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Different α_1-adrenoceptor subtypes mediate contraction in rabbit aorta and urethra

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Abstract

The α_1-adrenoceptor subtypes mediating contraction of rabbit aorta and urethra were pharmacologically characterized using an isolated organ bath technique. Although aorta was as sensitive as urethra to the contractile action of methoxamine, phenylephrine was about 10 times more potent as a contractile agonist on aorta than on urethra. In aorta, the rank order of agonist sensitivity was norepinephrine > phenylephrine > clonidine > methoxamine whereas the rank order in urethra was clonidine > methoxamine ≥ phenylephrine > norepinephrine. A lack of significant correlation between the potency of different α_1-adrenoceptor antagonists tested against the phenylephrine-induced contraction in aorta and in urethra indicated that different α_1-adrenoceptor subtypes mediated the contractile response in the two preparations. The potency of different α_1-adrenoceptor antagonists tested in rabbit urethra was significantly correlated with their affinity for the cloned human α_{1c}-, but not α_{1a}- or α_{1b}-, adrenoceptor subtype. Such a clear correlation with the potency of different α_1-adrenoceptor antagonists tested in rabbit aorta and their affinity for one subtype of cloned human α_1-adrenoceptor was not found. Chlorethylclonidine, which produced a 10000-fold rightward shift in the phenylephrine concentration-response curve for rat aorta, had a weak inhibitory effect in rabbit aorta and urethra as well as in other rabbit tissues (spleen, fundus, renal artery, saphenous artery). The results indicate that significant heterogeneity exists among α_1-adrenoceptor in rabbit aorta and urethra (α_{1c}-adrenoceptor). However, chlorethylclonidine does not seem to be a suitable tool for the differentiation of α_1-adrenoceptor subtypes in the rabbit.

Keywords: α_1-Adrenoceptor; Aorta; Urethra

1. Introduction

Classical pharmacological studies have suggested that postjunctional α_1-adrenoceptors can be divided into different subtypes. More recently, genes encoding at least three distinct α_1-adrenoceptors, designated α_{1a} or α_{1d} or $\alpha_{1a/d}$ (see Schwinn and Lomasney, 1992; Kenny et al., 1994 and Ford et al., 1994 for discussion), α_{1b}, and α_{1c} (Cotechia et al., 1988; Lomasney et al., 1991; Schwinn et al., 1990) have been cloned. Conversely, pharmacological studies have defined α_{1A}-, α_{1B}-, α_{1H}-, α_{1L}-, and α_{1N}-adrenoceptor subtypes (see Ford et al., 1994; Bylund et al., 1994). It is now recognized that the α_{1b} clone corresponds to the pharmacological α_{1B}-adrenoceptor and the α_{1c} clone to the α_{1A}-adrenoceptor whereas the pharmacology of the $\alpha_{1a/d}$ clone remains to determined and the pharmacological α_{1L}-adrenoceptor remains to be cloned (see Ford et al., 1994; Bylund et al., 1994). α_{1A} Subtypes have been demonstrated to be more sensitive than α_{1B}-adrenoceptors to the antagonists WB 4101 and 5-methyl-urapidil (Morrow and Creese, 1986; Gross et al., 1988; Lomasney et al., 1991; Schwinn et al., 1990) whereas spiperone exhibits high affinity for the α_{1B}-adrenoceptors (Michel et al., 1989). In addition, the irreversible α-adrenoceptor ligand chloroethylclonidine inactivates the α_{1B}-adrenoceptor but is less effective on the α_{1A}-adrenoceptor (Han et al., 1987; Minneman et al., 1988; Schwinn et al., 1990). Therefore, chloroethylclonidine, originally introduced into pharmacology by Leclerc et al. (1980), has been used as a tool for differentiating α-adrenoceptor subtypes in various tissues.

