0026-895X/94/061168-09$03.00/0

MOLECULAR PHARMACOLOGY, 45:1168–1176, 1994.

α_2-Adrenergic Receptors of the α_{2C} Subtype Mediate Inhibition of Norepinephrine Release in Human Kidney Cortex

ANNE-ULRIKE TRENDELENBURG, NORBERT LIMBERGER, and LARS C. RUMP

Pharmakologisches Institut (A.-U.T., N.L.) and Innere Medizin IV (L.C.R.), Universitätsklinikum, D-79104 Freiburg, Germany

Received September 15, 1993; Accepted February 21, 1994

SUMMARY

The pharmacological properties of the α_2-adrenergic receptors regulating the release of norepinephrine were investigated in human kidney cortex. Slices were preincubated with [^{3}H]norepinephrine, superfused in the presence of desipramine, and stimulated electrically. Two procedures were used to estimate the affinity of α-adrenergic antagonists at the autoreceptors. First, pEC_{30} values (negative logarithms of antagonist concentrations that increased the electrically evoked overflow of tritium by 30%) were determined. Second, antagonist pK_d values were determined against the overflow-inhibiting effect of the α_2 receptor-selective agonist UK 14,304. Antagonist pEC_{30} values correlated well with the respective pK_d values ($r = 0.96$, $p < 0.01$). The site of action of the phenylethylamine norepinephrine, as well as of the imidazoline derivative UK 14,304, is an α_2-adrenergic receptor. Neither the cyclooxygenase inhibitor indomethacin nor the adenosine receptor antagonist 8-sulfophenyltheophylline changed the concentration-inhibition curve of UK 14,304. When compared with binding data from the literature, the pEC_{30} values correlated best with the antagonist affinities at α_{2C} binding sites in an opossum kidney cell line and rat brain cortex ($r \geq 0.95$, $p < 0.001$) and at the affinities of α_{2C} sites obtained in COS cells transfected with either the human α_2-C4 or rat RG10-α_2 gene ($r \geq 0.95$, $p < 0.01$). In contrast, the correlations with α_{2A}, α_{2B}, and α_{2D} sites were not as good. Moreover, the α_2-autoreceptors in human kidney cortex were very similar to the α_{2C}-adrenergic receptors mediating prostaglandin synthesis in rabbit aorta but differed from α_{2A}- and α_{2D}-autoreceptors in rabbit and rat tissues. It is concluded that in human kidney cortex prejunctional autoreceptors are α_{2C}.

α_2-Adrenergic receptor activation inhibits neurotransmitter release in many tissues of various species (see Ref. 1), including rat (2) and human (3) kidney. Studies on inhibitory prejunctional α_2-adrenergic autoreceptors were the first to indicate a heterogeneity of α_2-adrenergic receptors (4–6). A species difference, for example, was repeatedly observed in the action of α-adrenergic receptor agonists and antagonists at α_2-autoreceptors in rat and rabbit vas deferens (7, 8) and brain cortex (9–11). A difference may also exist between the inhibitory α_2-adrenergic receptors in rat and human kidney (12). A systematic subclassification of the α_2-adrenergic receptors has been suggested on the basis of radioligand binding studies (13, 14). It is now widely accepted that these receptors can be divided into four pharmacological subtypes, $\alpha_{2A\text{-}D}$ (15, 16). This view has recently been supported by molecular genetic studies in humans and rats. In either species, three genes coding for different α_2-adrenergic receptors have been identified so far (17–19). Norepinephrine release-inhibiting α_2-autoreceptors have been subclassified almost exclusively in isolated tissues of laboratory animals. They have been shown to belong to either the α_{2A} or α_{2D} subtype (11, 20–26; but see Ref. 27), including in rat kidney (28, 29), and it has been suggested that α_2-autoreceptors may generally be of the α_{2A} or α_{2D} subtype (11). Little systematic work has been done in humans, but it has been proposed that α_2-autoreceptors that mediate inhibition of norepinephrine release in human brain cortex also belong to either the α_{2A} or α_{2D} subtype (30).

The aim of the present study was to characterize the norepinephrine release-inhibiting α_2-adrenergic receptors in human kidney cortex by determining antagonist and agonist affinity estimates for compounds that distinguish between the four α_2 subtypes. Endogenous norepinephrine can reduce its own release either by activation of prejunctional α-autoreceptors or by transjunctional prostaglandin- and adenosine-mediated mechanisms in rabbit (31) and rat (2, 32) kidney. The possible involvement of such a 'transjunctional' modulation of norepinephrine release was also investigated. Modulation of the electrically evoked overflow of tritium after preincubation of kidney slices with [^{3}H]norepinephrine was the response measured.

Materials and Methods

Human renal tissue. The present *in vitro* study was approved by the local ethics committee. It is based on 20 kidneys that were obtained from patients undergoing renal surgery because of hypernephroma (16 cases), epithelial carcinoma of the urinary tract (three cases), or metastasis of a bronchial carcinoma (one case). The age of the patients averaged 64.1 ± 3.2 years (range, 24–83 years). Only macroscopically healthy renal cortex tissue was used. None of the patients had been treated with drugs known to interfere with either the storage or release mechanisms of norepinephrine.

Superfusion experiments. About 30 cortical slices (0.4-mm thick,

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 325 and Ru 401/1-3).

3 mm in diameter) were prepared from each kidney. Half of the slices were kept at 2° (generally for about 5 hr) before being used in a superfusion experiment. The remaining slices were immediately incubated with 0.1 μM [^{3}H]norepinephrine, in 2 ml of medium, for 30 min at 37°. In each experiment 12 kidney slices were superfused in parallel, at a rate of 1 ml/min for 200 min, with medium containing 1 μM desipramine. There were five periods of electrical stimulation (rectangular pulses, 2-msec width, 15-V/cm voltage drop, 24-mA current strength). The first stimulation period was delivered after 30 min of superfusion (180 pulses at 3 Hz) and was not used for determination of tritium overflow. Stimulation periods were applied after 60 (S_1), 100 (S_2), 140 (S_3), and 180 min (S_4). After 50 min of superfusion successive 5-min samples of the superfusate were collected. Drugs were added either 20 min before S_2, S_3, and S_4 at increasing concentrations or from the beginning of superfusion onwards at a constant concentration (see below). At the end of the experiment, each slice was dissolved in tissue solubilizer. The tritium contents of the superfusate samples and of the tissue were determined.

Media. The superfusion medium contained 118 mM NaCl, 4.8 mM KCl, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, 11 mM glucose, 0.57 mM ascorbic acid, 0.03 mM disodium EDTA, and 0.001 mM desipramine. For incubation with [^{3}H]norepinephrine and for tissue storage the Ca^{2+} concentration of the medium was reduced to 0.2 mM and desipramine was omitted.

Calculation of tritium outflow. The outflow of tritium from the tissue was calculated as a fraction of the tritium content of the slice at the onset of the respective collection period and is expressed as fractional rate (in minutes^{-1}). The overflow of tritium elicited by electrical stimulation was calculated by subtraction of the estimated basal outflow from the total tritium outflow during the 5-min collection period in which the stimulation was applied plus the two 5-min periods thereafter. The overflow of tritium was then expressed as a percentage of the tritium content of the slice at the time of stimulation. For further evaluation of basal tritium outflow, ratios of the fractional rate during the 5 min before S_2, S_3, and S_4 to the fractional rate during the 5 min before S_1 were calculated. For further evaluation of the electrically evoked tritium overflow, ratios of the overflow evoked by S_2, S_3, and S_4 to the overflow evoked by S_1 (S_2/S_1, S_3/S_1, and S_4/S_1) were calculated. Moreover, effects of α-adrenergic receptor agonists and antagonists on the electrically evoked overflow were calculated, in each single slice, as a percentage of the corresponding average control (no agonist and no antagonist) S_2/S_1, S_3/S_1, and S_4/S_1 ratios.

Potency of α-adrenergic receptor antagonists in increasing [^{3}H]norepinephrine release. The effects of α-adrenergic receptor antagonists on the electrically evoked overflow of tritium were determined. Each of the four stimulation periods (S_1–S_4) consisted of 60 pulses at 1 Hz. Antagonists were added at increasing concentrations from 20 min before S_2, S_3, and S_4 onwards, to yield cumulative antagonist concentration-response curves (see Fig. 1A). Antagonist pEC_{30} values (negative logarithms of concentrations that increased the evoked overflow of tritium by 30%) were interpolated from the averaged concentration-response curves.

