Protean Agonism at $\alpha_{2A}$-Adrenoceptors

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ABSTRACT

The coupling of the endogenously expressed $\alpha_{2A}$-adrenoceptors in human erythroleukemia cells (HEL 92.1.7) to $\mathrm{Ca}^{2+}$- mobilization and inhibition of forskolin-stimulated cAMP production was investigated. The two enantiomers of medetomidine ([±]-4-(1-[2,3-dimethylphenyl]ethyl)-1H-imidazole) produced opposite responses. Dexametomidine behaved as an agonist in both assays (i.e., it caused $\mathrm{Ca}^{2+}$ mobilization and depressed forskolin-stimulated CAMP production). Levo- medetomidine, which is a weak agonist in some test systems, reduced intracellular $\mathrm{Ca}^{2+}$ levels and further increased forskolin-stimulated CAMP production and therefore can be classified as an inverse agonist. A neutral ligand, MPV-2088, antagonized responses to both ligands. Several other, chemically diverse $\alpha_{2}$-adrenergic ligands also were tested. Ligands that could promote increases in $\mathrm{Ca}^{2+}$ levels and inhibition of CAMP production could be classified as full or partial agonists. Their effects could be blocked by the $\alpha_{2}$-adrenoceptor antagonist rauwolscine and by pertussis toxin treatment. Some typical antagonists such as rauwolscine, idazoxan, and atipamezole had inverse agonist activity like levomedetomidine. The results suggest that the $\alpha_{2A}$-adrenoceptors in HEL 92.1.7 cells exist in a precoupled state with pertussis toxin-sensitive G proteins, resulting in a constitutive mobilization of intracellular $\mathrm{Ca}^{2+}$ and inhibition of CAMP production in the absence of agonist. This constitutive activity can be antagonized by inverse agonists such as levomedetomidine and rauwolscine. Levomedetomidine can be termed a “protean agonist” because it is capable of activating uncoupled $\alpha_{2A}$-adrenoceptors in other systems and inhibiting the constitutive activity of precoupled $\alpha_{2A}$-adrenoceptors in HEL 92.1.7 cells. With this class of compounds, the inherent receptor “tone” could be adjusted, which should provide a new therapeutic principle in receptor dysfunction.