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The development of α_1-adrenoceptor subtype-specific agents is considered as goal for improving therapeutic efficacy and lowering the incidence of adverse reactions. For example, the benefit of α_1-adrenoceptor antagonists, which have been found effective in the treatment of the dynamic component of urethral obstruction caused by benign prostatic hyperplasia, despite significant side effects, such as vasodilating effects, might be improved by a greater α_1-adrenoceptor subtype selectivity (Caine, 1990; Monda and Oesterling, 1993; Jiménez Cruz, 1993). However, only few data have been reported showing a clear dissociation among α_1-adrenoceptor subtypes mediating contraction of the lower urinary tract and blood vessels. Using the specific α_1-adrenoceptor antagonist YM-12617, Honda and Nakagawa (1986) reported that the characteristics of α_1-adrenoceptors are not significantly different in rabbit isolated aorta and urethra. Functional studies, mainly based on the use of chloroethylclonidine, indicated that α_1 contractile responses in rabbit urethra are mediated by α_{1A} and α_{1B} subtypes (Yoshida et al., 1991) as in rabbit aorta (Takayanagi et al., 1991; Oriowo and Ruffolo, 1992). However, a more recent study reported a good correlation between the potency of different α_1-adrenoceptor antagonists tested against the noradrenaline-induced contraction of rabbit urethra and their affinity for α_{1A}-receptors, with no correlation with α_{1B}-adrenoceptor subtypes (Testa et al., 1993). Thus, the aim of the present study was to compare the pharmacological characteristics of the α_1-adrenoceptor subtype mediating contraction in rabbit aorta and urethra using (i) different agonists, (ii) the correlation between the potency of different antagonists on contractile response and the binding properties of the cloned human α_1-adrenoceptor (Forray et al., 1994; Weinberg et al., 1994) and (iii) the ability of chloroethylclonidine to discriminate between α_1-adrenoceptor subtypes in different rabbit tissues.

2. Materials and methods

2.1. Tissue bath experiments

The aorta, renal artery, lateral saphenous artery, spleen, fundus and urethra were removed from male New Zealand rabbits (2.5–3.5 kg, Dombes, Romans, France) and the aorta from male Sprague-Dawley rats (280–330 g, Charles River, France) after cervical dislocation and cleaned of the surrounding connective tissue. Endothelium-denuded rings (2–3 mm wide) of aorta, renal and saphenous arteries, strips of prostatic urethra and fundus (3 mm wide, 10–15 mm long) and rings (4 mm wide) of spleen were suspended in organ baths containing 10 or 20 ml of physiological solution (for composition see below) under a tension of 2, 1.5, 1, 1, 1 and 1 g, respectively, at 37°C and gassed with 95% O_2-5% CO_2. Contractile responses were measured using force-displacement transducers coupled to a Gould 8000S polygraph or connected to a data collection system (IOS, Dei Lierre, Mitry-Mory, France). A 1-h equilibration period was allowed before experimentation. Physiological solution was composed of (mM): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25; glucose, 11. After equilibration, the preparations were sensitized by a submaximal concentration of phenylephrine. When the contraction elicited by phenylephrine was stable, carbachol (10 μM) was tested in order to verify the destruction of the endothelium of the arteries. 45 min after sensitization, a concentration-effect curve for an agonist was established. To determine the effect of antagonists, different preparations from the same animal were used in parallel. One served as control and the other received one concentration of antagonist (3 μM except for abanoquil and WB4101 10 nM and prazosin 0.1 μM) introduced into the bath 30 min before phenylephrine. The effect of chloroethylclonidine was examined after sensitization of the preparation with phenylephrine; 100 μM chloroethylclonidine was introduced into the bath for 30 min and the maximal contraction elicited by chloroethylclonidine during this period of time was recorded. Then the drug was washed-out extensively throughout a 60-min rest period during which the tone of the preparation returned to the basal level. The concentration-response curve for phenylephrine was then constructed. A time-matched control was also performed.