Dissociation constants of α-adrenergic receptor antagonists. The effects of adrenergic receptor antagonists against the α_2-receptor-selective agonist UK 14,304 were determined. The four stimulation periods (S_1–S_4) each consisted of five trains of six pulses at 50 Hz (train interval, 60 sec). UK 14,304 was added at increasing concentrations from 20 min before S_2, S_3, and S_4 onwards, to yield cumulative agonist concentration-inhibition curves. Antagonists, when used, were present throughout superfusion (see Fig. 1B). Antagonist dissociation constants (K_d values) were determined as described previously (23). Briefly, concentration-inhibition curves of UK 14,304 (weighted mean values) obtained in the absence and in the presence of antagonist were evaluated by logistic curve fitting using eq. 25 of Ref. 33. This yielded the maximal effect and the EC_{50} (concentration at which half-maximal inhibition was observed) of UK 14,304 given alone and of UK 14,304 in the presence of an antagonist. The negative logarithm of the dissociation constant of the antagonist (pK_d) was calculated using eq. 4 of Ref. 34.

Dissociation constant of the α-adrenergic receptor agonist oxymetazoline. The agonist dissociation constant (K_d) of oxymetazoline was determined by comparison of its concentration-inhibition curve in solvent-treated tissues and in tissues in which a fraction of the receptors had been blocked by the irreversible α-adrenergic receptor antagonist phenoxybenzamine (35). In each experiment, half of the slices were exposed to phenoxybenzamine (3 μM), whereas the other half were exposed to solvent. Phenoxybenzamine or solvent was added 10 min after the beginning of superfusion, was present for the following 10 min, and was then washed out. Each of the four stimulation periods (S_1–S_4) consisted of five trains of six pulses at 50 Hz (train interval, 60 sec). Cumulative concentration-inhibition curves of oxymetazoline were determined in phenoxybenzamine- and solvent-treated slices. The K_d of oxymetazoline was calculated according to the following steps (36): (a) fitting a logistic function (eq. 25 of Ref. 33) to the concentration-inhibition curve obtained in solvent-treated slices, (b) calculating oxymetazoline concentrations that were equieffective to oxymetazoline concentrations used in phenoxybenzamine-treated slices (concentrations causing <40% and >90% of the maximal inhibition obtained in phenoxybenzamine-treated slices were excluded; see Ref. 37), (c) fitting a hyperbola to the resulting pairs of equieffective concentrations, and (d) estimating the K_d and fraction of receptors still active after phenoxybenzamine treatment from the parameters of the hyperbola (see Ref. 11).

Effects of indomethacin and 8-sulfophenyltheophylline against UK 14,304. For these experiments the same protocol was used as for the determination of the α-adrenergic receptor antagonist dissociation constants. Either indomethacin or 8-sulfophenyltheophylline was present throughout superfusion, and UK 14,304 was added at increasing concentrations from 20 min before S_2, S_3, and S_4 onwards, to yield cumulative concentration-inhibition curves. Logistic curve fitting was carried out as described above, but no pK_d values were determined.

Statistics. Results are expressed as arithmetic mean ± standard error. Standard errors for maximal agonist effects and EC_{50} values derived from curve fitting were calculated as defined in Ref. 33. Groups were tested for significant differences with the Mann-Whitney test and Bonferroni correction. The n values indicate the number of slices.

Drugs. Purchased drugs were (−)-[*ring*-2,5,6-^{3}H]norepinephrine (specific activity, 56.7 Ci/mmol; DuPont, Dreieich, Germany), spiroxatrine, 8-*para*-sulfophenyltheophylline, and WB 4101 [(±)-2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane HCl] (Biotrend, Köln, Germany), oxymetazoline HCl (Merck, Darmstadt, Germany), rauwolscine HCl (Roth, Karlsruhe, Germany), and corynanthine HCl, indomethacin, and tetrodotoxin (Sigma, Deisenhofen, Germany). The following drugs were kindly provided by the producers: desipramine HCl and phentolamine methanesulfonate (Ciba-Geigy, Basel, Switzerland), (+)-mianserin maleate and (−)-mianserin maleate (Organon, Oss, The Netherlands), prazosin HCl and UK 14,304 [5-bromo-6-(2-imidazolin-2-ylamino)quinoxaline tartrate] (Pfizer, Karlsruhe, Germany), phenoxybenzamine HCl (Röhm, Weiterstadt, Germany), BRL 44408 [(±)-2-[2*H*-(1-methyl-1,3-dihydroisoindole)methyl]-4,5-dihydroimidazoline] and BRL 41992 [(−)-1,2-dimethyl-2,3,9,13b-tetrahydro-1*H*-dibenzo(*c,f*)imidazo 1,5)azepine] (SmithKline Beecham, Great Burgh, UK), SKF 104078 [6-chloro-9-[(3-methyl-2-butenyl)oxy]-3-methyl-1*H*-2,3,4,5-tetrahydro-3-benzazepine maleate] (SmithKline Beecham, King of Prussia, PA), and ARC 239 [2-[2-[4-(*o*-methoxyphenyl)piperazin-1-yl]ethyl]-4,4-dimethyl-1,3 (2*H*,4*H*)-isoquinolinedione 2HCl] (Thomae, Biberach an der Riss, Germany). Drugs were dissolved in distilled water, except for WB 4101 (1 mM HCl), spiroxatrine, BRL 44408, and BRL 41992 (10 mM HCl), tetrodotoxin (0.1 M phosphate buffer, pH 4.8), phenoxybenzamine (10 mM tartaric acid), and indomethacin (ethanol; final ethanol concentration, 6 μM), before being added to the superfusion medium. None of the solvents had any effect.

Results

Potencies of α-adrenergic receptor antagonists in increasing [^{3}H]norepinephrine release. The four stimulation periods (S_1–S_4) each consisted of 60 pulses at 1 Hz, and α-adrenergic receptor antagonists were added at increasing concentrations before S_2, S_3, and S_4. Basal outflow of tritium in the 5 min immediately before the first stimulation period S_1 was 0.89 ± 0.04 nCi, corresponding to a fractional rate of outflow of 0.00145 ± 0.00004 min^{-1} (n = 89). Electrical stimulation greatly accelerated the outflow of tritium (Fig. 1A). The tritium overflow evoked by S_1 was 1.16 ± 0.04 nCi, corresponding to 0.968 ± 0.027% of the tritium content of the tissue (n = 89). In control experiments (no antagonist added), the evoked overflow was reproducible over the four stimulation periods. The S_2/S_1, S_3/S_1, and S_4/S_1 ratios averaged 1.05 ± 0.01, 1.01 ± 0.02, and 0.98 ± 0.02 (n = 21). The α-adrenergic receptor antagonists rauwolscine, WB 4101, phentolamine, BRL 44408, (+)-mianserin, ARC 239, prazosin, SKF 104078, and corynanthine increased the evoked overflow of tritium, indicating the development of autoinhibition under these conditions of electrical stimulation. Fig. 1A illustrates an experiment with rauwolscine. Concentration-response curves for the antagonists are shown in Fig. 2 [except for BRL 44408, (+)-mianserin, and SKF 104078], and calculated pEC_{30} values are summarized in Table 1. BRL 41992 at up to 1 μM and (−)-mianserin at up to 10 μM only slightly enhanced the evoked tritium overflow (by about 15%). None of the antagonists caused any major change of basal tritium outflow at concentrations close to the calculated EC_{30}.