Many G protein-coupled receptors undergo spontaneous activation in the absence of agonist (Allen et al., 1991; Lefkowitz et al., 1993; Barker et al., 1994; Samama et al., 1994; Tian et al., 1994; Tiberi and Caron, 1994; Milligan et al., 1995). Basal activity has previously been detected in cells and in membrane preparations from cells endogenously expressing opioid receptors (Costa and Hertz, 1989). In experimental systems, spontaneous activity can be achieved through receptor mutation, overexpression, or G protein overexpression (Lefkowitz et al., 1993; Milligan et al., 1995) as has been shown for adrenergic, dopaminergic, and 5-hydroxytryptamine receptors (Allen et al., 1991; Ren et al., 1993; Barker et al., 1994; Samama et al., 1994; Tiberi and Caron, 1994; Chidic et al., 1996). The spontaneous receptor activity can be demonstrated using antagonists that produce an inverse response. Two types of antagonists are found, neutral antagonists and negative antagonists (Costa and Hertz, 1989). Antagonists with negative activity have also been termed inverse agonists. A new class of G protein-coupled receptor ligands called “protean agonists” has been proposed on theoretical grounds. Depending on the basal ABBREVIATIONS: [Ca$^{2+}$], intracellular free Ca$^{2+}$ concentration; IBMX, 3-isobuthyl-1-methyl-xanthine; TES, 2-(2-hydroxy-1,1-bis(hydroxymethyl)ethanesulfonic acid; TBM, TES-buffered medium; clonidine, 2-(2,6-dichloro-3-[[1-[(2,3-dihydrophenyl)ethyl]-2-imidazol-2-yl]-[1H-imidazole; guanabenz, 1-(2,6-dichloro-3-[[1-[(2,3-dihydrophenyl)ethyl]-2-imidazol-2-yl]-[1H-imidazole HCl; oxytetracobalamin, 3-[2,5-dihydro-1H-imidazol-2-yl]-[1H-imidazole]; guanabenz, 1-(2,6-dichloro-3-[[1-[(2,3-dihydrophenyl)ethyl]-2-imidazol-2-yl]-[1H-imidazole HCl; propranolol, (±)-[1-(isopropylamino)-3-(1-naphthyloxy)-2-propanol HCl; naphazoline, 2-(1,4-benzodioxan-2-yl)-2-imidazol-2-yl)-imidazole HCl; oxymetazoline, (3-[4-(1,3-thiazin-2-yl)-4-(1H-imidazol-2-yl)-6-(1,1-dimethylimidazolyl)-2,4-dimethylphenol] HCl; propranolol, (±)-[1-(isopropylamino)-3-(1-naphthyloxy)-2-propanol HCl; naphazoline, 4,5-dihydro-2-(1-naphthalenylmethyl)-1H-imidazole; xylazine, N-(2,6-dimethylphenyl)-6,6-dihydro-4H,1,3-thiazin-2-amine HCl; rauwolscine, 17α-hydroxy-20α-yohimb-16β-carboxylic acid methyl ester HCl; tizanidine, 5-chloro-4-[2-imidazol-2-yl-amino]-2,1,3-benzothiadiazole; atipamezole, (S)-[2-ethyl-2-indan-2-yl]-imidazole HCl; detomidine, (S)-[2,3-dimethylbenzyl]imidazole HCl; medetomidine, (±)-(4-(1H-imidazol-2-yl)-[1H-imidazole HCl; dexametomidine, (±)-(S)-4-(1H-imidazol-2-yl)-1H-imidazole HCl; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N′,N′'-tetraacetic acid; HEPES, 2-(1H-imidazole) HCl; 2-adrenoceptor antagonist; MPV-2088; atipamezole, 4(5)-(2-ethyl-indan-2-yl)imidazole HCl; detomidine, 4(5)-(2,3-dimethylbenzyl)imidazole HCl; idazoxan, 1-(2,6-dichloro-3-[[1-[(2,3-dihydrophenyl)ethyl]-2-imidazol-2-yl]-[1H-imidazole HCl; levomedetomidine, (±)-(R)-4-(1H-imidazol-2-yl)-1H-imidazole HCl; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N′,N′'-tetraacetic acid; HEPES, 2-(1H-imidazole) HCl; 2-adrenoceptor antagonist.
activity of the receptor, these should either act as agonists or inverse agonists (Kenakin, 1995). The rationale put forward by Kenakin (1995) has been that a protean agonist is a weak agonist (i.e., having a low efficacy). The basal receptor activity produces an active receptor state (R*) that competes with the ligand-induced active state (AR*) for the G protein. As AR* has a lower affinity for the G protein than R* a factual decrease (i.e., inverse agonism by the ligand) will be observed when there is a substantial basal formation of the R*. In the absence of a marked spontaneous production of R*, a protean agonist will act like a normal agonist. The results of Chidiac et al. (1996) have recently shown the existence of protean agonists in practice.

The HEL 92.1.7 cell line shows robust increases in [Ca\(^{2+}\)], (Michel et al., 1989; Musgrave and Seifert, 1995), as well as inhibition of adenylyl cyclase in response to agonist stimulation of the endogenous \(\alpha_2\)adrenoceptor (McKernan et al., 1987). Both responses are sensitive to pertussis toxin and hence mediated by \(\text{G}_{\text{i/o}}\)-type G protein. In this study, precoupling of the receptors was probed by measurement of changes in [Ca\(^{2+}\)], and cAMP levels in response to a variety of \(\alpha_2\)adrenoceptor ligands.

