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Inverse agonism at α_2-adrenoceptors in native tissue

L. Charles Murrin *, Moira E. Gerety, H. Kevin Happe, David B. Bylund

Department of Pharmacology, University of Nebraska College of Medicine, 986260 Nebraska Medical Center, Omaha, NE 68198-6260, USA

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Abstract

Several α_2-adrenoceptor antagonists have inverse agonist properties in cell culture systems, usually expressing high levels or a constitutively active form of α_2-adrenoceptors. In characterizing the binding of α_2-adrenoceptor agonists to rat brain tissue sections, we found that conditions known to alter agonist affinity for these receptors, particularly the addition of 100 μM GTP, altered the binding of the α_2-adrenoceptor antagonist, [^{3}H](1,4-benzodioxan-2-methoxy-2-yl)-2-imidazoline hydrochloride (RX821002). In further studies, we found that under our conditions [^{3}H]RX821002 demonstrates inverse agonist properties at α_2-adrenoceptors. This is the first demonstration of inverse agonism at α_2-adrenoceptors in native tissue. We found that the α_2-adrenoceptor antagonist, (2S,12bS)1′,3′-dimethylspiro(1,3,4,5′,6,6′,7,12b-octahydro-2H-benzo(b)furo(2,3-a)quinazoline)-2,4′-pyrimidin-2′-one (MK-912), did not have clearly discernible inverse agonist properties and acted as a neutral antagonist in these studies. On the other hand, the antagonist rauwolscine actually displayed partial agonist properties in our studies. These findings indicate that the inverse agonist properties of α_2-adrenoceptor antagonists can be demonstrated in native tissue, as well as in tissue culture, and they strengthen the idea that inverse agonist properties may be of physiological and pharmacological importance.

Keywords: α_2-Adrenoceptor; Agonist, Inverse; G protein-coupled receptor; RX821002; MK-912; GTP; Rauwolscine

1. Introduction

The α_2-adrenoceptor is one of the three major classes of receptors for norepinephrine and epinephrine (Bylund, 1988; Bylund et al., 1994). α_2-Adrenoceptors are widely distributed in the body and play important roles in a variety of physiological and pathological processes, including regulation of blood pressure, nociception, locomotion, and processing of stressful stimuli (Ruffolo et al., 1993, 1995). α_2-Adrenoceptor agonists have been used to ameliorate withdrawal symptoms from opiates and alcohol, as anesthetic adjuvants in surgery, and may be of some benefit in treating cognitive deficits in the elderly (Ruffolo et al., 1995). These actions and uses point to the importance of understanding the role of α_2-adrenoceptors in CNS function.

α_2-Adrenoceptors are members of the G protein-coupled receptor superfamily and appear to interact primarily with $G_{i/o}$ proteins (Chabre et al., 1994). When α_2-adrenoceptors are stimulated, GDP is released from the α subunit of the heterotrimeric G protein complex, allowing GTP to bind in its place. This leads to the dissociation of the α from the $\beta\gamma$ subunits and the subsequent regulation of signal transduction systems within the cell. This interaction with G proteins is an integral part of α_2-adrenoceptor function and provides a measure of the functional activity of these receptors in tissue.

Characterization of the pharmacological properties of α_2-adrenoceptor ligands indicates that some agonists act as partial agonists and some antagonists appear to have inverse agonist properties at these receptors (Tian et al., 1994; Jansson et al., 1998; Cayla et al., 1999; Wurch et al., 1999). Inverse agonists have a higher affinity for their receptors in the presence of high GTP concentrations, the opposite to what is found with agonists, and they are thought to reduce the functional activity of the receptors below the baseline activity observed in the absence of any ligand. Neutral antagonists, on the other hand, block receptors without affecting basal function and their affinity is not affected by GTP. Inverse agonist properties for some α_2-adrenoceptor antagonists have been demonstrated in cell lines, but not yet in native tissue. In preparation for autoradiography studies, we characterized agonist binding

* Corresponding author. Tel.: +1-402-559-4552; fax: 1-402-559-7495.
E-mail address: cmurrin@unmc.edu (L.C. Murrin).

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to α_2-adrenoceptor in tissue sections, including GTP-induced shifts in α_2-adrenoceptor agonist affinities. Unexpectedly, GTP also induced a change in binding of the α_2-adrenoceptor antagonist, [^{3}H](1,4-benzodioxan-2-methoxy-2-yl)-2-imidazoline hydrochloride (RX821002). In the current study, we examine the change in RX821002 binding in detail, as well as the binding characteristics of two other α_2-adrenoceptor antagonists, (2S,12bS)1′,3′-dimethylspiro(1,3,4,5′,6,6′,7,12b-octahydro-2H-benzo(b)-furo(2,3-a)quinazoline)-2,4′-pyrimidin-2′-one (MK-912) and rauwolscine, and report inverse agonist activity at α_2-adrenoceptor in native tissue for the first time. A preliminary report of these data has appeared in abstract form (Murrin et al., 1999).

2. Materials and methods

2.1. Materials

[^{3}H]RX821002 (59 Ci/mmol) was obtained from Amersham Pharmacia (Arlington Heights, IL). [^{3}H]MK-912 (80.5 Ci/mmol) and [^{3}H]rauwolscine (78 Ci/mmol) were obtained from NEN Life Science Products (Boston, MA). GTP (sodium salt) was purchased from Sigma (St. Louis, IL). Rauwolscine HCl and RX821002 HCl were purchased from Research Biochemical (Natick, MA). MK-912 HCl was a gift from Merck, Sharpe and Dohme (Rahway, NJ). All other chemicals were of research grade.

2.2. Animals

Adult Sprague–Dawley rats, 180–250 g (SASCO, Kingston, NY) were housed three or four per cage and fed ad libitum. Animals were sacrificed by decapitation under halothane anesthesia. Brains were removed, immediately frozen on dry ice, and stored at −80°C until use. Fresh–frozen brains from Sprague–Dawley rats were obtained from Zivic-Miller (Porterville, PA). Procedures were in strict accordance with The National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the local Animal Care Committee.

2.3. Methods

Tissue sections (16 μm) were cut using a cryostat, thaw-mounted on subbed slides and stored at −20°C until use. Coronal sections centered on the striatum were used (plates 13–18; Paxinos and Watson, 1986). Serial tissue sections from an individual animal were used in a randomized fashion within a single experiment to reduce the variance inherent in using brain tissue sections. Prior to assay, tissue sections were brought to room temperature for 45–60 min. Sections were incubated with various concentrations of tritiated ligand and unlabeled ligand (both saturation and homologous competition assays) in 50 mM Tris HCl, 3 mM $MgCl_2$, 1 mM EDTA buffer, pH 7.4 (Tris–Mg–EDTA), at room temperature for 60–90 min, unless stated otherwise. After incubation, sections were washed twice for 5 min in an ice-cold buffer and drained. Following washing, sections were wiped from slides with filter papers (25 mm P5; Fisher, St. Louis, MO) and bound radioactivity was determined by liquid scintillation spectrometry using Econosafe scintillation fluid (RPI, Mt. Prospect, IL).

2.4. Data analysis

Data from experiments using the same labeled and unlabeled ligand (mixed homologous saturation assays) were converted to total disintegrations per minute bound as if all ligand was radiolabeled at the same specific activity as the radiolabeled ligand. These data were then analyzed as saturation experiments using Prism 3 (GraphPad, San Diego, CA). The studies using [^{3}H]RX821002 as ligand were also analyzed using LIGAND (Munson and Rodbard, 1980) and KELL (Biosoft). Similar results were obtained with all three programs. Subsequent studies used only Prism because of ease of use. Unless otherwise stated, all curves were best fit by a one-site analysis. Statistical comparisons used the unpaired Student's *t*-test (InStat, GraphPad) and K_D values were converted to log values to more closely approximate a Gaussian distribution.