2.2. Drugs

Carbachol, clonidine hydrochloride, methoxamine hydrochloride, norepinephrine hydrochloride, phentolamine hydrochloride, phenylephrine hydrochloride, prazosin hydrochloride, yohimbine hydrochloride, and WB4101 hydrochloride were purchased from Sigma Chemical Co. (Clery en Vexin, France). Benoxathian hydrochloride, chlorethylclonidine 2 hydrochloride, 5-methyl-urapidil, and spiperone hydrochloride were purchased from Research Biochemical (Strasbourg, France). Abanoquil terazosin, REC 15\2739 (8-{3-[4-(2-methoxyphenyl)-1-piperazinyl]-propylcarbamoyl}-3-methyl-4-oxo-2-phenyl-4*H*-1-benzopyran) and alfuzosin were synthetized at the Chemistry Department, Institut Henri Beaufour Research Laboratories, Le Plessis-Robinson, France.

2.3. Statistics

Results are expressed as means ± S.E.M. of force (g) or percentage of the contraction. The maximal contractile response of each preparation that had been

subjected to antagonist or agonist other than phenylephrine was determined by comparing the response evoked by the application of the sensitizing concentration of phenylephrine with the response evoked in the control preparation (which received only phenylephrine). Comparisons were made using analysis of variance, P values < 0.05 being considered as significant. EC_{50} values were calculated from the maximum contractile response to each agonist by computer analysis using linear regression. The antagonist dissociation constants (K_b) were determined for each antagonist according to the following equation: $K_b = [B]/(\text{dose ratio} - 1)$; where [B] is the antagonist concentration and (dose ratio) is the EC_{50} of the agonist in the presence of the antagonist divided by the control EC_{50}. These results were then expressed as the negative logarithm of the K_b (apparent pK_b). The Schild plot parameter (pA_2) was evaluated by linear regression analysis according to Arunlakshana and Schild (1959) and was represented as pK_B.

Correlation analyses were assessed by least-squares linear regression.

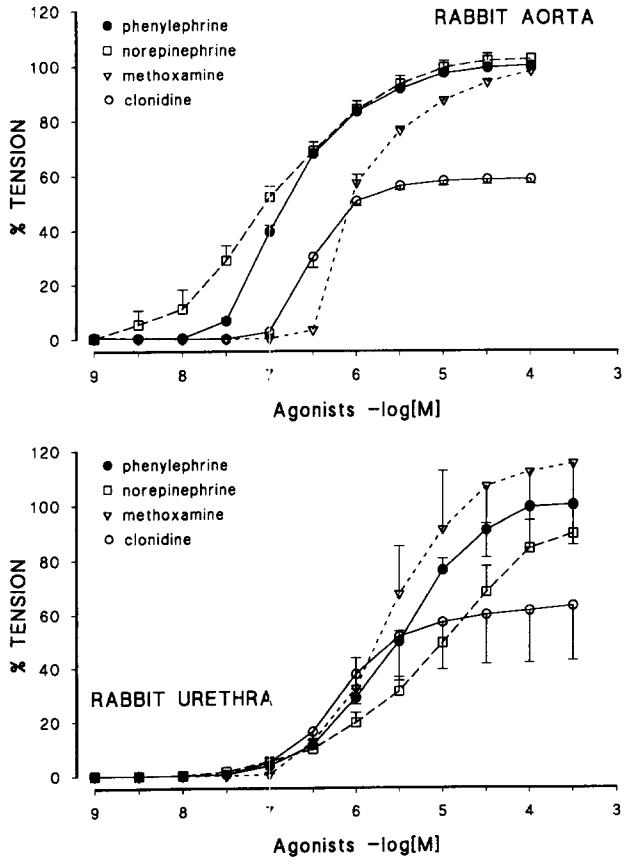

Fig. 1. Log concentration-response curves for the cumulative addition of norepinephrine; phenylephrine; methoxamine; clonidine in rabbit aorta (upper panel) and in urethra (lower panel). Data from six preparations taken from different rabbits are presented as means and S.E.M., shown by vertical bars.