**Materials and Methods**

**Drugs**

\(^{3}\text{H}\)Adenine and \(^{14}\text{C}\)cAMP were from Amersham (Buckinghamshire, UK). (-)-Epinephrine, clonidine, desipramine (10,11-dihydro-N-methyl-5H-di-benz[k,l]azepine-5-propanamide), guanabenz, guanfacine, idazoxan, IBMX, naphazoline, (-)-norepinephrine, prazosin, oxymetazoline, pertussis toxin, propranolol, quinacrine (6-chloro-9\(\text{-methyl}\)-5\(\text{-dibenz[H,N]}\)imidazole\(\text{HCl}\)), rauwolscine, and UK14,304 (5-bromo-6-\(\text{[2-imidazoline-2-ylamino]}\)quinoxaline) were from RBI (Natick, MA). Fura-2 acetoxymethyl ester was from Molecular Probes (Eugene, OR). Atipamezole, detomidine, MPV-2088 (6-chloro-9\(\text{-bromo-6-\[2-imidazoline-2-ylamino]}\)imidazoline HCl), rauwolscine, and UK14,304 (5-bromo-6-\(\text{[2-imidazoline-2-ylamino]}\)quinoxaline) were from RBI (Natick, MA). Fura-2 acetoxymethyl ester was from Molecular Probes (Eugene, OR). Atipamezole, detomidine, MPV-2088 ((1-4-(5-fluoro-2,3-dihydro-1H-inden-2-yl)-1H-imidazole\(\text{HCl}\)), medetomidine, and its two isomers dexmedetomidine and levomedetomidine were from Orion-Corporation, Orion-Pharma (Turku, Finland).

**Cell Culture**

HEL 92.1.7 cells obtained from the American Culture Collection (Rockville, MD) were grown in suspension culture in RPMI-1640 medium supplemented with 5 \(\mu\text{Ci/ml}\) \(^{3}\text{H}\)adenine. After incubation for 2 hr, the cells were collected, pelleted, and washed once with TBM, supplemented with 1 mM CaCl\(_2\). Thereafter, the cells were resuspended and divided into aliquots of \(-10^{6}\) cells in 0.8 ml of the same medium. The cells were preincubated with 0.5 mM IBMX (a phosphodiesterase inhibitor), 100 \(\mu\text{M}\) propranolol (a \(\beta\)-adrenoceptor antagonist), and 150 \(\mu\text{M}\) quinacrine (a phospholipase A\(_2\) inhibitor) for 10 min at 37\(^\circ\). Forskolin (10 \(\mu\text{M}\)) and agonists in different concentrations (1 nM to 100 \(\mu\text{M}\)) were added. After 10 min, the cells were centrifuged for 1 min at 10,000 \(\times\) g, the medium was immediately removed, and the reaction was terminated by resuspension in 1 ml of 0.3 m perchloric acid, containing about 1600 cpm \(^{14}\text{C}\)cAMP. The extent of conversion of \(^{3}\text{H}\)ATP to \(^{14}\text{C}\)cAMP was determined by sequential Dowex/alumina ion exchange to isolate CAMP (Salomon et al., 1974). Conversion to \(^{14}\text{C}\)cAMP was expressed as a percentage of total eluted tritium and was normalized to the recovery of \(^{14}\text{C}\)cAMP tracer (generally 70%). Radioactivity was determined by liquid scintillation counting (Wallac 1410; Wallac Oy, Turku, Finland) in Optiphase HiSafe 3.

**Radioligand Binding**

Preparation of the homogenates. Recombinant Shionogi S115 mouse mammary tumor cell lines expressing the human \(\alpha_2\)A (\(\alpha_2\)-C10), \(\alpha_2\)B (\(\alpha_2\)-C2), or \(\alpha_2\)C-adrenoceptor (\(\alpha_2\)-C4) subtypes were cultured in a hollow fiber bioreactor, harvested, and washed by centrifugation as described by Ala-Uotila et al. (1994). The cell pellet was suspended in 50 mM Tris/5 mM EDTA (pH 7.5 at 4\(^\circ\)) and homogenized in Teflon-glass homogenizer (10 strokes with 1000 rpm, Potter S). Cell homogenate was then centrifuged twice at 47,800 \(\times\) g for 30 min at 4\(^\circ\) with resuspension by two strokes in Tris/EDTA buffer. The final pellet was resuspended in incubation buffer (50 mM KH\(_2\)PO\(_4\) buffer, pH 7.5 at 25\(^\circ\)) and distributed in aliquots to be stored at \(-80^\circ\) for later use.

