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α_{2C}-Adrenoceptor-modulated release of noradrenaline in human right atrium

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1 The aim of the present study was to characterize the presynaptic α_2-autoreceptors in human right atrium in terms of the $\alpha_{2A\text{-}D}$ system. Segments of atrial appendages were preincubated with [^{3}H]-noradrenaline and then superfused in the presence of cocaine and stimulated electrically. $pEC_{30\%}$ values of eight α-adrenoceptor antagonists with discriminatory power were determined. $pEC_{30\%}$ is the negative logarithm of the antagonist concentration that increased the stimulation-induced overflow of tritium by 30%. For four antagonists, the dissociation constant K_D was determined, in addition to $pEC_{30\%}$, against the overflow-inhibiting effect of 5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline (UK 14,304) under autoinhibition-free conditions.

2 $pEC_{30\%}$ and K_D values yielded identical rank orders of antagonist affinity (rauwolscine > WB 4101 > phentolamine > prazosin) suggesting that both released noradrenaline and the exogenous agonist UK 14,304 activated the same receptor to inhibit release.

3 The eight antagonist $pEC_{30\%}$ values obtained in right atrium correlated significantly with their $pEC_{30\%}$ values, reported in the literature, at the presynaptic α_{2C}-autoreceptors in human kidney ($r = 0.817$; slope of the regression line 1.03). No significant correlation was obtained between $pEC_{30\%}$ values at atrial autoreceptors and pK_D values at previously characterized α_{2A}-autoreceptors in rabbit and α_{2D}-autoreceptors in rat, mouse and guinea-pig tissues.

4 Comparison of antagonist $pEC_{30\%}$ values with their pK_D values at native α_2 binding sites in cells or tissues that express a single subtype only, and with pK_D values at α_2 binding sites in membranes of COS cells transfected with human α_2 subtype genes confirms the α_{2C} character of the atrial autoreceptors: significant correlations were obtained exclusively with the α_{2C} binding sites.

5 Ratios of K_D values were computed for α_2-autoreceptors in human right atrium and for binding sites in COS cells transfected with human α_2 subtype genes. The autoreceptor ratios corresponded well with the respective ratios for the α_{2C} binding sites (maximal three fold deviation) but were, in part, markedly different from ratios calculated for α_{2A} and α_{2B} binding sites (up to 166 fold deviation). This outcome supports the α_{2C} designation of the autoreceptors.

6 In conclusion, the presynaptic α_2-autoreceptors in human right atrium are α_{2C}. In this they agree with the previously characterized α_2-autoreceptors in human kidney. The α_{2C} classification possibly separates, in general, human α_2-autoreceptors from those in lagomorph (rabbit) and rodent (rat, mouse, guinea pig) species that have been proposed to be predominantly α_{2A} or α_{2D}.

Keywords: Human heart atrium; sympathetic nervous system; noradrenaline release; α_2-adrenoceptor subtypes; presynaptic α_2-autoreceptors; α_{2C}-adrenoceptors

Introduction

α_2-Adrenoceptors are not a homogeneous class of receptors. Based on radioligand binding, four subtypes have been described: α_{2A}, α_{2B}, α_{2C}, and α_{2D} (Simonneaux *et al.*, 1991). Little is known about the functions the various subtypes subserve. So far, the presynaptic α_2-autoreceptors, the prototype α_2-adrenoceptors, have been most investigated. In rat brain cortex, hypothalamus, vas deferens, submaxillary gland and kidney the α_2-autoreceptors are all α_{2D} (Connaughton & Docherty, 1990; Limberger *et al.*, 1992; Schwartz & Malik, 1992; Smith & Docherty, 1992; Smith *et al.*, 1992; Bohmann *et al.*, 1993; 1994; Trendelenburg *et al.*, 1993; Millan *et al.*, 1994). An exception to the subtype homogeneity within the rat has been observed only for the atrial α_2-autoreceptors. The pharmacology of these autoreceptors deviates from that in other rat tissues and they have been classified as either α_{2B} or α_{2D}-like (Connaughton & Docherty, 1990; Limberger *et al.*, 1992; Smith *et al.*, 1992; see also Smith *et al.*, 1995). In fact, the first evidence for heterogeneity of the α_2-adrenoceptor class was obtained when presynaptic α_2-autoreceptors were compared in rat heart and vas deferens (Doxey & Everitt, 1977). In rabbit brain cortex, pulmonary artery, kidney and atria the α_2-autoreceptors have been characterized as α_{2A} (Limberger *et al.*, 1991; 1995a; Molderings & Göthert, 1992; Trendelenburg *et al.*, 1993). Based on these results, it has been proposed that 'the majority of α_2-autoreceptors generally are α_{2D} in the rat and α_{2A} in the rabbit. Moreover, receptors of the $\alpha_{2A/D}$ group generally may be the main mammalian α_2-autoreceptors' (Trendelenburg *et al.*, 1993).