Table 1
Apparent pK_b values for the different antagonists in inhibiting contractions induced by phenylephrine in isolated rabbit aorta and urethra

	Aorta	Urethra
Abanoquil	10.7±0.11	8.6±0.3
Alfuzosin	8.2±0.11	6.6±0.08
Benoxathian	8.5±0.05	8.0±0.1
Phentolamine	8.0±0.07	7.4±0.08
Prazosin	8.7±0.15	8.1±0.10
REC 15\2739	7.8 [a]	8.5 [a]
Spiperone	8.0±0.12	7.7±0.17
Terazosin	7.5±0.18	7.2±0.18
WB4101	9.0±0.25	8.7±0.19
5-Methyl-urapidil	7.8±0.13	8.0±0.24

Means±S.E.M.; $n = 4–7$. [a] Evaluated from the Schild plot (slope of 1.06 and 1.29, not significantly different from 1 for urethra and aorta, respectively; $n = 12$).

3. Results

3.1. Contractile responses to different agonists

Norepinephrine, phenylephrine, methoxamine and clonidine caused concentration-dependent contractions of rabbit aorta and urethra (Fig. 1). In both tissues, the amplitude of clonidine-induced contractions was significantly smaller than those induced by other agonists, indicating that clonidine acts as partial agonist, whereas norepinephrine, methoxamine and phenylephrine are full agonists. In aorta, the EC_{50} values were in the following rank order: norepinephrine (0.14 ± 0.033 μM) > phenylephrine (0.22 ± 0.009 μM) > clonidine (0.37 ± 0.052 μM) > methoxamine (1.4 ± 0.15 μM), whereas in urethra, the rank order was clonidine (0.98 ± 0.018 μM) > methoxamine (2.7 ± 0.38 μM) ≥

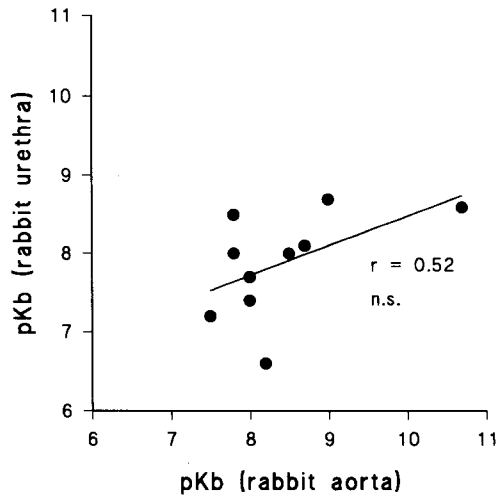

Fig. 2. Correlations between the potency (apparent pK_b values Table 1) of the α_1-adrenoceptor antagonists tested on phenylephrine-induced contractions in rabbit aorta and in rabbit urethra.

phenylephrine (2.85 ± 0.55 μM) > norepinephrine (7.4 ± 2.70 μM). Thus, among the specific α_1-adrenoceptor agonists tested, methoxamine had the same potency in aorta and in urethra, whereas phenylephrine was 10-fold less sensitive in urethra than in aorta.

3.2. Effects of antagonists

The effect of the α_2-adrenoceptor antagonist yohimbine was tested on phenylephrine-induced contractions in the two preparations. The apparent pK_b values were comparable in the two tissues (6.4 ± 0.03 for aorta and 5.95 ± 0.19 for urethra; $n = 4$) and similar to the pA_2 values obtained in urethra by Honda et al. (1985) using noradrenaline, phenylephrine and clonidine as agonists. The different α_1-adrenoceptor antagonists, which were relatively selective for the α_{1A}-adrenoceptors (5-methyl-urapidil; WB 4101) or α_{1B}-adrenoceptors (spiperone), caused parallel shifts to the

