100 mM NaCl, 4.6 mM KCl, 10 mM glucose; L-ascorbic acid was added just prior to use to a final concentration of 1 mM. Aliquots (1.9 ml) of this suspension were added to tubes containing 50 μl of 8 mM nialamide solution and 25μl of distilled water (blank) or test compound or compound vehicle and mixed. Each concentration was assayed in triplicate. The mixture was incubated at 37° for 10 min; the tubes were then removed and cooled in ice for 10 min, after which 25 μl of (^{3}H)-amine (final concentration 0.1 μM) was added and the tubes re-incubated for a further 20 min. Blank tubes were kept in ice following the addition of the (^{3}H)-amine. The mixture was rapidly filtered through GFB filters, washing with 4 × 4 ml of ice-cold saline. The filters were immersed in scintillation fluid and the radioactivity was counted.

Measurement of NA and DA turnover rates. Apparent rates of turnover were determined for control and drug-treated rats by following the rates of decline of the catecholamine concentrations at intervals over a period of 2 hr, following intraperitoneal injection of D,L-α-methyl-*p*-tyrosine methyl ester HCl (α-MPT) 320 mg/kg, in saline at a dosage volume of 2 ml/kg. Idazoxan, 5–80 mg/kg in distilled water, or yohimbine, 1.25–20 mg/kg in 2% Tween 80/distilled water, or vehicle was administered orally at a dosage volume of 5 ml/kg. Because of the rapid absorption of idazoxan, this was dosed directly after α-MPT whereas yohimbine which is absorbed more slowly was dosed 30 min prior to α-MPT. Fractional rate constants, pool sizes and apparent turnover rates were determined from analysis of the data using the 'SAS General Linear Models' procedure [12].

Catecholamine assay. Catecholamines in rat cortex/striatum were extracted using the alumina method of Wagner *et al.* [13]. Noradrenaline and dopamine were measured by HPLC on a 10 × 0.46 cm (i.d.) stainless steel column packed with 3 μm Hypersil-ODS (Shandon). The sample was introduced via a Rheodyne 7120 valve injector fitted with a 20 μl loop. The mobile phase consisted of 35 ml of acetonitrile (HPLC grade, Rathburn Chemicals) and 965 ml of an aqueous buffer containing 70 mM NaH_2PO_4, 2 mM heptane sulphonic acid sodium salt and 1 mM Na_2 EDTA; this was adjusted to pH 6.4 with 3 M NaOH prior to mixing with the acetonitrile. The mobile phase was degassed by bubbling with helium for 3 min before use. Detection was carried out using a Bioanalytical Systems Model LC4 amperametric detector equipped with a model TL-5 flow cell and glassy carbon electrode maintained at a potential of +550 mV versus an Ag^+/AgCl reference electrode.

MHPG assay. MHPG was extracted from rat cortex, hydrolysed and extracted with ethyl acetate [14]. The residue was resuspended in 70 μl of a buffer containing 70 mM NaH_2PO_4, 2 mM heptane sulphonic acid sodium salt and 1 mM Na_2EDTA and containing 2 pM D,L-normetanephrine HCl internal standard. The HPLC conditions were similar to those for NA except that 2% acetonitrile was used and the electrochemical detector was set at a potential of +800 mV.

RESULTS

(a) *α-Adrenoceptor selectivity of idazoxan.* Radioligand binding studies were used to assess the α-adrenoceptor selectivity of idazoxan. The α_1-sites

Table 1. Apparent affinities (K_i-values) of α-antagonists for the binding sites in rat cortex P_2-fraction labelled by (^{3}H)-idazoxan (α_2-site) and (^{3}H)-prazosin (α_1-site)

	K_i vs (^{3}H)-idazoxan (nM)	K_i vs (^{3}H)-prazosin (nM)	α_2-Selectivity (index)
Idazoxan	3.1 ± 0.4 (1.08)	142 ± 27 (0.92)	46
Yohimbine	40 ± 5.5 (0.82)	230 ± 16 (0.82)	5.7
Mianserin	82 ± 2.4 (0.89)	103 ± 4 (0.80)	1.3
Phentolamine	7.6 ± 0.9 (0.81)	9.1 ± 0.9 (0.79)	1.2
WB 4101	129 ± 51 (1.02)	0.62 ± 0.15 (0.77)	0.0048
Prazosin	1827 ± 707 (0.95)	0.31 ± 0.02 (0.79)	0.00017

The compounds are arranged in descending order of the α_2-selectivity index (the mean K_i-value versus (^{3}H)-prazosin divided by the mean K_i-value versus (^{3}H)-idazoxan. K_i-values are the mean ± S.E.M. of 3 determinations. Mean Hill slopes are shown in parentheses.

Table 2. % Displacement of the binding of (^{3}H)-DHA (1nM) and (^{3}H)-DOM (1.5 nM) and % inhibition of the uptake of (^{3}H)-NA (0.1 μM) and (^{3}H)-DA (0.1 μM) produced by idazoxan compared with standard agents

System	Idazoxan concentration (μM)	Inhibition (%)	Reference compounds	IC_{50} (μM)
(^{3}H)-DHA binding	10	30	Propranolol	0.001
(^{3}H) DOM binding	20	30	D-Butaclamol	0.0019
			Yohimbine	1.30
(^{3}H)-NA uptake	31	50	Desipramine	0.038
(^{3}H)-DA uptake	800	50	Nomiphensine	0.410

were labelled with (^{3}H)-prazosin and the α_2-sites were labelled with (^{3}H)-idazoxan. In both assays, rat cortex P_2-fractions were prepared and the membranes were incubated in a physiological salt solution: in this medium, each radioligand appeared to label a single site; (^{3}H)-prazosin (0.06–5 nM), B_{max} = 158 ± 16 fmole/mg protein, K_D = 0.69 ± 0.21 nM; (^{3}H)-idazoxan (0.1–35 nM), B_{max} = 153 ± 15 fmole/mg protein, K_D = 4.6 ± 0.6nM [9]. Mean K_i-values (±S.E.M.) of the compounds for the two binding sites are given in Table 1. Mean Hill slopes are shown in parentheses. Selectivity indices obtained by dividing the mean α_1-inhibitor constant by the mean α_2-inhibitor constant are also presented in Table 1. Under the described experimental conditions, idazoxan had about six times the selectivity of yohimbine for the α_2-adrenoceptor and had about ten times higher affinity. A study carried out using whole brain minus cerebellum, in which (^{3}H)-clonidine was used to label α_2-adrenoceptors, gave similar results: the mean K_i-value of idazoxan versus (^{3}H)-clonidine was 5.8 and versus (^{3}H)-prazosin was 547, an α_2-selectivity value of 94 [7]. In the same study, the α_2-selectivity value of yohimbine was 14, about 7-fold less than that of idazoxan.

(b) *Specificity of idazoxan.* In contrast to its high affinity for α_2-binding sites, idazoxan exhibited only weak affinity for β-adrenergic and dopamine binding sites and was only a weak inhibitor of the uptake of (^{3}H)-NA and (^{3}H)-DA (Table 2).

(c) *Effects on NA and DA turnover.* The concentrations of noradrenaline and dopamine in rat cortex/striatum were reduced following treatment with α-MPT. Over the first 4 hr, the data were adequately explained by single exponential models [15]. The mean ± S.E.M. of the fractional rate constant for NA was −0.186 ± 0.014 per hr and for DA was −0.318 ± 0.01 per hr (N = 6).

Following treatment with idazoxan, 10–40 mg/kg po, there was a dose related, and statistically sig-

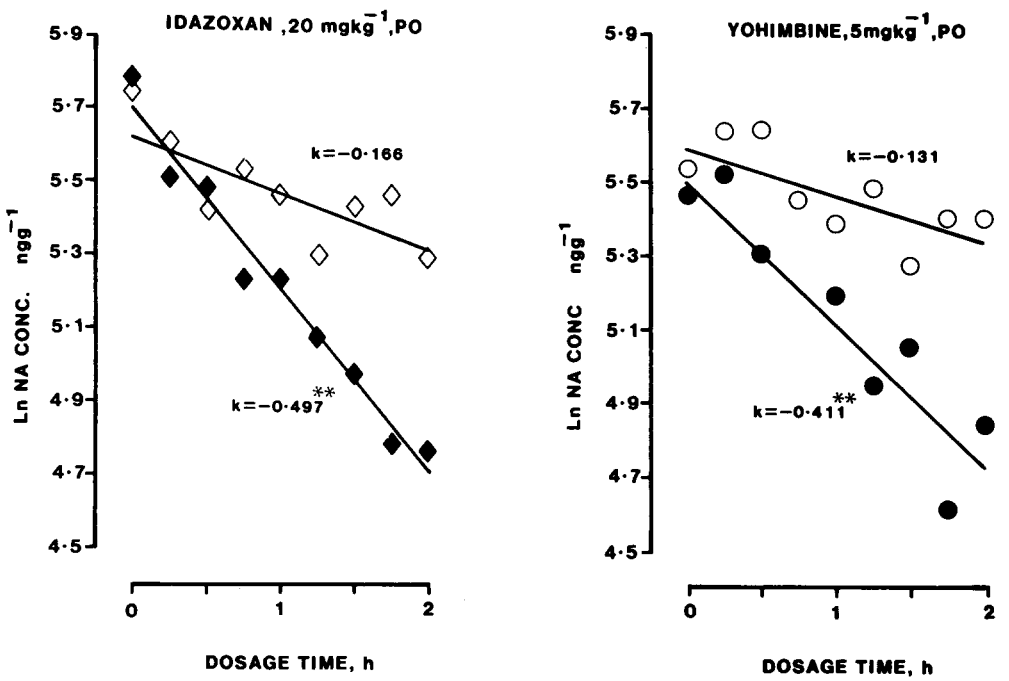

Fig. 1. α-MPT-induced decline in the concentration of NA in rat cortex/striatum with time following idazoxan, 20 mg/kg, p.o. (◆) compared with vehicle treatment (◇) and following yohimbine, 5 mg/kg, p.o. (●) compared with vehicle (○).** $P < 0.01$ versus vehicle fractional rate.

Table 3. The effect of idazoxan, 2.5–40 mg/kg, p.o. on the concentration of MHPG (free and hydrolysed conjugate) in rat cerebral cortex

Treatment	Dose (mg/kg, p.o.)	Mean [MHPG] ± S.E.M. (ng/g) (N = 6)
Distilled water		95 ± 8
Idazoxan	2.5	89 ± 7
	5	123 ± 7 *
	10	133 ± 6 **
	20	158 ± 11**
	40	201 ± 20**

The dosage time was 1 hr. Dunnett's *t*-test was used to test for statistical significance [34].
* $P < 0.05$, ** $P < 0.01$.

nificant increase in the fractional rate constant. The magnitude of the increases were large: 59% increase in the fractional rate constant after 10 mg/kg, po ($P < 0.05$); 199% ($P < 0.01$) after 20 mg/kg, po; 172% ($P < 0.01$) after 40 mg/kg, po. The data for idazoxan, 20 mg/kg, po are shown in Fig. 1. Similar increases in the NA fractional rate constant were observed following yohimbine (5, 10 and 20 mg/kg, po); 156% increase ($P < 0.01$) after 5 mg/kg po; 171% increase ($P < 0.01$) after 10 mg/kg, po, 149% increase ($P < 0.01$) after 20 mg/kg, po. The data for yohimbine, 5 mg/kg, po are shown in Fig. 1.

A further demonstration that idazoxan produced an increase in the turnover of noradrenaline was obtained by measuring the concentrations of MHPG. One hour after an oral dose of idazoxan there was a dose-related increase in the concentration of MHPG in rat cortex (Table 3).

In contrast to its effects on the NA turnover rate idazoxan, (20–80 mg/kg, po) produced no significant changes in the dopamine fractional rate constants compared with control: 22% increase following 20 mg/kg po (NS); 1% increase following 40 mg/kg, po (NS); 1% increase following 80 mg/kg, po (NS). Yohimbine (5–20 mg/kg, po) however did produce increases in the fractional rates of decline of dopamine: 35% increase following 5 mg/kg, po (NS); 39% increase following 10 mg/kg, po ($P < 0.01$); 69% increase following 20 mg/kg, po ($P < 0.01$).

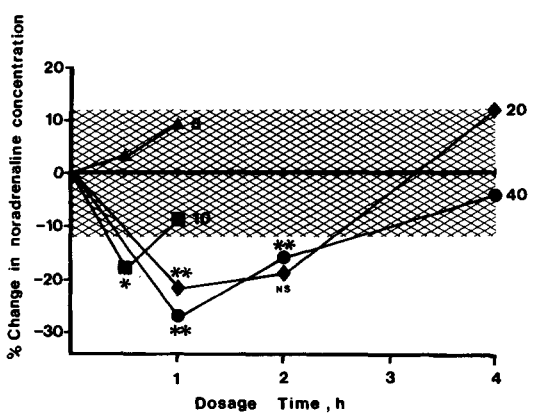

Fig. 2. Percentage change in the steady state concentration of NA in rat cortex/striatum between 30 min and 4 hr following dosing with idazoxan, 5–40 mg/kg, p.o. Each point was obtained from a comparison of 6 drug treated and 6 control rats. Dunnett's *t*-test was used to assign statistical significance.* $P < 0.05$, ** $P < 0.01$. The hatched area shows ± standard deviation of the control NA concentration.

(d) *Effects on steady-state concentrations of noradrenaline and dopamine in rat cortex/striatum.* The mean noradrenaline and dopamine concentrations in rat cortex/striatum were found to be 289 ± 6 ng/g and 1116 ± 47 ng/g respectively (N = 24). Idazoxan, 10–40 mg/kg, po (in the absence of α-MPT) produced small but significant reductions (15–25%) in the noradrenaline concentration (Fig. 2). The duration of the reduction was dose-related. Yohimbine, 10 and 20 mg/kg, po produced similar (24 and 19%) reductions in the NA concentrations 2 hr following dosing. The duration of the effect was not measured.

No statistically significant changes in the dopamine concentration were observed with idazoxan 10–80 mg/kg, for example: −5% and +1% change 1 and 2 hr following 20 mg/kg, po; +3% and −1% change 1 and 2 hr following 40 mg/kg, po. No effect on the dopamine concentration was observed with yohimbine except in one experiment following 10 mg/kg, po (18% reduction, $P < 0.01$).

DISCUSSION

This paper describes a number of findings about the novel compound idazoxan. Firstly, using *in vitro* tests to examine catecholamine binding sites and uptake sites in the rat CNS, idazoxan exhibited marked specificity for α-adrenoceptor binding sites and had 40–50-fold selectivity for the α_2-type. Secondly, idazoxan increased the apparent turnover rate of NA in rat cortex as judged by the increased rate of depletion of the NA concentration following inhibition of synthesis by α-MPT and by the increased levels of the NA metabolite MHPG. Thirdly, idazoxan, unlike the α_2-adrenoceptor antagonist yohimbine, did not increase the rate of turnover of DA in rat brain.

Others have demonstrated the specificity and selectivity of idazoxan in functional studies [5, 6]. In addition, a number of studies have examined the ability of idazoxan to increase the release of radioactive NA following electrical stimulation of vas deferens [16] and brain slices [17, 18]. All these, and the present study point to the fact that idazoxan is a potent and specific α_2-adrenoceptor antagonist. The suggestion that idazoxan exhibits some agonism at α_1-adrenoceptors [19, 20] may be correct but would not be identified in the present study.

The most important finding of this work is the specificity of action of idazoxan on NA turnover [8]. Since this observation, others have shown by the α-MPT method and by measuring metabolites of NA and DA that idazoxan increases the turnover of NA but does not affect the turnover of DA in rat brain [21, 22]. The increase in NA turnover rate produced by α_2-antagonists has been attributed to blockade of α_2-adrenoceptors either at the nerve ending or at the locus coeruleus [23–27], where α_2-blockade prevents the feedback inhibition by the natural transmitter NA released from the main or recurrent collateral branches [28, 29]. The increase in DA turnover rate shown by yohimbine led to the suggestion that this was produced by blockade of α_2-adrenoceptors located on DA neurones [3]. This is now unlikely since the more selective α_2-antagonists idazoxan did not increase DA turnover. Scatton *et al.* [4] have proposed that yohimbine has a direct effect on dopamine receptors. The (^{3}H)-DOM binding data presented here indicates that yohimbine does have a higher affinity than idazoxan for these dopamine binding sites, but is still relatively weak.

Whilst the evidence is good for α_2-adrenoceptors being involved in the regulation of NA release, other studies have implicated α_1-adrenoceptors in a similar role [30–32]. It is unlikely, however, that idazoxan increased NA turnover rate via α_1-adrenoceptors for two reasons. Firstly idazoxan has a 45-fold selectivity for α_2-adrenoceptors in the rat CNS and secondly the doses which affected NA turnover in this study were similar to those producing antagonism of the centrally-mediated mydriatic effect of α_2-adrenoceptor agonists [33].

In the absence of α-MPT, small dose-related reductions in the steady state concentrations of NA were observed following idazoxan. The most probable explanation for this was that the rate of NA synthesis could not keep pace with the increased transmitter release caused by idazoxan. The data from the α-MPT studies have shown that the apparent rate of turnover of NA is increased about 3-fold and to maintain a constant tissue concentration NA synthesis would have to increase at a similar rate. These findings are in contrast to those of Scatton *et al.* [4] who observed no reductions in the steady state concentration of NA. In agreement with the observed lack of effect of idazoxan on dopamine turnover, the compound produced no significant changes in the steady state dopamine concentrations.

In conclusion, in the CNS the novel α_2-adrenoceptor antagonist idazoxan had a marked specificity of action at α-adrenoceptors with 46-fold selectivity for the α_2-type. It showed only weak affinity for β-adrenoceptors and dopamine receptors and was not an inhibitor of the noradrenaline or dopamine uptake mechanisms. The lack of effect on dopamine systems *in vitro* was mirrored *in vivo* where idazoxan produced no increase in the turnover rate of dopamine but had a marked effect on the noradrenaline turnover rate. This was in contrast to yohimbine which increased the turnover rates of both catecholamines.

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