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[^{3}H]Dexmedetomidine, an α_2-adrenoceptor agonist, detects a novel imidazole binding site in adult rat spinal cord

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Abstract

Binding properties of [^{3}H]dexmedetomidine [(+)-(*S*)-4-[1-(2,3-dimethylphenyl)ethyl]-1H-imidazole] as an agonist-type radioligand for α_2-adrenoceptors were characterised for the first time in tissues relevant to its analgesic (spinal cord from neonatal or adult rats) and behavioural (rat cerebral cortex) actions. In membranes of rat cerebral cortex (K_d *High* 0.2 ± 0.03 nM, K_d *Low* 8.8 ± 1.4 nM with B_{max} *High* 130 ± 11 fmol/mg protein, R_{High} 16%) and neonatal spinal cord (K_d *High* 0.3 ± 0.04 nM, K_d *Low* 14 ± 3.7 nM with B_{max} *High* 290 ± 40 fmol/mg protein, R_{High} 25%) Gpp(NH)p modifies the biphasic binding to monophasic and binding is competed with specifically by α_2-adrenoceptor compounds. Binding to rat cerebral cortex is not modified by pretreatment with the noradrenergic neurotoxin, DSP-4 (*N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine). In contrast, [^{3}H]dexmedetomidine binding to adult rat spinal cord membranes is more complex and both saturation analysis and competition experiments indicate the presence of a non-adrenergic component of binding (about 40% of total binding) which is sensitive to imidazole-type compounds. This non-adrenergic component of [^{3}H]dexmedetomidine binding can be defined as a novel type of imidazole binding site such that, of the imidazoline I_1 or I_2 receptor ligands, only cimetidine has relatively high affinity. In conclusion, [^{3}H]dexmedetomidine shows very complex binding characteristics that limit its use as an agonist-type radioligand for α_2-adrenoceptors but it may be a useful tool for imidazoline receptor characterisation.

Keywords: α_2-Adrenoceptor; Spinal cord, neonatal; Spinal cord, adult; Cerebral cortex; Imidazole receptor; Imidazole-preferring binding site

1. Introduction

Dexmedetomidine, (+)-(*S*)-4-[1-(2,3-dimethylphenyl)-ethyl]-1H-imidazole, is the pharmacologically active *d*-enantiomer of medetomidine, a potent α_2-adrenoceptor agonist. Dexmedetomidine has high affinity for α_2-adrenoceptors as assessed by [^{3}H]clonidine and [^{3}H]rauwolscine displacement in rat brain membranes. [^{3}H]Prazosin is displaced only weakly by dexmedetomidine, indicating a low affinity for α_1-adrenoceptors, the overall selectivity to α_2-adrenoceptors being approximately 1300. Dexmedetomidine is also devoid of affinity for putative neurotransmitter receptors, such as histamine H_1 or H_2 receptors, dopamine D_1 or D_2 receptors, serotonin 5-HT_1 or 5-HT_2 receptors, benzodiazepine, μ- or α-opioid receptors (Savola et al., 1986; Virtanen et al., 1988; Scheinin et al., 1989; Savola and Virtanen, 1991). In addition, binding studies with transfected mammalian cells expressing exclusively only one human or rat α_2-adrenoceptor subtype indicate that dexmedetomidine has equal affinity for the α_2-adrenoceptor subtypes (Harrison et al., 1991, Jansson et al., 1994). The affinity of medetomidine and its enantiomers for previously described imidazoline I_1 and I_2 receptors (imidazol(in)e-preferring receptors, idazoxan receptors) is remarkably less than the affinity for the α_2-adrenoceptors (Wikberg et al., 1991; Remaury and Paris, 1992; Piletz and Sletten, 1993).

In vivo dexmedetomidine, but not its *l*-enantiomer, induces a dose-dependent CNS depression characterised by a decrease of vigilance and motor activity (Savola and Virtanen, 1991). High doses of dexmedetomidine cause sleep and anaesthesia via activation of α_2-adrenoceptors in rats (Doze et al., 1989) which is caused predominantly by activation of the α_{2A}-adrenoceptors in locus coerulaeus (Correa-Sales et al., 1992a,b; Hayashi et al., 1995). In addition, dexmedetomidine is analgesic in humans and produces antinociception in various experimental animal models at the spinal cord level (Kalso et al., 1991; Aho et

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al., 1991; Pertovaara et al., 1991). Based on these features, medetomidine and its active enantiomer, dexmedetomidine, have been used widely to study the mechanism of α_2-adrenoceptor-mediated antinociception (Kendig et al., 1991; Sullivan et al., 1991; Takano and Yaksh, 1993; Sabbe et al., 1994; Hämäläinen and Pertovaara, 1995).

Being a potent and efficient α_2-adrenoceptor agonist, dexmedetomidine would be valuable as an agonist-type radioligand. In the present study, properties of [^{3}H]dexmedetomidine were characterised in pharmacologically relevant target tissues, i.e. cerebral cortex (related to its behavioural actions) and spinal cord (related to its antinociceptive actions) of rats.

2. Materials and methods

2.1. Membrane preparations

Adult (female Sprague-Dawley, 250–350 g, Alab, Sweden) or neonatal (1–2-day-old neonatal Sprague-Dawley, both sexes, born at Orion Corporation Orion-Farmos, Finland) rats were decapitated, spinal cords and whole brains were rapidly excised and rinsed in ice-cold 50 mM Tris-HCl containing 0.8 mM EDTA (pH 7.5 at 4°C) buffer followed by careful dissection of cerebrocortical tissue. A group of female rats was pretreated with 50 mg/kg *N*-2-chloroethyl-*N*-ethyl-2-bromobenzylamide (DSP-4) i.p. 10 days before decapitation while the control group rats received the vehicle (saline 1 ml/kg i.p.) only. The tissues were either homogenised immediately or frozen in liquid nitrogen and stored at −70°C until homogenised and used. Homogenisation was done in a minimum of 20 vols. of ice-cold 50 mM Tris-HCl/0.8 mM EDTA buffer using a Teflon glass homogeniser (Potter S, Medical Brown, Germany) at 1000 rpm, 10 strokes. Suspensions were then centrifuged at 500 × *g* for 5 min at 4°C; supernatants were filtered through cheesecloth and centrifuged again at 47 800 × *g* for 15 min. The pellets were then washed twice by repeating centrifugation in the above buffer and the final pellets were suspended in 50 mM potassium phosphate buffer (pH 7.5 at 25°C). the suspensions were frozen in liquid nitrogen and stored at −70°C. Protein concentrations were determined using the colorimetric reaction of Bradford (Bradford, 1976) with bovine serum albumin as a reference standard.

2.2. Binding assays

Pilot experiments indicated that binding of [^{3}H]dexmedetomidine was sensitive to buffer composition. Kinetic studies at 25°C showed that in 50 mM potassium phosphate buffer containing 2 mM $MgCl_2$ (pH 7.5 at 25°C) binding was most appropriate, association being rapid and equilibrium being achieved within 20 min (Fig. 1). Dissociation induced by the addition of an excess of unlabelled dexmedetomidine was also rapid and was complete within 30 min when approximately 0.5 nM [^{3}H]dexmedetomidine was used.

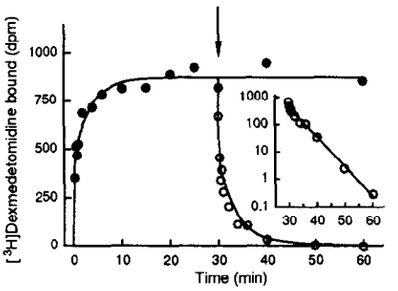

Fig. 1. Kinetics of [^{3}H]dexmedetomidine binding in rat cerebrocortical membranes. In association experiments (●), tissue homogenate was incubated in 0.3 nM [^{3}H]dexmedetomidine at 25°C with either buffer or 1 μM dexmedetomidine to measure total and non-specific binding, respectively. Data shown are from a typical experiment and represent the mean of triplicate determinations. Two binding components were derived with $k_{obs1} = 0.281\ min^{-1}$ and $k_{obs2} = 5.59\ min^{-1}$. In dissociation experiments (○), tissue homogenate was incubated for 30 min with 0.3 nM [^{3}H]dexmedetomidine at 25°C, then an excess of dexmedetomidine (final concentration 1 μM, arrow) was added to initiate dissociation of binding. The data shown are from a typical experiment and represent the mean of triplicate determinations. Specific binding was defined as above. The two dissociation rate constants from this experiments were as follows: $k_{-11} = 0.01\ min^{-1}$ and $k_{-12} = 0.26\ min^{-1}$. Assuming binding extensively to a single site at this [^{3}H]dexmedetomidine concentration, the resulting kinetic K_d is 0.15 nM. Inset shows log of [^{3}H]dexmedetomidine bound (mean dpm, ordinate) vs. time (min, abscissa).

Binding assays were performed by incubating 100 μl [^{3}H]dexmedetomidine to equilibrium (30 min at 25°C) in a shaking water bath with 140 μl membrane suspension (about 100 μg protein) in the absence or presence of various competing drugs in a final assay volume of 250 μl. Non-specific binding was defined with 100 μM (−)-adrenaline, 1 μM dexmedetomidine or 10 μM detomidine in cerebral cortex and spinal cords, respectively, as indicated in the text. Specific binding was calculated as the difference between total and non-specific binding. Saturation experiments were also performed in the presence of 10 μM Gpp(NH)p (guanyl-5′-yl-imidodiphosphate) to evaluate possible high- and low-affinity sites of the [^{3}H]dexmedetomidine binding. Competition experiments were performed in duplicate at [^{3}H]dexmedetomidine concentrations close to the K_d of the high-affinity sites. Specific binding was defined with unlabelled 1 μM dexmedetomidine or 10 μM detomidine in cerebral cortex and spinal cords, respectively.

Bound ligand was separated from free by rapid filtration over Whatman GF/B glass fibre filters and 3 × 5 ml washes with ice-cold 50 mM potassium buffer, using a cell harvester (Brandel, MD). Filters were counted in a β-counter (Wallac 1410, Wallac-Pharmacia, Finland), using 3 ml Opti-Phase II scintillation cocktail (Fisons Chemicals, UK). (−)-Adrenaline was diluted into 0.01 N HCl to avoid degradation and 10 μM pargyline was added to the incubations when the affinity of serotonin was studied.

2.3. Data analysis

Data from saturation studies were analysed to establish K_d and B_{max} values using LIGAND, a non-linear least squares computer program (McPherson, 1985). In competition studies, the IC_{50} values and maximal inhibition of the radioligand by competing drugs were analysed using non-linear regression procedures in Inplot (GraphPad Software, 1990). All data are expressed as means ± S.E.M.