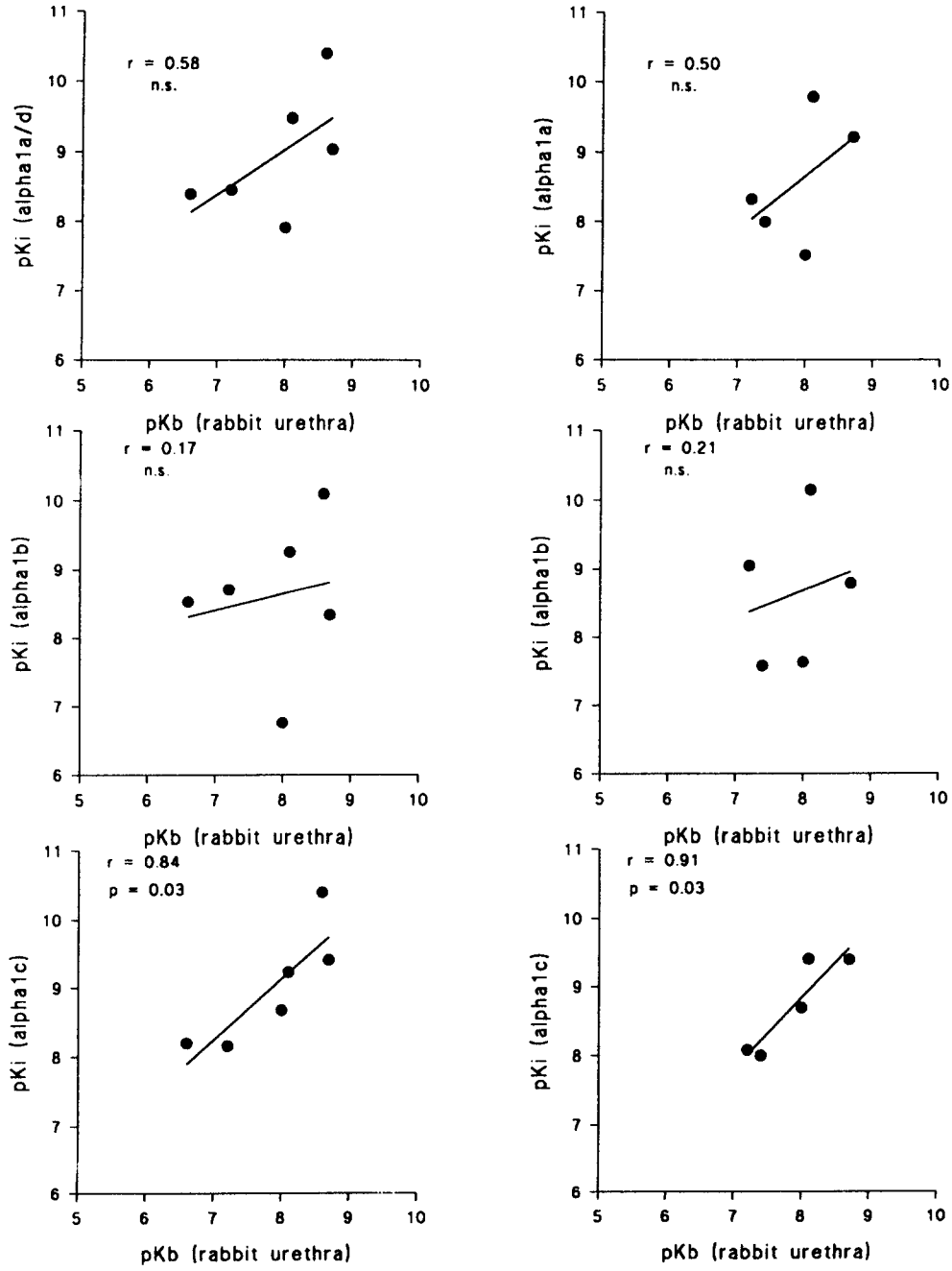

Fig. 3. Correlations between the potency (apparent pK_b values) of the α_1-adrenoceptor antagonists tested on phenylephrine-induced contractions in rabbit urethra and the affinity (pK_i values) for the different cloned human α_1-adrenoceptor subtypes: $\alpha_{1a/d}$- and α_{1a}-adrenoceptor (upper panel); α_{1b}-adrenoceptor (middle panel); α_{1c}-adrenoceptor (lower panel). pK_i values for abanoquil, alfuzosin, 5-methyl-urapidil, prazosin, terazosin and WB4101 on cloned human α_1-adrenoceptor subtypes were taken from Forray et al. (1994) (left panels) and for 5-methyl-urapidil, prazosin, phentolamine, terazosin and WB4101 were taken from Weinberg et al. (1994) (right panels).

