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ACCELERATED COMMUNICATION

Oxymetazoline Inhibits Adenylate Cyclase by Activation of Serotonin-1 Receptors in the OK Cell, an Established Renal Epithelial Cell Line

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SUMMARY

The nonselective α-adrenergic agonist oxymetazoline inhibits parathyroid hormone (PTH)-stimulated cAMP production in intact OK cells, an epithelial cell line derived from an American opossum kidney. This inhibition, however, is not blocked by α_2-adrenergic receptor antagonists. After excluding several alternate hypotheses to explain this anomolous activity of oxymetazoline, we hypothesized that oxymetazoline activates a receptor in OK cells that is negatively coupled to adenylate cyclase but distinct from the α_2-adrenergic receptor. Prior exposure of OK cells to pertussis toxin blocks the inhibitory response to oxymetazoline, suggesting involvement of a guanine nucleotide-binding regulatory protein. Screening various compounds for attenuation of PTH-stimulated adenylate cyclase showed that serotonin (5HT) is a potent and fully efficacious agonist. Desensitization of α_2-receptor-mediated inhibition of cAMP production by epinephrine did not alter the response to either 5HT or oxymetazoline, indicating that these compounds do not produce their effect by activating α_2-adrenergic receptors. The $5HT_1$ receptor-selective antagonist methiothepin, but not ketanserin ($5HT_2$-selective) or ICS-205,930 ($5HT_3$-selective), blocked the response to both 5HT and oxymetazoline. The potency of methiothepin for antagonizing oxymetazoline-induced inhibition of PTH-stimulated cAMP production was not significantly different from its potency for the 5HT-induced effect. These data indicate that OK cells express a $5HT_1$ receptor that is negatively coupled to adenylate cyclase and that oxymetazoline is an agonist at these receptors.

Oxymetazoline is a member of a family of sympathomimetic and sympatholytic imidazolines whose biological activity has been recognized for almost 50 years (1). Oxymetazoline and several other imidazolines appear to exert their sympathomimetic actions by activation of α-adrenergic receptors (2, 3), for which they are generally partial agonists (4). α-Adrenergic receptors can be subclassified into α_1- and α_2-adrenergic receptor subtypes (5). Oxymetazoline is generally a partial agonist at both of these α-adrenergic receptor subtypes (6–9). More recently, oxymetazoline has been useful in discriminating subtypes of the α_2-adrenergic receptors (10). In radioligand binding studies using [^{3}H]yohimbine, an α_2-adrenergic-selective radioligand, oxymetazoline is 50-fold more potent at α_{2A}-adrenergic receptor binding sites than at α_{2B} sites and has therefore been proposed to be an α_{2A}-adrenergic receptor subtype-selective compound (11).

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The OK cell line is derived from the kidney of an American opossum and retains several properties of proximal tubular epithelial cells in culture (12, 13). In competition binding studies using [^{3}H]yohimbine, oxymetazoline binds to a single class of α_2 adrenergic binding sites. Furthermore, the affinity of oxymetazoline for this binding site is not significantly decreased in the presence of GTP. Because inhibition binding curves for other α_2-adrenergic agonists are better fit by two-site binding models in the absence of GTP and because the affinity of these agonists is decreased in the presence of GTP, it appears that oxymetazoline is an antagonist at the OK cell α_2-adrenergic receptor. However, oxymetazoline is a potent and fully efficacious agonist at inhibiting PTH-stimulated cAMP production in intact cell adenylate cyclase assays, a response expected for α_2-adrenergic receptor activation (14). Unlike other α_2-adrenergic receptor agonists, however, the response to oxymetazoline is not blocked by α_2-adrenergic antagonists. We undertook the present study to explain the anomolous behavior of oxymetazoline in the OK cell line. Our results indicate that

ABBREVIATIONS: OK cell, opossum kidney cell line; PTH, bovine parathyroid hormone peptide fragment 1-34; BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle's medium; 5HT, 5-hydroxytryptamine (serotonin); UK-14,304; 5-bromo-6-[2-imidazoline-2-y1-amino]quinoxaline; MR30143, 5-carboxamidotryptamine; RU24969; 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridyl)indole; ICS-205,930, *endo*-8-methyl-8-azabicyclo[3.2.1.]oct-3-ol indol-3-y1-carboxylate; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Ro20-1724, 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone; 8-OH-DPAT, 8-hydroxy-2-(di-*N*-propylamino)tetralin.

oxymetazoline is an agonist at a receptor negatively coupled to adenylate cyclase, which is distinct from the α_2-adrenergic receptor. The data indicate that this inhibitory receptor belongs to the $5HT_1$ class of serotonin receptors.