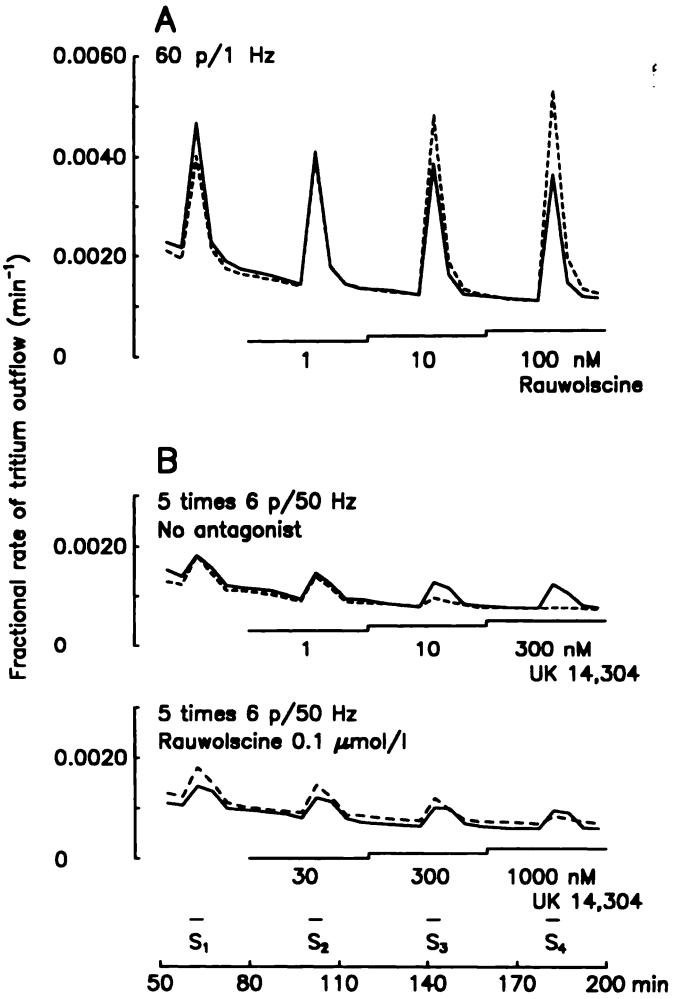

Fig. 1. Time course of the outflow of tritium from human kidney cortex slices preincubated with [^{3}H]norepinephrine, showing the effects of rauwolscine (A) and the interaction of UK 14,304 with rauwolscine (B). Slices were superfused in the presence of desipramine (1 μM). There were four stimulation periods (S_1–S_4), each consisting of 60 pulses at 1 Hz (A) or five trains of six pulses at 50 Hz (B) (train interval, 60 sec). Solvent (———) or increasing concentrations of rauwolscine (A) or UK 14,304 (B) (– – –) were added as indicated. In interaction experiments, rauwolscine (0.1 μM) was present throughout superfusion, and increasing concentrations of UK 14,304 were added as indicated. *Abscissae*, minutes of superfusion. A, Results are from four slices obtained from one kidney (from which a total of 12 slices were used) and are means of two slices for each curve. B, Results are from six (of 12) slices from a second kidney and are values from one slice for each curve in the absence of rauwolscine and means of two slices for each curve in the presence of rauwolscine.

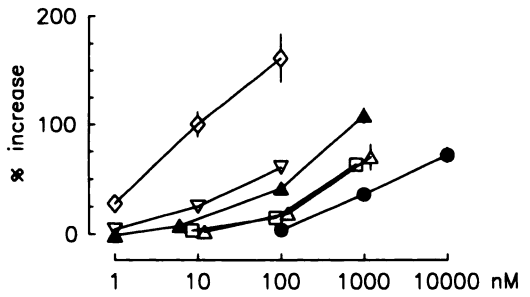

Fig. 2. Effects of α-adrenergic receptor antagonists on electrically evoked tritium overflow from human kidney cortex slices preincubated with [^{3}H]norepinephrine. Slices were superfused in the presence of desipramine (1 μM). The four stimulation periods (S_1–S_4) each consisted of 60 pulses at 1 Hz. Rauwolscine (◇), WB 4101 (▽), phentolamine (▲), prazosin (□), ARC 239 (△), or corynanthine (●) was added at increasing concentrations before S_2, S_3, and S_4. *Abscissae*, antagonist concentration. *Ordinates*, percentage increase caused by the antagonists, calculated from S_n/S_1 values. Data points are means ± standard errors of six slices obtained from two or three kidneys.

TABLE 1

Potencies of α-adrenergic receptor antagonists in increasing the stimulation-evoked overflow of tritium (pEC_{30}) and in antagonizing the overflow-inhibiting effect of UK 14,304 (pK_d) in slices from human kidney cortex preincubated with [^{3}H]norepinephrine

The pEC_{30} values are negative logarithms of antagonist concentrations that increased the evoked tritium overflow (60 pulses at 1 Hz) by 30%. The pK_d values were calculated from antagonism of the inhibitory effect of UK 14,304 on the evoked tritium overflow (five trains of six pulses at 50 Hz; train interval, 60 sec). Antagonist concentrations used to determine pEC_{30} values were those of Fig. 2 and 1–10,000 nM for antagonists not shown in Fig. 2. Antagonist concentrations used to determine pK_d values were those indicated in Table 2. Each pEC_{30} is based on six to nine slices obtained from two or three kidneys, and each pK_d is based on 12–18 slices obtained from two or three kidneys (controls and slices that received agonist only not included).

α-Adrenergic antagonist	pEC_{30}	pK_d
Rauwolscine	9.0	9.0
WB 4101	7.9	7.9
Phentolamine	7.3	7.7
BRL 44408	7.0	—
(+)-Mianserin	6.9	—
ARC 239	6.8	6.3
Prazosin	6.7	6.2
SKF 104078	6.4	—
Corynanthine	6.2	6.2
BRL 41992	<6	—
(−)-Mianserin	<5	—
Spiroxatrine	—	7.7

Dissociation constants of α-adrenergic receptor antagonists. The four stimulation periods (S_1–S_4) each consisted of five trains of six pulses at 50 Hz (train interval, 60 sec). Under these conditions, tetrodotoxin (0.3 μM) abolished the evoked overflow of tritium (data not shown). Rauwolscine (1 μM), when added before S_2, did not significantly increase the evoked overflow (S_2/S_1 ratio, 1.09 ± 0.06 in the absence of rauwolscine and 1.20 ± 0.05 in the presence of rauwolscine; n = 8 and 9, respectively) indicating a lack of autoinhibition under these conditions of electrical stimulation. To determine dissociation constants, the antagonists were present throughout superfusion and the selective α_2-adrenergic receptor agonist UK 14,304 was added at increasing concentrations before S_2, S_3, and S_4. An experiment with rauwolscine is shown in Fig. 1B. Values for basal outflow and stimulation-evoked overflow in the absence and in the presence of antagonists are summarized in Table 2. None of the antagonists altered basal outflow of tritium except for prazosin (1 μM) and spiroxatrine (0.3 μM), which caused accelerations of about 97% and 35%, respectively. The antagonists did not consistently change the overflow evoked by S_1, in accordance with the lack of effect of rauwolscine (1 μM) when added before S_2. In control experiments (no agonist and no antagonist), the evoked overflow was reproducible and the S_2/S_1, S_3/S_1, and S_4/S_1 ratios averaged 1.00 ± 0.03, 0.96 ± 0.03, and 0.93 ± 0.03 (n = 24), respectively. When antagonists were present throughout superfusion the S_n/S_1 ratios did not differ significantly from the respective control S_n/S_1 ratios (n = 5 for each antagonist). UK 14,304, given alone, reduced the evoked tritium overflow with an EC_{50} of 4.7 ± 0.6 nM and a maximal inhibition of 96.6 ± 1.3%. All antagonists tested, i.e., rauwolscine, WB 4101, phentolamine, ARC 239, prazosin, corynanthine, and spiroxatrine, shifted the concentration-inhibition curve of UK 14,304 to the right in a parallel manner (Fig. 3). Calculated pK_d values are summarized in Table 1. The pK_d values of rauwolscine, WB 4101, phentolamine, ARC 239, prazosin, and corynanthine were similar to the respective pEC_{30} values, and regression analysis indicated a highly significant correlation (r = 0.96, $p < 0.01$).

Effects of indomethacin and 8-sulfophenyltheophylline against UK 14,304. The four stimulation periods (S_1–S_4) each consisted of five trains of six pulses at 50 Hz. UK 14,304 was added at increasing concentrations before S_2, S_3, and S_4 and indomethacin (10 μM) or 8-sulfophenyltheophylline (100 μM), when used, was present throughout superfusion. Neither indomethacin nor 8-sulfophenyltheophylline altered basal outflow of tritium or the overflow evoked by S_1 (data not shown). S_n/S_1 ratios obtained in the presence of indomethacin or 8-sulfophenyltheophylline were not significantly different from the respective control S_n/S_1 ratios (n = 5 for each). As shown in Fig. 4, neither indomethacin nor 8-sulfophenyltheophylline affected the concentration-inhibition curve of UK 14,304.