right of the phenylephrine concentration-response curves without affecting, to any noticeable extent, the maximum contraction caused by the agonist in both tissues (data not shown). The potency of the antagonists in inhibiting phenylephrine-induced contractions in aorta and urethra is shown in Table 1 and indicates that the apparent pK_b values of several compounds studied are in accordance with the pA_2 values estimated from the Schild plot, for both aorta (Furchgott, 1980; Chiang et al., 1991; Takayanagi et al., 1991) and urethra (Honda et al., 1985; Testa et al., 1993). Abanoquil and alfuzosin were about 100- and 40-fold more potent on aorta than on urethra, respectively. Conversely, REC15\2739 was the only α_1-adrenoceptor antagonist significantly more potent on urethra than on aorta (5-fold).

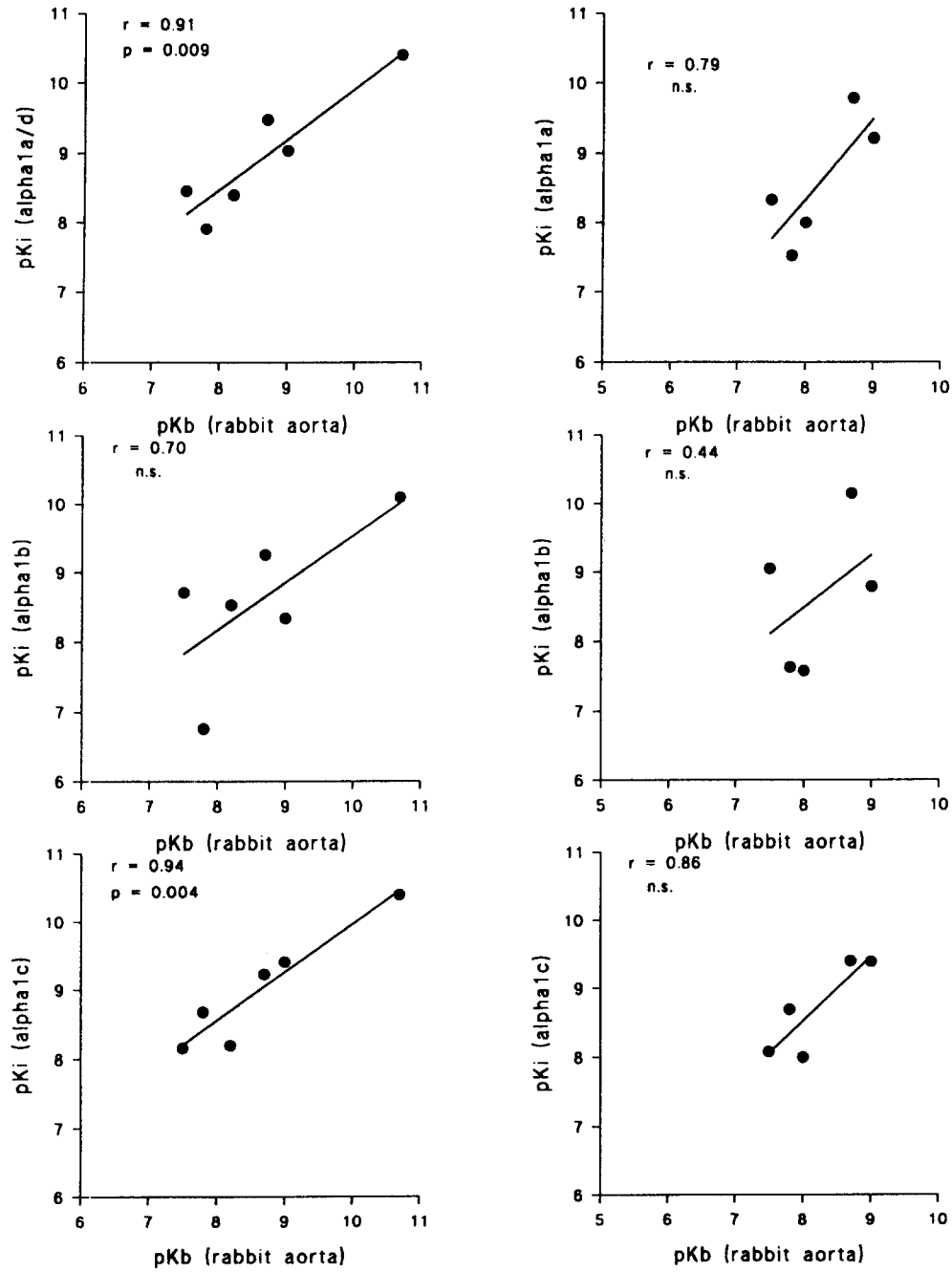

Fig. 4. Correlations between the potency (apparent pK_b values) of the α_1-adrenoceptor antagonists tested on phenylephrine-induced contractions in rabbit aorta and the affinity (pK_i values) for the different cloned human α_1-adrenoceptor subtypes: $\alpha_{1a/d}$- and α_{1a}-adrenoceptor (upper panel); α_{1b}-adrenoceptor (middle panel); α_{1c}-adrenoceptor (lower panel). pK_i values for abanoquil, alfuzosin, 5-methyl-urapidil, prazosin, terazosin and WB4101 on cloned