Experimental Procedures

Materials. The following chemicals were obtained from Sigma Chemical Co. (St. Louis, MO): (−)-epinephrine, (−)-norepinephrine, 5HT, BSA (radioimmunoassay grade) penicillin, 3-methyl-isobutylxanthine, Tris-HCl, PTH (bPTH 1-34), and glycylglycine. Forskolin was purchased from Calbiochem (San Diego, CA) and pertussis toxin from List Biological Laboratories (Wayland, MA). Trypsin (0.25%) and DMEM were obtained from GIBCO Laboratories (Grand Island, NY). Fetal bovine serum was purchased from Sigma, GIBCO, and Cell Culture Laboratories (Cleveland, OH). [^{3}H]Rauwolscine (88 Ci/mmol) was purchased from New England Nuclear (Boston, MA) and [^{3}H] adenine (35 Ci/mmol) from ICN Radiochemicals (Irvine, CA). The following drugs were generous donations from their respective companies: ICS-205,930 (Sandoz Ltd., Basel, Switzerland), ketanserin (Janssen Pharmaceutica, Beerse, Belgium), Ro20-1724 (Hoffman Larouche, Inc., Nutley, NJ), oxymetazoline (Schering Corp., Kenilworth, NJ), UK-14,304 (Pfizer, Inc., Groton, CT, rauwolscine (Accurate Scientific Chemical Corp., Westbury, NY), and MR30143 (Glaxo, Inc., Hertfordshire, UK). We are grateful to Dr. Stephen Peroutka (Stanford University, Stanford, CA) for his generous donation of methiothepin. Generally, all drugs and radioligands were made up in 5 mM HCl or in 70% ethanol with subsequent dilution in 5 mM HCl. The concentration of ethanol in a particular assay did not exceed 0.07%.

Cell culture and binding assay. Growth and subculture of OK cells were performed essentially as previously described (12). Briefly, OK cells were grown on plastic tissue culture dishes at 37° in 95% air/5% CO_2, in high glucose, bicarbonate-buffered DMEM supplemented with 5% fetal bovine serum. Twenty hours before harvest or assay of confluent cells, growth media were exchanged for serum-free DMEM containing 0.1% BSA and 100 units/ml penicillin. To prepare crude membranes for radioligand binding studies, cells were harvested from 150-mm dishes (Falcon, Inc., Oxnard, CA) in ice-cold phosphate-buffered saline and pelleted by centrifugation at 3000 × *g* at 4° for 5 min. The pellet was resuspended in 40 volumes of ice-cold 50 mM Tris-HCl, pH 8.0, and disrupted with a Tekmar Tissuemizer at a setting of 90 for 20 sec. The homogenate was centrifuged at 35,000 × *g* at 4° for 10 min. This wash step was repeated once. Membrane pellets were stored for up to 2 months at −80° until used in radioligand binding assays. For these assays, pellets were thawed and resuspended as 1 g of wet weight/500 ml of 25 mM glycylglycine buffer, pH 7.6, and [^{3}H] rauwolscine saturation binding assays were performed as previously described (15) at 22° in a 2.0-ml assay volume (170 μg of protein/tube). Protein was determined by the method of Lowry *et al.* (16) using BSA as standard. Saturation binding data were analyzed by computer-assisted nonlinear regression as previously described (12) to obtain K_D and B_{max} values.

cAMP production assay. We used the [^{3}H]adenine prelabeling technique (17) as an assessment of adenylate cyclase activity in intact OK cell monolayers grown on 35-mm plastic tissue culture dishes (Falcon). The assay was initiated by pretreating cells for 30 min with 3 μCi of [^{3}H]adenine in 1.0 ml of HEPES-buffered DMEM at 37°. In selected experiments, other drugs were included during, or before, this pretreatment step. The cells were then washed twice with 2.0 ml of HEPES-DMEM followed by a 3-min, 37°, incubation with HEPES-DMEM containing drugs to stimulate cAMP production. The assay was terminated by aspiration of the drug-containing media and the addition of 1.0 ml of 5% trichloroacetic acid. [^{3}H]ATP and [^{3}H]cAMP fractions were isolated by ion-exchange column chromatography and radioactivity was assessed as previously described (12). cAMP production was calculated as a percentage of the conversion of ATP to cAMP: % conversion = [^{3}H]cAMP/([^{3}H]cAMP + [^{3}H]ATP). Due to day-to-day variations in the per cent conversion of ATP to cAMP elicited by PTH or forskolin, results are presented as a percentage of the maximum PTH- or forskolin-stimulated per cent conversion in the absence of inhibitory agonists. In general, the variation (standard error) for triplicate determinations within a particular treatment did not exceed 10% of the mean.

Data analysis and statistical methods. Dose-response curves to inhibitory agonists were analyzed by computer-assisted nonlinear regression using a least-squares parametric curve fitting program (CDATA; EMF Software, Inc., Knoxville, TN) to obtain EC_{50} values. All data points from a given experiment (generally 33 points) were entered as per cent conversion values and were fit to a one-site model floating all adjustable parameters. For those experiments subjected to statistical analysis, treatments were randomized to dish and order in assay using random number tables (18). Parametric statistical methods were therefore used to obtain experimental Student's *t* values or Fischer ratios and compared with tabulated values (18) with the level of significance chosen as the probability of a Type I error rate of less than 5%.

Results

The effects of inhibitory agonists on PTH-stimulated cAMP production in OK cell monolayers are presented in Fig. 1. In these experiments, 100 nM PTH was utilized to stimulate adenylate cyclase. This concentration of PTH gave an optimum signal with which to study receptor-mediated inhibition of cAMP production, inasmuch as the level of stimulation over basal was routinely 8- to 15-fold. The α_2-adrenergic-selective agonist UK-14,304 (19) and oxymetazoline inhibited PTH-stimulated cAMP production with EC_{50} values of 32 and 0.7 nM, respectively. The maximum response to UK-14,304, and also (−)-epinephrine and (−)-norepinephrine (12), was a 60–65% attenuation of PTH-stimulated cAMP production, whereas the response to oxymetazoline resulted in a 75–80% reduction in cAMP production. Also shown in Fig. 1 are the dose-dependent inhibitory effects of 5HT (EC_{50} = 23 nM), MR30143 (EC_{50} = 0.34 nM), and RU24969 (EC_{50} = 0.08 nM)

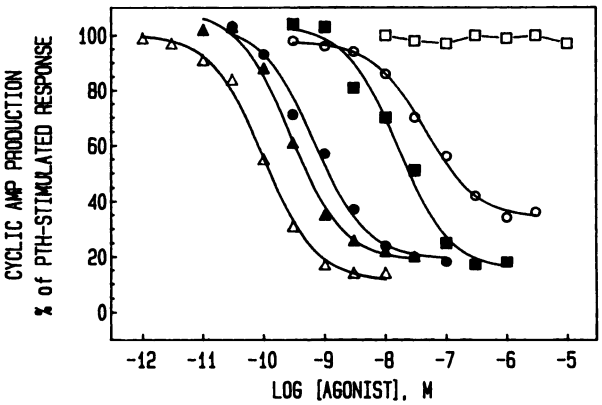

Fig. 1. Inhibition of PTH-stimulated cyclic AMP production in OK cell monolayers. Monolayers on 35-mm dishes were pretreated for 30 min with 3 μCi of [^{3}H]adenine at 37°. At the end of this period, the cells were washed and exposed to solutions containing 100 nM PTH and the indicated concentrations of drugs for 3 min at 37°. [^{3}H]ATP and [^{3}H] cAMP fractions were collected and stimulation was assessed as a percentage of conversion of [^{3}H]ATP to [^{3}H]cAMP. Each *point* represents the mean of two to five experiments performed in triplicate expressed as a percentage of the response to PTH (0.28 ± 0.06% conversion, mean ± SE; 16 determinations) in the absence of inhibitory agonists. Basal cAMP production was usually 7–10% of the PTH-stimulated response. ▲, MR30143; ●, oxymetazoline; ■, 5HT; ○, UK-14,304; △, RU24969; and □, 8-OH-DPAT.

and the lack of an inhibitory effect by 8-OH-DPAT. Each of the active agonists inhibited PTH-stimulated cAMP production by 75–80%. We have shown previously that inhibition of cAMP production in OK cells by UK-14,304 is blocked by α-adrenergic antagonists with a rank order of potency indicating an α_2-adrenergic receptor-mediated response (12). However, α-adrenergic antagonists did not reverse oxymetazoline-induced inhibition of PTH-stimulated cAMP production (for example, see Fig. 5).

We considered several alternate hypotheses to explain this lack of effect of α-adrenergic antagonists on the oxymetazoline-induced inhibition. First, it is possible that the failure of α-adrenergic antagonists to reverse the oxymetazoline-induced response resulted from irreversible binding of this agonist to the α_2-adrenergic receptor. To test this hypothesis, OK cells were preincubated for 30 min with 1.0 μM phenoxybenzamine, an irreversible α-antagonist, before subsequent challenge with oxymetazoline. Phenoxybenzamine pretreatment failed to block the oxymetazoline-induced inhibition of PTH-stimulated cAMP production, whereas the response to UK-14,304 was completely antagonized by this pretreatment (data not shown). To further rule out the possibility that oxymetazoline is an irreversible agonist at the OK cell α_2-adrenergic receptor, we performed α_2-adrenergic receptor saturation binding studies in OK cell membranes using [^{3}H]rauwolscine in the absence or presence of 7, 20, and 100 nM oxymetazoline (Fig. 2). In the presence of oxymetazoline the K_D of [^{3}H]rauwolscine for the α_2-adrenergic receptor was lower but the B_{max} was unchanged, suggesting that oxymetazoline behaves in a competitive manner at the α_2-adrenergic receptor.

A second hypothesis that could explain the anomolous behavior of oxymetazoline in the OK cell is that this compound is an antagonist at PTH receptors. To test this hypothesis, we assessed oxymetazoline-induced inhibition of forskolin-stimulated cAMP production. We reasoned that if the mechanism of action of oxymetazoline were limited to a PTH antagonist, then oxymetazoline should not inhibit forskolin-stimulated activity. However, as shown by the dose-response curves in Fig. 3, the inhibitory effect of oxymetazoline on forskolin-stimulated cAMP production was similar to its effect on the PTH-stimulated response. These data also exclude the possibility that the effect of oxymetazoline results from its activation of a cAMP phosphodiesterase, because the experiments shown in Fig. 3 were conducted in the presence of Ro20-1724, a phosphodiesterase inhibitor. Similar results were obtained when 1-methyl-3-isobutylxanthine was used to inhibit phosphodiesterase activity (data not shown).

Together these results indicate that oxymetazoline is not an agonist at the OK cell α_2-adrenergic receptor or a PTH-receptor antagonist and suggest its effects on PTH-stimulated cAMP production could result from a specific interaction with a receptor that is negatively coupled to adenylate cyclase through G_i, but which is distinct from the α_2-adrenergic receptor. To investigate this possibility, OK cell monolayers were pretreated with 200 ng/ml pertussis toxin for 19 hr. Pertussis toxin catalyzes the ADP-ribosylation of G_i, resulting in the uncoupling of the effects of inhibitory agonists on adenylate cyclase (20). As shown in Table 1, pertussis toxin pretreatment completely attenuated UK-14,304-, oxymetazoline-, and 5HT-induced inhibition of PTH-stimulated cAMP production.

Several classes of compounds were screened for their inhibition of PTH-stimulated cAMP production in OK cell monolayers. Among the inactive compounds were histamine, carbachol, 8-OH-DPAT, and dopamine. By contrast, as shown in Fig. 1, both 5HT and the 5HT$_1$ receptor-selective agonists MR30143 and RU24969 inhibited PTH-stimulated cAMP production to an extent similar to that seen with oxymetazoline but greater than for UK-14,304.

To test the possibility that 5HT exerts its effects through the α_2-adrenergic receptor, we designed a cross-desensitization experiment. OK cell monolayers were pretreated for 2.5 hr without or with epinephrine. After extensive washing to remove epinephrine, cAMP production was assessed (Fig. 4). In control cells, UK-14,304 inhibited PTH-stimulated cAMP production by 60% whereas oxymetazoline and 5HT were more efficacious. In cells pretreated with epinephrine, the inhibitory responses

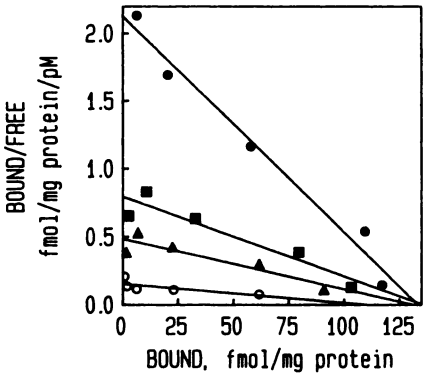

Fig. 2. Rosenthal plots of [^{3}H]rauwolscine saturation binding to OK cell membranes in the presence of oxymetazoline. Membranes (0.17 mg of protein) in 2.0 ml of 25 mM glycylglycine buffer were incubated with 3–800 pM [^{3}H]rauwolscine in the absence (●) or presence of 7 nM (■), 20 nM (▲), or 100 nM (○) oxymetazoline for 30 min at 22°. Specific binding was calculated for each concentration of radioligand as the difference between total and nonspecific binding (0.1 mM (−)-norepinephrine). Computer-assisted nonlinear regression was used to obtain [^{3}H]rauwolscine K_d and B_{max} values. Oxymetazoline reduced the apparent affinity of the receptor for [^{3}H]rauwolscine (K_d = 66, 162, 251, and 1104 pM, for 0, 7, 20, and 100 nM oxymetazoline, respectively) without significantly changing the total number of binding sites labeled by the radioligand (B_{max} = 130, 130, 124, and 113 fmol/mg of protein, respectively). The figure is representative of three separate experiments performed in duplicate or triplicate.

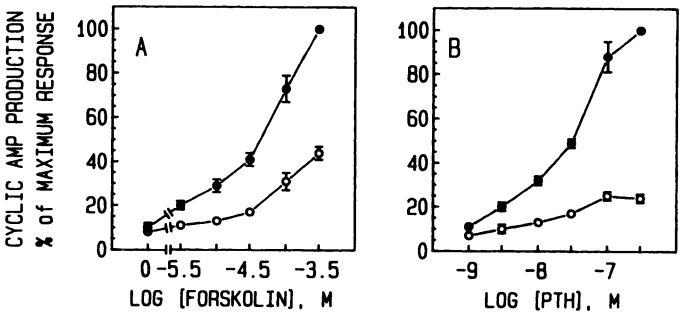

Fig. 3. Dose-response curves to (A) forskolin or (B) PTH in the absence (●) or presence (○) of 1 μM oxymetazoline. Cell monolayers were pretreated with 3 μCi of [^{3}H]adenine for 30 min at 37° in media containing 0.1 mM Ro20,1724, washed, and exposed to solutions containing the indicated concentrations of agonists. [^{3}H]ATP and [^{3}H]cAMP fractions were collected and cAMP production was assessed as a percentage of conversion of [^{3}H]ATP to [^{3}H]cAMP. Each *point* represents the mean ± standard error of three experiments performed in triplicate and are expressed as a percentage of the maximum response (0.77 ± 0.09% conversion or 0.85 ± 0.09% conversion for 0.3 mM forskolin or 100 to 300 nM PTH, respectively) observed in the absence of oxymetazoline.

TABLE 1

Attenuation by pertussis toxin of the inhibition of PTH-stimulated cAMP production by UK-14,304, 5HT, and oxymetazoline

OK cell monolayers in 35-mm dishes were pretreated with 200 ng/ml pertussis toxin in serum-free DMEM for 19 hr. The cells then were washed with HEPES-DMEM and pretreated for 30 min with 3 μCi of [^{3}H]adenine at 37°. At the end of this period, the cells were stimulated for 3 min at 37° with 100 nM PTH without or with other drugs. [^{3}H]ATP and [^{3}H]cAMP fractions were collected and cAMP production was determined as the percentage of conversion of [^{3}H]ATP to [^{3}H]cAMP. Each value represents the mean ± standard error of three experiments performed in triplicate and is expressed as a percentage of the control PTH-stimulated cAMP production in the absence of other drugs (0.27 ± 0.04% conversion; mean ± SE; three experiments).

Drug in Addition to 100 nM PTH	Pretreatment	
	No Pertussis toxin	200 ng/ml Pertussis toxin
	% of control	
None	100	104 ± 4
UK-14,304 (10 μM)	34 ± 1	104 ± 5
Oxymetazoline (1 μM)	17 ± 2	106 ± 5
5HT (1 μM)	18 ± 3	116 ± 5

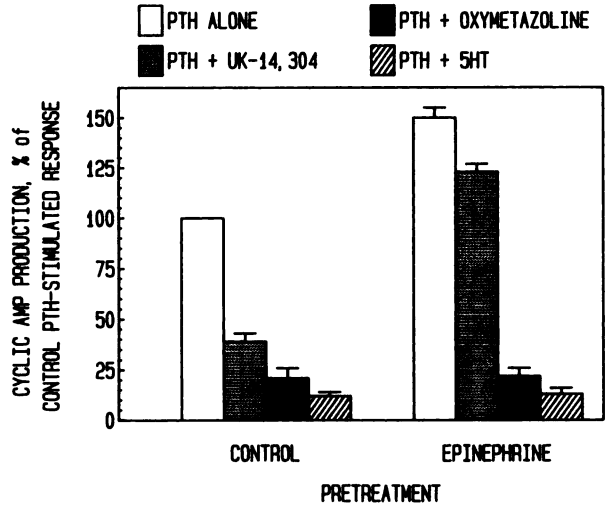

Fig. 4. Effect of (−)-epinephrine pretreatment on inhibition of PTH-stimulated cAMP production by UK-14,304, oxymetazoline, and 5HT. Cell monolayers were pretreated for 2.5 hr in the presence of 28 μM (−)-epinephrine (added at a 100-fold higher concentration in 5 mM HCl vehicle) or vehicle for control. [^{3}H]Adenine (1–3 μCi) was added to monolayers for the final 30 min of this pretreatment period. After this, the cells were extensively washed and incubated for 3 min at 37° in solutions containing 100 nM PTH without or with 10 μM inhibitory agonists. Basal cAMP production was unaffected by this pretreatment (not shown). [^{3}H]ATP and [^{3}H]cAMP fractions were collected and cAMP production was assessed as a percentage of conversion of [^{3}H]ATP to [^{3}H]cAMP (0.32 ± 0.05% conversion for control PTH-stimulated group). Each *bar* represents the mean ± standard error of four experiments performed in triplicate and expressed as a percentage of the response to PTH alone for the control pretreatment.

to oxymetazoline and 5HT were similar to those in control cells, inhibiting 80–85% of the PTH-stimulated response. In contrast, UK-14,304 inhibited PTH-stimulated cAMP production by only 16%. This experiment indicates that epinephrine pretreatment results in a marked desensitization of subsequent α_2-adrenergic receptor-mediated inhibition of PTH-stimulated cAMP production but does not desensitize the inhibitory response to 5HT or oxymetazoline.

Interestingly, homologous desensitization of the OK cell α_2-adrenergic receptor with epinephrine was accompanied by a sensitization of the PTH-stimulated responsiveness inasmuch as PTH-stimulated cAMP production in the epinephrine-treated group was enhanced by 50% over that seen in the control group. A more robust sensitization of the response to adenylate cyclase activators by α_2 agonist pretreatment is seen in the HT29 cell ine (21).

These data led us to hypothesize that oxymetazoline is an agonist at a 5HT receptor in the OK cell line. In order to test this postulate we assessed a series of 5HT receptor antagonists for their ability to reverse both oxymetazoline- and 5HT-induced inhibition of PTH-stimulated cAMP production. As shown in Fig. 5, neither the $5HT_2$ receptor-selective antagonist ketanserin, nor the $5HT_3$ receptor-selective antagonist ICS-205,930 had an appreciable effect on the response elicited by submaximally effective concentrations of oxymetazoline or 5HT. Also shown is the lack of antagonism by the α_2 adrenergic-selective antagonist rauwolscine. However, the $5HT_1$ receptor-selective antagonist methiothepin completely reversed the inhibition elicited by both 5HT and oxymetazoline. Additionally, these antagonists by themselves neither stimulated nor attenuated PTH-stimulated cAMP production (data not shown).

In order to demonstrate that oxymetazoline is an agonist at the 5HT receptor, we generated a series of dose-response curves for both oxymetazoline and 5HT inhibition of PTH-stimulated cAMP production in the absence and presence of 0.1, 0.3, and 3 μM methiothepin. The effect of methiothepin in these experiments was a dose-dependent rightward shift in the agonist dose-response curves (Fig. 6). Analysis of these data by Schild regression (22) demonstrated that the pA_2 of methiothepin for the oxymetazoline-induced effect (pA_2 = 7.44 ± 0.13, slope = 1.3 ± 0.1; mean ± SE three experiments) was not significantly different ($p < 0.05$, Student's t test) from that for the 5HT-induced effect (pA_2 = 7.12 ± 0.14, slope = 1.0 ± 0.1; three experiments). The slopes of these regressions were near unity,

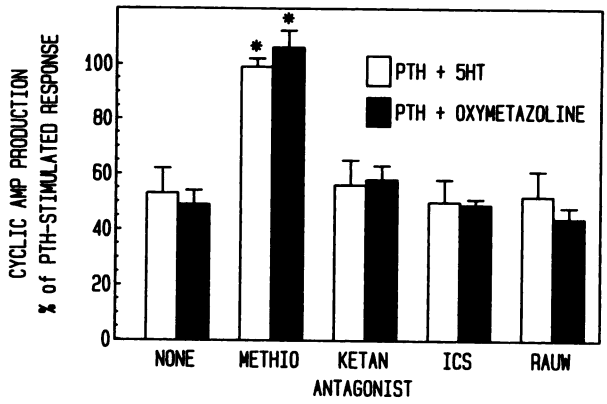

Fig. 5. Effect of antagonists on inhibition of PTH-stimulated cAMP production by oxymetazoline and 5HT. Monolayers were pretreated for 30 min with 3 μCi of [^{3}H]adenine at 37°, washed, and exposed for 3 min to solutions containing 100 nM PTH in the absence or presence of either 3 nM oxymetazoline or 30 nM 5HT. Also included in these incubations were either no antagonists (*NONE*), 3 μM methiothepin, (*METHIO*), 10 μM ketanserin (*KETAN*), 10 μM ICS-205-930 (*ICS*), or 1 μM rauwolscine (*RAUW*). [^{3}H]ATP and [^{3}H]cAMP fractions were collected and cAMP production was assessed as a percentage of the conversion of [^{3}H]ATP to [^{3}H]cAMP. Each *bar* represents the mean ± standard error of three experiments performed in triplicate and are expressed as a percentage of the response elicited by PTH in the absence of other drugs (0.18 ± 0.06% conversion, three determinations, data not shown). The antagonists had no effect on the level of cAMP production elicited by PTH alone nor did they affect basal cAMP production (not shown). *, Significantly different ($p < 0.05$) from the response to PTH in the presence of oxymetazoline or 5HT using Waller's treatment of the LSD rule (18). Five independent comparisons were made in this analysis.

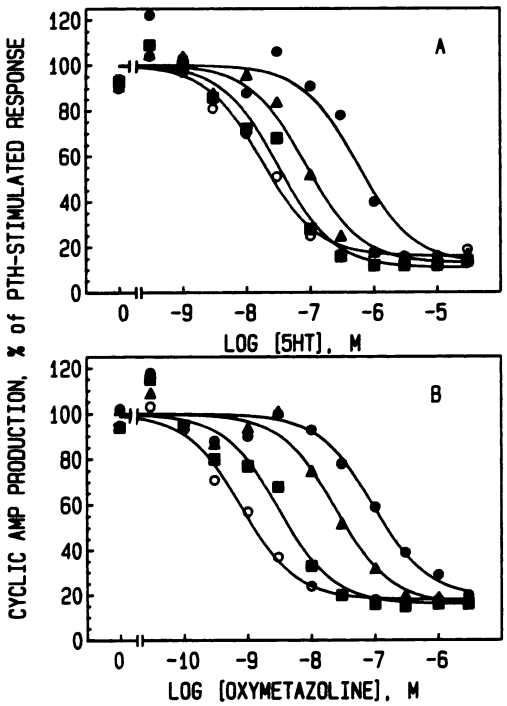

Fig. 6. Dose-response curves for (A) 5HT- or (B) oxymetazoline-induced inhibition of PTH-stimulated cAMP production in the presence of 0 (○), 0.1 μM (■), 0.3 μM (▲), and 3.0 μM (●) methiothepin. Monolayers were pretreated for 30 min with 3 μCi of [^{3}H]adenine at 37°, washed, and exposed to solutions containing the indicated concentrations of drugs for 3 min at 37°. [^{3}H]ATP and [^{3}H]cAMP fractions were collected and cAMP production was assessed as a percentage of conversion of [^{3}H]ATP to [^{3}H]cAMP. Each *point* represents the mean of three experiments performed in triplicate and is expressed as a percentage of the PTH-stimulated response (0.27 ± 0.03% conversion) in the absence of inhibitory agonists. Methiothepin alone neither stimulated nor inhibited PTH-stimulated or basal cAMP production.

indicating that the interaction of methiothepin with these agonists is competitive. Because the pA$_2$ values were not different, we conclude that oxymetazoline is an agonist at a 5HT$_1$ receptor in the OK cell line.

Discussion

There are two major findings from this study. The first is the demonstration of agonist-induced desensitization of the OK cell α_2-adrenergic receptor-mediated inhibition of PTH-stimulated cAMP production. This desensitization appears to be homologous for the α_2-adrenergic receptor, because 5HT inhibition of adenylate cyclase was unaffected by this pretreatment. An understanding of mechanisms of regulation for receptors negatively coupled to adenylate cyclase lags significantly behind that for receptors that stimulate adenylate cyclase activity. One limitation for α_2-adrenergic receptor studies has been the lack of cell lines that express these receptors and in which agonist-induced desensitization can be demonstrated (23, 24). The OK cell line should prove valuable in further studying mechanisms of agonist-induced regulation of α_2-adrenergic receptors.

The second principal finding of our study is that oxymetazoline is an agonist at a 5HT$_1$ receptor negatively coupled to adenylate cyclase in the OK cell line. Several lines of evidence converge to support this finding. 1) Oxymetazoline inhibition of PTH-stimulated cAMP production is refractory to blockade by α-adrenergic antagonists but is reversed by methiothepin, a 5HT receptor antagonist. 2) The lack of α-adrenergic antagonism is not due to irreversible binding of oxymetazoline to the α_2-adrenergic receptor. 3) Oxymetazoline is not an antagonist at PTH receptors. 4) The inhibitory response to oxymetazoline was blocked by pertussis toxin but was unaffected by α_2-adrenergic receptor desensitization. 5) Finally, methiothepin has the same affinity for blocking both the oxymetazoline and the 5HT response.

Various sympathomimetic imidazolines are known to exert physiological responses through activation of receptors other than α-adrenergic receptors. Tolazoline and tetrahydrozoline, but not oxymetazoline, activate the H$_2$-histaminergic receptors in guinea pig heart (5, 25). Thus, compounds closely related to oxymetazoline, but not oxymetazoline itself, have been shown to possess activity for at least one other receptor class. To our knowledge, the activity of the sympathomimetic imidazolines at receptors other than α-adrenergic and histaminergic has not been reported. The possibility of a broader spectrum of activity for these compounds needs to be considered.

Our findings raise the question as to whether the serotonergic activity of oxymetazoline can explain some of its diverse and varied physiological effects. The affinity of oxymetazoline for the 5HT receptor in the OK cell (5 nM as determined by radioligand binding in the presence of GTP)[1] is similar to its affinity for the α_2 adrenergic receptor (20 nM; Ref. 12). It has been recognized for some time that the intrinsic activity of oxymetazoline at various peripheral α-adrenergic receptors is different (26). To a large degree, these effects have been explained by preferential activation of either α_1- or α_2-adrenergic receptors present in the preparations but activity at other receptor classes has not been rigorously excluded in most of these studies. In dogs and cats, intracisternal injection of oxymetazoline usually, but not always, lowers blood pressure and reduces efferent sympathetic nerve traffic, similar to the effect of clonidine (27). In rats, stereotaxic infusion of oxymetazoline into the anterior hypothalamus failed to cause hypotension, although injection of clonidine did (3). One possible explanation for these contradictory findings is that oxymetazoline acts at other receptors in these preparations to oppose, facilitate, or mimic central α-adrenergic receptor-mediated responses. Indeed, evidence suggests central 5HT receptors may play a role in modulating peripheral cardiovascular responses (28, 29).

The heterogeneity of 5HT receptors is well known (30). Peroutka and Snyder (31) proposed the subclassification of 5HT receptors into 5HT$_1$ and 5HT$_2$ subtypes. Bradley *et al.* (32) have recently extended this classification to include 5HT$_3$ receptors. Although it appears that the 5HT$_2$ binding site as defined by Peroutka and Snyder represents a receptor coupled to phosphoinositide hydrolysis (33), definitive evidence for a biochemical effector system associated with 5HT$_1$ binding sites is still lacking. One complicating factor is the apparent heterogeneity of 5HT$_1$ binding sites. Pedigo *et al.* (34) have suggested these binding sites can be subclassified into 5HT$_{1a}$ and 5HT$_{1b}$ sites. A third 5HT$_1$ binding site, 5HT$_{1c}$, has been identified in mammalian choroid plexus epithelium (35) and also represents a receptor coupled to phosphoinositide hydrolysis (36). Additionally, a 5HT$_{1d}$ binding site has been identified in bovine brain membranes (37).

[1] Unpublished observations.

5HT receptor-mediated regulation of adenylate cyclase has been reported in several systems. Stimulation of adenylate cyclase in rat (38) and guinea pig hippocampus (39) has been ascribed to activation of $5HT_{1a}$ receptors. However, $5HT_{1a}$ receptor-mediated inhibition of adenylate cyclase in guinea pig hippocampus (40, 41) and in murine striatal and cortical neurons (42) has also been reported. It is unclear why this discrepancy exists. One problem has been the lack of specific and selective high affinity antagonists for subtypes of the $5HT_1$ receptor. In these studies, characterization of the receptors mediating both the inhibitory and stimulatory responses were largely based upon differential potencies of agonists. Because the preparations used in these studies represent complex mixtures of multiple neuronal cell types and their associated receptors, it is conceivable that the agonists used to activate a response were working through a variety of receptors located on different cells. Attempts to define biochemical effectors for $5HT_{1b}$ and $5HT_{1d}$ binding sites have not been reported, although the $5HT_{1b}$ sites appear to be associated with the inhibitory autoreceptor modulating evoked 5HT overflow from rat brain cortex (43) and norepinephrine overflow from rat vena cava (44).

Bradley *et al.* (32) have proposed a classification system for 5HT receptors with particular emphasis on the need for caution in subclassification of $5HT_1$ receptors. Recognizing the heterogeneity of $5HT_1$ receptors but noting the lack of selective antagonists for these receptors, they have proposed that receptors that do not fulfill criteria for classification as $5HT_2$ or $5HT_3$ receptors be determined $5HT_1$-like receptors. Responses elicited by $5HT_1$-like receptors should be antagonized by methiothepin but not by $5HT_2$ antagonists (ketanserin) or by $5HT_3$ antagonists (ICS-205,930). Additionally, MR30143 should be at least as potent as 5HT in eliciting responses mediated by the $5HT_1$-like receptors. Our study indicates that the 5HT receptor in the OK cell fulfills these criteria for classification as a $5HT_1$-like receptor. We have tentatively classified the OK 5HT receptor as $5HT_{1b}$ receptor, based upon the following logic: the $5HT_{1a}$-selective agonist 8-OH-DPAT is inactive; ketanserin, which blocks $5HT_{1c}$ responses, is without effect; and RU24969, which is potent at the OK cell 5HT receptor, is relatively weak at $5HT_{1d}$ receptors. Preliminary data with additional drugs and radioligand binding studies using ^{125}I-cyanopindolol support this conclusion (45).

The identification of cell lines that express a single subtype of the $5HT_1$-like receptors will be important tools in defining biochemical effector systems and functional responses coupled to these receptors. Continuous cell lines offer the advantage of being relatively homogeneous populations of cells in which receptor-mediated phenomena can be studied under well controlled conditions. Receptor subtype pharmacology, biochemical effector systems, receptor-mediated cellular responses, and regulation can be much more rigorously defined in the absence of multiple neurotransmitter receptor species associated with more heterogeneous tissue preparations. The OK cell line should be useful in characterizing $5HT_1$ receptor pharmacology and function.

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