3. Results

To optimize conditions for the analysis of agonist affinity states of α_2-adrenoceptors with quantitative autoradiography, we characterized the binding of agonists to α_2-adrenoceptors in the presence and absence of added GTP using rat brain tissue sections. These studies used a Tris–Mg–EDTA buffer since previous studies have shown this to be an optimum buffer for agonist binding to α_2-adrenoceptors (Deupree et al., 1996). We used [^{3}H]RX821002 as the radioligand, assuming it was an antagonist at α_2-adrenoceptors. In the course of these studies, we found that addition of GTP to the buffer increased [^{3}H]RX821002 binding. Because the affinity of [^{3}H]RX821002 for α_2-adrenoceptors is a factor in the calculation of agonist K_i values for the various affinity states of the receptor, we examined the binding of [^{3}H]RX821002 in greater detail to determine if the increase in binding was due to a change in antagonist K_D, B_{max} or both. Subsequently, we examined the binding of two other commercially available α_2-adrenoceptor radioligands, [^{3}H]MK-912 and [^{3}H]rauwolscine, which are usually assumed to be neutral antagonists at the α_2-adrenoceptor.

3.1. [^{3}H]RX821002 binding

RX821002 saturation studies used a mixed saturation-homologous competition protocol, which combines a typi-

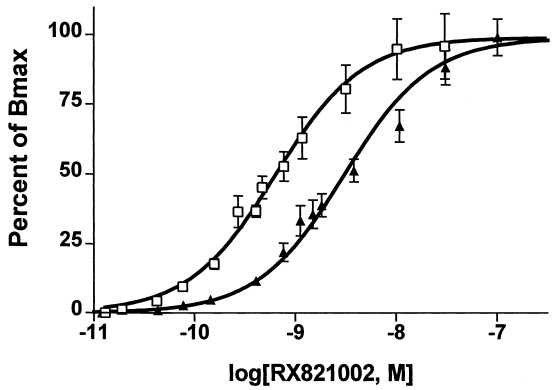

Fig. 1. RX821002 mixed homologous saturation study. Tissue sections were incubated with [^{3}H]RX821002 (0.01–1.5 nM; saturation part), or with unlabeled RX821002 (0–300 nM) plus [^{3}H]RX821002 (0.2–2 nM; homologous competition part), without (▲) or with (□) GTP (100 μM). Data were converted to total moles RX821002 bound and analyzed with Prism. Data are presented as percent of B_{max} and are the mean ± S.E.M. of four experiments. K_D values for RX821002 from analysis of these mean data are 0.6 nM in the presence of GTP and 2.4 nM in the absence of added GTP.

cal saturation experiment (increasing concentrations of radioligand) to define the saturation curve at lower ligand concentrations and a homologous competition experiment to define the curve at higher ligand concentrations. RX821002 studies were carried out with [^{3}H]RX821002 concentrations from 10^{-11} to 1.5×10^{-9} M in the saturation part of the assay and with unlabeled RX821002 concentrations from 10^{-11} to 3×10^{-7} M, competing against a single radioligand concentration (0.2–2 nM, depending on the assay) for the homologous competition part. In studies carried out in Tris–Mg–EDTA buffer without added GTP, RX821002 binding was best fit by a one-site model with a K_D of 5 nM (Fig. 1, Table 1). The K_D value is higher (lower affinity) than is found under conditions optimized for antagonist binding, but consistent with previous results (Deupree et al., 1996). The addition of high concentrations of GTP reduces the affinity of agonists for their receptors, increases inverse agonist receptor affinity and has no effect on antagonist affinity. We found that RX821002 has a fivefold higher affinity, 0.9 nM, for α_2-adrenoceptors when 100 μM GTP was added to the assay (Fig. 1, Table 1). This statistically significant change indicates that RX821002 has inverse agonist properties at α_2-adrenoceptors in rat brain tissue sections under these conditions. We also found that addition of GTP decreased the B_{max} compared to assays in the absence of GTP. In preliminary studies, 5′-guanylylimidodiphosphate (GppNHp), a GTP analog, produced a similar effect on RX821002 binding, whereas ATP and 5′-adenylylimidodiphosphate (AppNHp) had little or no effect (data not shown).

Table 1
Mixed homologous saturation assays at α_2-adrenoceptors
Data are means ± S.E.M. ($n = 3$–4) from the individual analysis of each mixed homologous saturation assay for each ligand. Data were analyzed with Prism. See Section 2.3 for details.

Ligand	−GTP		+GTP	
	K_D (nM)	B_{max} (fmol/section)	K_D (nM)	B_{max} (fmol/section)
RX821002	5.0 ± 0.4	64 ± 3	0.9 ± 0.3*	49 ± 6*
MK-912	7.3 ± 3.0	84 ± 9	2.3 ± 0.4	83 ± 4
Rauwolscine	6.6 ± 1.0	44 ± 3	41 ± 13*	81 ± 7*

* Statistically significantly different from −GTP assays, $P < 0.05$.

3.2. [^{3}H]MK-912 binding

We next examined another α_2-adrenoceptor antagonist, MK-912, for binding properties to α_2-adrenoceptors in rat brain tissue sections under similar conditions. MK-912 studies were carried out with [^{3}H]MK-912 concentrations from 10^{-11} to 10^{-8} M for the saturation part and with unlabeled ligand in concentrations from 10^{-9} to 10^{-7} M competing against a single radioligand concentration (0.7–0.9 nM) for the homologous competition part. In studies carried out in Tris–Mg–EDTA buffer without added GTP, MK-912 binding was best fit by a one-site model with a K_D of 7.3 nM (Fig. 2, Table 1). When 100 μM GTP was added to the assay buffer, MK-912 had a slightly (threefold) higher affinity, 2.3 nM, for α_2-adrenoceptors (Fig. 2, Table 1), but this was not a statistically significant difference. This indicates that MK-912 acts as a neutral antagonist at α_2-adrenoceptors in rat brain tissue sections under these conditions, although MK-912 may have weak inverse agonist properties that could not be clearly discerned. For the purposes of subsequent experiments, we considered MK-912 to be a neutral antagonist. As might be expected,

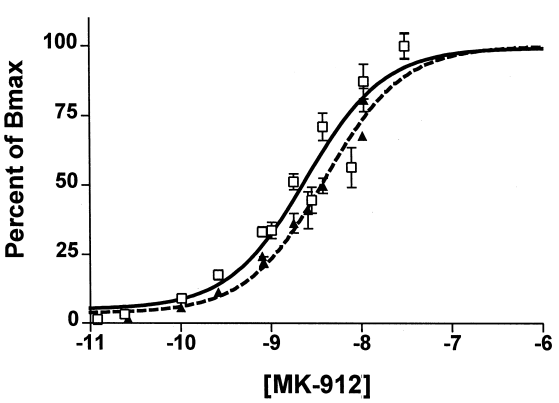

Fig. 2. MK-912 mixed homologous saturation study. Tissue sections were incubated in Tris–Mg–EDTA buffer with [^{3}H]MK-912 (0.01–10 nM; saturation part), or with unlabeled MK-912 (0–300 nM) plus [^{3}H]MK-912 (0.7–0.9 nM; homologous competition part), without (▲) or with (□) GTP (100 μM). Data were converted to total moles MK-912 bound and analyzed with Prism. Data are presented as percent of B_{max} and are the mean ± S.E.M. of three experiments. K_D values for MK-912 from analysis of these mean data are 1.9 nM in the presence of GTP and 3.6 nM in the absence of added GTP.

there was no change in the B_{max} of MK-912 binding in the presence or absence of added GTP.

3.3. [^{3}H]Rauwolscine binding

Studies with a third α_2-adrenoceptor antagonist, rauwolscine, were carried out with [^{3}H]rauwolscine concentrations from 10^{-11} to 1.2×10^{-8} M for the saturation experiments, and with unlabeled ligand in concentrations from 10^{-9} to 3×10^{-7} M competing against a single radioligand concentration (0.75–1.2 nM) for the homologous competition experiments. In studies carried out in Tris–Mg–EDTA buffer without added GTP, rauwolscine binding was best fit by a one-site model with a K_D of 6.6 nM (Fig. 3, Table 1). When 100 μM GTP was added to the assay buffer, rauwolscine had a sixfold lower affinity, 41 nM, for α_2-adrenoceptors (Fig. 3, Table 1). The shift in binding was in the opposite direction from the shift with RX821002 and indicates that rauwolscine has partial agonist properties at α_2-adrenoceptors in rat brain tissue sections under these conditions. We also found with this ligand that addition of GTP led to a higher B_{max} compared to assays in the absence of GTP, again, different from the findings with RX821002. In these studies, we used RX821002 to define non-specific binding to eliminate from consideration potential interaction of rauwolscine with serotonin receptors.