Preparation of the rat and rabbit liver followed what has been described previously (Garcia-Sainz et al., 1992). After removal, tissue was cleaned from the connective tissue and washed in buffer of 0.25 m sucrose with 5 mM HEPES (pH 7.4 at 4\(^\circ\)), minced with scissors and frozen in liquid nitrogen to be stored at \(-80^\circ\) for later use. Liver suspension was centrifuged for 5 min at 600 \(\times\) g at 4\(^\circ\), supernatant was poured through a double cheesecloth, and the pellet was homogenized, centrifuged, and filtered as above. The supernatants were combined and centrifuged for 15 min at 47,800 \(\times\) g at 4\(^\circ\). After resuspension in ice-cold 50 mM Tris buffer with 10 mM EDTA (pH 7.4 at 30\(^\circ\)), the homogenate was...
incubated for 30 min at 30° to remove endogenous catecholamines and then centrifuged as above.

**Binding assays.** In saturation binding experiments with S115 cells, 10 concentrations of [3H]rauwolscine (77.9 or 80.5 Ci/mmol; New England Nuclear Research Products, Boston, MA) were incubated with the S115 cell homogenate (20–50 μg of total protein per tube) in a total volume of 0.25 ml in triplicate with or without presence of 100 μM oxymetazoline (to indicate nonspecific binding of the radioligand) in 50 mM KH2PO4 (pH 7.5 at 25°). In competition binding experiments, the radioligands were incubated at concentrations close to their KD value with the cell and tissue suspension (protein concentration as above) with or without presence of a test compound in a total volume of 0.25 ml. Nonspecific binding was determined using 100 μM oxymetazoline as described above. After a 30-min incubation at 25°, incubation was terminated by rapid filtration (TomTec 96 harvester) through prewet GF/B glass-fiber filter mats (Wallac Oy) and three washes with ice-cold 10 mM Tris (pH 7.7 at 4°). After drying, a solid scintillate (Meltilex; Wallac) was melted on filter mats, and the radioactivity was measured (BetaPlate; Wallac Oy) with 35% efficiency for tritium.

In a saturation binding experiment with rat and rabbit liver, 10 concentrations of [3H]prazosin (74.4 Ci/mmol; New England Nuclear) were incubated with the liver homogenate (100–200 μg of total protein per tube) in a total volume of 0.25 ml in triplicate with or without presence of 10 μM phenolamine (Sigma; to indicate nonspecific binding of the radioligand) in 50 mM Tris (pH 7.7 at 25°). In competition binding experiments, the radioligand was incubated at concentrations close to their KD value with the tissue suspension (protein concentration as above) with or without presence of a test compound in a total volume of 0.25 ml. Nonspecific binding was determined using 10 μM phenolamine as described above. After a 30-min incubation at 25°, incubation was terminated by rapid filtration, the filters were washed and dried, and the radioactivity was measured as above. For all the binding experiments, protein determinations were done using a colorimetric assay (Bradford, 1976) with bovine serum albumin as standard.

**Twitch Responses in Vas Deferens**

All animal experimentation was approved by the local laboratory animal care committee (approval no. 123). Rats of Sprague-Dawley strain were from B&K (Stockholm, Sweden). The rats were housed in groups of four or five in the same cage, under standard conditions (20–22°, light/dark cycle with lights on between 6 a.m. and 6 p.m.) with free access to water and food. Action of levomedetomidine on peripheral presynaptic α2-adrenoceptors in vitro was investigated in the prostatic portion of the rat vas deferens as described previously (Virtanen et al., 1988). In brief, the prostatic portions of the vas deferens of male rats (200–300 g) were suspended under a resting tension of 0.5 g weight in an organ bath containing Kreb’s solution supplemented with 1 μM (β)-propranolol and 20 mM desipramine. Twitch responses were induced by stimulation of intramural nerves (0.2 Hz, 2 msec, supramaximal voltage) and recorded as above.

**Calculations**

All results were analyzed using Prism (GraphPAD Software, San Diego, CA). Statistical analysis was carried out by one-way analysis of variance followed by Dunnett’s test. p < 0.05 was considered to be statistically significant. The results are expressed as mean ± standard error.

**Results**

**Effects on [Ca2+]i.** The [Ca2+]i in resting HEL 92.1.7 cells was 97 ± 7 nM (13 batches of cells). The two enantiomers of medetomidine had opposite effects on [Ca2+]i; dexmedetomidine caused a transient increase in [Ca2+]i, and levomedetomidine caused a transient reduction (Fig. 1). The dose-response relation of these two opposite effects is shown in Fig. 2. In both cases, an apparent saturation of the response was seen with calculated EC50 values of 18 ± 6 and 761 ± 502 nM for dexmedetomidine and levomedetomidine, respectively. The effect of levomedetomidine was preserved after several washes of the cells (data not shown), indicating that the basal receptor activity was not caused by the presence of an endogenous α2 agonist.

Twenty different α2-adrenoceptor ligands were tested for effects on [Ca2+]i. Most of the agonists tested gave significant transient increases in [Ca2+]i, (Fig. 3). To investigate whether the rise in [Ca2+]i, is a result of influx from the external medium or mobilization from intracellular stores, some of the experiments were performed in Ca2+-free medium (nominally Ca2+-free TBM plus 100 μM EGTA). The increases induced by epinephrine, norepinephrine, dexmedetomidine, and clonidine were reduced by 30–40% by Ca2+ removal (data not shown). For all ligands tested, the changes in [Ca2+]i were dose dependent (data not shown). Epinephrine was most active although it was closely followed by norepinephrine and UK14,304 (Fig. 3). The increases in [Ca2+]i, by 10 μM concentration of the different agonists could be blocked by the α2-adrenoceptor antagonist prazosin (1 μM) and by pertussis toxin pretreatment (500 ng/ml, 24 hr) (data not shown), whereas essentially no effect was seen with the α2-antagonist prazosin; its affinity was calculated to be >2 μM with respect to noradrenaline-induced Ca2+ elevation. Two of the tested antagonists, rauwolscine and idazoxan, caused a reduction in [Ca2+]i, in a similar manner as levomedetomidine and were thus classified as inverse agonists (Fig. 3). One α2-adrenoceptor ligand, MPV-2088, had no effect on [Ca2+]i.

Oxymetazoline, an α2-selective ligand with a weak agonistic effect, caused a small Ca2+ elevation. Prior (5 min in advance) application of oxymetazoline inhibited both the Ca2+-decreasing effect of levomedetomidine and the Ca2+-elevating effect of dexmedetomidine. The calculated affinities for the inhibition of the responses were not significantly different (10 ± 1 and 50 ± 25 nM, respectively; three determinations).

**Effects on cAMP.** In nonstimulated HEL 92.1.7 cells, the conversion of [3H]ATP to [3H]cAMP was 0.21 ± 0.06% (16 batches of cells), and this conversion was stimulated 7–10-fold by forskolin. The same ligands that were used in the Ca2+ experiments were tested for their ability to modify forskolin-stimulated cAMP production. In the same way as with the Ca2+ measurements, the ligands could be classified as agonists, neutral ligands, and inverse agonists. The rela-
tive maximum effects obtained in the cAMP assay closely followed those obtained in Ca$^{2+}$ measurements, with epinephrine, norepinephrine, and UK14,304 being full agonists and the typical antagonists rauwolscine, atipamezole, and idazoxan being inverse antagonists (Fig. 3). For the agonists detomidine, tizanidine, guanabenz, guanfacine, xylazine, oxymetazoline, and naphazoline, no significant effects on cAMP production could be seen. The inhibitory effects by 10 μM of the agonists could be blocked by rauwolscine (10 μM), MPV-2088 (10 μM), and pertussis toxin pretreatment (500 ng/ml, 24 hr).

Again, the two enantiomers of medetomidine had opposite effects regarding intracellular cAMP level. Dexmedetomidine inhibited forskolin-stimulated cAMP production, but with levomedetomidine, a potentiation of cAMP production was seen (Fig. 4). The EC$_{50}$ values for dexmedetomidine and levomedetomidine were 17 ± 0.9 and 390 ± 200 nM respectively (six batches of cells). MPV-2088 had no effect on the forskolin-stimulated cAMP production, but it blocked the effects of both levomedetomidine and dexmedetomidine (Figs. 3 and 5). Pertussis toxin pretreatment abolished the stimulation of cAMP production by levomedetomidine, as well as the inhibitory effect by dexmedetomidine, suggesting that both effects are transduced through G$_{i/o}$-type G proteins (Fig. 5).

**Agonist activity of levomedetomidine in vas deference.** Twitch responses of the prostatic portion of the rat vas deferens were inhibited by cumulative concentrations (at 2-min interval) of levomedetomidine with a pD$_2$ value of 7.76 ± 0.19 (five batches of cells). The maximum response to levomedetomidine was 30% of the response to dexmedetom-

MPV-2088. Because MPV-2088 was the only tested α$_{2}$ antagonist that was neutral, its effect on the levomedetomidine-induced elevation and dexmedetomidine-induced decrease of cAMP level was determined. As expected, it blocked the increase in cAMP production by levomedetomidine as well as the reduction in cAMP production by dexmedetomidine (Fig. 5). In addition, MPV-2088 showed high selectivity for α$_{2}$-adrenoceptors in binding experiments: in recombinant S115 cells, its binding affinity (K$_{D}$) was 0.30 ± 0.07 nM for α$_{2A}$ (six determinations in triplicate), 1.7 ± 0.14 nM for α$_{2B}$ (five determinations in triplicate), and 0.95 ± 0.03 nM for α$_{2C}$ (three determinations in triplicate). The binding affinity for MPV-2088 for the α$_{1}$-adrenoceptor was 169 ± 21 nM as determined in rat liver (four determinations in triplicate) and

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**Fig. 2.** The dose-response curves for dexmedetomidine (○) and levomedetomidine (■) with respect to the modulation of the [Ca$^{2+}$]. The basal level of [Ca$^{2+}$], is subtracted from each point. The values are mean ± standard error from three independent experiments. Observe the nonlinear ordinate.

**Fig. 3.** Effects of 10 μM of different α$_{2}$-adrenoceptor ligands on the intracellular level of Ca$^{2+}$ (left ordinate, □) and on the forskolin-stimulated cAMP production (right ordinate, ■). The basal [Ca$^{2+}$], and the forskolin-stimulated increase in cAMP production are taken as 0; negative Ca$^{2+}$ elevation and negative cAMP decrease therefore mean Ca$^{2+}$ decrease and cAMP elevation, respectively. The values are mean ± standard error from three independent experiments performed in triplicate. Ca$^{2+}$ responses to medetomidine and atipamezole were not tested. Observe the nonlinear left ordinate.

**Fig. 4.** The effects of dexmedetomidine (○) and levomedetomidine (■) on the cAMP production in the presence of 10 μM forskolin. The forskolin-stimulated increase in cAMP production is taken as 0. Negative values indicate inhibition and positive values further stimulation of forskolin-stimulated cAMP production. The values are mean ± standard error from three independent experiments performed in triplicate.

**Fig. 5.** Effect of 10 μM levomedetomidine (levomed), 10 μM dexmedetomidine (dexmed), and 10 μM MPV-2088 on cAMP production in the absence and presence of 10 μM forskolin in the control cells (□) and in the cell pretreated with pertussis toxin (500 ng/ml, 24 hr; ■). The values are mean ± standard error from three independent experiments performed in triplicate. Statistical significance was assessed against the basal level in control cells (a, p > 0.05, not significant), to the forskolin-stimulated level in control cells (b, p > 0.05, not significant; c, p < 0.05; d, p < 0.01), and to the forskolin-stimulated level in pertussis toxin-pretreated cells (e, p > 0.05, not significant).
Discussion

The results of the current study show that the two isomers of medetomidine, dexmedetomidine and levomedetomidine, have opposite effects on signal transduction through the endogenous $\alpha_2$-adrenoceptors in HEL 92.1.7 cells. Dexmedetomidine acted as an agonist (by elevating $[\text{Ca}^{2+}]$i and inhibiting cAMP production) as has been shown in many other systems (Savola and Virtanen, 1991; Jansson et al., 1994a, 1994b, 1995; Pohjanoksa et al., 1997). On the contrary, levomedetomidine acted as an inverse agonist by reducing $[\text{Ca}^{2+}]$i and enhancing cAMP production. In many other systems, levomedetomidine has been a weak partial $\alpha_2$-adrenoceptors agonist on the level of adenylyl cyclase ($\alpha_\text{2A}, \alpha_\text{2C}$ in S115 cells: Jansson et al., 1994a; $\alpha_\text{2A}, \alpha_\text{2C}$ in PS12 cells: Jansson et al., 1994b; $\alpha_\text{2B}$ in S9 cells: Jansson et al., 1995; $\alpha_\text{2C}$ in CHO cells: Pohjanoksa et al., 1997). In vivo levomedetomidine has shown agonistic effect by increasing sleeping time (Savola and Virtanen, 1991). The agonistic character of levomedetomidine was also seen in the inhibition of the contraction of rat vas deferens (effect on $\alpha_2\text{A,D}$-adrenoceptors; Smith and Docherty, 1992; Smith et al., 1992) shown in the current study. On the contrary, in HEL 92.1.7 cells levomedetomidine was an inverse agonist. The inverse agonistic effect seen in here was sensitive to rauwolscine and MPV-2088 and to pertussis toxin. This together with the previously reported high selectivity of levomedetomidine for $\alpha_2$ receptors (Virtanen et al., 1988) suggests that the inverse effect reported in the current study is indeed transduced by $\alpha_2$-adrenoceptors.

The antagonists rauwolscine, atipamezole, and idazoxan induced similar responses in the $[\text{Ca}^{2+}]$i, and/or cAMP accumulation assays as levomedetomidine. Because these antagonists showed negative activity, they can be classified as inverse agonists. The antagonist MPV-2088 showed no activity, although it has nanomolar or even subnanomolar affinity for $\alpha_2$-adrenoceptors. Both the agonistic and inversely agonistic effects were blocked by MPV-2088 and by pertussis toxin treatment, indicating that these signals are transmitted through $\alpha_2$-adrenoceptors and G$i/o$-type G proteins. The effects of the inverse agonists on cAMP production could only be seen after forskolin treatment. This is probably due to the low basal adenylyl cyclase activity and correspondingly small effect of the active G$i/o$-type G proteins in the absence of forskolin. After activation of adenylyl cyclase with forskolin, the constitutive activity of the G$i/o$-type G proteins and the subsequent effect of inverse agonists on it are revealed.

The reason for the transient nature of the inverse $\text{Ca}^{2+}$ signal to inverse $\alpha_2$ agonists is unknown. We, however, assume this to be a result of a feedback regulatory mechanism activated to protect the cells from the possibly noxious $[\text{Ca}^{2+}]$i caused by the constitutive receptor activity. The $\text{Ca}^{2+}$ levels would remain elevated due to the capacitative $\text{Ca}^{2+}$ entry (Putney and Bird, 1993) but would be limited by the activation of the plasma membrane $\text{Ca}^{2+}$-ATPase by $\text{Ca}^{2+}$-calmodulin (Scharff, 1981). If this was the case with HEL 92.1.7 cells, the cells would not have to have a notably elevated $[\text{Ca}^{2+}]$i despite the constitutive receptor activity. Binding of inverse agonist would thus lead to a decrease in spontaneous receptor activity, resulting in an undershoot of $[\text{Ca}^{2+}]$i due to the activated $\text{Ca}^{2+}$-ATPase. The slow return to the basal level would be due to the dissociation of the calmodulin from the $\text{Ca}^{2+}$-ATPase, which has a half-time of minutes (Scharff and Foder, 1982; Åkerman et al., 1985). Whether this is the actual mechanism can only be speculated. However, it is clear that the inverse $\text{Ca}^{2+}$ signal is caused by $\alpha_2$-receptor inhibition as (1) it is observed with both rauwolscine and levomedetomidine and (2) the signal to levomedetomidine is inhibited both by rauwolscine and MPV-2088.

