Affinity of detomidine, medetomidine and xylazine for alpha-2 adrenergic receptor subtypes

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α2-Adrenergic receptor agonists are widely used in veterinary medicine as sedative/hypnotic agents. Four pharmacological subtypes of the α2-adrenergic receptor (A, B, C and D) have been identified based primarily on differences in affinity for several drugs. The purpose of this study was to examine the affinities of the sedative agents, xylazine, detomidine and medetomidine at the four α2-adrenergic receptor subtypes. Saturation and inhibition binding curves were performed in membranes of tissues containing only one subtype of α2-adrenergic receptor. The Kᵩ for the α2-adrenergic receptor radioligand, [3H]-MK-912, in HT29 cells (α2A-), neonatal rat lung (α2B+), OK cells (α2C- and PC12 cells transfected with RG20 (α2D+)) were 0.38 ± 0.08 nM, 0.70 ± 0.5 nM, 0.07 ± 0.02 nM and 0.87 ± 0.03 nM, respectively. Detomidine and medetomidine had approximately a 100 fold higher affinity for all the α2-adrenergic receptors compared to xylazine but neither agonist displayed selectivity for the α2-adrenergic receptor subtypes. These data suggest that available sedative/hypnotic α2-adrenergic receptor agonists can not discriminate between the four known α2-adrenergic receptor subtypes.

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INTRODUCTION

The sedative effects of α2-adrenergic receptor agonists are mediated by receptors located primarily on locus coeruleus neurons in the pons of the lower brainstem (Svensson et al., 1975; Cedarbaum & Aghajanian, 1976). Brainstem locus coeruleus contains α2-adrenergic receptors and microinjection of α2-adrenergic receptor agonists into the locus coeruleus produced sedation and analgesia (DeSarro et al., 1987). The sedative and analgesic properties of these agents have found prominent clinical applications in both veterinary and human medicine.

Pharmacologically, four subtypes (A, B, C and D) of the α2-adrenergic receptor have been reported based primarily on differences in affinity for several drugs (Bylund et al., 1992, 1988). Genetically, however, there appears to be only three receptor subtypes corresponding to the A, B and C subtypes of α2-adrenergic receptor. The α2B-adrenergic receptor subtype (Rat RG20) (Lanier et al., 1988) as identified in bovine pineal and rat submaxillary glands is considered to be a species homologue of the α2A-adrenergic receptor (Blaxall et al., 1993). These two receptors possess ≈89% sequence homology but differ in their pharmacological properties. The genes for three human α2-adrenergic receptors have been cloned and are designated α2-C10, α2-C2 and α2-C4 and correspond to the A, B and C subtypes, respectively (Bylund et al., 1992). Homologues in rat and pig have also been cloned (Bylund et al., 1992).

α2-Adrenergic receptors are distributed throughout the central nervous system in a non-homogeneous fashion. The α2A- and α2C-subtypes predominate throughout the brain (Rosin et al., 1993; MacDonald & Scheinin, 1995) and the α2D-subtype is reported to be present in the thalamus (Scheinin et al., 1994). In the rat, the locus coeruleus is abundant in the mRNA coding for the α2A-adrenergic receptor (Scheinin et al., 1994) and studies using α2A-adrenergic receptor antisense oligodeoxynucleotides have reported that the hypnotic response to the α2-adrenergic receptor agonist dexmedetomidine in the locus coeruleus of the rat is mediated by the α2A-adrenergic receptor (Mizobe et al., 1996).

α2-Adrenergic receptor agonists are widely used in veterinary medicine as sedative/hypnotic agents. Xylazine was the first centrally active α2-adrenergic receptor agonist used as an anaesthetic adjunct. More recently, the selective α2-adrenergic receptor agonists detomidine and medetomidine have become widely available. To date, no study has systematically examined the pharmacological properties of all three sedative agents on all four α2-adrenergic receptor subtypes. Therefore, the aim of this study was to examine the pharmacological properties of the sedative/hypnotic agents xylazine, detomidine and medetomidine at the four α2-adrenergic receptor subtypes using tissues.
that contain only one subtype of \( \alpha_2 \)-adrenergic receptor (HT29, \( \alpha_{2A} \); rat neonatal lung, \( \alpha_{2B} \); OK cells, \( \alpha_{2C} \); PC12 cells transfected with RG20, \( \alpha_{2D} \)). Northern blot analysis, ribonuclease protection assays and radioligand binding studies have established the existence of only one subtype of \( \alpha_2 \)-adrenergic receptor in these tissues (Zeng et al., 1990; Byland et al., 1992; Sun et al., 1992).