3.4. Competition studies using [^{3}H]MK-912 as a neutral antagonist

In order to confirm these findings, we carried out competition studies under identical conditions using RX821002 and rauwolscine as competing ligands and

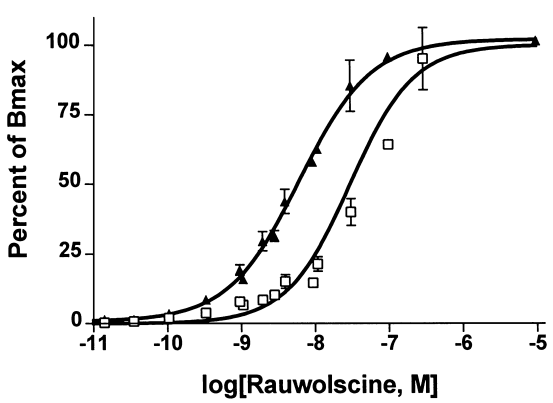

Fig. 3. Rauwolscine mixed homologous saturation study. Tissue sections were incubated in Tris–Mg–EDTA buffer with [^{3}H]rauwolscine (0.01–10 nM; saturation part), and with unlabeled rauwolscine (0–300 nM) plus [^{3}H]rauwolscine (0.75–1.2 nM; homologous competition part), without (▲) or with (□) GTP (100 μM). Data were converted to total moles rauwolscine bound and analyzed with Prism. Data are presented as percent of B_{max} and are the mean ± S.E.M. of three experiments. K_D values for rauwolscine from analysis of these mean data are 22.3 nM in the presence of GTP and 6.6 nM in the absence of added GTP.

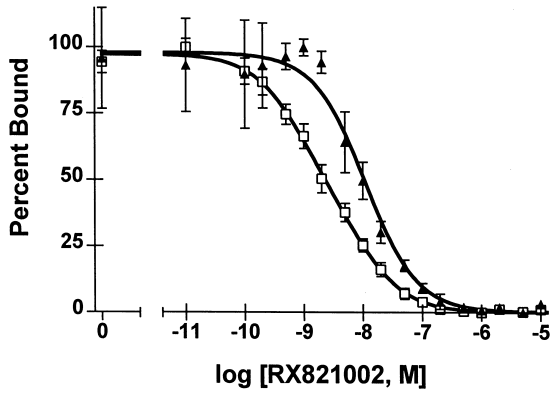

Fig. 4. Competition studies with RX821002, using [^{3}H]MK-912 as radioligand. Tissue sections were incubated in Tris–Mg–EDTA buffer with [^{3}H]MK-912 as radioligand (1 nM) and various concentrations of RX821002 without or with GTP (100 μM). Data are the mean ± S.E.M. of three experiments and were analyzed with Prism. RX821002 was used at 17 concentrations (0.01 nM–100 μM). In the presence of added GTP (□), RX821002 competition data were best fit by a two-site analysis, with K_i values of 0.6 (53%) and 5.8 nM. In the absence of added GTP (▲), RX821002 data were best fit by a one-site model with a K_i value of 6.0 nM.

[^{3}H]MK-912 as the radioligand, considering it to be a neutral antagonist. The results were consistent with our mixed homologous binding studies described above, although in these studies, a two-site fit was sometimes found to be best for fitting the data. This is consistent with previous studies in our hands, in that we find it difficult to detect two affinity states when using the same labeled and unlabeled ligand (homologous studies), and much easier to detect two sites when the ligands are different (heterologous studies).

In studies with RX821002, we used 18 concentrations of unlabeled RX821002 from 10^{-11} to 10^{-4} M in competition with 1.0 nM [^{3}H]MK-912. In the absence of added GTP, the data were best fit by a one-site model with a K_i for RX821002 of 8.7 nM (Fig. 4). When 100 μM GTP was added to the assay, the data were best described by a two-site fit (Fig. 4), the low affinity site having an affinity of 5.6 nM and the high affinity site having an affinity of 0.6 nM and accounting for 53% of the binding sites. The affinities of RX821002 for α_2-adrenoceptors found in these experiments are consistent with the values found in the RX821002 saturation-homologous competition studies above. In these studies, addition of GTP to the assay shifted about half of the receptors to a higher affinity state for RX821002, again pointing to its inverse agonist properties.