Dissociation constant of the α-adrenergic receptor ag-

TABLE 2

Basal outflow (b_1) and electrically evoked overflow (S_1) of tritium from human kidney cortex slices preincubated with [^{3}H] norepinephrine

Slices were superfused in the presence of 1 μM desipramine plus the α-adrenergic receptor antagonist indicated. b_1 represents the outflow of tritium in the collection period immediately before the first stimulation period (from 55 to 60 min of superfusion) and is expressed as a fractional rate. S_1 represents the tritium overflow elicited by the first period of electrical stimulation with five trains of six pulses at 50 Hz, expressed as a percentage of the tritium content of the tissue. Values are means ± standard errors of n slices.

Drug present throughout superfusion	b_1	S_1	n
μM	min^{-1}	%	
—	0.00104 ± 0.00003	0.371 ± 0.016	58
Rauwolscine, 0.1	0.00111 ± 0.00004	0.474 ± 0.025[a]	15
WB 4101, 0.1	0.00103 ± 0.00003	0.427 ± 0.019	17
Phentolamine, 0.3	0.00096 ± 0.00003	0.400 ± 0.020	16
ARC 239, 1	0.00112 ± 0.00004	0.368 ± 0.009	17
Prazosin, 1	0.00205 ± 0.00004[b]	0.414 ± 0.023	19
Corynanthine, 3	0.00102 ± 0.00002	0.602 ± 0.057[b]	17
Spiroxatrine, 0.3	0.00140 ± 0.00007[b]	0.329 ± 0.017	20

[a] Significant differences from experiments with desipramine only, $p < 0.05$.
[b] $p < 0.01$.

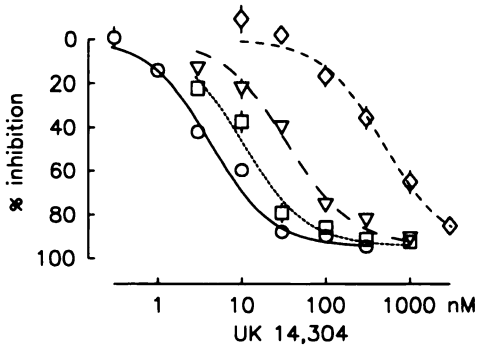

Fig. 3. Effect of UK 14,304 on electrically evoked tritium overflow from human kidney cortex slices preincubated with [^{3}H]norepinephrine, and its interaction with α-adrenergic receptor antagonists. Slices were superfused either in the presence of desipramine (1 μM) only (○) or in the presence of desipramine plus rauwolscine (0.1 μM) (◇), WB 4101 (0.1 μM) (▽), or prazosin (1 μM) (□). The four stimulation periods (S_1–S_4) each consisted of five trains of six pulses at 50 Hz (train interval, 60 sec). UK 14,304 was added at increasing concentrations before S_2, S_3, and S_4. *Abscissae*, concentration of UK 14,304; *ordinates*, percentage inhibition caused by UK 14,304, calculated from S_n/S_1 values. Data points are means ± standard errors of six to 11 slices obtained from at least two kidneys.

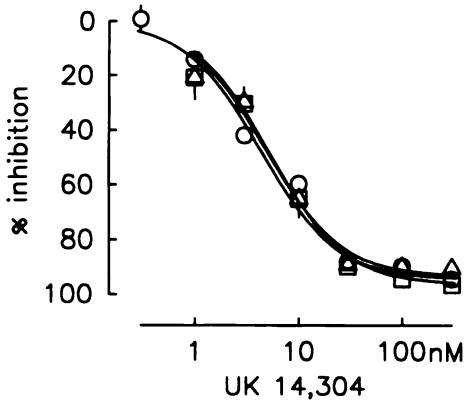

Fig. 4. Effect of UK 14,304 on electrically evoked tritium overflow from human kidney cortex slices preincubated with [^{3}H]norepinephrine, and its interaction with indomethacin and 8-sulfophenyltheophylline. Slices were superfused either in the presence of desipramine (1 μM) only (○) or in the presence of desipramine plus indomethacin (10 μM) (△) or 8-sulfophenyltheophylline (100 μM) (□). The four stimulation periods (S_1–S_4) each consisted of five trains of six pulses at 50 Hz. UK 14,304 was added at increasing concentrations before S_2, S_3, and S_4. *Abscissae*, concentration of UK 14,304; *ordinates*, percentage inhibition caused by UK 14,304, calculated from S_n/S_1 values. The concentration-inhibition curve for UK 14,304 in the presence of desipramine only is that of Fig. 3. Data points are means ± standard errors of six to 11 slices obtained from at least two kidneys.

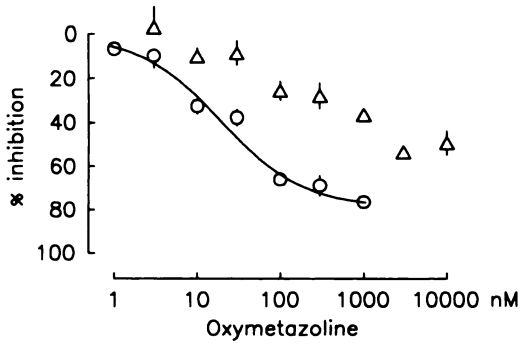

Fig. 5. Effect of oxymetazoline on electrically evoked tritium overflow from human kidney cortex slices preincubated with [^{3}H]norepinephrine, and its interaction with phenoxybenzamine. Slices were superfused in the presence of desipramine (1 μM). Either solvent (○) or phenoxybenzamine (3 μM) (△) was added 10 min after the beginning of superfusion, was present for the following 10 min, and was then washed out. The four stimulation periods (S_1–S_4) each consisted of five trains of six pulses at 50 Hz. Oxymetazoline was added at increasing concentrations before S_2, S_3, and S_4. *Abscissae*, concentration of oxymetazoline; *ordinates*, percentage inhibition caused by oxymetazoline, calculated from S_n/S_1 values. Data points are means ± standard errors of five or six slices obtained from four kidneys.

onist oxymetazoline. The four stimulation periods (S_1–S_4) each consisted of five trains of six pulses at 50 Hz. Oxymetazoline was added at increasing concentrations before S_2, S_3, and S_4. Pretreatment with phenoxybenzamine (3 μM) for 10 min affected neither basal outflow nor the electrically evoked overflow by S_1. S_n/S_1 ratios obtained after phenoxybenzamine treatment were not significantly different from the respective control S_n/S_1 ratios. In solvent-treated slices, oxymetazoline (1–1000 nM) reduced the evoked tritium overflow with an EC_{50} of 19.5 ± 4.2 nM and a maximal inhibition of 79.0 ± 2.6%. In phenoxybenzamine-pretreated slices, the concentration-inhibition curve of oxymetazoline was shifted to the right and the maximal inhibition was reduced by 35% (Fig. 5). Using the logistic function (eq. 25 of Ref. 33), oxymetazoline concentrations for solvent-treated slices were computed that were equieffective to oxymetazoline (30, 100, 300, and 1000 nM) after phenoxybenzamine treatment. The resulting four pairs of equieffective concentrations were fitted to a hyperbola. From the parameters of the hyperbola the fraction of α_2-adrenergic receptors still active after phenoxybenzamine treatment (0.06) and the pK_d value of oxymetazoline (6.1) were calculated. With the dissociation constant known, the law of mass action can be applied to compute the fraction of α_2-adrenergic receptors occupied at a given oxymetazoline concentration. At 300 nM, for instance, oxymetazoline occupied <30% of the receptors but produced almost 90% of its maximal inhibitory effect.