Levomedetomidine differs markedly from rauwolscine and idazoxan in that the latter two compounds, tested in many different systems, always act as antagonists. The inverse response to a ligand that in some systems acts as a weak agonist on the same subtype is possible to explain by using the model of Kenakin (1995). A protein agonist will normally produce an activated receptor state that has, however, lower affinity for the G protein than spontaneously produced active receptor state. In systems in which there is little spontaneous formation of the activated receptor state, the receptor-activating property of the ligand will therefore be seen and positive agonism will be observed. On the other hand, when there is a considerable spontaneous formation of the active receptor state, the lower efficacy of the ligand will result in inverse agonism (Kenakin, 1995). Altogether, the results with levomedetomidine indicate that this compound is a “protean” ligand, having the ability to act as an agonist (a weak such) in systems with little or no constitutive receptor activity and as an inverse agonist in systems with receptors expressing high constitutive activity. The possibility that a coupling to different G proteins in different cells would be causing the normal versus inverse agonism observed cannot be excluded. This, however, might also be the case in a system where both agonistic and inversely agonistic responses are seen in the same cells (e.g., at different receptor expression levels). Both the agonistic (S115 cells, Jansson et al., 1994a; S9 cells, Jansson et al., 1994b) and inverse agonistic effects (the current study) are seen as an effect on the adenylyl cyclase function, suggesting that levomedetomidine can transduce both agonistic and antagonistic signals through $\alpha_2\text{A}$ receptors and $\alpha_i$ G proteins, although the subtypes of $\alpha_i$ are unknown. Also, the high affinity of oxymetazoline for inhibition of levomedetomidine- and dexmedetomidine-induced signals and the low affinity of prazosin suggest the involvement of $\alpha_2\text{A}$ receptors both in the $\text{Ca}^{2+}$ decrease and increase in HEL 92.1.7 cells.

The structural basis for the opposite actions of levomedetomidine and dexmedetomidine should be considered. In the case of the rigid agonist UK14,304 (a full $\alpha_2\text{A}$-adrenoceptor agonist) and other conformationally restricted $\alpha_2$-adrenoceptor agonists, a left-handed kink with the chiral center between the imidazoline ring and the quinoxaline nucleus is seen (Hancock et al., 1988; Munck et al., 1994). This defines that the activated receptor must accommodate for a pronounced left-handed kink of the rigid ligand. Because the two enantiomers, which are mirror images, produce opposite effects, the conformation of the binding cavity should change drastically on transition from the inactivated to the activated state. This model is in agreement with recent studies on the $\beta_2$-adrenoceptors using fluorescence tagging in which a range of ligands produced spectral shifts that correlated with...
their functional activity (Gether et al., 1995). Compounds usually considered to be antagonists behaved as inverse agonists and showed opposite fluorescence changes compared with agonists. This suggests that native receptors can access a neutral conformation when no ligand is bound. These conformational changes in the binding cavity may well be related to the previously reported conformational changes in rhodopsin involving helix number III (Alkorta and Du, 1994; Farahbakhsh et al., 1995) and to the cis/trans-isomerization of retinal in rhodopsin where the kinked cis conformation is associated with the active state of the G protein and the linear trans conformation is silent (Arns and Hofmann, 1995).

The protean agonistic properties of levmedetomidine [i.e., weak agonism in some systems (Jansson et al., 1994a, 1994b, 1995) and inverse agonism in others] can be explained by the inherent tonus of the receptor-signal transduction pathway (Kenakin, 1995). With the recent realization that receptors can have spontaneous activity both in normal and in pathological situations (Milano et al., 1994), protean agonists represent a new and promising class of targeted drug therapy. This applies to cases in which a constitutively active mutated receptor or possible overactivity of G protein-modulatory proteins (Sato et al., 1996; Dohlan and Thorner, 1997) can cause pathophysiological elements of disease. It will be beneficial to be able to restore the tonus of receptor activation without interfering significantly with the normal activation of the receptor. There is a basic difference in the action of the antagonist-type inverse agonists and protean agonists: the former inhibit the activation of the silent, “normal,” receptors, whereas the latter will activate silent receptors. Both types of inverse agonism may be beneficial in different pathological situations.

References


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