In a similar set of studies, we used rauwolscine as competing ligand against [^{3}H]MK-912 with identical conditions as for RX821002 competition studies. The results were less dramatic than those with RX821002 but consistent with our saturation studies with rauwolscine. In the absence of GTP, rauwolscine binding was best described by a two-site fit, with K_i values of 6.4 and 164 nM, 51%

of the receptor sites being in the high affinity state. Upon addition of 100 μM GTP, about half of the high affinity α_2-adrenoceptors sites were shifted to the low affinity state for rauwolscine. The K_i values for rauwolscine were 3.5 and 467 nM, with the high affinity state accounting for 28% of the sites. This is consistent with partial agonist properties for rauwolscine in rat brain sections under our experimental conditions.

4. Discussion

While examining the agonist affinity states of α_2-adrenoceptors in tissue sections in preparation for autoradiographic studies, we found differences in the binding of the radiolabeled antagonist, [^{3}H]RX821002, in the presence and absence of GTP. Since we were using [^{3}H]RX821002 as a base for characterizing agonist affinity for α_2-adrenoceptors in these competition assays, it was necessary to examine these changes in detail because the affinity of the radioligand has an impact on data analysis. We found that in rat brain under the conditions used in our study, [^{3}H]RX821002 has properties of an inverse agonist. Although this has been reported previously for RX821002 in cell culture systems with transfected receptors, this is the first time such properties have been demonstrated in native tissue expressing normal levels of receptors and G proteins. We also examined two other commercially available α_2-adrenoceptor radioligands and found that [^{3}H]MK-912 acts as a neutral antagonist, whereas [^{3}H]rauwolscine acts as a partial agonist in our preparations.

The concept of inverse agonism arose initially in studies of the benzodiazepine receptors (Braestrup et al., 1982; Polc et al., 1982), which form part of the GABA receptor complex (Braestrup et al., 1982). In general, agonists stimulate their receptors and increase the receptor's regulatory action on intracellular processes. Inverse agonists, most of which were originally thought to be neutral antagonists, reverse the active state of the receptor and so produce effects opposite those of agonists, decreasing the receptor's regulatory actions. Antagonists have no effect on their own, but will block the actions of agonists and inverse agonists. In the case of the benzodiazepine receptor, agonists potentiate the actions of GABA and so lead to greater chloride ion channel activity while inverse agonists decrease the actions of GABA and lead to less ion channel activity.

It was later proposed that inverse agonists could exist in other receptor systems, including G protein-coupled receptor systems (Wreggett and De Léan, 1982). Confirming this idea, Costa and Herz (1989) demonstrated that under proper conditions, delta opioid receptors had a basal activity in the absence of agonist, which was inhibited by inverse agonists but not by neutral antagonists. In characterizing the interaction of a variety of drugs with these receptors, it was found that drugs traditionally considered opioid antagonists were acting as inverse agonists, neutral antagonists or as partial agonists, similar to what was found in the present study. These findings also suggested that unless the system was sensitive enough, the full range of activities of drugs at receptors would be hidden, an idea supported by other studies (Chidiac et al., 1994).

Since that time, many studies have demonstrated inverse agonism at G protein-coupled receptors, including 5-HT$_{2C}$ (Barker et al., 1994), α_2-adrenergic (Tian et al., 1994; Jansson et al., 1998; Cayla et al., 1999; Wurch et al., 1999), β_2-adrenergic (Chidiac et al., 1994; Bond et al., 1995), dopamine D2 (Nilsson et al., 1996) and retinoic acid receptors (Klein et al., 1996). In most of these cases, it was possible to demonstrate inverse agonism because of the overexpression of the receptor in question or because a constitutively active receptor mutant was used. In a few cases, it was possible to demonstrate inverse agonism in cell lines that naturally expressed the receptor in question (Barker et al., 1994; Cayla et al., 1999).

In this paper, we demonstrate for the first time the inverse agonist properties of RX821002 in native tissue that normally expresses α_2-adrenoceptors. This provides further support for the idea that the inverse agonist properties of drugs may be functionally relevant in the whole animal. Indeed, this has been demonstrated for β_2-adrenoceptors in transgenic animals expressing a constitutively active mutant of the β_2-adrenoceptor (Bond et al., 1995). For α_2-adrenoceptors, most previous demonstrations of the inverse agonist properties of putative antagonists have used transfected cell lines expressing high densities of wild type α_2-adrenoceptors or constitutively active α_2-adrenoceptors. An exception to this used cultured cells, which normally express α_2-adrenoceptors (Cayla et al., 1999). The most parsimonious explanation of the difficulty in demonstrating these properties in native tissue is that α_2-adrenoceptors and/or the relevant G proteins are expressed in low numbers relative to the cell culture systems, and that normally the balance between receptors in the active and inactive state is heavily in favor of inactive receptors (Kenakin, 1997). As a result, there is relatively little positive signal to suppress. In preliminary studies on neonatal tissue, we have found further evidence for the inverse agonist properties of RX821002, using the agonist-stimulated [^{35}S]GTPγS binding assay. Because of the low signal to noise ratio in this assay in adult tissue, it is more difficult to demonstrate this in adult tissue.

The α_2-adrenoceptor antagonist MK-912 is a neutral antagonist in our hands. Whereas there was a small increase in MK-912 affinity for α_2-adrenoceptors in the presence of added GTP compared to its absence, suggesting weak inverse agonist properties, this change was not statistically significant. This is consistent with the previous analysis of MK-912 (Wurch et al., 1999).

Perhaps the most surprising finding in our studies was the partial agonist properties of rauwolscine. Under our conditions, rauwolscine had a lower affinity for α_2-adren-

oceptors in the presence of GTP than in its absence, similar to what we have found for α_2-adrenoceptor agonists, such as brimonidine (Bylund et al., 1998). This is in contrast to the findings in several other studies using cell culture systems, in which rauwolscine has been shown to be an inverse agonist (Tian et al., 1994; Jansson et al., 1998). The reasons for this discrepancy are not clear, although the most obvious explanation is the differences in preparations assayed or receptor subtype. On the other hand, it is not unprecedented for a drug to exhibit a range of properties from inverse agonist to partial agonist, and there is a theoretical basis for such properties (Chidiac et al., 1994; Kenakin, 1995, 1997). Atipamezole has been shown to have this complete range of properties at α_2-adrenoceptors, depending on variations in the preparations used (Jansson et al., 1998; Wurch et al., 1999), as has levomedetomidine (Jansson et al., 1998). A similar range of effects has been found for labetalol and dichloroisoproterenol at β_2-adrenoceptors (Chidiac et al., 1994, 1996). It has been proposed that the agonist/antagonist/inverse agonist properties of a ligand with respect to a particular receptor depend on many variables, including level of expression of the receptor, level and type of G proteins present, and the assay being used to measure these differences (Costa et al., 1992; Chidiac et al., 1994, 1996; Kenakin, 1995; Wurch et al., 1999). As a result, whereas the findings with rauwolscine were unexpected, they are not unprecedented. In addition, the competition studies using unlabeled rauwolscine and [^{3}H]MK-912 as radioligand support the findings using only rauwolscine in saturation studies.

In summary, we have examined how the properties of three putative α_2-adrenoceptor antagonists are altered by the presence or absence of added GTP in native tissue that normally expresses these receptors. We have found that RX821002 has inverse agonist properties, consistent with the findings in cell culture systems. We found that MK-912 acts as a neutral antagonist, although in a more sensitive assay it may be found to be a weak inverse agonist, and that rauwolscine acts as a partial agonist. The latter is surprising and contrasts with the findings in several cell culture systems. This is most probably explained by the differences in preparations used, including the possible differences between native tissue and cell culture systems. This is the first demonstration of inverse agonist properties of α_2-adrenoceptor drugs in native tissue. These findings further support the existence of inverse agonists and support the idea that inverse agonists at α_2-adrenoceptors may have physiological and clinical relevance.

Acknowledgements

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