human α_1-adrenoceptor subtypes were taken from Forray et al. (1994) (left panels) and for 5-methyl-urapidil, prazosin, phentolamine, terazosin and WB4101 were taken from Weinberg et al. (1994) (right panels).

3.3. Correlation studies

There was no significant correlation between the apparent pK_b values obtained in aorta and in urethra (Fig. 2). The functional potencies of various α_1-adrenoceptor antagonists were compared with their affinity for human α_1-adrenoceptor subtypes cloned and expressed by two different teams using [^{3}H]prazosin binding to a membrane preparation of CHO (Chinese hamster ovary) cells (Forray et al., 1994) and [^{125}I]HEAT (2-[β-{4-hydroxy-3-[^{125}I]iodophenyl}ethylamino-methyl]tetralone) binding to membranes of COS-7 (monkey kidney) cells (Weinberg et al., 1994) (Figs. 3 and 4). The results indicate that there was a close correlation between the potency of α_1-adrenoceptor antagonists to inhibit contraction of the urethra and their affinity for human α_{1c}-adrenoceptor. In contrast, the potency of α_1-adrenoceptor antagonists to inhibit contraction of the aorta was significantly correlated with their affinity for both α_{1a}- and α_{1c}-adrenoceptors for one study (Forray et al., 1994) or for any human α_1-adrenoceptor subtype in the other study (Weinberg et al., 1994).