Discussion

Electrical stimulation of slices from human kidney cortex preincubated with [^{3}H]norepinephrine evokes an overflow of tritium that consists mainly of unmetabolized [^{3}H]norepinephrine. Moreover, the evoked tritium overflow depends on the presence of extracellular Ca^{2+} and is sensitive to tetrodotoxin when slices are stimulated with low-frequency pulse trains (3). Tetrodotoxin also abolishes the overflow of tritium from slices stimulated with trains of electrical pulses at high frequency (50 Hz), as used in the present study. Hence, in human kidney cortex the electrically evoked tritium overflow reflects action potential-induced release of [^{3}H]norepinephrine and is taken

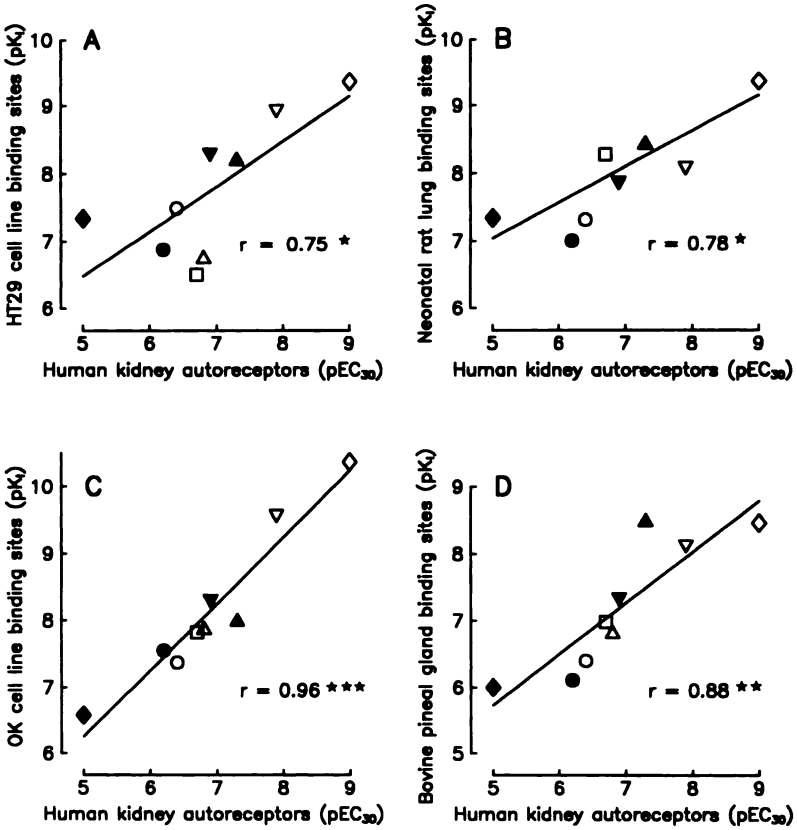

Fig. 6. Correlation between antagonist affinity estimates for α_2-adrenergic autoreceptors and for the [^{3}H]rauwolscine binding sites in human colonic adrenocarcinoma (HT-29) cells (A), in neonatal rat lung (B), in an opossum kidney (*OK*)-derived cell line (C), and in bovine pineal gland (D). Autoreceptor affinity estimates are pEC_{30} values (from Table 1). Binding site affinity estimates are pK_i values determined from inhibition of the binding of [^{3}H]antagonist (from Table 2 of Ref. 46). ◇, Rauwolscine; ▽, WB 4101; ▲, phentolamine; ▼, (+)-mianserin; △, ARC 239; □, prazosin; ○, SKF 104078; ●, corynanthine; ♦, (−)-mianserin.

TABLE 3

Correlation between antagonist affinity estimates for α_2-adrenergic autoreceptors in human kidney cortex and for various α_2-adrenergic sites

Shown are correlation coefficients (r) and slopes of the regressions for pK_i at α_2-adrenergic binding sites and pK_d at α_2-adrenergic receptors versus pEC_{30} at human kidney α_2-adrenergic autoreceptors. pEC_{30} values at human kidney autoreceptors were those of Table 1. pK_i and pK_d values for α_2-adrenergic binding sites and autoreceptors, respectively, were taken from the references indicated.

	r	Slope	Number of antagonists
Correlation with α_2-adrenergic binding sites			
HT-29 cell line (α_{2A})[a]	0.75[j]	0.67	9
Neonatal rat lung (α_{2B})[a]	0.78[j]	0.55	9
Opossum kidney cell line (α_{2C})[a]	0.96[k]	0.99	9
Rat brain cortex (α_{2C})[b]	0.95[k]	0.74	10
Bovine pineal gland (α_{2D})[a]	0.88[j]	0.77	9
Rat submaxillary gland (α_{2D})[c]	0.66	0.62	6
Rat brain cortex (α_{2D})[b]	0.37	0.28	10
α_2-C10 (α_{2A})[d]	0.72	0.72	6
α_2-C2 (α_{2B})[d]	0.72	0.55	6
α_2-C4 (α_{2C})[d]	0.95[j]	0.62	6
RG10-α_2[b]	0.96[k]	0.75	10
Correlation with α_2-adrenergic receptors			
Rabbit brain cortex autoreceptors (α_{2A})[e]	0.74[j]	0.73	9
Rabbit aorta (α_{2C})[f]	1.00[k]	0.95	6
Rat kidney autoreceptors (α_{2A}/α_{2D})[g,h]	0.79	0.50	6
Rat submaxillary gland autoreceptors (α_{2D})[i]	0.54	0.51	8
Rat brain cortex autoreceptors (α_{2D})[e]	0.39	0.40	8

[a] From Ref. 46.
[b] From Ref. 47.
[c] From Ref. 48.
[d] From Ref. 17.
[e] From Ref. 11.
[f] From Ref. 38.
[g] From Ref. 29.
[h] From Ref. 28.
[i] From Ref. 23.
[j] Significant difference from 0: [j] $p < 0.05$; [k] $p < 0.001$; [l] $p < 0.01$.

as an index of endogenous norepinephrine release from sympathetic nerve terminals.

As in many animal tissues, the release of [^{3}H]norepinephrine in slices of human kidney cortex is reduced through activation of α_2-adrenergic receptors by the endogenous agonist norepinephrine, which is secreted during the course of electrical stimulation, or by exogenous agonists such as UK 14,304 and oxymetazoline (present study and Refs. 3 and 12). The α_2-adrenergic receptors are most probably located in the surface membrane of the axon terminals, i.e., they are prejunctional receptors. However, there are other possibilities. Activation of postjunctional α_2 receptors might cause the release of secondary modulators such as prostaglandins (38) and adenosine, which then diminish the release of norepinephrine. Such transjunctional modulation has been proposed in rabbit (31) and rat (2, 32) kidney. In human kidney cortex, neither the prostaglandin synthesis inhibitor indomethacin nor the adenosine receptor antagonist 8-sulfophenyltheophylline altered the concentration-inhibition curve of UK 14,304, suggesting that the α_2-selective agonist reduced the release of [^{3}H]norepinephrine through activation of prejunctional α_2-adrenergic receptors; prostaglandins and adenosine were not involved in the effect of UK 14,304. This conclusion is supported by the finding that activation of only α_1-adrenergic receptors, and not α_2-adrenergic receptors, induces prostaglandin synthesis in rat kidney (39). Similarly, it has been shown that activation of α_1-adrenergic receptors evokes the release of adenosine or adenine nucleotides from sympathetically innervated tissues (40, 41), but so far no results have been published demonstrating that α_2-adrenergic receptor activation does so as well.

The aim of the present study was to subclassify the norepinephrine release-inhibiting prejunctional α_2-adrenergic receptors. A functional receptor is best characterized by the relative affinities of agonists and, preferably, antagonists (42). In the present study, two procedures were used to estimate affinities of α-adrenergic receptor antagonists for the prejunctional α_2-autoreceptors. First, the tritium overflow-enhancing potency of 11 antagonists was assessed under conditions of pronounced autoinhibition. With this method, antagonist affinities were estimated against the action of released norepinephrine. Second, the potency of seven antagonists against the release-inhibiting effect of UK 14,304 was assessed, yielding the dissociation constant (K_d) of the antagonist/α_2-autoreceptor complex. The dissociation constant of the α-adrenergic receptor agonist oxymetazoline was also determined, because oxymetazoline was shown to be the best single compound to differentiate between α_{2A}, α_{2B}, and α_{2C} binding sites (19). The measurement of agonist and antagonist affinities for prejunctional autoreceptors can be disturbed by the endogenous agonist (1). For instance, under conditions of autoinhibition the dissociation constant of an antagonist against an exogenous agonist is underestimated (see, for example, Ref. 10). To avoid a distortion, release of [^{3}H]norepinephrine was elicited with five trains of six pulses at 50 Hz, with the trains being separated by 60 sec. This pattern was used because the overflow was sufficiently high to allow reliable determination of concentration-inhibition curves of agonists (see Fig. 1B). On the other hand, under these conditions autoinhibition did not develop. Blockade of prejunctional autoreceptors by rauwolscine at a concentration 1000 times its dissociation constant did not increase the release of [^{3}H]norepinephrine. Hence, agonist and antagonist affinities were determined under optimal conditions (1, 43).