Molecular genetics indicate that this $\alpha_{2A/D}$ concept may be restricted even to a single adrenoceptor subtype: α_{2A}- and α_{2D}-adrenoceptors are structurally very similar across species, the amino acid identity being 89 to 96% in man, rat, mouse and pig (see Bylund *et al.*, 1994; O'Rourke *et al.*, 1994). Despite the small structural differences, the receptor proteins have different pharmacological properties: in man and pig the pharmacology is α_{2A} whereas in rat and mouse it is α_{2D}. α_{2A} and α_{2D} may be considered as 'orthologous' α_2-adrenoceptors only one being present in any given species. Hence, across all mammalian species, noradrenergic neurones probably predominantly express the same gene, the $\alpha_{2A/D}$ ortholog, to control transmitter

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release through presynaptic autoreceptors (see Limberger *et al.*, 1995a). In agreement with the $\alpha_{2A/D}$ hypothesis, the α_2-autoreceptors in mouse and guinea-pig brain cortex as well as in guinea-pig atria and ileum have recently been subclassified as α_{2D} (Funk *et al.*, 1994; Trendelenburg *et al.*, 1994b; Limberger *et al.*, 1995b). There is also some evidence that α_2-autoreceptors in human brain cortex may belong to the α_{2A} subtype (Raiteri *et al.*, 1992). The hypothesis, however, has been challenged when the α_2-autoreceptors in human kidney have been shown to conform to the α_{2C} subtype (Trendelenburg *et al.*, 1994a).

Isolated cardiac tissue has been used successfully to study the release, and modulation of release, of noradrenaline from postganglionic sympathetic nerve endings. Guinea-pig atria and rabbit heart were among the tissues in which presynaptic release-inhibiting α_2-autoreceptors (Langer *et al.*, 1971; Starke, 1971; 1972; McCulloch *et al.*, 1972) as well as presynaptic release-enhancing β-adrenoceptors (Adler-Graschinsky & Langer, 1975) and angiotensin II receptors (Schümann *et al.*, 1970) were first described. Recently, we have shown that, in human right atrium, the release of noradrenaline is similarly modulated through presynaptic α_2- and β-adrenoceptors and angiotensin II receptors (Rump *et al.*, 1994; 1995). The present study was devised to characterize the α_2-adrenoceptors in terms of the $\alpha_{2A\text{-}D}$ system. Moreover, we were interested to see whether the α_2-autoreceptors in human cardiac tissue fit into the $\alpha_{2A/D}$ concept of presynaptic α_2-autoreceptors.

Methods

Human atrial tissue

The present *in vitro* study on human right atrial appendages was approved by the local ethics committee. Tissues were taken from 26 patients undergoing open heart surgery for coronary bypass grafting (24 patients) and aortic valve replacement (2 patients). The age of the patients averaged 60.8 ± 2.2 years (range: 35 to 78). None of the patients had been treated with drugs known to interfere with either the storage or release mechanism of noradrenaline.

Experimental protocol

Right atrial appendages were dissected free from connective tissue and four to six segments per atrium were prepared. The tissue segments, weighing on average 15.6 ± 0.5 mg ($n = 138$), were incubated with [^{3}H]-noradrenaline (0.5 μM) for 60 min in Krebs-Henseleit solution, which was continuously bubbled with 5% CO_2 in 95% O_2. After incubation, each of the segments was placed in one of six parallel chambers (250 μl volume) between platinum electrodes and then superfused at 37°C with tritium-free Krebs-Henseleit solution (flow rate 2.5 ml min^{-1}). Cocaine 10 μM was added to the solution from 105 min of superfusion onwards. After 141 min of superfusion, the superfusion solution was collected in 28 or 35 fractions. At the end of the experiment, the tissue segments were dissolved; tritium content was determined in superfusate samples and tissue.

The Krebs-Henseleit solution contained (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 0.45, $NaHCO_3$ 25, KH_2PO_4 1.0, glucose 11.1, ascorbic acid 0.07, disodium EDTA 0.067, corticosterone 0.02.

During superfusion, five periods of electrical stimulation were applied. An initial priming stimulation was delivered after 125 min of superfusion consisting of rectangular pulses at 10 Hz for 60 s (45 mA current strength, 1 ms pulse width). The following four periods of electrical stimulation (S_1 to S_4) each consisted either of 300 pulses at 5 Hz or of 5 trains of 10 pulses at 100 Hz, train interval 50 s (45 mA, 1 ms-pulses).

When the effects of α-adrenoceptor antagonists on the stimulation-induced overflow of tritium were determined (S_1 to S_4) each consisted of 300 pulses at 5 Hz. Antagonists were added at increasing concentrations from 12 min before S_2, S_3 and S_4 onwards in order to yield cumulative antagonist concentration-response curves (see Figure 1a below). Antagonist $pEC_{30\%}$ values (negative logarithms of concentrations that enhanced the stimulation-induced overflow of tritium by 30%) were interpolated from the averaged concentration-response curves.

For some of the antagonists, the α_2-autoreceptor/antagonist dissociation constant, K_D, was determined in addition to the $pEC_{30\%}$ value. In these experiments, S_1 to S_4 each consisted of 5 trains of 10 pulses at 100 Hz, train interval 50 s. Antagonists when used, were added to the superfusion solution at a constant concentration 23 min before S_1. The α_2-selective agonist, 5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline (UK 14,304) was added at increasing concentrations from 16 min before S_2, S_3 and S_4 onwards in order to yield cumulative concentration-inhibition curves (see Figure 1b below). A single concentration-inhibition curve for UK 14,304 in the absence of antagonists served for comparison with the effect of UK 14,304 in the presence of an antagonist. The concentration of UK 14,304 causing 50% inhibition, in the absence and in the presence of antagonist, was interpolated from the respective averaged concentration-inhibition curve. The negative logarithm of the dissociation constant (pK_D) was then calculated according to equation 4 of Furchgott (1972).

The outflow of tritium from the tissue was calculated as a fraction of the tritium content of the tissue at the start of the respective collection period and is expressed as fractional rate (min^{-1}). The stimulation-induced overflow of tritium elicited by electrical stimulation was calculated by subtraction of the estimated basal outflow from the total tritium outflow during the collection period in which the stimulation was applied plus the three periods thereafter; the basal outflow of tritium was assumed to decline linearly from the collection period before to the fourth collection period after stimulation. The overflow of tritium was then expressed as a percentage of the tritium content of the tissue segment at the time of stimulation. For further evaluation of the stimulation-induced overflow of tritium, ratios were calculated for the overflow induced by S_2, S_3 and S_4 and the overflow induced by S_1 (S_2/S_1, S_3/S_1, S_4/S_1). Moreover, effects of α-adrenoceptor antagonists and of UK 14,304 on the stimulation-induced overflow were calculated, in each single tissue segment, as a percentage of the corresponding average control (no antagonist, no agonist) S_2/S_1, S_3/S_1 and S_4/S_1 ratios.

Statistics

Results are expressed as arithmetic mean $\pm$ s.e.mean. Groups were tested for significant differences with Student's t test and Bonferroni correction. n is the number of atrial segments.

Drugs

Purchased drugs were (−)-[ring-2,5,6-^{3}H]-noradrenaline, specific activity 1.6 TBq mmol^{-1}; (DuPont, Dreieich, Germany), (±)-2-(2,6-dimethoxy-phenoxyethyl)aminomethyl-1,4-benzodioxane HCl (WB 4101; Biotrend, Köln, Germany), cocaine HCl (Merck, Darmstadt, Germany), rauwolscine HCl (Roth, Karlsruhe, Germany), corticosterone, corynanthine HCl, (Sigma, Deisenhofen, Germany). The following drugs were kindly provided by the producers: phentolamine methanesulphonate (Ciba-Geigy, Basel, Switzerland); prazosin HCl, 5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline tartrate (UK 14,304; Pfizer, Sandwich, Kent, UK); (±)-2-[2H-(1-methyl-1,3-dihydroisoindole)methyl]-4,5-dihydroimidazoline (BRL 44408) and (−)-1,2-dimethyl-2,3,9,13b-tetrahydro-1H-dibenzo(c,f)imidazo(1,5-)azepine (BRL 41992; SmithKline Beecham, Great Burgh, Epsom, Surrey, UK); 6-chloro-9-[(3-methyl-2-butenyl)oxy]-3-methyl-1H-2,3,4,5-tetrahydro-3-benzazepine maleate (SKF 104078; SmithKline Beecham, King of Prussia, PA, U.S.A.). Drugs were dissolved in distilled water except corticosterone (ethanol), WB 4101 (HCl 1 mM), BRL 44408

and BRL 41992 (HCl 10 mM) before being added to the superfusion medium. None of the solvents had any effect on either spontaneous efflux or stimulation-induced overflow of tritium.

Results

As shown in Figure 1, electrical stimulation induced a reproducible increase in the outflow of tritium from segments of the right atrium, preincubated with [^{3}H]-noradrenaline. When 300 pulses were applied at 5 Hz, the overflow of tritium induced by the first stimulation period S_1 was 191 ± 14 Bq, corresponding to 0.554 ± 0.039% of the tritium content of the tissue (n = 69). The α-adrenoceptor antagonists, rauwolscine (1 to 100 nM), WB 4101 (10 nM to 1 μM), phentolamine (1 nM to 1 μM), prazosin, corynanthine, BRL 41992, SKF 104078 and BRL 44408 (each 10 nM to 1 μM) increased concentration-dependently the stimulation-induced overflow of tritium. This is shown for rauwolscine in Figure 1a. Concentration-response curves for rauwolscine, WB 4101, phentolamine, prazosin and corynanthine are illustrated in Figure 2. From the averaged concentration-response curves, $pEC_{30\%}$ values (negative logarithms of antagonist concentrations that increased the stimulation-induced overflow by 30%) were interpolated; they are summarized in Table 1.

For rauwolscine, WB 4101, phentolamine and prazosin dissociation constants K_D were also determined. The antagonists were present from 23 min before S_1 onwards and the α_2-selective agonist, UK 14,304, was added at increasing concentrations before S_2, S_3 and S_4. Figure 1b illustrates an experiment with rauwolscine. S_1 to S_4 each consisted of 5 trains of 10 pulses at 100 Hz. In the absence of antagonists, the stimulation-induced overflow of tritium at S_1 was 0.167 ± 0.012% of the tritium content of the tissue (n = 25). The antagonists did not significantly change the overflow of S_1 indicating that autoinhibition did not develop under these conditions of electrical stimulation (compare overflow peaks at S_1 in Figure 1b upper panel with those in the lower panel illustrating the lack of effect of rauwolscine). UK 14,304 concentration-dependently reduced the stimulation-induced overflow of tritium. As shown in Figure 3, rauwolscine (10 nM), WB 4101 (300 nM), phentolamine (300 nM) and prazosin (100 nM) shifted the concentration-inhibition curve for UK 14,304 to the right. From these shifts dissociation constants K_D were calculated; their negative logarithms are summarized in Table 1. For each antagonist, the pK_D value was higher than its $pEC_{30\%}$ value, the difference being greatest for phentolamine.

Prazosin 1 μM when added before S_4, accelerated basal outflow of tritium by 56%. None of the other compounds including UK 14,304 changed significantly basal tritium efflux

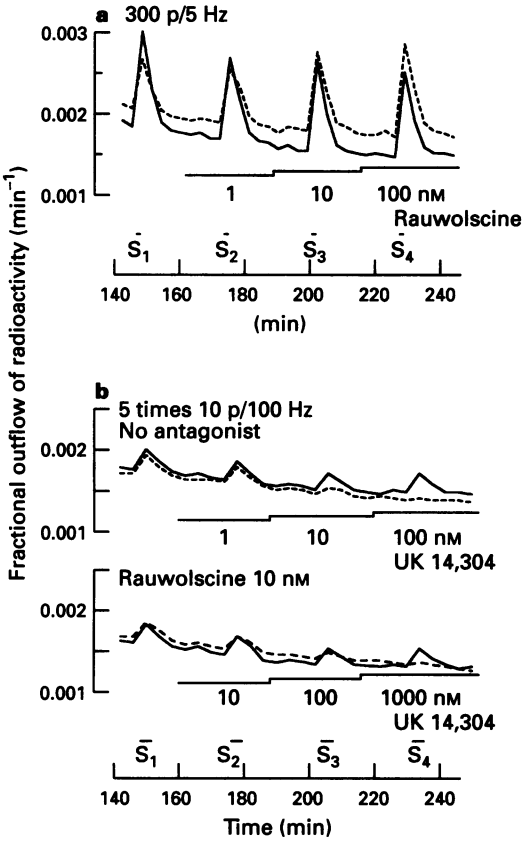

Figure 1 Fractional outflow of radioactivity from human right atrium preincubated with [^{3}H]-noradrenaline; (a) effect of rauwolscine and (b) interaction of UK 14,304 with rauwolscine. Atrial segments were superfused in the presence of cocaine 10 μM. S_1 to S_4 each consisted of (a) 300 pulses at 5 Hz or (b) 5 trains of 10 pulses at 100 Hz, train interval 50 s. Solvent (solid lines) or increasing concentrations of (a) rauwolscine or (b) UK 14,304 (dashed lines) were added as indicated. In interaction experiments, rauwolscine 10 nM was present from 23 min before S_1. Abscissae, minutes of superfusion. Each line represents the mean of n = 6 segments from 2 atria.

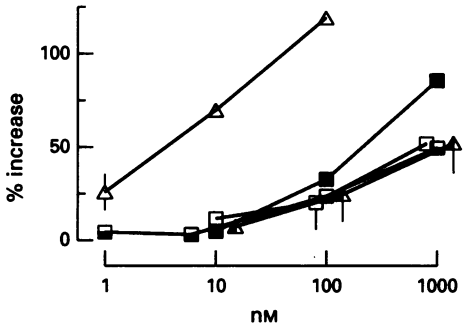

Figure 2 Effects of α-adrenoceptor antagonists on stimulation-induced overflow of radioactivity from human right atrium preincubated with [^{3}H]-noradrenaline. Atrial segments were superfused in the presence of cocaine 10 μM. S_1 to S_4 each consisted of 300 pulses at 5 Hz. Rauwolscine (△), WB 4101 (■), phentolamine (▬), prazosin (□), 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. Means ± s.e.mean of n = 6 segments from 2 atria.

Table 1 $pEC_{30\%}$ and pK_D values of α-adrenoceptor antagonists at presynaptic α_2-autoreceptors in human right atrium

α-Adrenoceptor antagonist	*$pEC_{30\%}$*	*pK_D*
Rauwolscine	8.9	9.1
WB 4101	7.2	7.9
Phentolamine	6.8	7.8
Prazosin	6.7	7.2
Corynanthine	6.7	–
BRL 41992	6.5	–
SKF 104078	6.4	–
BRL 44408	6.1	–

$pEC_{30\%}$ values are negative logarithms of antagonist concentrations that increased the stimulation-induced overflow of tritium (300 pulses at 5 Hz) by 30%. pK_D values are calculated from antagonism against the inhibitory effect of UK 14,304 on the stimulation-induced overflow of tritium (5 trains of 10 pulses at 100 Hz; train interval 50 s). Each $pEC_{30\%}$ value is based on 6 to 9 segments from 2 atria, and each pK_D on 10 to 12 segments from 2 or 3 atria, controls and segments that received agonist only not included.

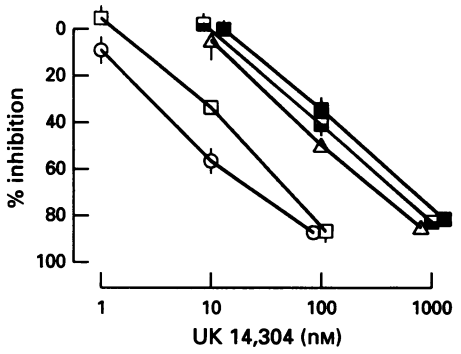

Figure 3 Effect of UK 14,304 on stimulation-induced overflow of tritium from human right atrium preincubated with [^{3}H]-noradrenaline, and its interaction with α-adrenoceptor antagonists. Atrial segments were superfused either in the presence of cocaine 10 μM only (○), or in the presence of cocaine plus rauwolscine (10 nM; △), WB 4101 (300 nM; ■), phentolamine (300 nM; ⬓), or prazosin (100 nM; □). S_1 to S_4 each consisted of 5 trains of 10 pulses at 100 Hz, train interval 50 s. 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. Means ± s.e.mean of $n=6$ to 11 segments from at least 2 atria.

when added before S_2, S_3 and S_4. Rauwolscine, WB 4101, phentolamine and prazosin, when given 23 min before S_1, did not alter basal outflow.

Discussion

Small segments of human right atrium appendages, when incubated with [^{3}H]-noradrenaline, specifically accumulate and store radioactivity (Rump *et al.*, 1994). Electrical stimulation evokes a reproducible overflow of tritium that is abolished by tetrodotoxin as well as by removal of Ca^{2+}. Hence, the stimulation-induced overflow of tritium reflects action-potential evoked release of [^{3}H]-noradrenaline and may be taken as an index of noradrenaline release from postganglionic sympathetic nerve endings.

Recently, we have shown that in human right atrium the release of noradrenaline is reduced by activation of presynaptic α_2-autoreceptors (Rump *et al.*, 1995). The aim of the current study was to subclassify these autoreceptors in terms of the $\alpha_{2A\text{-}D}$ system. A functional receptor is best characterized by the relative affinities of agonists and, preferably, antagonists (Kenakin *et al.*, 1992; for some methodological comments on autoreceptor classification see Limberger *et al.*, 1995a). In the present study, eight α-adrenoceptor antagonists with discriminatory power were chosen. The release-enhancing potencies of these antagonists were assessed as affinity estimates; the antagonists competed with released noradrenaline for the autoreceptor. $pEC_{30\%}$ values (i.e. negative logarithms of the antagonist concentrations that increased release of noradrenaline by 30%) were calculated to quantify antagonist affinities. For some of the antagonists, the potency against the release-inhibiting effect of the α_2-selective agonist, UK 14,304, was assessed in addition, yielding the dissociation constant K_D of the antagonist/autoreceptor complex as a more direct measure of affinity. K_D values were determined in the absence of presynaptic autoinhibition. This was achieved by stimulating the tissue with short (91 ms) trains of electric pulses, too short to allow autoinhibition to develop (Singer, 1988). Autoinhibition-free conditions are required for valid determination of dissociation constants (Fuder *et al.*, 1983; Limberger *et al.*, 1989; see Starke *et al.*, 1989).

When both $pEC_{30\%}$ and pK_D were determined for an antagonist, the pK_D value was higher than the $pEC_{30\%}$ value, indicating that more than 50% receptor occupation by an antagonist was necessary for a 30% increase in noradrenaline release. In this respect the human right atrium differs from human kidney in which, for a given antagonist, $pEC_{30\%}$ and pK_D values correspond well (Trendelenburg *et al.*, 1994a). The human atrium also differs from rabbit and rat atria, rabbit kidney and rat submaxillary gland. In these tissues $pEC_{30\%}$ values are higher than the respective pK_D value indicating that less than 50% receptor occupation sufficed for a 30% increase in noradrenaline release (Limberger *et al.*, 1992; 1995a). Most importantly, in human right atrium, as in the tissues mentioned, the rank order of the $pEC_{30\%}$ values is identical to the rank order of the pK_D values (rauwolscine > WB 4101 > phentolamine > prazosin) suggesting that both released noradrenaline and the exogenous agonist, UK 14,304, inhibited transmitter release through the same α_2-receptor.

In a first attempt to classify the presynaptic α_2-autoreceptors in human right atrium, the eight antagonist $pEC_{30\%}$ values were compared with their $pEC_{30\%}$ values previously reported for the α_{2C}-autoreceptors in human kidney (Trendelenburg *et al.*, 1994a). As shown in Figure 4, the $pEC_{30\%}$ values in the two tissues are significantly correlated with the regression line passing almost exactly through the origin of the coordinate system and the slope of the line being almost unity. The close correlation suggests that atrial and renal α_2-autoreceptors belong to the same subtype, namely α_{2C}. The α_{2C} designation gains further, though indirect, support by the non-identity of the atrial autoreceptors with the α_{2A}- and α_{2D}-autoreceptors previously described in rabbit, rat, mouse and guinea-pig tissues (see Introduction for references). There is no significant correlation between antagonist $pEC_{30\%}$ values in human right atrium on the one hand and, on the other hand, antagonist pK_D values at either α_{2A}-autoreceptors in rabbit brain cortex, atria and kidney, or α_{2D}-autoreceptors in rat, mouse and guinea-pig brain cortex, rat kidney and submaxillary gland, and guinea-pig atria (5 to 8 antagonists entered into each regression analysis; the best correlation was obtained with the α_{2A}-autoreceptors in rabbit kidney; $r=0.848$; 5 antagonists).

In order to determine the subtype of the α_2-autoreceptors in human right atrium more convincingly, the antagonist $pEC_{30\%}$ values were also compared with their pK_D values (i) at native

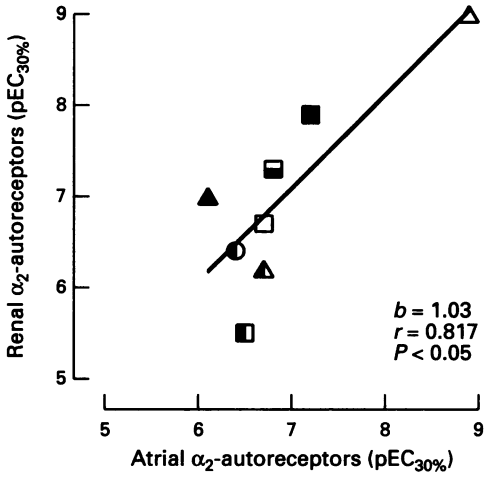

Figure 4 Correlation between antagonist $pEC_{30\%}$ values at presynaptic α_2-autoreceptors in human right atrium and antagonist $pEC_{30\%}$ values at presynaptic α_2-autoreceptors in human kidney. Atrial $pEC_{30\%}$ values from Table 1, kidney $pEC_{30\%}$ values from Trendelenburg *et al.* (1994a). Symbols: rauwolscine (△); WB 4101 (■); phentolamine (⬓); prazosin (□); corynanthine (◭); BRL 41992 (◧); SKF 104078 (◐); BRL 44408 (▲).

prototype α_2 binding sites in cells or tissues that express a single subtype only, and (ii) at α_2 binding sites in membranes of COS cells transfected with either the human α_2-C10 (α_{2A}), the human α_2-C2 (α_{2B}) or the human α_2-C4 (α_{2C}) gene. The binding site pK_D values were taken from the literature. Linear regression analyses were carried out to determine the degree of similarity between α_2-autoreceptors and α_2 binding sites. As shown in Figures 5 and 6, the comparison confirms the initial α_{2C} suggestion: the $pEC_{30\%}$ values at the atrial α_2-autoreceptors are significantly correlated exclusively with the pK_D values at the α_{2C} binding sites expressed in OK cells and those expressed in COS cells transfected with the human α_2-C4 gene. However, one might object that the mere fact of a significant correlation does not prove the identity of the atrial autoreceptors with the α_{2C} binding sites. In case of identity, the slope of the regression line should be equal to unity and the regression line should start from the origin. Figures 5c and 6c show that these two prerequisites for absolute agreement were not fulfilled. The reasons are not known. Variations in experimental conditions (isolated tissues versus cultured cells) and in parameters measured (competition of radioligand binding versus disinhibition of release) may be an explanation. Absolute antagonist affinity estimates at binding sites are often higher than absolute affinity estimates at functioning receptors. Systematic differences in absolute ligand affinity estimates at identical binding sites are also observed in binding studies when carried out in different laboratories. For instance, Bylund *et al.* (1992) reported about 10 times higher ligand pK_D values at α_{2C} sites in membranes obtained from COS-7 cells transfected with the human α_2-C4 gene than did Devedjian *et al.* (1994); moreover, there is only a poor correlation between the affinity estimates at the α_{2C} sites of the two studies. Systematic deviations of experimental data seem to be quite common and similar results have been explained by the use of different assay buffers (Bylund *et al.*, 1992). When looking at the regression analyses between $pEC_{30\%}$-values at atrial autoreceptors and pK_D-values at the various α_2 binding sites presented in Figures 5 and 6, it is obvious that the correlation is best between autoreceptors and α_{2C} binding sites despite the deviation from absolute agreement. For α_{2C} sites, the pairs of antagonist $pEC_{30\%}/pK_D$-values are lined up along the regression line, whereas for α_{2A}, α_{2B} and α_{2D} sites the pairs of $pEC_{30\%}/pK_D$-values are much more scattered about the coordinate system. The identification of the atrial autoreceptors as α_{2C} is supported by the significant correlation between the antagonist $pEC_{30\%}$-values and their pK_D values at α_{2C} binding sites in rat brain cortex ($r=0.838$;

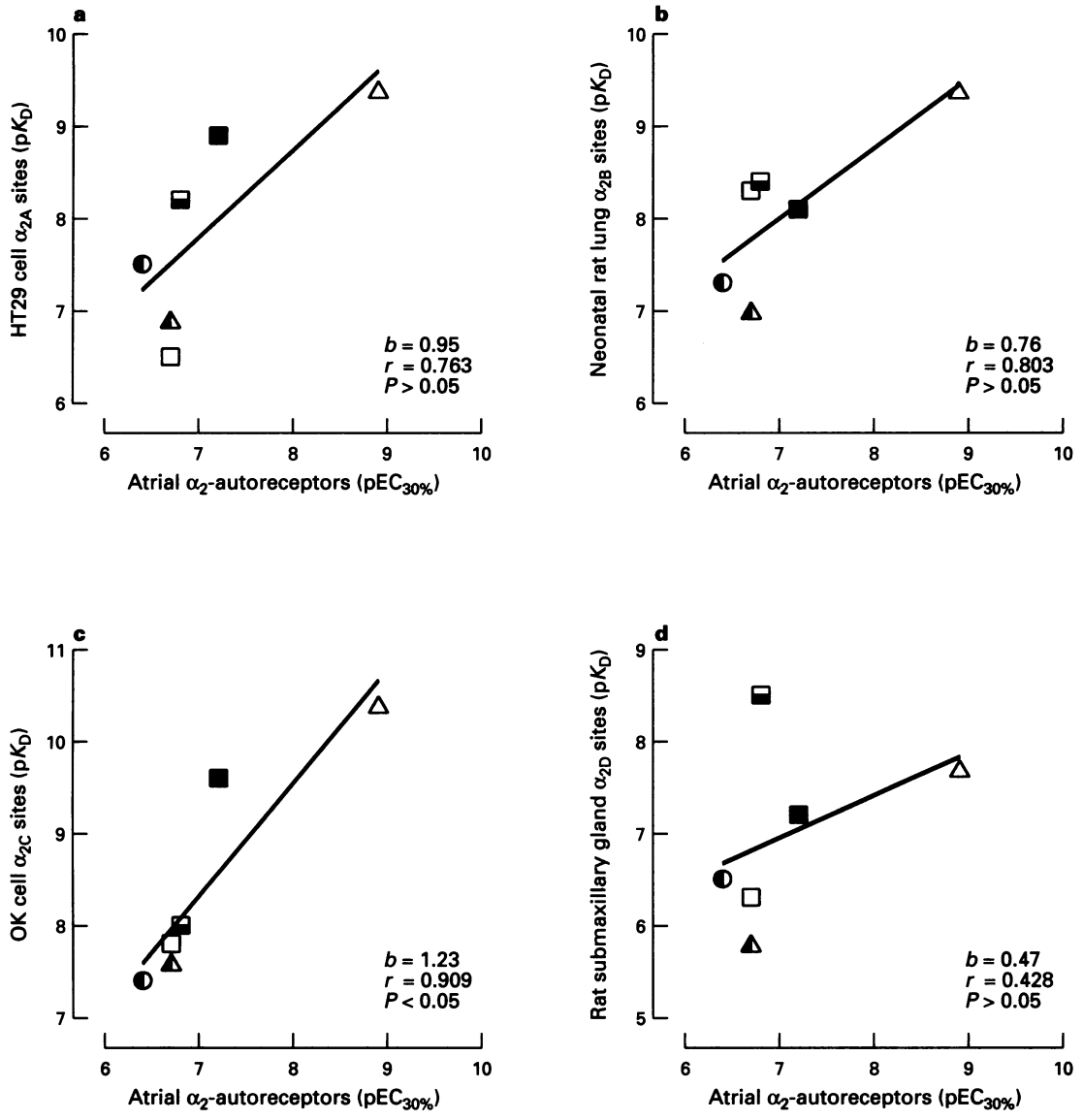

Figure 5 Correlation between antagonist $pEC_{30\%}$ values at presynaptic α_2-autoreceptors in human right atrium and antagonist pK_D values at [^{3}H]-rauwolscine binding sites in (a) human colonic adenocarcinoma (HT29) cells, (b) neonatal rat lung, (c) an opossum kidney (OK) derived cell line, and (d) rat submaxillary gland. $pEC_{30\%}$ values from Table 1. pK_D values from inhibition of [^{3}H]-rauwolscine binding (a,b,c from Simonneaux *et al.*, 1991 and (d) from Michel *et al.*, 1989). Symbols as in Figure 4.

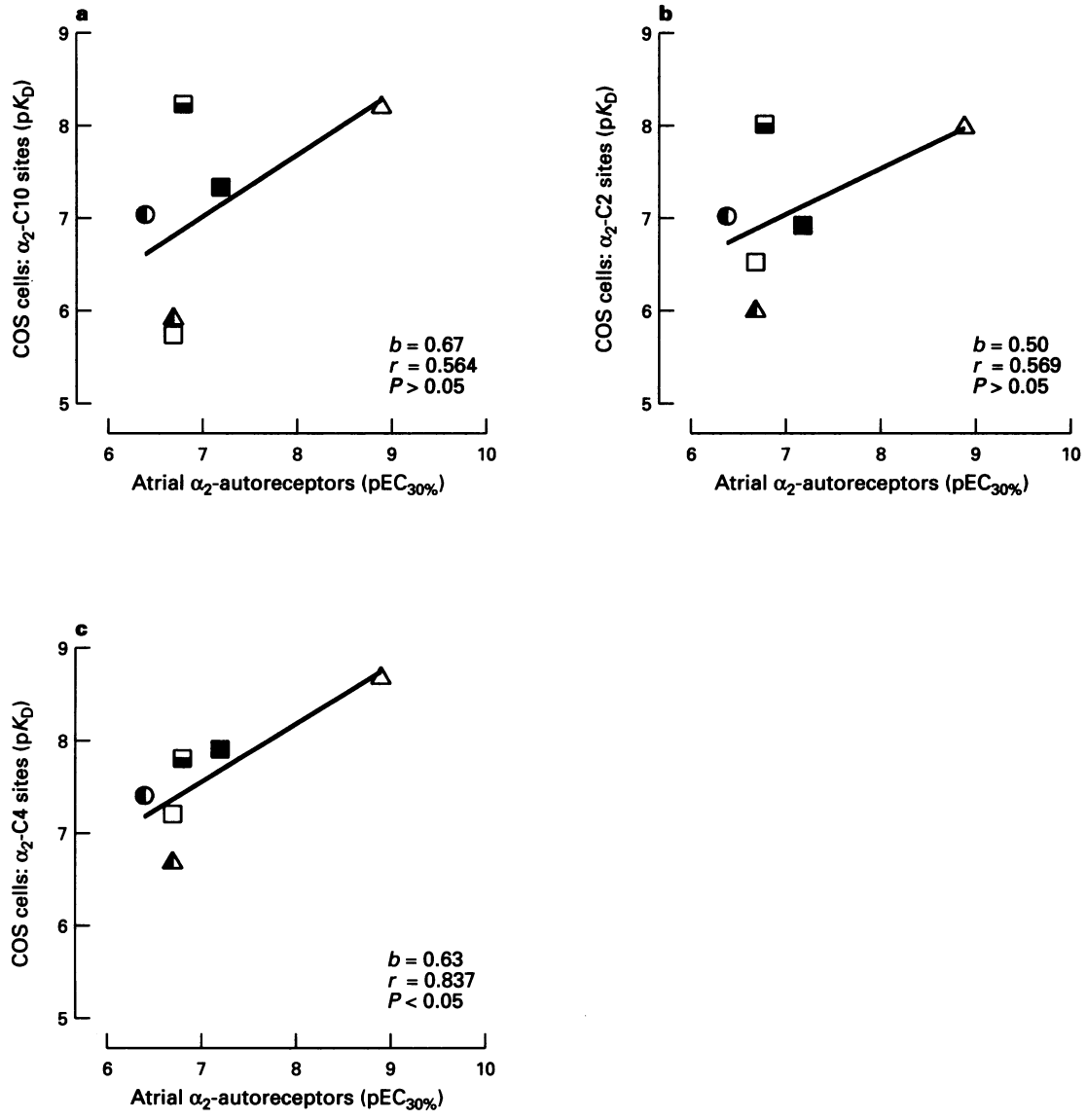

Figure 6 Correlation between antagonist pEC$_{30\%}$ values at presynaptic α_2-autoreceptors in human right atrium and antagonist pK_D values at [^{3}H]-yohimbine binding sites in membranes of COS cells transfected with (a) human α_2-C10 gene (α_{2A}), (b) human α_2-C2 gene (α_{2B}) or (c) human α_2-C4 gene (α_{2C}). pEC$_{30\%}$ values from Table 1. pK_D values from inhibition of [^{3}H]-yohimbine binding (from Lomasney *et al.*, 1991). Symbols as in Figure 4.

Table 2 Ratios of dissociation constants K_D of α-adrenoceptor antagonists

Tissue	*WB 4101/ phentolamine*	*WB 4101/ prazosin*	*Prazosin/ phentolamine*	*Rauwolscine/ phentolamine*
Human right atrium	0.79	0.20	3.98	0.05
α_2-C10 (α_{2A})	6.30	0.013	659	1.15
α_2-C2 (α_{2B})	14.3	0.36	42.3	1.20
α_2-C4 (α_{2C})	0.53	0.11	6.15	0.15

Shown are ratios for human atrial presynaptic α_2-autoreceptors (calculated from pK_D values of Table 1) and for α_2 binding sites in membranes of COS cells transfected with either the human α_2-C10, α_2-C2 or α_2-C4 gene (from Lomasney *et al.*, 1991 and Devedjian *et al.*, 1994; mean ratio if K_D values were reported in both studies).

$P<0.05$; 7 antagonists), whereas the correlation between pEC$_{30\%}$-values and the pK_D values at α_{2D} sites in the same tissue did not reach a level of significance ($r=0.151$; $P>0.05$; 7 antagonists; binding data from Uhlén *et al.*, 1992).

The problem of variability of experimental data shows that multiple comparisons are mandatory for a reliable subtype identification. To validate further the classification, ratios of antagonist dissociation constants K_D were computed for human right atrium and for binding sites in COS cells transfected with one of the three human α_2 genes. The ratio of the K_Ds of two antagonists, a measure of the relative affinity of these two antagonists at a given receptor, can be a sensitive indicator of receptor subtypes (Bylund *et al.*, 1992). Assuming that a more than tenfold difference in the K_D ratio of a pair of antagonists indicates a true difference in their relative affinity, the analysis favours the conclusion that the atrial autoreceptors belong to

the α_{2C} subtype: Three of the four ratios calculated for the atrial autoreceptors deviate more than ten fold from the respective ratios calculated for the α_{2A} binding sites expressed in COS cells that had been transfected with the α_2-C10 gene (maximal deviation 166 fold; see Table 2). The same holds true for the four ratios calculated for the α_{2B} binding sites in COS cells transfected with the α_2-C2 gene (maximal deviation 24 fold). In contrast, the four autoreceptor ratios deviate maximally three fold from the respective ratios for the α_{2C} binding sites in COS cells transfected with the α_2-C4 gene. It should be pointed out that, apart from $pEC_{30\%}$, the K_D is a second, independently obtained affinity estimate for the antagonists at atrial α_2-autoreceptors. Therefore, the outcome of the K_D ratio comparison strongly supports the α_{2C} designation of the autoreceptors reached by the correlation analyses of the $pEC_{30\%}$ values.

So far, in man presynaptic α_2-autoreceptors have been subclassified in brain cortex (Raiteri *et al.*, 1992), kidney (Trendelenburg *et al.*, 1994a) and right atrium (present study). In brain cortex, it has been suggested that they are α_{2A}. This contrasts with the α_{2C} classification in the kidney and heart atrium. One explanation may be that, in man, the α_2-autoreceptors in the central nervous system differ from those in the postganglionic sympathetic nervous system. This, however, is unlikely because in other species a difference between central and peripheral α_2-autoreceptors has not been observed. Within the rabbit, for instance, there is an impressive α_{2A} subtype homogeneity of central and peripheral α_2-autoreceptors, and the same holds true for the rat and the guinea-pig with their α_{2D} homogeneity (see Introduction). The brain cortex results are open to an additional explanation. A major argument for the α_{2A} classification of the autoreceptors was the observation that prazosin and ARC 239, antagonists with high affinity for the α_{2B} and α_{2C} subtypes as compared to α_{2A} and α_{2D}, were ineffective at 300 nM against the release-inhibiting effect of clonidine (Raiteri *et al.*, 1992). However, in absolute terms the affinities of prazosin and ARC 239 seem to be lower at human α_{2C}-autoreceptors (pK_D of prazosin; 7.2 in right atrium and 6.2 in the kidney; pK_D of ARC 239: 6.3 in the kidney; Table 1 and Trendelenburg *et al.*, 1994a) than at α_{2C} binding sites in OK cells (pK_D 7.8 for prazosin and 7.9 for ARC 239; Simonneaux *et al.*, 1991). With similar low affinities of prazosin and ARC 239 at the brain cortex α_2-autoreceptors, 300 nM probably was too low a concentration to counteract the inhibitory effect of clonidine. Only further studies, including discriminative compounds, will show whether or not the α_2-autoreceptors in brain cortex are similar to those in right atrium and kidney.

In conclusion, the presynaptic α_2-autoreceptors in human right atrium are α_{2C}. In this they agree with the autoreceptors in the human kidney. It has been proposed that noradrenergic neurones predominantly express the ortholog $\alpha_{2A/D}$ gene to control transmitter release through presynaptic α_2-autoreceptors, in all mammals. The two-fold α_{2C} classification in human tissue seems to exclude the human species from this rule. The general $\alpha_{2A/D}$ character of the presynaptic α_2-autoreceptors is probably true only for species of the order *Lagomorpha* (e.g. rabbit) and of the order *Rodentia* (e.g. rat, mouse, guinea-pig). In order to obtain any general rule concerning the subtype of the human presynaptic α_2-autoreceptor, additional tissues need to be investigated.

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