2.4. Drugs and chemicals

[^{3}H]Dexmedetomidine [(+)-(*S*)-4-[1-(2,3-dimethylphenyl)ethyl]-1H-imidazole hydrochloride, specific activity 35.1 and 67.5 Ci/mmol for two different batches] was synthesised for Orion Corporation Orion-Farmos by Amersham (UK) and its purity was > 98%, verified by thin-plate chromatography (89 : 10 : 1 dichloro methane : methanol : ammonium as a solvent system). Dexmedetomidine, detomidine (4-(2,3-dimethylbenzyl)imidazole), *l*-medetomidine [(−)-(R)-4-[1-(2,3-dimethylphenyl)ethyl]-1H-imidazole], atipamezole [4-(2-ethylindan-2-yl)imidazole] and idazoxan were synthesised as hydrochlorides by Orion Corporation Orion-Farmos (Finland). (−)-Adrenaline, clonidine, guanabenz, oxymetazoline, phenylephrine, corynanthine, amiloride, cimetidine, imidazole, imidazole-4-acetic-acid, isoprenaline, (±)-propranolol, serotonin, pargyline and Gpp(NH)p were from Sigma (MO). DSP-4 (*N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine), α-methylnoradrenaline, *p*-aminoclonidine, phentolamine, prazosin, rauwolscine, RX-821002 [(1,4-benzodioxan-2-methoxy-2-yl)-2-imidazoline] and dopamine were from Research Biochemicals (MA). Yohimbine was from Carl Roth (Germany) and histamine from Merck (Germany).

All other reagents were of the highest analytical grade available and were purchased from commercial suppliers.

3. Results

3.1. Saturation analysis

3.1.1. Rat cerebral cortex

Saturation experiments with [^{3}H]dexmedetomidine in rat cerebrocortical membranes indicated binding to two affinity sites with different capacities (Fig. 2A; Table 1). When 100 μM (−)-adrenaline was used to define non-specific binding the proportion of specific [^{3}H]dexmedetomidine binding ranged between 60 and 70% of total [^{3}H]dexmedetomidine binding and B_{max} was about 300 fmol/mg protein. When 1 μM unlabelled dexmedetomidine was used to define non-specific binding the proportion of specific [^{3}H]dexmedetomidine binding was increased to 90–95% and B_{max} was increased to about 900 fmol/mg protein. Regardless of how non-specific binding was defined, approximately 10–15% of the total binding of [^{3}H]dexmedetomidine was at the high-affinity site (Table 1). In the presence of 10 μM Gpp(NH)p, saturation analysis revealed only one class of binding sites and Rosenthal plots were linear (Fig. 2A).

After pretreatment of the rats with i.p. 50 mg/kg DSP-4, the affinity of [^{3}H]dexmedetomidine was similar (K_i *High* 0.29 ± 0.01 nM; K_d *Low* 14 ± 0.85 nM; $n = 3$) to those of the control group (K_i *High* 0.18 ± 0.02 nM; K_d *Low* 12 ± 3.1 nM; $n = 3$) and 21 ± 2% of the binding was at the high-affinity site with B_{max} *High* 160 ± 7 fmol/mg protein (controls: 20 ± 2 high-affinity site with B_{max} *High* 150 ± 15 fmol/mg protein).

3.1.2. Neonatal rat spinal cord

Saturation experiments with [^{3}H]dexmedetomidine and neonatal rat spinal cord membranes indicated binding to two affinity sites with different capacities (Fig. 2B; Table 1). The proportion of specific binding of [^{3}H]dexmede-

Table 1
Affinity of [^{3}H]dexmedetomidine binding in rat cerebrocortical and spinal cord membranes, and effect of Gpp(NH)p

Tissue and non-specific binding definition	K_d *High* (nM)	K_d *Low* (nM)	B_{max} *High* (fmol/mg)	B_{max} *Low* (fmol/mg)	R_{High} (%)	*n*
Rat cerebral cortex						
Adrenaline	0.39 ± 0.02	4.9 ± 1.6	23 ± 8.6	280 ± 42	7.6 ± 2.1	4
Dexmedetomidine	0.20 ± 0.03	8.8 ± 1.4	130 ± 11	790 ± 130	16 ± 2.3	4
+Gpp(NH)p		5.5 ± 0.2		790 ± 42		4
Neonatal rat spinal cord						
Adrenaline	0.08 ± 0.06	5.2 ± 4.3	90 ± 77	581 ± 316	10 ± 5.0	3
Detomidine	0.27 ± 0.04	14 ± 3.7	290 ± 40	860 ± 123	25 ± 2.4	10
+Gpp(NH)p		3.1 ± 0.85		340 ± 54		4
Adult rat spinal cord						
Adrenaline	0.36 ± 0.13	13 ± 2.3	59 ± 27	200 ± 31	23 ± 11	4
+Gpp(NH)p		11 ± 5.9		280 ± 100		2
Detomidine	0.32 ± 0.05	13 ± 0.9	68 ± 14	1200 ± 130	5.7 ± 1.0	9
+Gpp(NH)p		9.4 ± 1.1		1100 ± 68		2

Affinity values were obtained from saturation of binding of [^{3}H]dexmedetomidine in rat cerebrocortical and spinal cord membranes in absence or presence of 10 μM Gpp(NH)p as described in Materials and methods. Each value represents mean ± S.E.M. of *n* determinations performed in duplicate.

tomidine was high (82–88%) when 100 μM (−)-adrenaline was used to define non-specific binding. Using 10 μM detomidine to define non-specific binding, the proportion of specific binding of [^{3}H]dexmedetomidine was slightly increased being 93–98% of total binding and these saturation results were best-fitted to a two-site model (Table 1). In the presence of 10 μM Gpp(NH)p, saturation analysis revealed only one class of the binding sites and Rosenthal plots were linear (Fig. 2B; Table 1).

3.1.3. Adult rat spinal cord

Also in adult rat spinal cord preparations, saturation experiments with [^{3}H]dexmedetomidine indicated binding to two affinity sites with different capacities (Fig. 2C; Table 1). The proportion of specific binding of [^{3}H]dexmedetomidine was low (23–45%) when 100 μM (−)-adrenaline was used to define non-specific binding. When non-specific binding was defined with 10 μM detomidine the proportion of specific binding of [^{3}H]dexmedetomidine was increased to 82–95% of the total [^{3}H]dexmedetomidine binding. In both cases, the saturation data for [^{3}H]dexmedetomidine binding were best-fitted to two sites with different affinities and capacities (Fig. 2C; Table 1). Saturation experiments performed in the presence of 10 μM Gpp(NH)p yielded only one binding site and Rosenthal plots were linear (Fig. 2C).

3.2. Competition of [^{3}H]dexmedetomidine binding

3.2.1. Rat cerebral cortex

The ability of various adrenoceptor agonists and antagonists as well as compounds known to bind to imidazoline receptors (Table 2) to inhibit 0.3–0.4 nM [^{3}H]dexmedetomidine binding was studied. Specific binding was defined with 1 μM unlabelled dexmedetomidine and was about 90% of the total binding. Most of the compounds inhibited [^{3}H]dexmedetomidine in a manner consistent with a single binding site but the imidazoline compounds, phentolamine and oxymetazoline, inhibited [^{3}H]dexmedetomidine in a biphasic manner (Table 2). The specific binding of [^{3}H]dexmedetomidine to rat cerebrocortical membranes was most effectively inhibited by known α_2-adrenoceptor agonists and antagonists (Fig. 3A, Fig. 4A) while, e.g. β-adrenergic and serotonergic agents as well as cimetidine had low affinity (Table 2). Of the specific binding, 80–90% was sensitive to α_2-adrenoceptor ligands, while the remaining 10–20% was sensitive only to dexmedetomidine or its analogs and clonidine, oxymetazoline and dopamine (Table 2).

3.2.2. Neonatal rat spinal cord

Adrenoceptor agonists and antagonists from different chemical classes were tested for their ability to inhibit the

Table 2
Affinity values of competing drugs at [^{3}H]dexmedetomidine binding sites in rat cerebrocortical membranes

Competitor	n_H	$IC_{50(1)}$ (nM)	$IC_{50(2)}$ (nM)	$R_{(1)}$ (%)	I_{max} (%)	n
α_1 and α_2-adrenoceptor agonists						
Dexmedetomidine	1.0 ± 0.11	0.99 ± 0.2				3
Oxymetazoline	0.94 ± 0.03	8.3 ± 3.5	990 ± 590	87 ± 5		4
Detomidine	1.0 ± 0.26	9.4 ± 2.9				3
Clonidine	0.81 ± 0.06	15 ± 3.1				5
(−)-Adrenaline	1.1 ± 0.04	37 ± 6.6			83 ± 1	3
α-Methylnoradrenaline	0.98 ± 0.06	65 ± 7.9			80 ± 2	4
l-Medetomidine	0.89 ± 0.10	94 ± 14				4
Phenylephrine	0.74 ± 0.10	1 900 ± 320			87 ± 3	5
α_1 and α_2-adrenoceptor antagonists						
Atipamezole	0.83 ± 0.09	1.9 ± 0.1				3
Phentolamine	0.58 ± 0.03	24 ± 5.3	6 800 ± 1 600	80 ± 1		4
Yohimbine	0.71 ± 0.19	100 ± 6.9			88 ± 1	3
Rauwolscine	0.72 ± 0.10	110 ± 47			88 ± 3	3
Prazosin	0.87 ± 0.08	1 400 ± 280			83 ± 6	3
Corynanthine	0.72 ± 0.05	8 800 ± 61				4
Others						
Dopamine	0.75 ± 0.10	1 500 ± 120				4
Methysergide	0.77 ± 0.06	6 500 ± 1 200			89 ± 2	4
Isoprenaline	0.71 ± 0.82	25 000 ± 5 000			89 ± 1	5
(±)-Propranolol	0.78 ± 0.05	28 000 ± 6 500			89 ± 3	5
Serotonin	1.09 ± 0.12	77 000 ± 9 300			81 ± 4	4
Cimetidine		n.d.			62 ± 2	5

Affinity values (IC_{50}, nM) were obtained from analyses of competition for binding of 0.2–0.3 nM [^{3}H]dexmedetomidine from rat cerebrocortical membranes as described in Materials and methods (n.d., not determinable). n_H and $R_{(1)}$ refer to Hill slope and proportion (%) of high-affinity binding site. Each value represents mean ± S.E.M. of n determinations performed in duplicate. Maximal inhibition (I_{max}; plateau or response to 200 μM, highest concentration used) is given if < 100%.

binding of 0.3–0.4 nM [^{3}H]dexmedetomidine to membranes of neonatal spinal cord. The specificity of [^{3}H]dexmedetomidine binding was defined with 10 μM detomidine and was about 95% of the total binding. All compounds studied were best-fitted to a one-site model. The specific binding of [^{3}H]dexmedetomidine to rat neonatal spinal cord membranes was most effectively inhibited by known α_2-adrenoceptor agonists and antagonists (Fig. 3B, Fig. 4B) while, e.g. β-adrenergic and serotonergic agents and cimetidine had low affinity (Fig. 5A; Table 2). The maximal responses to amiloride, cimetidine, histamine, imidazole and imidazole-4-acetic acid (at 200 μM) were as follows: 42, 43, 65, 22 and 0%, respectively.

3.2.3. Adult rat spinal cord

Adrenoceptor agonists and antagonists from different chemical classes were tested for their ability to inhibit the binding of 0.3–0.4 nM [^{3}H]dexmedetomidine to membranes of adult spinal cord. The specificity of [^{3}H]dexmedetomidine binding was defined with 10 μM detomidine and was about 90% of the total binding. Known α_2-adrenergic compounds, such as (−)-adrenaline, α-methylnoradrenaline, rauwolscine and yohimbine, had good affinity and known α_1-adrenoceptor compounds, i.e. prazosin, corynanthine and phenylephrine, had low affinity, but all were able to inhibit specific [^{3}H]dexmedetomidine binding only by about 60% (Fig. 3C, Fig. 4C; Table 3). β-Adrenergic and serotonergic compounds had only very low affinity (Table 3). Known α_2-adrenoceptor antagonists and agonists with imidazoli(di)ne structure (idazoxan, RX-821002, phentolamine, clonidine and *p*-aminoclonidine) and guanabenz competed for the binding of [^{3}H]dexmedetomidine in a biphasic manner (Fig. 3C, Fig. 4C, Fig. 5B), the proportion of high-affinity site being about 60% (Table 3). Cimetidine (Fig. 5B) inhibited [^{3}H]dexmedetomidine binding in a biphasic manner also, the affinity for the high-affinity site being IC_{50} 140 ± 30 nM ($n = 3$). Histamine, imidazole-4-acetic acid and amiloride had low affinity (Fig. 5B; Table 3).

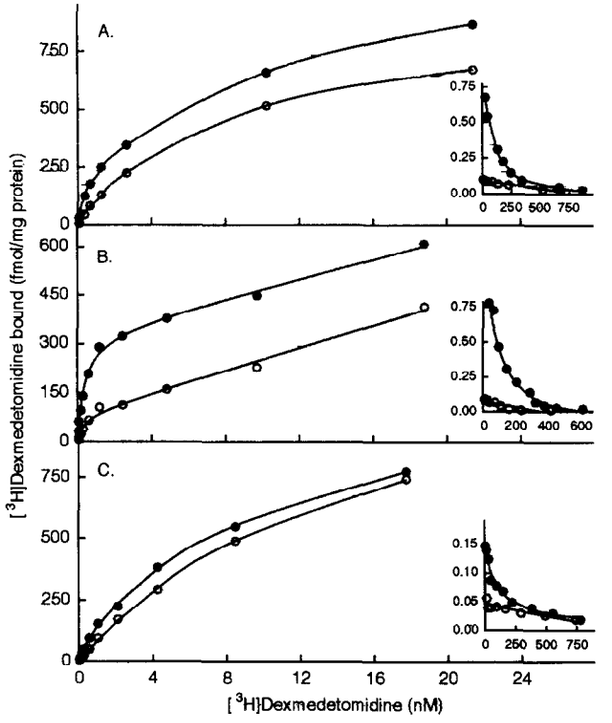

Fig. 2. Equilibrium-saturation binding of [^{3}H]dexmedetomidine in membranes of rat cerebral cortex (A), neonatal rat spinal cord (B) and adult rat spinal cord (C). Membranes were incubated with the indicated concentrations of [^{3}H]dexmedetomidine in the absence (●) or presence (○) of 10 μM Gpp(NH)p for 30 min at 25°C. Data are from a typical experiment and represent the mean of triplicate determinations. Insets show respective Rosenthal plots with bound/free (ordinate) vs. bound ligand (fmol/mg protein, abscissa) of [^{3}H]dexmedetomidine binding.

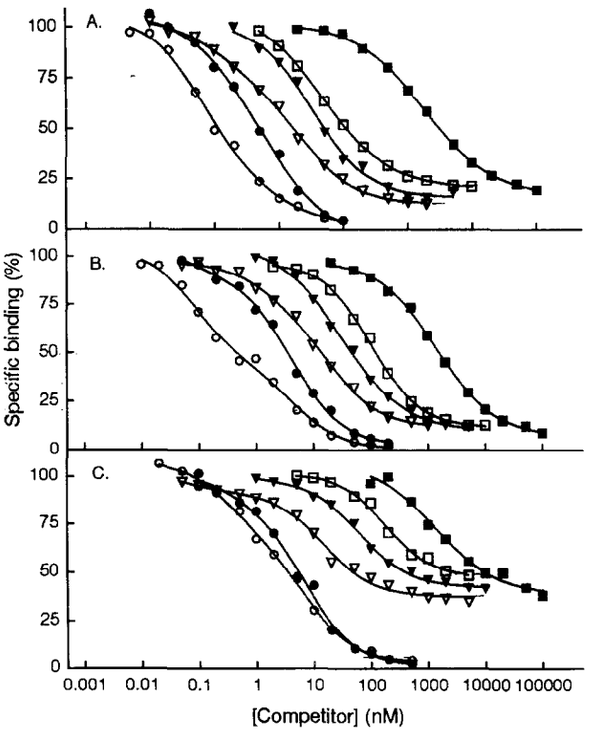

Fig. 3. Competition of α_1- and α_2-adrenoceptor agonists for specific [^{3}H]dexmedetomidine binding in rat cerebrocortical (A), neonatal rat spinal cord (B) and adult rat spinal cord (C) membranes. Membranes were incubated with 0.3–0.4 nM [^{3}H]dexmedetomidine and the following competing drugs: dexmedetomidine (○), detomidine (●), clonidine (▽), adrenaline (▼), α-methylnoradrenaline (□) and phenylephrine (■). Data shown are mean values of at least three separate experiments, each performed in duplicate. S.E. values (< 10%) are omitted for clarity.

4. Discussion

This study has shown that [^{3}H]dexmedetomidine binds with high affinity to α_2-adrenoceptors but with very low affinity to α_1-adrenoceptors and other putative neurotransmitter receptors in cerebral cortex, confirming the previously reported high specificity of dexmedetomidine (Savola

and Virtanen, 1991). The α_2-adrenoceptors labelled by [^{3}H]dexmedetomidine in cerebral cortex appear to locate to a major extent on non-adrenergic neurones because pretreatment with DSP-4, an effective noradrenergic-selective neurotoxin (Berridge and Dunn, 1990), does not modify the binding of [^{3}H]dexmedetomidine.

The specificity of [^{3}H]dexmedetomidine for α_2-adrenoceptors appears to be the case also in the neonatal rat spinal cord but certainly not in adult rat spinal cord. During the maturation process of the rat spinal cord the appearance of non-adrenergic binding sites, remarkably labelled by [^{3}H]dexmedetomidine, is evident, supporting the notion that these sites have a physiological role. The profile of this non-adrenergic binding site of [^{3}H]dexmedetomidine is different from those previously described for imidazoline receptor ligands (see below).

The most important direct impact of these results is on studies of spinal effects of dexmedetomidine. Unfortunately, in most experiments where dexmedetomidine has been used as a tool to study α_2-adrenoceptor-mediated antinociception at the spinal cord, pharmacological specificity was verified only with atipamezole, an α_2-adrenoceptor antagonist (Virtanen et al., 1989) that also has marked non-adrenergic binding characteristics (Sjöholm et al., 1992, 1995). In the present study, atipamezole competes completely for [^{3}H]dexmedetomidine in the adult rat spinal cord, a property which is not shared with the 'conventional' α_2-adrenoceptor antagonists, rauwolscine and yohimbine (Table 3), indicating that atipamezole has, additionally, good affinity to the imidazole binding site labelled by [^{3}H]dexmedetomidine.

The possible role of spinal imidazoline receptors in antinociceptive actions of clonidine has already been challenged (Monroe et al., 1995). However, as this study indicates, the imidazoline receptors of [^{3}H]clonidine have quite different characteristics compared to these imidazole-sensitive sites detected by [^{3}H]dexmedetomidine. Spinal imidazole binding sites of [^{3}H]dexmedetomidine

Table 3
Affinity values of competing drugs at [^{3}H]dexmedetomidine binding sites in spinal cord membranes of neonatal or adult rats

Competitor	Neonatal rat spinal cord			Adult rat spinal cord					
	n_H	IC_{50} (nM)	n	n_H	$IC_{50(1)}$ (nM)	$IC_{50(2)}$ (nM)	R_{High} (%)	I_{max} (%)	n
α_1 and α_2-adrenoceptor agonists									
Dexmedetomidine	0.66 ± 0.09	0.60 ± 0.2i	5	0.77 ± 0.01	3.0 ± 0.2				3
Guanabenz	0.65 ± 0.08	2.9 ± 0.4	4	0.17 ± 0.10	3.2 ± 0.3	14 000 ± 1 100	58 ± 1		4
Detomidine	0.83 ± 0.05	3.4 ± 0.1	3	0.87 ± 0.06	5.1 ± 0.3				3
Oxymetazoline	0.78 ± 0.03	5.5 ± 1.4	4	0.62 ± 0.04	26 ± 7.7				6
p-Aminoclonidine	0.84 ± 0.03	8.4 ± 2.0	5	0.38 ± 0.09	9.2 ± 2.3	n.d.	6.3 ± 2		4
Clonidine	0.75 ± 0.11	11 ± 0.9	3	0.38 ± 0.04	15 ± 5.3	n.d.	65 ± 4		4
(−)-Adrenaline	0.85 ± 0.05	34 ± 3.3	6	0.86 ± 0.04	67 ± 12			59 ± 3	6
l-Medetomidine	0.85 ± 0.07	75 ± 9.4	3	0.88 ± 0.10	32 ± 6.8				4
α-Methylnoradrenaline	1.1 ± 0.19	97 ± 14	5	1.0 ± 0.16	200 ± 18			54 ± 3	3
Phenylephrine	0.81 ± 0.03	1 400 ± 50	3	0.72 ± 0.06	2 900 ± 1100			69 ± 4	3
α_1 and α_2-adrenoceptor antagonists									
RX-821002	0.83 ± 0.03	2.8 ± 0.4	4	0.28 ± 0.07	1.3 ± 0.3	9 500 ± 1 300	62 ± 5		4
Atipamezole	0.73 ± 0.03	2.8 ± 1.0	6	0.73 ± 0.03	2.1 ± 0.2				4
Phentolamine	1.0 ± 0.07	29 ± 3.7	5	0.24 ± 0.04	8.7 ± 2.3	11 000 ± 1 400	51 ± 3		7
Idazoxan	0.74 ± 0.05	35 ± 13	4	0.38 ± 0.05	15 ± 2.3	7 700 ± 1 300	59 ± 4		5
Rauwolscine	0.68 ± 0.07	140 ± 43	5	0.50 ± 0.10	110 ± 17			62 ± 7	5
Yohimbine	0.66 ± 0.07	160 ± 62	5	0.58 ± 0.04	74 ± 30			57 ± 5	5
Prazosin	0.77 ± 0.10	1 800 ± 80	3	0.57 ± 0.11	3 100 ± 440			79 ± 6	3
Corynanthine	0.78 ± 0.05	25 000 ± 4 800	5	0.78 ± 0.05	13 000 ± 1200			64 ± 5	3
Others									
Dopamine	1.1 ± 0.09	1 100 ± 180	3	1.1 ± 0.16	1 500 ± 290			57 ± 2	3
Methysergide	0.92 ± 0.04	5 500 ± 450	3	0.62 ± 0.05	8 600 ± 1 100			78 ± 4	5
Isoprenaline	0.88 ± 0.05	20 000 ± 1 800	3	0.94 ± 0.19	64 000 ± 20 000			65 ± 1	4
(±)-Propranolol	0.61 ± 0.10	29 000 ± 4 000	3	0.80 ± 0.10	21 000 ± 4 900			63 ± 5	6
Serotonin	0.97 ± 0.07	48 000 ± 11 000	3	1.1 ± 0.25	28 000 ± 5 700			55 ± 3	6
Amiloride		n.d.	6		n.d.			55 ± 9	5
Cimetidine		n.d.	3	0.26 ± 0.02	136 ± 30	n.d.		73 ± 2	3
Histamine		n.d.	5		n.d.			73 ± 5	4
Imidazole		n.d.	3		n.d.			65 ± 3	4
Imidazole-4-acetic acid		n.d.	3		n.d.				4

Affinity values (IC_{50}, nM) were obtained from analyses of competition for binding of 0.2–0.3 nM [^{3}H]dexmedetomidine from rat spinal membranes as described in Materials and methods (n.d., not determinable). n_H and R_{High} refer to Hill slope and proportion (%) of high-affinity binding site. Each value represents mean + S.E.M. of n determinations performed in duplicate. Maximal inhibition (I_{max}; plateau or response to 200 μM, highest concentration used) is given if < 100%.

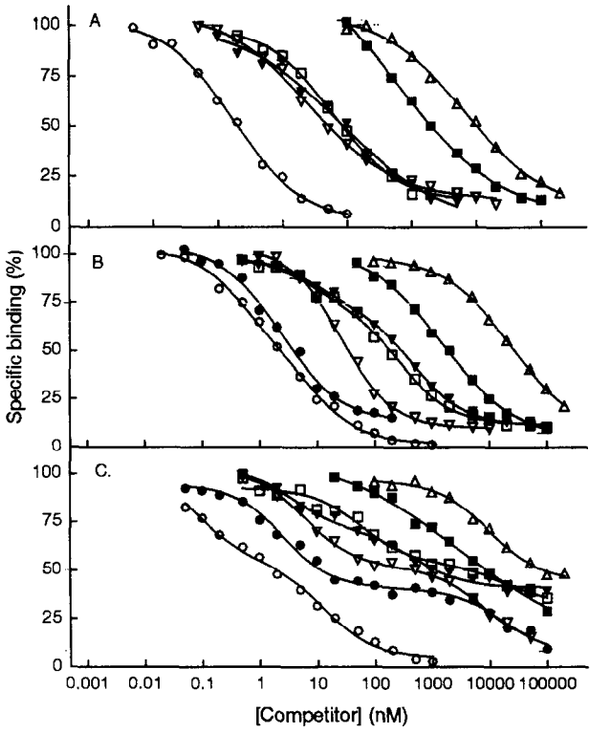

Fig. 4. Competition of α_1- and α_2-adrenoceptor antagonists for specific [^{3}H]dexmedetomidine binding in rat cerebrocortical (A), neonatal rat spinal cord (B) and adult rat spinal cord (C) membranes. Membranes were incubated with 0.3–0.4 nM [^{3}H]dexmedetomidine and the following competing drugs: RX821002 (●), atipamezole (○), phentolamine (▽), yohimbine (▼), rauwolscine (□), prazosin (■) and corynanthine (△). Data shown are mean values of at least three separate experiments, each performed in duplicate. S.E. values (< 10%) are omitted for clarity.

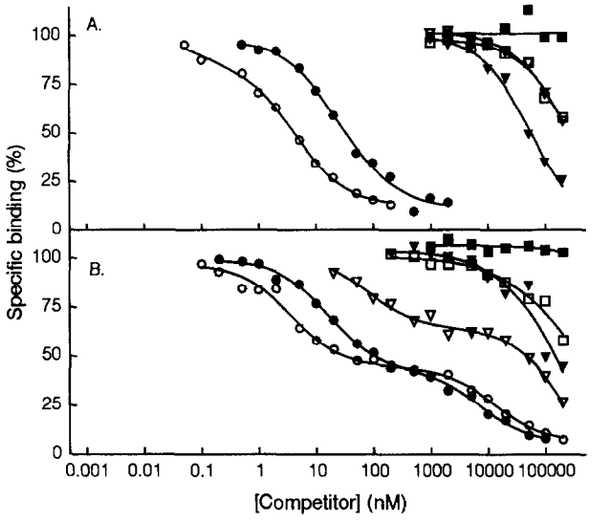

Fig. 5. Competition of drugs with known affinity to previously characterised I receptors for specific [^{3}H]dexmedetomidine binding in neonatal rat spinal cord (A) and adult rat spinal cord (B) membranes. Membranes were incubated with 0.3–0.4 nM [^{3}H]dexmedetomidine and the following competing drugs: idazoxan (○), guanabenz (●), cimetidine (▽), histamine (▼), amiloride (□) and imidazole-4-acetic acid (■). Data shown are mean values of at least three separate experiments, each performed in duplicate. S.E. values (< 10%) are omitted for clarity.

are probably not linked to a major extent to antinociceptive actions of dexmedetomidine because the spinal effects of dexmedetomidine can also be antagonised with reasonable doses of idazoxan (Fisher et al., 1991) which shows only low affinity for [^{3}H]dexmedetomidine's imidazole binding sites (Table 3).

In this study, [^{3}H]dexmedetomidine was used for the first time as a radioligand. The results indicate that [^{3}H]dexmedetomidine binds with high affinity, in a saturable and reversible manner, to membranes prepared from rat cerebral cortex, neonatal rat spinal cord and adult rat spinal cord, albeit with very complex binding characteristics.

The heterogeneity of [^{3}H]dexmedetomidine binding is dependent on the tissue and the developmental stage of the rat CNS. Both in adult rat cerebrocortical and neonatal rat spinal cord membranes [^{3}H]dexmedetomidine labels a higher number of receptors when some imidazole derivative are used instead of (−)-adrenaline to define non-specific binding. This difference most probably does not reflect marked binding to imidazole binding sites because α-methylnoradrenaline and many other ligands known not to bind to imidazoline I_1 or I_2 receptors (and which fail to completely compete for the binding of [^{3}H]dexmedetomidine in the adult spinal cord) are potent competitors in these tissues, although the non-specific binding was defined using the imidazole derivatives.

α_2-Adrenoceptors are members of G-protein-coupled receptors. In this superfamily of receptors, the presence of Mg^{2+} ion and subsequent G-protein-induced formation of two affinity conformations of the receptor typically results in binding isotherms of agonist which are best-fitted to two-site models that incorporate one high-affinity and one low-affinity binding site (Birnbaumer et al., 1990). It has been also shown that imidazoline I_1 receptors share this property of G-protein-coupled receptors (Ernsberger, 1992). In contrast, imidazoline I_2 receptors are devoid of G-protein coupling because experimental modifications modulating G-protein receptor axes have no manifest effect (Michel and Insel, 1989). To evaluate if the two-site binding isotherms of [^{3}H]dexmedetomidine were due to G-protein-related high- and low-affinity agonist sites, a non-hydrolyzable GTP analog (Gpp(NH)p) was used to uncouple the G-protein from the receptor and force the receptor to the low-affinity agonist state. In the presence of Gpp(NH)p, the high-affinity state binding of [^{3}H]dexmedetomidine is completely abolished in cerebrocortical, neonatal spinal cord and adult spinal cord membranes, suggesting that [^{3}H]dexmedetomidine is binding to two different G-protein-dependent affinity states.

The imidazole binding site for [^{3}H]dexmedetomidine differs from those described for other imidazoline receptor ligands, such as [^{3}H]*p*-aminoclonidine, [^{3}H]clonidine, [^{3}H]idazoxan and [^{3}H]atipamezole. There are two major classes of imidazoline receptors classified at the moment. First, the imidazoline I_1 receptors labelled by [^{3}H]*p*-

aminoclonidine or [^{3}H]clonidine (Ernsberger et al., 1987) and second, the imidazoline I_2 receptors labelled by [^{3}H]idazoxan (Coupry et al., 1987). In addition, Sjöholm and co-workers have described a novel type of non-adrenergic binding site in neonatal rat lung labelled by [^{3}H]atipamezole (Sjöholm et al., 1995), another imidazole derivative. Imidazoline I_1 and I_2 receptors have special pharmacological characteristics so that clonidine, imidazole-4-acetic acid and cimetidine are potent competitors at imidazoline I_1 receptors (Ernsberger et al., 1987, 1988). In contrast, imidazoline I_1 receptors are not recognised by guanabenz or cirazoline which have high affinity for imidazoline I_2 receptors (Ernsberger et al., 1988). In addition, imidazoline I_2 sites have a high affinity for idazoxan and other imidazolines (Parini et al., 1989; Langin and Lafontan, 1989; Zonnenschein et al., 1991; Yablonsky et al., 1991) but either high or low affinity for amiloride (Michel and Ernsberger, 1992).

The arguments for characterising the non-adrenergic binding of [^{3}H]dexmedetomidine as a novel type of a non-adrenergic binding site are as follows. First, the low affinities of clonidine and phentolamine indicate that this binding is not to the imidazoline I_1 receptors. Second, idazoxan and guanabenz have only moderate affinity, indicating that this binding is not to the previously described I_2 receptors. Of the other imidazoline receptor ligands, only cimetidine but not histamine, imidazole-4-acetic acid or imidazole competes for this non-adrenergic binding site of [^{3}H]dexmedetomidine. This non-adrenergic binding site of [^{3}H]dexmedetomidine has some similarity to that non-adrenergic binding site detected with [^{3}H]atipamezole in neonatal rat lung (Sjöholm et al., 1992, 1995), so that compounds having an imidazole structure (dexmedetomidine, detomidine, *l*-medetomidine, atipamezole) inhibit non-adrenergic binding most effectively. In contrast with the [^{3}H]dexmedetomidine binding site, the binding site labelled by [^{3}H]atipamezole in neonatal rat lung has a low affinity for oxymetazoline, cimetidine and guanabenz. Taking all these results together, the non-adrenergic binding site of [^{3}H]dexmedetomidine can be classified as a cimetidine-sensitive imidazole-preferring site which might represent the one described previously for the rat CNS (Sastry and Phillis, 1977; Karppanen, 1981).

In conclusion, the results of this study indicate that [^{3}H]dexmedetomidine has very complex binding characteristics, which limits its usefulness as a radioligand at α_2-adrenoceptors. In contrast, [^{3}H]dexmedetomidine might be a useful tool for the characterisation of imidazoline receptors and their possible physiological functions.

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