α-Adrenergic receptor antagonist pEC_{30} values correlated well with the respective pK_d values ($r = 0.96$, $p < 0.01$). The identical rank orders of pEC_{30} and pK_d values show that re-

TABLE 4

Ratios of dissociation constants of α-adrenergic receptor ligands

Ratios were calculated for the α_2-adrenergic autoreceptors in human kidney and for various α_2-adrenergic binding sites. Antagonist pK_d values were those of Table 1, and the K_d of oxymetazoline was 6.1. The pK_i values were determined from inhibition of [^{3}H]antagonist binding (from Table 2 of Ref. 46).

Tissue	Oxymetazoline/ spiroxatrine	Oxymetazoline/ rauwolscine	ARC 239/ WB 4101	Prazosin/ WB 4101	Prazosin/ rauwolscine
Human kidney	40	790	40	50	631
HT-29 cell line (α_{2A})	0.062	1.8	320	263	724
Neonatal rat lung (α_{2B})	210	160	0.18	0.62	12
Opossum kidney cell line (α_{2C})	120	1600	48	56	355
Bovine pineal gland (α_{2D})	0.16	0.43	20	14	31

leased norepinephrine, a phenylethylamine, and the exogenous agonist UK 14,304, an imidazoline derivative, inhibited the release of [^{3}H]norepinephrine through the same receptor subtype. UK 14,304 did not act through imidazoline receptors. In rat kidney, it has been postulated that prejunctional α_1-adrenergic receptors, in addition to α_2-adrenergic receptors, are involved in the modulation of norepinephrine release (2, 32, 44, 45). However, in human kidney cortex the identity of the rank order of antagonist affinities determined with the nonselective compound norepinephrine (pEC_{30}) and the rank order of antagonist affinities determined with the α_2-selective compound UK 14,304 (pK_d) argues against the involvement of prejunctional α_1-adrenergic receptors.

To which of the four α_2 subtypes defined by radioligand binding studies do the prejunctional autoreceptors of human kidney belong? To answer this question, antagonist affinity estimates for the autoreceptors were compared with respective affinities at radioligand binding sites in membrane fragments obtained from cell lines and tissues containing only one α_2 subtype. The cells and tissues were the human colonic adenocarcinoma cell line HT-29 for α_{2A}, neonatal rat lung for α_{2B}, a cell line derived from opossum kidney for α_{2C}, and bovine pineal gland for α_{2D} (46). pEC_{30} values were used as antagonist affinity estimates for autoreceptors because a larger number of antagonists entered into the regression analysis. The affinities for the human kidney cortex autoreceptors correlated significantly with the affinities (pK_i) for all four α_2 binding sites, although the α-adrenergic receptor antagonists were selected to discriminate well between the four α_2 subtypes. This was not completely unexpected, because the autoreceptors as well as the binding sites all have α_2 character. The best correlation was obtained with the affinities for the α_{2C} site in opossum kidney cells. However, there was also a good correlation with the affinities of the α_{2D} site in the bovine pineal gland (Fig. 6; Table 3). To assign the kidney autoreceptors to one of these two subtypes, they were also compared with an α_{2C} and an α_{2D} binding site in rat brain cortex (47), with the α_{2D} binding site in rat submaxillary gland (48), and with binding sites in membranes of COS cells transfected with either human or rat α_2-adrenergic receptor genes. Table 3 shows that autoreceptor affinities correlated excellently with the affinities of the brain cortex α_{2C} binding site and with the affinities of the products of the human α_2-C4 and rat RG10 genes, which both code for the α_{2C}-receptor subtype (17, 19, 47). In contrast, the correlation with the affinities of the α_{2D} sites in brain cortex and submaxillary gland did not reach a level of significance. These results support the classification of kidney autoreceptors as α_{2C} and exclude an α_{2D} subtype. Moreover, currently there is no clear evidence in the literature for an α_{2D}-adrenergic receptor or for a gene coding for an α_{2D} subtype in humans. Finally, comparison of human kidney autoreceptors with previously classified α_2-adrenergic receptors clearly differentiated the autoreceptors from the α_{2A} and α_{2D} subtype. Table 3 shows that autoreceptor affinities did not correlate with those of the prejunctional α_{2D}-autoreceptors in rat brain cortex, submaxillary gland, and kidney and correlated only weakly with those of the α_{2A}-autoreceptors in rabbit brain cortex. There was excellent agreement with the affinities of the α_{2C}-adrenergic receptors mediating prostaglandin synthesis in smooth muscle cells of rabbit aorta. However, it should be noted that, at human kidney autoreceptors, BRL 44408 was a potent antagonist against endogenous norepinephrine, whereas BRL 41992 was ineffective at up to 1 μM. On the other hand, in rabbit aorta muscle cells BRL 41992 was a potent antagonist against UK 14,304 but BRL 44408 seemed to have no effect (38). The reason for this striking difference is not known. Contrasting results with BRL 41992 have been reported previously (see Ref. 49). In addition, the ineffectiveness of BRL 44408 in the rabbit aorta muscle cells was not convincingly documented; it was mentioned only in the Summary and Discussion and the concentration at which BRL 44408 was investigated was not indicated (38). Due to these uncertainties, the affinity estimates for BRL 41992 were not considered when kidney autoreceptors were compared with α_{2C}-adrenergic receptors mediating prostaglandin synthesis in rabbit aorta. Our results obtained with the two BRL compounds are in line with data obtained in binding studies on α_{2C} sites (47). For instance, WB 4101 has a much higher affinity than does BRL 41992 (at least 45-fold) at the α_{2C} binding sites, in close agreement with the kidney α_2-autoreceptors but in contrast to rabbit aorta muscle cells, where the affinity of BRL 41992 is similar to the affinity of WB 4101. Also, BRL 44408 has higher affinity than does BRL 41992 at both kidney autoreceptors and α_{2C} binding sites.[1]

The correlation analyses suggest that the kidney α_2-autoreceptor is an α_{2C}-adrenergic receptor. To increase the reliability of the subclassification, the second independently determined set of affinity estimates, the dissociation constants (K_d), were used to compute relative affinities of α_2-adrenergic receptor ligands. The affinity ratio of two compounds can be a sensitive indicator of pharmacological differences among receptor subtypes (11, 19, 23, 46). Five selected antagonists and oxymetazoline were used to differentiate between the four α_2-adrenergic receptors. Table 4 summarizes the ratios of K_d values. They support the α_{2C} character of the autoreceptors. Moreover, the ratios clearly differentiate the autoreceptors from the α_{2A}, α_{2B}, and α_{2D} subtypes. For example, the oxymetazoline/spiroxatrine K_i ratio is 0.062 at α_{2A} binding sites but the K_d ratio is 40 at kidney autoreceptors, a 645-fold difference. The ARC 239/WB 4101 K_i ratio is 0.18 at α_{2B} sites but the K_d ratio is 40 at kidney autoreceptors, a 222-fold difference. The oxymetazoline/rauwolscine ratio is 0.43 at α_{2D} sites but 790 at kidney autoreceptors, a 1837-fold difference. On the other hand, the five K_d

[1] Linear regression has been used to compare the kidney α_2-autoreceptors with α_2-adrenergic receptor subtypes. To quantify the closeness of the linear relationship between antagonist affinity estimates for kidney autoreceptors and antagonist affinity estimates for the various α_2 sites, correlation coefficients were calculated. However, a simple correlation does not prove the identity of the autoreceptors with a binding site. In the case of identity, the slope of the regression line should be equal to, or at least near, unity. Moreover, if dissociation constants are compared for identical receptor subtypes there should be agreement, i.e., the regression line should start from the origin. Table 3 shows that the slopes of the lines obtained from the regression for rat brain cortex α_{2C} binding sites and for the α_2-C4 and RG10-α_2 gene products versus kidney α_2-autoreceptors are less than unity. In addition, the antagonists have a 12–15-fold lower affinity for the kidney autoreceptors than for the α_{2C}-adrenergic receptors mediating prostaglandin synthesis in rabbit aorta muscle cells and the α_{2C} binding sites in cells from opossum kidney (Fig. 6). The reasons for this deviation from absolute agreement are not known. Variation in experimental conditions (e.g., use of cultured cells versus tissue slices) and in parameters measured (competition of radioligand binding versus disinhibition of release) may explain these deviations. Absolute antagonist affinities at binding sites are often higher than absolute affinities at functioning receptors (see, for example, Fig. 7 of Ref. 50). Systematic differences in absolute ligand affinities at identical receptor subtypes were also obtained in binding studies carried out in different laboratories. For instance, α-adrenergic receptor antagonists have higher affinity at α_{2C} binding sites in opossum kidney cells (46) than at α_{2C} binding sites in rat brain cortex (47) or at α_{2C} sites expressed in COS cells transfected with either RG10-α_2 (47) or α_2-C4 (17). Similar results have been explained by the use of different assay buffers (19).

ratios at kidney autoreceptors, given in Table 4, differ maximally 3-fold from the corresponding K_i ratios calculated for α_{2C} binding sites in cells derived from the opossum kidney.

As mentioned in the introduction, prejunctional α_2-autoreceptors have been subclassified almost exclusively in laboratory animals. They belong to either the α_{2A} or α_{2D} subtype (an exception is that in cat and bovine cerebral arteries α_2-autoreceptors have been proposed to be α_{2B}) (27). Molecular cloning indicates that the α_{2A}-adrenergic receptor exhibits a high degree (about 90%) of amino acid identity with the α_{2D} receptor, suggesting that the two subtypes are species homologues. This relationship led to the speculation that early in evolution the α_{2A}/α_{2D} ancestor gene was expressed in adrenergic neurons to yield prejunctional and, presumably, soma-dendritic α_2-autoreceptors and that it is this branch of the α_2-adrenoceptor tree to which the mammalian α_2-autoreceptors, including those in humans, belong (see Ref. 11). The present characterization of human kidney autoreceptors as α_{2C} requires a correction of the speculation, to the extent that probably all α_2 receptor subtypes can be expressed in mammalian adrenergic neurons to yield prejunctional autoreceptors.

The question arises as to whether, in a single species, the prejunctional α_2-autoreceptors generally belong to the same subtype. In rats, the autoreceptors in brain cortex (11), heart atrium, submaxillary gland (23), kidney (28, 29), and presumably vas deferens (26) are all α_{2D}. However, the pharmacological properties of atrial and kidney autoreceptors differ in some respects from those of α_{2D} receptors, leaving some doubt that, in rats, the receptors are generally α_{2D} (23, 28, 29) (see Ref. 11). In humans, so far only two tissues have been investigated and the α_2-autoreceptors in brain cortex (α_{2A} or α_{2D}) (30) seem to differ from those in kidney cortex (α_{2C}).

In conclusion, the release of norepinephrine is modulated by prejunctional α_2-autoreceptors in human kidney cortex. Prostaglandins and adenosine are not involved in the α_2 receptor-mediated inhibition of norepinephrine release. Neuronally released norepinephrine and the exogenous agonist UK 14,304 inhibit the release via the same α_2-adrenoceptor subtype, namely α_{2C}. There is no evidence for prejunctional α_1-adrenergic or imidazoline receptors. This is the first study to describe an α_2-autoreceptor of the α_{2C} subtype.

Acknowledgments

We thank Ulrike Schaible for excellent technical assistance and Ciba-Geigy, Organon, Pfizer, Röhm, SmithKline Beecham, and Thomae for gifts of drugs. The renal tissue was kindly provided by the Department of Urology (Prof. Dr. Sommerkamp).

References

1. Starke, K. Presynaptic α-autoreceptors. *Rev. Physiol. Biochem. Pharmacol.* **107**:73–146 (1987).
2. Rump, L. C., and H. Majewski. Modulation of norepinephrine release through α_1- and α_2-adrenoceptors in rat isolated kidney. *J. Cardiovasc. Pharmacol.* **9**:500–507 (1987).
3. Rump, L. C., E. Schwertfeger, M. J. Schuster, U. Schaible, A. Frankenschmidt, and P. J. Schollmeyer. Dopamine DA_2-receptor activation inhibits norepinephrine release in human kidney slices. *Kidney Int.* **43**:197–204 (1993).
4. Doxey, J. C., and J. Everitt. Inhibitory effects of clonidine on responses to sympathetic nerve stimulation in the pithed rat. *Br. J. Pharmacol.* **61**:559–566 (1977).
5. Dubocovich, M. L. Pharmacological differences between the *alpha*-presynaptic adrenoceptors in the peripheral and the central nervous systems, in *Presynaptic Receptors* (S. Z. Langer, K. Starke, and M. L. Dubocovich, eds.). Pergamon, Oxford, UK, 29–36 (1979).
6. Reichenbacher, D., W. Reimann, and K. Starke. α-Adrenoceptor-mediated inhibition of noradrenaline release in rabbit brain cortex slices. *Naunyn-Schmiedebergs Arch. Pharmacol.* **319**:71–77 (1982).
7. Lattimer, N., and K. F. Rhodes. A difference in the affinity of some selective α_2-adrenoceptor antagonists when compared on isolated vasa deferentia of rat and rabbit. *Naunyn-Schmiedebergs Arch. Pharmacol.* **329**:278–281 (1985).
8. Alabaster, V. A., R. F. Keir, and C. J. Peters. Comparison of potency of α_2-adrenoceptor antagonists *in vitro*: evidence for heterogeneity of α_2-adrenoceptors. *Br. J. Pharmacol.* **88**:607–614 (1986).
9. Ennis, C. Comparison of the α_2-adrenoceptors which modulate norepinephrine release in rabbits and rat occipital cortex. *Br. J. Pharmacol.* **85**:318P (1985).
10. Limberger, N., A. Mayer, G. Zier, B. Valenta, K. Starke, and E. A. Singer. Estimation of pA_2 values at presynaptic α_2-autoreceptors in rabbit and rat brain cortex in the absence of autoinhibition. *Naunyn-Schmiedebergs Arch. Pharmacol.* **340**:639–647 (1989).
11. Trendelenburg, A. U., N. Limberger, and K. Starke. Presynaptic α_2-autoreceptors in brain cortex: α_{2D} in the rat and α_{2A} in the rabbit. *Naunyn-Schmiedebergs Arch. Pharmacol.* **348**:35–45 (1993).
12. Rump, L. C., E. Schwertfeger, U. Schaible, M. J. Schuster, A. Frankenschmidt, and P. Schollmeyer. Dopamine receptor modulation of noradrenaline release by carmoxirole in human cortical kidney slices. *Eur. J. Clin. Pharmacol.* **44**:S47–S49 (1993).
13. Bylund, D. B. Heterogeneity of *alpha*-2 adrenergic receptors. *Pharmacol. Biochem. Behav.* **22**:835–843 (1985).
14. Nahorski, S. R., D. B. Barnett, and Y. D. Cheung. α-Adrenoceptor-effector coupling: affinity states or heterogeneity of the α_2-adrenoceptor? *Clin. Sci.* **68** (Suppl. 10):39s-42s (1985).
15. Bylund, D. B. Subtypes of α_1- and α_2-adrenergic receptors. *FASEB J.* **6**:832–839 (1992).
16. Ruffolo, R. R., A. J. Nichols, J. M. Stadel, and J. P. Hieble. Pharmacologic and therapeutic applications of α_2-adrenoceptor subtypes. *Annu. Rev. Pharmacol. Toxicol.* **32**:243–279 (1993).
17. Lomasney, J. W., S. Cotecchia, R. J. Lefkowitz, and M. G. Caron. Molecular biology of α-adrenergic receptors: implications for receptor classification and for structure-function relationships. *Biochim. Biophys. Acta* **1095**:127–139 (1991).
18. Harrison, J. K., D. D. D'Angelo, D. Zeng, and K. R. Lynch. Pharmacological characterization of rat α_2-adrenergic receptors. *Mol. Pharmacol.* **40**:407–412 (1991).
19. Bylund, D. B., H. S. Blaxall, L. J. Iversen, M. G. Caron, R. J. Lefkowitz, and J. W. Lomasney. Pharmacological characteristics of α_2-adrenergic receptors: comparison of pharmacologically defined subtypes with subtypes identified by molecular cloning. *Mol. Pharmacol.* **42**:1–5 (1992).
20. Gobbi, M., E. Frittoli, and T. Mennini. The modulation of [^{3}H]noradrenaline and [^{3}H]serotonin release from rat brain synaptosomes is not mediated by the α_{2B}-adrenoceptor subtype. *Naunyn-Schmiedebergs Arch. Pharmacol.* **342**:382–386 (1990).
21. Shen, K. Z., C. Barajas-Lopez, and A. Surprenant. Functional characterization of neuronal pre- and postsynaptic α_2-adrenoceptor subtypes in guinea-pig submucosal plexus. *Br. J. Pharmacol.* **101**:925–931 (1990).
22. Limberger, N., L. Späth, and K. Starke. Subclassification of the presynaptic α_2-autoreceptors in rabbit brain cortex. *Br. J. Pharmacol.* **103**:1251–1255 (1991).
23. Limberger, N., A. U. Trendelenburg, and K. Starke. Pharmacological characterization of presynaptic α_2-autoreceptors in rat submaxillary gland and heart atrium. *Br. J. Pharmacol.* **107**:246–255 (1992).
24. Alberts, P. Subtype classification of the prejunctional α-adrenoceptors which regulate [^{3}H]-noradrenaline secretion in guinea-pig isolated urethra. *Br. J. Pharmacol.* **105**:142–146 (1992).
25. Molderings, G., and M. Göthert. Pharmacological characterization of the inhibitory α_2-autoreceptors on the sympathetic nerves of the rabbit pulmonary artery. *Naunyn-Schmiedebergs Arch. Pharmacol.* **346**:R64 (1992).
26. Smith, K., and J. R. Docherty. Are the prejunctional α_2-adrenoceptors of the rat vas deferens and submandibular gland of the α_{2A}- or α_{2D}-subtype? *Eur. J. Pharmacol.* **219**:203–210 (1992).
27. Arribas, S., R. Galvan, M. Ferrer, M. J. Herguido, J. Marin, and G. Balfagón. Characterization of the subtype of presynaptic α_2-adrenoceptors modulating noradrenaline release in cat and bovine cerebral arteries. *J. Pharm. Pharmacol.* **43**:855–859 (1991).
28. Schwartz, D. D., and K. U. Malik. Characterization of prejunctional *alpha*-2 adrenergic receptors involved in modulation of adrenergic transmitter release in the isolated perfused rat kidney. *J. Pharmacol. Exp. Ther.* **261**:1050–1055 (1992).
29. Bohmann, C., P. Schollmeyer, and L. C. Rump. α_2-Autoreceptor subclassification in rat isolated kidney by use of short trains of electrical stimulation. *Br. J. Pharmacol.* **108**:262–268 (1993).
30. Raiteri, M., G. Bonanno, G. Maura, M. Pende, G. C. Andrioli, and A. Ruelle. Subclassification of release-regulating α_2-autoreceptors in human brain cortex. *Br. J. Pharmacol.* **107**:1146–1151 (1992).
31. Hedqvist, P. Trans-synaptic modulation versus α-autoinhibition of noradrenaline secretion, in *Chemical Neurotransmission: 75 Years* (L. Stjärne, P. Hedqvist, H. Lagercrantz, and A. Wennmalm, eds.). Academic Press, London, 223–233 (1981).
32. Rump, L. C., and H. Majewski. Methoxamine inhibits noradrenaline release in rat isolated kidney through release of prostaglandins and direct activation