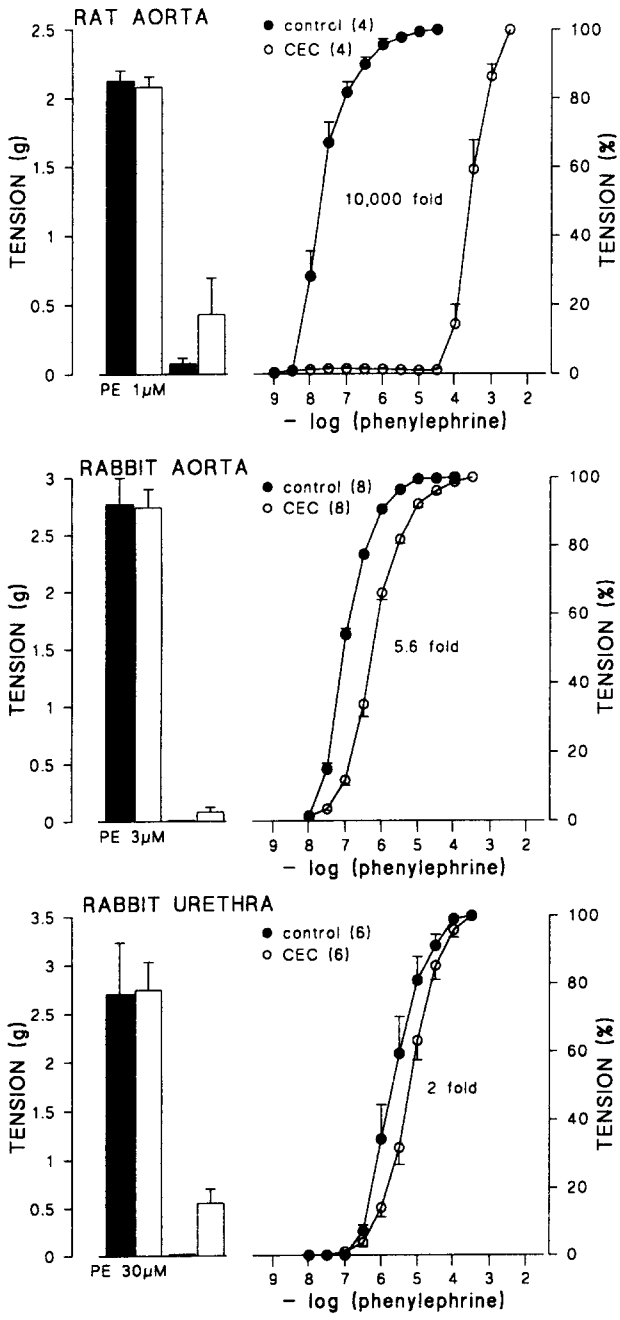

Fig. 5. Effect of chloroethylclonidine (CEC, 100 μM) on phenylephrine-induced contractile responses in rat aorta (upper panel); in rabbit aorta (middle panel) and in urethra (lower panel). Histograms depict the maximal tension induced by the sensitizing concentration of phenylephrine (PE) for the two groups and the maximal contractile response elicited by chloroethylclonidine in the treated group during the 30-min application period (see Methods). Each data point represents the mean and S.E.M., shown by vertical bars (n).

3.4. Use of chlorethylclonidine for determination of α_1-adrenoceptor subtypes

In rat aorta, chloroethylclonidine (100 μM) caused a weak contraction per se, but produced a 10000-fold rightward shift of the phenylephrine concentration-response curve without depressing the maximal effect (Fig. 5). Chloroethylclonidine, which was without significant contractile effect, also produced a rightward shift of the phenylephrine concentration-response curve in rabbit aorta and urethra, but only by 5.6- and 2-fold, respectively (Fig. 5).

In other rabbit vascular and non-vascular tissues such as renal artery, spleen and gastric fundus, chloroethylclonidine elicited no significant contractile effect and caused a weak rightward shift of the concentration-response curve for phenylephrine by 1.4-, 3.9- and 3.2-fold, respectively (Fig. 6). In contrast, in rabbit saphenous artery chloroethylclonidine induced a potent contraction which was difficult to wash-out (data not shown), and caused a significant leftward shift of the phenylephrine concentration-response curve by 0.4-fold (Fig. 6).

4. Discussion

On the basis of both functional and binding experiments and the use of various agonists and antagonists, the present study shows that the α_1-adrenoceptor subtypes which induce contraction are different in the rabbit aorta and urethra. Heterogeneity of α_1-adrenoceptors has been already demonstrated. However, especially in vascular tissues, determination of the α_1-adrenoceptor subtypes generally distinguishes between α_{1A}- and α_{1B}-adrenoceptors (Takayanagi et al., 1991; Oriowo and Ruffolo, 1992; Satoh et al., 1993; Hoo et al., 1994). