ELSEVIER

European Journal of Pharmacology 277 (1995) 215–221

ejp

Receptor binding and functional evidence suggest that postsynaptic α_2-adrenoceptors in rat brain are of the α_{2D} subtype

David J. Heal *, Sharon C. Cheetham, Sarah A. Butler, Jane Gosden, Michael R. Prow, W. Roger Buckett

Boots Pharmaceuticals Research Department, Nottingham NG2 3AA, UK

Received 25 October 1994; revised 24 January 1995; accepted 31 January 1995

Abstract

This study has determined the subtype(s) of postsynaptic α_2-adrenoceptors in rat brain. This question has been addressed by using two separate approaches, i.e. ligand displacement of [^{3}H]2-(2-methoxy)-1,4-benzodioxan-2-yl)-2-imidazoline ([^{3}H]RX 821002) from membranes prepared from rat cortex after noradrenergic denervation and, secondly, by antagonism of clonidine-induced mydriasis. After rats had been lesioned using *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4; 100 mg/kg i.p., 30 min after zimeldine 10 mg/kg i.p.), noradrenaline was undetectable in the cortex 3 days later. Displacement of [^{3}H]RX 821002 with a range of agonists and antagonists which distinguish between the known α_2-adrenoceptor subtypes ($\alpha_{2A\text{-}2D}$) yielded pK_i values which correlated very well with reported values for the α_{2D}-adrenoceptor ($r = 0.929$; $P < 0.001$), but not the α_{2A} ($r = 0.450$; $P = 0.192$), α_{2B} ($r = 0.280$, $P = 0.434$) or α_{2C} ($r = 0.283$; $P = 0.460$) subtypes. Similarly, the potencies of various α_2-adrenoceptor antagonists to inhibit clonidine (0.03 mg/kg i.p.)-induced mydriasis in conscious rats correlated strongly with their pK_i values for α_{2D}-adrenoceptors ($r = 0.899$; $P = 0.015$) but not α_{2A}- ($r = 0.369$; $P = 0.472$), α_{2B}- ($r = -0.224$; $P = 0.670$) or α_{2C}-adrenoceptors ($r = 0.253$; $P = 0.584$). These data are, therefore, consistent and argue strongly that postsynaptic α_2-adrenoceptors in the rat cortex and Edinger-Westphal nucleus are of the α_{2D} subtype.

Keywords: DSP-4 (*N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine); α_2-Adrenoceptor, postsynaptic; Brain, rat; [^{3}H]RX 821002 binding; Clonidine-induced mydriasis; α_{2D}-Adrenoceptor

1. Introduction

In 1974, Langer proposed that α_2-adrenoceptors should be subclassified as α_1 and α_2 (Langer, 1974). More recently, evidence has been provided to show that neither α_1- nor α_2-adrenoceptors comprise a homogeneous population of receptors. In the latter case, four subtypes have been identified on the basis of molecular biological and radioligand receptor binding techniques, viz. α_{2A}, α_{2B}, α_{2C} and α_{2D} (see Bylund et al., 1991 for review). Prototypically, the α_{2A} subtype has been identified in human platelets, rabbit spleen and the HT29 human adrenocarcinoma cell line (Turner et al., 1985; Bylund et al., 1988; Michel et al., 1989), the α_{2B} subtype in rat neonatal lung, rat kidney and the NG108 cell line (Latifpour et al., 1982; Bylund et al., 1988; Michel et al., 1989), the α_{2C} subtype in the opossum kidney (OK) cell line (Murphy and Bylund, 1988; Bylund et al., 1991) and the α_{2D} subtype in rat submaxillary gland (Michel et al., 1989).

In the rat central nervous system (CNS), it is clear that there are at least two distinct populations of α_2-adrenoceptors. The first consists of the autoreceptors on noradrenergic neurones which control the presynaptic release of noradrenaline (Starke and Montel, 1973; Dennis et al., 1987) and noradrenergic neuronal firing (Cedarbaum and Aghajanian, 1978). The second population of α_2-adrenoceptors is located on sites postsynaptic to noradrenergic neurones (U'Prichard et al., 1980; Dooley et al., 1983). The latter has been defined as a consequence of selective noradrenergic lesioning experiments and, until recently, they were believed to form the overwhelming majority of α_2-adrenoceptors in the CNS (U'Prichard et al., 1980; Dooley et al., 1983). Recently, we have shown that postsynaptic α_2-adrenoceptor proliferation in response to noradrenergic neuronal lesioning has led to a marked

* Corresponding author. Tel. 0115 949 2149, fax 0115 949 2330.

SSDI 0014-2999(95)00078-X

underestimate of the contribution of presynaptic α_2-adrenoceptors to the total number. The latter, in fact, comprises 20–40% of the population present in rat brain (Heal et al., 1993).

In the present study, we have lesioned noradrenergic neurones in rat brain to eliminate α_2-adrenoceptor autoreceptors by administration of the selective neurotoxin, *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4) (Jonsson et al., 1981; Hallman and Jonsson, 1984) which binds covalently to electrophilic centres (Dudley et al., 1990) and inactivates these neurones after being transported into them via the high affinity reuptake system (Cho et al., 1980; Hallman and Jonsson, 1984). We have subsequently determined the affinities of a range of agonists and antagonists for α_2-adrenoceptors in cortical membranes prepared from DSP-4-treated rats to define the subtype(s) of the remaining α_2-adrenoceptors which are located postsynaptically. These α_2-adrenoceptors were labelled using the antagonist ligand, [^{3}H]2-(2-methoxy-1,4-benzodioxan-2-yl)-2-imidazoline ([^{3}H]RX 821002); this radioligand exhibits very high affinity and specificity for α_2-adrenoceptors with 1000- and 300-fold lower affinity for non-adrenergic imidazoline binding sites (NAIBS) and α_1-adrenoceptors than [^{3}H]idazoxan (Langin et al., 1989,1990; Hudson et al., 1992).

The subtype(s) of postsynaptic α_2-adrenoceptors has also been determined using a functional model. It has clearly been demonstrated using both anaesthetised and conscious rats that the mydriasis (pupil dilatation) evoked by α_2-adrenoceptor agonists is exclusively mediated by postsynaptic α_2-adrenoceptors in the CNS (Gherezghiher and Koss, 1979; Hey et al., 1985; Christensen et al., 1990; Heal et al., 1995). Consequently, we have also defined the relative potencies of various α_2-adrenoceptor antagonists to inhibit clonidine-induced mydriasis in conscious rats to define postsynaptic α_2-adrenoceptor subtypes.

2. Materials and methods

2.1. Animals, drugs and administration protocols

Adult male CD rats (Charles River, Margate, UK), weighing 180–200 g at the start of treatment were used. They were housed two or three per cage on a 12 h light/dark cycle (lights on 07.00 h) at 21° C and 55% humidity and allowed CRM (cubed rat and mouse) diet and water ad libitum.

Drugs were obtained from the following sources: corynanthine HCl, oxymetazoline HCl and piperoxan HCl (Sigma, Poole, UK); clonidine HCl, DSP-4 HCl, efaroxan HCl, fluparoxan HCl, idazoxan HCl, phentolamine mesylate, prazosin HCl, 2-(2-methoxy-1,4-benzodioxan-2-yl)-2-imidazoline hydrochloride (RX 821002 HCl), rauwolscine HCl, spiroxatrine, 5-bromo-6-(2-imidazolin-2-ylamino)quinoxaline (UK 14 304), *N*-[2-(2,6-dimethoxyphenoxy)ethyl]-1,4-benzodioxan-2-methylamine hydrochloride (WB 4101 HCl), yohimbine HCl and zimeldine 2HCl (Research Biochemicals, Natick, MA, USA); 1-(2-pyrimidinyl)piperazine dihydrochloride (1-PP 2HCl), 6-chloro-2,3,4,5-tetrahydro-3-methyl-1*H*-3-benzazepine hydrochloride (SKF 86466 HCl) and *N*-methyl-*N*-(1,3,4,6,7,11*b*α-hexahydro-(2*H*)-benzo-[*a*]-quinolizin-2β-yl)propan-1-sulphonamide hydrochloride (WY 26392 HCl) (Boots Pharmaceuticals Medicinal Chemistry Department, UK). All other reagents were obtained from Fisons (Loughborough, UK), Sigma (Poole, UK) and BDH (Poole, UK).

[^{3}H]RX 821002 [(1,4-[6,7(*n*)-^{3}H]benzodioxan-2-methoxy-2-yl)-2-imidazole] (40–60 Ci/mmol) was obtained from Amersham International UK.

To eliminate the contribution of central presynaptic α_2-adrenoceptors, all radioligand-receptor binding studies were conducted using cortices taken from rats which had been lesioned using the selective neurotoxin, DSP-4. For these studies, rats were pretreated with zimeldine (10 mg/kg i.p.) to protect central 5-HT-containing neurones. DSP-4 (100 mg/kg i.p.) was injected 30 min later. Controls received zimeldine followed by saline (2 ml/kg). All rats were then dosed twice daily for 3 days with 1 ml of 2% glucose (p.o.) and 0.5 ml of 0.9% (w/v) saline (s.c.) and received wet mash and 1% glucose (w/v) ad libitum. Animals were killed 72 h after DSP-4 treatment.

2.2. Sample preparation

Rats were killed by cervical dislocation and their brains rapidly removed. The right halves of the cerebral cortex from six rats were used for the determination of monoamine concentrations by high performance liquid chromatography with electrochemical detection (HPLC-ECD). The remaining cortices were used for α_2-adrenoceptor binding. All brain samples were snap frozen in liquid nitrogen. Monoamine determinations were carried out immediately, whereas samples for α_2-adrenoceptor binding were stored at −20° C until assayed.

2.3. [^{3}H]RX 821002 binding assay

Tissue preparation

Cortical tissue was thawed and homogenized in ice-cold 0.25 M sucrose (1:30 w/v) and centrifuged at $1000 \times g$ for 10 min. The supernatant was removed, stored on ice and the pellet homogenized in 0.25 M sucrose (1:15 w/v) and centrifuged at $850 \times g$ for 10 min. Combined supernatants were made up to 1:80 w/v with 5 mM Tris-HCl buffer pH 7.5 (at 25° C) containing 5 mM EDTA and centrifuged at $39000 \times g$

for 12 min. The resulting pellet was resuspended in 50 mM Tris-HCl buffer pH 7.5 (1:80 w/v) containing 0.5 mM EDTA and 5.7 mM L-ascorbic acid and centrifuged at 39000 × *g* for 12 min. The final pellet was resuspended in 50 mM Tris-HCl buffer pH 7.5 (at 25° C) containing 0.5 mM EDTA and 5.7 mM L-ascorbic acid (equivalent to 12.5 mg wet weight of tissue/ml) and used immediately in the binding assays. All centrifugations were carried out at 4° C.

Binding assay

For drug displacement studies, an aliquot (400 μl) of freshly prepared membranes (equivalent to 5 mg wet weight tissue), 50 μl [^{3}H]RX 821002 (1 nM), 50 μl 50 mM Tris-HCl buffer pH 7.5 (at 25° C) containing 0.5 mM EDTA and 5.7 mM L-ascorbic acid (total binding) or 50 μl of drug solution at 10 concentrations ranging from 10^{-12} to 10^{-4} M or 50 μl of 5 μM phentolamine (non-specific binding) were incubated for 75 min at 0° C. Saturation studies were carried out at 8 concentrations of [^{3}H]RX 821002 (0.1–8 nM). Membrane-bound radioactivity was recovered by rapid filtration through Skatron 11734 filter mats using a Skatron cell harvester (setting 6,0,0) with ice-cold 50 mM Tris-HCl buffer pH 7.5. Radioactivity was determined by liquid scintillation counting using Packard emulsifier scintillator 299 at an efficiency of ~ 45%.

2.4. Measurement of brain monoamine concentrations

Measurement of monoamines (noradrenaline, 5-hydroxytryptamine (5-HT) and dopamine) in rat cortex was performed by HPLC with electrochemical detection (HPLC-ECD) to confirm the selectivity and extent of the DSP-4 lesioning procedure. Tissues were homogenized in 5 vols. (w/v) of 0.1 M perchloric acid containing 0.4 mM sodium metabisulphite (antioxidant) and 0.8 μM isoprenaline (internal standard) using a Polytron PT 10-35 disruptor (setting 5–6). After centrifugation at 1100 × *g* for 15 min at 4° C and 15000 × *g* for 5 min at 4° C, 30 μl of the resulting supernatant was injected onto the HPLC system. This comprised a Spectra Physics 8810 pump (flow rate 1 ml/min) connected via a WISP 712 refrigerated autoinjector to a 5 μm Hypersil ODS1 reversed-phase analytical column (length 250 × 4.6 mm i.d.) maintained at 45° C and protected by a Brownlee Aquapore RP-300 precolumn (length 30 × 4.6 mm i.d.). The HPLC mobile phase was 0.1 M sodium dihydrogen orthophosphate-orthophosphoric acid buffer (pH 3.2) containing 16% (v/v) methanol, 2.8 mM 1-octane sulphonic acid sodium salt and 0.7 mM EDTA. Noradrenaline, 5-HT and dopamine were detected by use of a BAS LC-4A amperometric detector connected to a TL-5 flow cell set at a potential of +0.75 V versus an Ag/AgCl reference electrode.

2.5. Measurement of clonidine-induced mydriasis

As clonidine-induced mydriasis is exclusively mediated by central postsynaptic α_2-adrenoceptors, these studies were conducted using unlesioned rats. For these experiments, animals were allowed at least 30 min to adapt to the low ambient lighting conditions. The following α_2-adrenoceptor antagonists (with doses in mg/kg i.p. shown in brackets) were each injected 30 min prior to clonidine (0.03 mg/kg i.p.): – RX 821002 (0.01–3), fluparoxan (0.03–3), efaroxan (0.03–3), idazoxan (0.03–3), yohimbine (0.03–3), SKF 86466 (0.1–10), WY 26392 (0.1–10), phentolamine (0.3–10), piperoxan (0.3–10), 1-PP (0.3–10), rauwolscine (0.3–10), WB 4101 (0.3–30), prazosin (1–30) and spiroxatrine (3–60). Pupil diameters were measured immediately prior to administration of both the antagonists and clonidine and again 10 min after the latter. Measurements were made using a Wild M1 binocular microscope containing a graticule scale in one eyepiece. The microscope was linked to a Swift light box (setting 1, light intensity 450 lux). The procedure was carried out under conditions of low ambient light in an artificially lit room (light intensity 20 lux). The rat was carefully held underneath the light source and its pupil diameter was read off the graticule scale in eyepiece units. This figure was then converted to millimetres.

2.6. Analyses of data and statistics

The concentration of drug required to inhibit binding of the radioligand by 50% (IC_{50}) was determined using the EBDA iterative curve fitting program (McPherson, 1983). These data were converted to inhibition constants (K_i values) using the Cheng and Prusoff (1973) equation. Values for the equilibrium dissociation constant (K_d) and the maximum number of binding sites (B_{max}) were determined by non-linear regression analysis fitted to a one-site binding model using the LIGAND program (Munson and Rodbard, 1980).

The dose of drug required to produce a 50% inhibition of clonidine mydriasis (ID_{50}) was calculated using linear regression of change in pupil diameter 10 min after clonidine plotted against log drug dose.

Correlation coefficients were determined by linear regression analysis. Comparisons between DSP-4-lesioned and non-lesioned animals were made using Student's unpaired *t*-test.

3. Results

3.1. Effects of DSP-4 lesioning on cortical monoamine concentrations

Three days after administration of DSP-4 (100 mg/kg i.p.), rats were killed and single cortices were

taken from six rats which were randomly selected from the group. This lesioning procedure resulted in an almost total obliteration of cortical noradrenaline with no significant effect on either 5-HT or dopamine (Table 1).

3.2. Inhibition constants of various ligands for postsynaptic α_2-adrenoceptors

Three days after DSP-4 lesioning, [^{3}H]RX 821002 binding was conducted in rat cortical membranes. Eighteen drugs were tested for their abilities to dis-

Table 1
Effects of DSP-4 lesioning on noradrenaline, 5-HT and dopamine concentrations in rat cortex

	Noradrenaline	5-HT	Dopamine
Saline	155 ± 6	349 ± 16	15 ± 2
DSP-4	1 ± 1 [a]	304 ± 22	21 ± 5
% Depletion	99	13	+40

Rats were given an i.p. injection of zimeldine (10 mg/kg) 30 min prior to DSP-4 (100 mg/kg i.p.) or saline. Three days later rats were killed and noradrenaline, 5-HT and dopamine concentrations were determined by HPLC-ECD as detailed in Materials and methods. Results are mean monoamine concentration (ng/g tissue wet weight) ± S.E.M. for groups of six rats. [a] Significantly different from saline control $P < 0.001$.

Table 2
Inhibition constants (K_i values) for a range of ligands for postsynaptic α_2-adrenoceptors in rat cortex and those previously reported for individual α_2-adrenoceptor subtypes in prototypical tissues and ID_{50} values for inhibition of clonidine-induced mydriasis in the rat

Drug	Rat cortex after DSP-4	Rabbit spleen	Rat kidney	OK cells	Rat submax	Mydriasis ID_{50}
1 RX 821002	0.69 ± 0.07 [9.16]	–	–	–	–	0.15 [0.82]
2 Fluparoxan	0.92 ± 0.07 [9.04]	–	–	–	–	0.18 [0.74]
3 Efaroxan	0.94 ± 0.13 [9.02]	–	–	–	–	0.20 [0.70]
4 Idazoxan	2.40 ± 0.11 [8.62]	76 [7.12]	51 [7.29]	0.56 [9.25]	5.6 [8.25]	0.46 [0.34]
5 SKF 86466	6.45 ± 0.71 [8.19]	–	–	–	–	1.1 [−0.04]
6 Phentolamine	9.69 ± 1.24 [8.01]	17 [7.77]	11 [7.96]	9.8 [8.01]	3.4 [8.47]	2.5 [−0.40]
7 Oxymetazoline	23.4 ± 3.8 [7.63]	7.8 [8.11]	251 [6.60]	10 [8.00]	8.7 [8.06]	–
8 WY 26392	28.3 ± 1.1 [7.55]	–	–	–	–	1.8 [−0.26]
9 Piperoxan	33.4 ± 6.4 [7.48]	–	–	–	–	3.1 [−0.49]
10 Clonidine	62.6 ± 6.3 [7.20]	155 [6.81]	58 [7.24]	–	63 [7.20]	–
11 1-PP	75.2 ± 2.7 [7.12]	–	–	–	–	4.2 [−0.62]
12 Rauwolscine	85.0 ± 8.5 [7.07]	8.7 [8.06]	3.5 [8.46]	0.05 [10.3]	19 [7.72]	5.1 [−0.71]
13 Yohimbine	105 ± 4 [6.98]	–	–	0.17 [9.77]	–	1.3 [−0.11]
14 WB 4101	128 ± 14 [6.89]	6.8 [8.17]	35 [7.46]	0.48 [9.32]	60 [7.22]	11 [−1.04]
15 Spiroxatrine	182 ± 31 [6.74]	93 [7.03]	3.4 [8.47]	0.49 [9.31]	251 [6.60]	31 [−1.49]
16 UK 14 304	274 ± 24 [6.56]	35 [7.46]	245 [6.61]	–	158 [6.80]	–
17 Prazosin	377 ± 25 [6.42]	4677 [5.33]	60 [7.22]	15 [7.82]	457 [6.34]	28.5 [−1.45]
18 Corynanthine	7533 ± 629 [5.12]	437 [6.36]	295 [6.53]	28 [7.55]	1549 [5.81]	–

K_i values (nM) for rat cortex (after DSP-4) are means ± S.E.M. for 3–4 independent determinations. The K_i values (nM) for rabbit spleen (α_{2A}), rat kidney (α_{2B}) and rat submaxillary gland (α_{2D}) are taken from Michel et al. (1989) and for OK cells (α_{2C}) are taken from Bylund et al. (1991). pK_i values (negative logarithm of the K_i values) are given in parentheses [ ]. ID_{50} values (mg/kg i.p.) for clonidine-induced mydriasis in the rat by a range of adrenoceptor antagonists were calculated using change in pupil diameter 10 min after clonidine at a range of doses. −Log ID_{50} values are given in parentheses [ ].

place [^{3}H]RX 821002 and of these, unlabelled RX 821002 was the most potent, whilst corynanthine was the least active. The K_i and pK_i values of these compounds are given in Table 2. The Hill coefficients for the displacements did not deviate from unity (data not shown).

To characterise the subtype of the cortical postsynaptic α_2-adrenoceptors, linear correlations were calculated for the pK_i values obtained here and those previously reported for individual α_2-adrenoceptor subtypes in prototypical tissues (data shown in Table 2). There was a highly significant correlation between the pK_i values of the ligands for postsynaptic α_2-adrenoceptors and the pK_i values for the α_{2D} subtype ($r = 0.929$, $P < 0.001$) (Fig. 1). There were only moderate to poor correlations with the pK_i values for the α_{2A} ($r = 0.450$, $P = 0.192$), α_{2B} ($r = 0.280$, $P = 0.434$) and α_{2C} ($r = 0.283$, $P = 0.460$) subtypes (Fig. 1).

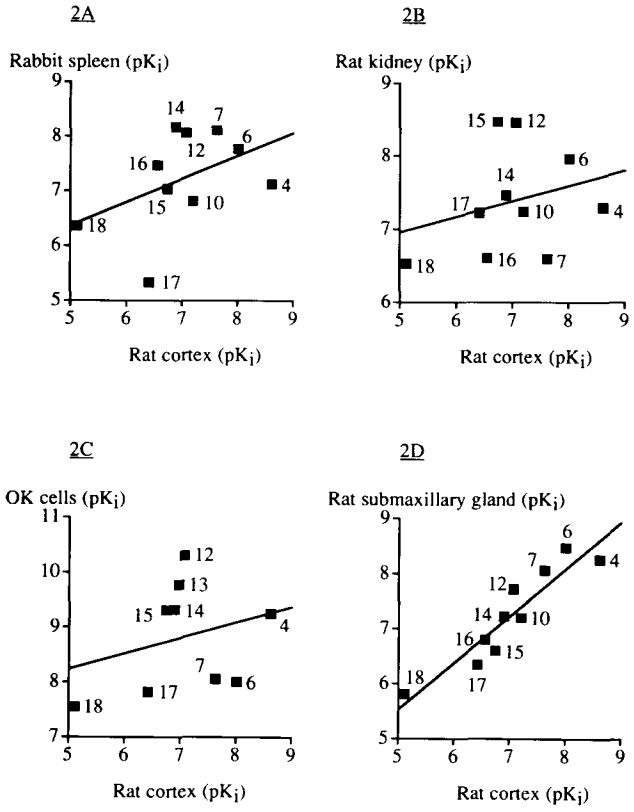

Fig. 1. Correlation plots of pK_i values of a range of ligands for postsynaptic α_2-adrenoceptors in rat cortex labelled with [^{3}H]RX 821002, and those previously reported for individual α_2-adrenoceptor subtypes in prototypical tissues, rabbit spleen (α_{2A}), rat kidney (α_{2B}), OK cells (α_{2C}) and rat submaxillary gland (α_{2D}) taken from Table 2. The numbers correspond to compound number in Table 2. The solid lines represent the regression lines. The data were best described by the expression $y = 0.424x + 4.246$ (α_{2A}), $y = 0.211x + 5.904$ (α_{2B}), $y = 0.285x + 6.814$ (α_{2C}) and $y = 0.850x + 1.274$ (α_{2D}). Correlation coefficients are: α_{2A} $r = 0.450$, $P = 0.192$; α_{2B} $r = 0.280$, $P = 0.434$; α_{2C} $r = 0.283$, $P = 0.460$; α_{2D} $r = 0.929$, $P < 0.001$.

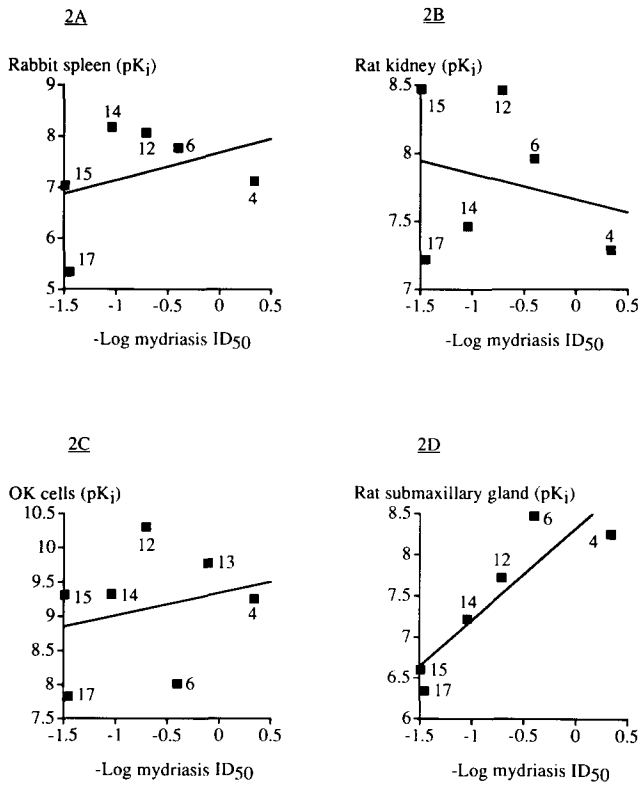

Fig. 2. Correlation plots of $-\log$ mydriasis ID_{50} values for a range of adrenoceptor antagonists to inhibit clonidine (0.03 mg/kg i.p.)-induced mydriasis in the rat and pK_i values for individual α_2-adrenoceptor subtypes in prototypical tissues, rabbit spleen (α_{2A}), rat kidney (α_{2B}), OK cells (α_{2C}) and rat submaxillary gland (α_{2D}) taken from Table 2. The numbers correspond to compound number in Table 2. The solid lines represent the regression lines. The data were best described by the expression $y = 0.541x + 7.675$ (α_{2A}), $y = -0.191x + 7.659$ (α_{2B}), $y = 0.330x + 9.341$ (α_{2C}) and $y = 1.118x + 8.318$ (α_{2D}). Correlation coefficients are: α_{2A} $r = 0.369$, $P = 0.472$; α_{2B} $r = -0.224$, $P = 0.670$; α_{2C} $r = 0.253$, $P = 0.584$; α_{2D} $r = 0.899$, $P = 0.015$.

3.3. Relative potencies of various adrenoceptor antagonists to inhibit clonidine-induced mydriasis

The potencies of 14 adrenergic antagonists to inhibit clonidine-induced mydriasis (0.03 mg/kg i.p.) were also determined. In this model, RX 821002 was again the most potent inhibitor with spiroxatrine being the least potent. The ID_{50} and $-\log ID_{50}$ values of these adrenergic antagonists are given in Table 2.

As before, to characterise the subtype of the postsynaptic α_2-adrenoceptor mediating mydriasis, linear correlations were calculated for the $-\log ID_{50}$ values to inhibit clonidine-induced mydriasis with the pK_i values previously reported for individual α_2-adrenergic subtypes (data shown in Table 2). There was a highly significant correlation between these $-\log ID_{50}$ values and the pK_i values for the α_{2D} subtype ($r = 0.899$, $P = 0.015$) (Fig. 2) with only poor correlations with the

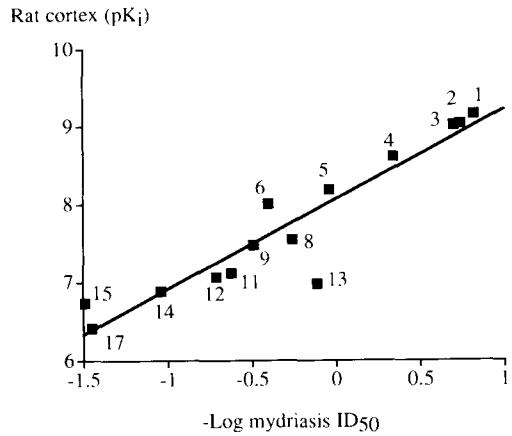

Fig. 3. Correlation plot of pK_i values of a range of adrenoceptor antagonists for postsynaptic α_2-adrenoceptors in rat cortex labelled with [^{3}H]RX 821002 and $-\log$ mydriasis ID_{50} values for inhibition of clonidine (0.03 mg/kg i.p.)-induced mydriasis in the rat taken from Table 2. The numbers correspond to compound number in Table 2. The solid line represents the regression line. The data were best described by the expression $y = 0.733x + 7.731$. The correlation coefficient is $r = 0.933$, $P < 0.001$.

pK_i values for the α_{2A} ($r = 0.369$, $P = 0.472$), α_{2B} ($r = -0.224$, $P = 0.670$) and the α_{2C} ($r = 0.253$, $P = 0.584$) subtypes (Fig. 2). There was also a highly significant correlation between the $-\log ID_{50}$ values for the antagonists and their pK_i values to displace [^{3}H]RX 821002 from postsynaptic α_2-adrenoceptors in rat cortex ($r = 0.933$, $P < 0.001$; Fig. 3).

4. Discussion

It has been previously demonstrated using functional studies that α_2-adrenoceptors exist as autoreceptors on the cell bodies and terminals of noradrenergic neurones (Cedarbaum and Aghajanian, 1978; Starke and Montel, 1973; Dennis et al., 1987). In addition, these receptors are also located on sites postsynaptic to noradrenergic neurones (U'Prichard et al., 1980; Dooley et al., 1983) where they comprise 60–80% of the total α_2-adrenoceptor population depending on the brain region (Heal et al., 1993). Recently, Trendelenburg et al. (1993) have postulated that the prejunctional α_2-adrenoceptors in rat cortex are of the α_{2D} subtype based on the potencies of a variety of α_2-adrenergic ligands to modulate the electrically evoked release of [^{3}H]noradrenaline from brain slices. However, until now, the postsynaptic α_2 subtype(s) existing in rat brain has not been characterised. To address this question using radioligand-receptor binding techniques, noradrenergic neurones have first been lesioned using the neurotoxin DSP-4 (Jonsson et al., 1981; Hallman and Jonsson, 1984). We have shown that, in addition to inactivating noradrenergic neurones, DSP-4 also eliminates presynaptic α_2-adrenoceptor binding in rat cortex (Heal et al., 1993). Using the depletion of noradrenaline to determine the extent of lesioning, the total loss of noradrenaline observed after DSP-4 treatment argues that the cortical membrane preparation was unlikely to have been contaminated by prejunctional α_2-adrenoceptors. In fact, the degree of cortical noradrenergic denervation obtained in these studies was greater than that previously reported in experiments to quantify the presynaptic α_2-adrenoceptor population (Heal et al., 1993). Under these conditions and using a range of ligands which distinguish between the α_2 subtypes, we obtained a highly significant correlation between their pK_i values to displace [^{3}H]RX 821002 from cortical membranes and those previously reported for the α_{2D} subtype (Michel et al., 1989). No significant correlation was obtained with any of the other α_2 subtypes. These results, therefore, argue that postsynaptic α_2-adrenoceptors in rat cortex are predominantly of the α_{2D} subtype. Furthermore, when taken together with the findings of Trendelenburg et al. (1993), they indicate that, in rat cortex, presynaptic and postsynaptic mechanisms are both mediated by the same α_2 subtype, viz. α_{2D}.

The situation that pre- and postsynaptic α_2-adrenoceptors are of the same subtype explains why MacKinnon et al. (1992) obtained evidence for a single population of α_2-adrenoceptors of the α_{2D} subtype in rat cortex using radioligand displacement techniques.

α_2-Adrenoceptor agonists evoke mydriasis in numerous species including rats (see Koss, 1986; Heal, 1990) and studies conducted in anaesthetised rats have shown that this response is exclusively mediated by α_2-adrenoceptors within the CNS (Hey et al., 1985). Furthermore, it has been postulated that these receptors are located in the Edinger-Westphal nucleus where they inhibit parasympathetic tone to the iris (Sharpe and Pickworth, 1981; Hey et al., 1985). Experiments conducted on both anaesthetised and conscious rats have demonstrated that these α_2-adrenoceptors are located postsynaptically to noradrenergic neurones (Hey et al., 1985; Heal et al., 1995). Using clonidine-induced mydriasis in conscious rats to monitor postsynaptic α_2-adrenoceptor function, the potencies of a range of antagonists to inhibit this response correlated well with their pK_i values for the α_{2D}-adrenoceptor (Fig. 2). Once again, there was no significant correlation with any other α_2 subtype.

Overall, the data argue that postsynaptic α_2-adrenoceptors in both the cortex and the Edinger-Westphal nucleus of the rat are predominantly of the α_{2D} subtype. It must be emphasised that correlation coefficients are not sufficiently definitive as a measure to conclude that all of the postsynaptic α_2-adrenoceptors

in these two brain regions are of this subtype. Moreover, these experiments cannot provide any conclusions about the subtype(s) of α_2-adrenoceptors occurring in other rat brain areas.

In conclusion, therefore, evidence has been obtained from both radioligand binding and functional studies to demonstrate that, in the cortex and Edinger-Westphal nucleus of the rat, postsynaptic α_2-adrenoceptors are predominantly of the α_{2D} subtype. These data complement the recent finding (Trendelenburg et al., 1993) that prejunctional α_2-adrenoceptors in rat cortex are also of this type.

Acknowledgements

The authors would like to thank Jean Smith and Michelle Byrne for typing the manuscript.

References

Bylund, D.B., C. Ray-Prenger and T.J. Murphy, 1988, Alpha-2A and alpha-2B adrenergic receptor subtypes: antagonist binding in tissues and cell lines containing only one subtype, J. Pharmacol. Exp. Ther. 245, 600.

Bylund, D.B., H.S. Blaxall, T.J. Murphy and V. Simmoneaux, 1991, Pharmacological evidence for alpha-2C and alpha-2D adrenergic receptor subtypes, in: Adrenoceptors: Structure, Mechanisms, Function, eds. E. Szabadi and C.M. Bradshaw (Birkhäuser Verlag, Basel) p. 27.

Cedarbaum, J.M. and G.K. Aghajanian, 1978, Afferent projections to the rat locus coeruleus as determined by a retrograde tracing technique, J. Comp. Neurol. 178, 1.

Cheng, Y.-C. and W.H. Prusoff, 1973, Relationship between the inhibition constant (K_i) and the concentration of the inhibitor which causes 50 percent inhibition (I_{50}) of an enzymatic reaction, Biochem. Pharmacol. 22, 3099.

Cho, A.K., R.W. Ransom, J.B. Fischer and R.C. Kammerer, 1980, The effects of xylamine, a nitrogen mustard on [^{3}H]norepinephrine accumulation in rabbit aorta, J. Pharmacol. Exp. Ther. 214, 324.

Christensen, H.D., M. Mutzig and M.C. Koss, 1990, CNS alpha$_2$-adrenoceptor induced mydriasis in conscious rats, J. Ocul. Pharmacol. 6, 123.

Dennis, T., R. L'Heureux, C. Carter and B. Scatton, 1987, Presynaptic alpha-2 adrenoceptors play a major role in the effects of idazoxan on cortical noradrenaline release (as measured by in vivo dialysis) in the rat, J. Pharmacol. Exp. Ther. 241, 642.

Dooley, D.J., H. Bittiger, K.L. Hauser, S.F. Bischoff and P.C. Waldmeier, 1983, Alteration of central alpha$_2$- and beta-adrenergic receptors in the rat after DSP-4, a selective noradrenergic neurotoxin, Neuroscience 9, 889.

Dudley, M.W., B.D. Howard and A.K. Cho, 1990, The interaction of the beta-haloethyl benzylamines, xylamine, and DSP-4 with catecholaminergic neurons, Annu. Rev. Pharmacol. Toxicol. 30, 387.

Gherezghiher, T. and M.C. Koss, 1979, Clonidine mydriasis in the rat, Eur. J. Pharmacol. 57, 263.

Hallman, H. and G. Jonsson, 1984, Pharmacological modifications of the neurotoxic action of the noradrenaline neurotoxin DSP4 on central noradrenaline neurons, Eur. J. Pharmacol. 103, 269.

Heal, D.J., 1990, The effects of drugs on behavioural models of central noradrenergic function, in: The Pharmacology of Noradrenaline in the Central Nervous System, eds. D.J. Heal and C.A. Marsden (Oxford Medical Publications, Oxford) p. 266.

Heal, D.J., S.A. Butler, M.R. Prow and W.R. Buckett, 1993, Quantification of presynaptic α_2-adrenoceptors in rat brain after short-term DSP-4 lesioning, Eur. J. Pharmacol. 249, 37.

Heal, D.J., M.R. Prow, S.A. Butler and W.R. Buckett, 1995, Mediation of mydriasis in conscious rats by central postsynaptic α_2-adrenoceptors, Pharmacol. Biochem. Behav. 50, 219.

Hey, J.A., T. Gherezghiher and M.C. Koss, 1985, Studies on the mechanism of clonidine-induced mydriasis in the rat, Naunyn-Schmied. Arch. Pharmacol. 328, 258.

Hudson, A.L., N.J. Mallard, R. Tyacke and D.J. Nutt, 1992, [^{3}H]-RX821002: a highly selective ligand for the identification of α_2-adrenoceptors in the rat brain, Mol. Neuropharmacol. 1, 219.

Jonsson, G., H. Hallman, F. Ponzio and S. Ross, 1981, DSP4 (*N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine) – a useful denervation tool for central and peripheral noradrenaline neurons, Eur. J. Pharmacol. 72, 173.

Koss, M.C., 1986, Pupillary dilation as an index of central nervous system α_2-adrenoceptor activation, J. Pharmacol. Methods 15, 1.

Langer, S.Z., 1974, Presynaptic regulation of catecholamine release, Biochem. Pharmacol. 23, 1793.

Langin, D., M. Lafontan, M.R. Stillings and H. Paris, 1989, [^{3}H]RX821002: a new tool for the identification of α_{2A}-adrenoceptors, Eur. J. Pharmacol. 167, 95.

Langin, D., H. Paris and M. Lafontan, 1990, Binding of [^{3}H]idazoxan and of its methoxy derivative [^{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.

Latifpour, J., S.B. Jones and D.B. Bylund, 1982, Characterization of [^{3}H]yohimbine binding to putative alpha-2 adrenergic receptors in neonatal rat lung, J. Pharmacol. Exp. Ther. 223, 606.

MacKinnon, A.C., A.T. Kilpatrick, B.A. Kenny, M. Spedding and C.M. Brown, 1992, [^{3}H]-15385-197, a selective and high affinity radioligand for α_2-adrenoceptors: implications for receptor classification, Br. J. Pharmacol. 106, 1011.

McPherson, G.A., 1983, A practical computer-based approach to the analysis of radioligand binding experiments, Comput. Programs Biomed. 17, 107.

Michel, A.D., D.N. Loury and R.L. Whiting, 1989, Differences between the α_2-adrenoceptor in rat submaxillary gland and the α_{2A}- and α_{2B}-adrenoceptor subtypes, Br. J. Pharmacol. 98, 890.

Munson, P.J. and D. Rodbard, 1980, LIGAND: a versatile computerised approach for characterization of ligand-binding systems, Anal. Biochem. 107, 220.

Murphy, T.J. and D.B. Bylund, 1988, Oxymetazoline inhibits adenylate cyclase by activation of serotonin-1 receptors in the OK cell, an established renal epithelial cell line, Mol. Pharmacol. 34, 1.

Sharpe, L.G. and W.B. Pickworth, 1981, Pupillary dilation after microinjections of morphine, normorphine and clonidine into the Edinger-Westphal nucleus (EW) in the cat, Neurosci. Abstr. 7, 609.

Starke, K. and H. Montel, 1973, Involvement of α-receptors in clonidine-induced inhibition of transmitter release from central monoamine neurones, Neuropharmacology 12, 1073.

Trendelenburg, A.-U., N. Limberger and K. Starke, 1993, Presynaptic α_2-autoreceptors in brain cortex: α_{2D} in the rat and α_{2A} in the rabbit, Naunyn-Schmied. Arch. Pharmacol. 348, 35.

Turner, J.T., C. Ray-Prenger and D.B. Bylund, 1985, α_2-Adrenergic receptors in the human cell line, HT29. Characterization with the full agonist radioligand [^{3}H]UK-14,304 and inhibition of adenylate cyclase, Mol. Pharmacol. 28, 422.

U'Prichard, D.C., T.D. Reisine, S.T. Mason, H.C. Fibiger and H.I. Yamamura, 1980, Modulation of rat brain α- and β-adrenergic receptor populations by lesion of the dorsal noradrenergic bundle, Brain Res. 187, 143.