of inhibitory prejunctional α_1-adrenoceptors. *J. Cardiovasc. Pharmacol.* **10** (Suppl. 4):S147–S149 (1987).
33. Waud, D. R. Analysis of dose-response relationships. *Adv. Gen. Cell. Pharmacol.* **1**:145–178 (1976).
34. Furchgott, R. F. The classification of adrenoceptors (adrenergic receptors): an evaluation from the standpoint of receptor theory. *Handb. Exp. Pharmacol.* **33**:283–335 (1972).
35. Furchgott, R. F. The use of β-haloalkylamines in the differentiation of receptors and in the determination of dissociation constants of receptor-agonist complexes. *Adv. Drug. Res.* **3**:21–55 (1966).
36. Parker, R. B., and D. R. Waud. Pharmacological estimation of drug-receptor dissociation constants: statistical evaluation. I. Agonists. *J. Pharmacol. Exp. Ther.* **177**:1–12 (1971).
37. Thron, C. D. Graphical and weighted regression analyses for the determination of agonist dissociation constants. *J. Pharmacol. Exp. Ther.* **175**:541–553 (1970).
38. Nebigil, C., and K. U. Malik. Prostaglandin synthesis elicited by adrenergic stimuli is mediated via *alpha*-2C and *alpha*-1A adrenergic receptors in cultured smooth muscle cells of rabbit aorta. *J. Pharmacol. Exp. Ther.* **260**:849–858 (1992).
39. Cooper, C. L., and K. U. Malik. Prostaglandin synthesis and renal vasoconstriction elicited by adrenergic stimuli are linked to activation of *alpha*-1 adrenergic receptors in the isolated rat kidney. *J. Pharmacol. Exp. Ther.* **233**:24–31 (1985).
40. Sedaa, K. O., R. A. Bjur, K. Shinozuka, and D. P. Westfall. Nerve and drug-induced release of adenine nucleosides and nucleotides from rabbit aorta. *J. Pharmacol. Exp. Ther.* **252**:1060–1067 (1990).
41. Shinozuka, K., K. O. Sedaa, R. A. Bjur, and D. P. Westfall. Participation by purines in the modulation of norepinephrine release by methoxamine. *Eur. J. Pharmacol.* **192**:431–434 (1991).
42. Kenakin, T. P., R. A. Bond, and T. I. Bonner. Definition of pharmacological receptors. *Pharmacol. Rev.* **44**:351–362 (1992).
43. Singer, E. A. Transmitter release from brain slices elicited by single pulses: a powerful method to study presynaptic mechanisms. *Trends Pharmacol. Sci.* **9**:274–276 (1988).
44. Murphy, T. V., and H. Majewski. Modulation of noradrenaline release in slices of rat kidney cortex through α_1- and α_2-adrenoceptors. *Eur. J. Pharmacol.* **169**:285–295 (1989).
45. Bohmann, C., P. Schollmeyer, and L. C. Rump. Methoxamine inhibits noradrenaline release through activation of α_1- and α_2-adrenoceptors in rat isolated kidney: involvement of purines and prostaglandins. *Naunyn-Schmiedebergs Arch. Pharmacol.* **347**:273–279 (1993).
46. Simonneaux, V., M. Ebadi, and D. B. Bylund. Identification and characterization of α_{2D}-adrenergic receptors in bovine pineal gland. *Mol. Pharmacol.* **40**:235–241 (1991).
47. Uhlén, S., Y. Xia, V. Chhajlani, C. C. Felder, and J. E. S. Wikberg. [^{3}H]-MK 912 binding delineates two α_2-adrenoceptor subtypes in rat CNS, one of which is identical with the cloned pA2d α_2-adrenoceptor. *Br. J. Pharmacol.* **106**:986–995 (1992).
48. Michel, A. D., D. N. Loury, and R. L. Whiting. Differences between the α_2-adrenoceptor in rat submaxillary gland and the α_{2A}- and α_{2B}-adrenoceptor subtypes. *Br. J. Pharmacol.* **98**:890–897 (1989).
49. Gleason, M. M., and J. P. Hieble. Ability of SK&F 104078 and SK&F 104856 to identify *alpha*-2 adrenoceptor subtypes in NCB20 cells and guinea pig lung. *J. Pharmacol. Exp. Ther.* **259**:1124–1132 (1991).
50. Schoeffter, P., and D. Hoyer. 5-Hydroxytryptamine (5-HT)-induced endothelium-dependent relaxation of pig coronary arteries is mediated by 5-HT receptors similar to the 5-HT_{1D} receptor subtype. *J. Pharmacol. Exp. Ther.* **252**:387–395 (1990).

Send reprint requests to: Norbert Limberger, Pharmakologisches Institut, Hermann-Herder-Strasse 5, D-79104 Freiburg im Breisgau, Germany.