METHODS

Membrane preparations

Harlan Sprague-Dawley rat pups (1–2 days old) were anaesthetized with Halothane and decapitated. The lungs (containing \( \alpha_{2B} \)-adrenergic receptors) were rapidly removed and snap frozen in liquid nitrogen. Lungs were homogenized in 10 volumes of homogenization buffer (50 mM Tris, 5 mM EDTA, pH 7.4) with a polytron (Kinematica GmbH, Kriens-Luzern, Switzerland) (setting #10, 2 × 30 s). HT-29 cells containing the \( \alpha_{2A} \)-adrenergic receptor (American Type Tissue Culture HTB-38) were grown in McCoy’s 5a medium, 90%, foetal bovine serum (FBS), 10%. OK cells containing the \( \alpha_{2C} \)-adrenergic receptor (American opossum proximal tubule, ATCC CRL-1840) were grown in Eagle’s MEM with Earle’s BSS, 90%; FBS; 10%. PC12 cells transfected with RG20 (a gift from Dr Stephen Lanier, Medical University of South Carolina) were grown in DMEM with high glucose, 90%, FBS 10% (Sato et al., 1995). Cells were rinsed with phosphate buffered saline, scraped and homogenized in homogenization buffer in a polytron (setting #10, 2 × 30 s). Homogenates from neonatal rat lung, HT-29, OK and PC12 cells were centrifuged at 45 000 × g 4 °C, 15 min and washed twice. The final pellet was suspended in homogenization buffer and protein concentration determined with the Bradford method. All procedures involving the use of animals were approved by the institution’s animal care and use committee.

Radioligand binding assays

Binding studies were carried out with the \( \alpha_2 \)-adrenergic receptor radioligand \([\text{H}]\)-MK-912 (76.5 Ci/mmol, New England Nuclear, Boston, MA) (Uhlen et al., 1994). Saturation experiments were carried out in assay buffer (50 nM Tris, 0.5 mM EGTA (pH 7.4)) containing either 5–10 \( \mu \)g protein (PC12 cells) or 100–200 \( \mu \)g protein (rat lung, HT29 and OK cells) and \([\text{H}]\)-MK-912 (0.2–3.0 nM) in a final volume of 250 \( \mu \)L. Non-specific binding was determined with the selective \( \alpha_2 \)-adrenergic receptor antagonist, RX-821002 hydrochloride \( [2H]-2\text{-(2-methoxy-1,4-benzodioxanyl)-[imidazoline hydrochloride} \] (2 \( \mu \)M) (O’Rourke et al., 1994). Membranes were incubated for 1 h at 25 °C. The experiment was terminated by rapid dilution of the suspension with ice cold assay buffer followed by vacuum filtration through GF/C filters (Whatman, Clifton, NJ) and washing with 2 × 4 mL assay buffer. Radioactivity on the filters was determined by liquid scintillation counting. The Kd values for RX-821002 determined from competition experiments with \([\text{H}]\)-MK-912 at the various \( \alpha_2 \)-adrenergic receptors were: \( \alpha_{2A} \), 1.1 ± 0.1 nM; \( \alpha_{2B} \), 11.1 ± 0.9 nM; \( \alpha_{2C} \), 0.6 ± 0.2 nM and \( \alpha_{2D} \), 0.6 ± 0.4 nM.

Inhibition experiments were performed in assay buffer containing either 5–10 \( \mu \)g protein (PC12 cells) or 100–200 \( \mu \)g protein (rat lung, HT29 and OK cells), a fixed concentration of \([\text{H}]\)-MK-912 (0.5 nM) and various concentrations of competing drug for 1 h at 25 °C in a volume of 250 \( \mu \)L. The experiment was terminated by rapid dilution of the suspension with ice cold assay buffer followed by vacuum filtration through GF/C filters (Whatman, Clifton, NJ) and washing with 2 × 4 mL assay buffer. Radioactivity on the filters was determined by liquid scintillation counting. The IC\(_{50}\) values were converted to Ki (inhibitor constant) values by the method of Cheng and Prusoff (1973).

Drugs and chemicals

\([\text{H}]\)-MK-912 (specific activity 76.5 Ci/mmol) was obtained from New England Nuclear, Boston, MA. Xylazine was obtained from Research Biochem Int., Natick, MA. Detomidine hydrochloride (Dormosedan) and medetomidine hydrochloride (Dormitor) were obtained from Pfizer Animal Health, West Chester, PA. RX-821002 was purchased from Research Biochemical International, Natick, MA. All tissue culture supplies were obtained from Sigma Chemical Co, St. Louis, MO.

RESULTS

Saturation binding of \([\text{H}]\)-MK-912 to HT-29, neonatal rat lung, OK and PC12 cells

To determine the Kd values of \([\text{H}]\)-MK-912 for the \( \alpha_{2A} \), \( \alpha_{2B} \), \( \alpha_{2C} \) and \( \alpha_{2D} \) adrenergic receptors, saturation binding curves were performed in membranes of tissues containing only one subtype of receptor (Fig. 1). Non-specific binding was determined in the presence of RX-821002 (2 \( \mu \)M). The Kd and the non specific binding of \([\text{H}]\)-MK-912 at the Kd for the \( \alpha_{2A} \), \( \alpha_{2B} \), \( \alpha_{2C} \) and \( \alpha_{2D} \) adrenergic receptors are depicted in Table 1. \([\text{H}]\)-MK-912 displayed approximately a 10 fold higher affinity for the \( \alpha_{2C} \) adrenergic receptor in the OK cells compared to the other receptor subtypes. There was no significant difference between the Kd’s of \([\text{H}]\)-MK-912 at the \( \alpha_{2A} \), \( \alpha_{2B} \) or \( \alpha_{2D} \) adrenergic receptor subtypes.

Inhibition of \([\text{H}]\)-MK-912 binding to HT-29, neonatal rat lung, OK and PC12 cell membranes by xylazine, detomidine and medetomidine

To determine the Kd values for xylazine, detomidine and medetomidine against \([\text{H}]\)-MK-912 for the \( \alpha_{2A} \), \( \alpha_{2B} \), \( \alpha_{2C} \) and \( \alpha_{2D} \) adrenergic receptors, inhibition binding curves were performed in membranes of tissues containing only one subtype of receptor (Fig. 2). The calculated Kd values and slope of the Hill plots are depicted in Table 2. Detomidine and medetomidine had approximately a 100 fold higher affinity for all four subtypes of one.
α2-adrenergic receptors compared to xylazine, but neither agonist displayed selectivity between subtypes.

**DISCUSSION**

Activation of α2-adrenergic receptors mediate a wide variety of responses including sedation, analgesia, vasoconstriction and hyperthermia. Pharmacologically, α2-adrenergic receptors can be divided into four distinct subtypes of receptors based on the affinities of various drugs (Bylund et al., 1988, 1992). Physiologically, however, the functional correlates for these subtypes are less clear (MacKinnon et al., 1994). The purpose of this study was to examine the pharmacological binding properties of three widely used sedatives, xylazine, detomidine and medetomidine at the four subtypes of α2-adrenergic receptors in tissues containing only one subtype of receptor. The data suggest that detomidine and medetomidine have a higher affinity for all the α2-adrenergic receptors subtypes vs. xylazine, although neither drug showed any selectivity for a particular α2-adrenergic receptor subtype.

In this study, tissues containing only one α2-adrenergic receptor subtype were used for radioligand binding studies. The human adenocarcinoma cell line, HT29, contains the α2A-adrenergic receptor that is found in human platelets and corresponds to the human α2-C10 gene (Bylund et al., 1992). Neonatal rat lung is a prototype for the α2B-adrenergic receptor and is analogous to the human α2-C2 adrenergic receptor (Bylund et al., 1988, 1992, 1994). The human α2-C4 gene codes for the α2C-adrenergic receptor has pharmacological properties similar to the α2-adrenergic receptor found in OK cells (Blaxall et al., 1991). In the present study, we have also used PC12 cells transfected with the cloned rat homologue of the human α2-C10 receptor (RG20) (Lanier et al., 1988). The human α2A-adrenergic receptor and the rat α2D-adrenergic receptor are structurally very similar (Blaxall et al., 1993), but have different ligand binding properties (Lanier et al., 1988).

The α2-adrenergic receptor radioligand [3H]MK-912 was used in the present study. [3H]MK-912 has been reported to be selective for the α2C-adrenergic receptor compared to the α2A- and α2B-adrenergic receptor (Uhlen et al., 1994). The present study confirms and extends the former study by examining the affinity of [3H]MK-912 for the α2D-adrenergic receptor. In the present study, [3H]MK-912 showed higher affinity (0.07 ± 0.02 nM) for the α2C- compared to the α2A-, α2B- or α2D-adrenergic receptor (0.38 ± 0.08 nM, 0.7 ± 0.05 nM, 0.87 ± 0.03 nM, respectively), confirming that this radioligand is selective for the α2C-adrenergic receptor subtype.

### Table 1

<table>
<thead>
<tr>
<th>Receptor Subtype</th>
<th>KD (nM)</th>
<th>Bmax (fmol/mg)</th>
<th>NSB at KD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α2A</td>
<td>0.38 ± 0.08</td>
<td>372 ± 75</td>
<td>3.1 ± 0.3%</td>
</tr>
<tr>
<td>α2B</td>
<td>0.70 ± 0.05</td>
<td>69 ± 17</td>
<td>15.7 ± 3.3%</td>
</tr>
<tr>
<td>α2C</td>
<td>0.07 ± 0.02</td>
<td>54 ± 15</td>
<td>5.3 ± 0.3%</td>
</tr>
<tr>
<td>α2D</td>
<td>0.87 ± 0.03</td>
<td>40 ± 15</td>
<td>2.48 ± 0.32</td>
</tr>
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</table>

NSB–Non-specific Binding.
The affinities of the sedative/analgesics xylazine, detomidine and medetomidine were determined at the four different α₂-adrenergic receptor subtypes with [³H]MK-912. Xylazine showed low affinity for all four subtypes (range 1200–1460 nM) whereas detomidine and medetomidine displayed much higher affinity (range 3–26 nM). This difference in affinities is reflected in the effective sedative dose of these agents. Detomidine and medetomidine are administered in the mg/kg intravenous (i.v.) dosage range whereas xylazine is administered in the mg/kg i.v. range (Plumb, 1995). Despite the difference in affinities of xylazine compared to detomidine and medetomidine for the α₂-adrenergic receptor, there was no selectivity for any of these agents for the different subtypes.

Species differences exist to the clinical response of α₂-adrenergic receptor agonists. For example, ruminants are extremely sensitive to the sedative and hypotensive effects of xylazine compared to other species (Plumb, 1995), and this difference cannot be explained by species difference in metabolism and/or excretion (García-Villar et al., 1981; Gross & Booth, 1995). Data from the present study suggest that differences in the affinity of the α₂-adrenergic receptor agonists for the various subtypes cannot explain this enhanced sensitivity in ruminants. The subtype of α₂-adrenergic receptor mediating sedation in the rat is reported to be the A subtype (Mizobe et al., 1996). Less is known about the subtype mediating sedation in other species. Bovine appear to possess the α₂D-adrenergic receptor subtype (retina (Berlie et al., 1995); pineal gland (O’Rourke et al., 1994). Assuming the α₂-adrenergic receptor mediating sedation in bovine is the D subtype, differences in the affinity cannot explain the species variability. Further studies are needed to determine whether the α₂-adrenergic receptor subtype mediating sedation in ruminants differs from the four described α₂-adrenergic receptors.

α₂-Adrenergic receptors also mediate antinociception and are used in combination with opiates for the chemical management of pain. The primary site of α₂-adrenergic receptor-mediated antinociception is the spinal cord (Yaksh, 1985). Pharmacologically, the α₂A-adrenergic receptor has been reported to mediate this antinociceptive effect in mice (Millan, 1992). In humans, α₂A-adrenergic receptor mRNA has been demonstrated in cervical spinal cord whereas thoracic, lumbar and sacral spinal cord contain α₂D-adrenergic receptor mRNA (Smith et al., 1995). Further studies are necessary to determine the role of these receptor subtypes in the antinociception response produced by α₂-adrenergic receptor agonists.

Table 2. \(K_i\) and Slope of the Hill Plot for xylazine, detomidine and medetomidine at the α₂-adrenergic receptor subtypes

<table>
<thead>
<tr>
<th></th>
<th>(\alpha_2A)</th>
<th>(\alpha_2B)</th>
<th>(\alpha_2C)</th>
<th>(\alpha_2D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylazine</td>
<td>1570 ± 240 (1.00 ± 0.06)</td>
<td>1360 ± 100 (1.26 ± 0.17)</td>
<td>1200 ± 223 (1.27 ± 0.09)</td>
<td>1460 ± 250 (1.21 ± 0.31)</td>
</tr>
<tr>
<td>Detomidine</td>
<td>6.16 ± 0.74 (1.00 ± 0.04)</td>
<td>26.7 ± 1.9 (1.04 ± 0.04)</td>
<td>14.0 ± 3.6 (1.07 ± 0.11)</td>
<td>7.90 ± 0.7 (1.01 ± 0.1)</td>
</tr>
<tr>
<td>Medetomidine</td>
<td>3.89 ± 1.46 (0.89 ± 0.06)</td>
<td>7.40 ± 2.61 (1.01 ± 0.06)</td>
<td>12.3 ± 5.07 (1.21 ± 0.07)</td>
<td>5.74 ± 0.73 (0.76 ± 0.06)</td>
</tr>
</tbody>
</table>
In conclusion, the sedative/hypnotic agents, xylazine, detomidine and medetomidine do not show selectivity for any of the four described $\alpha_2$-adrenergic receptors determined by radioligand binding studies in tissues containing only one subtype of $\alpha_2$-adrenergic receptor.

REFERENCES


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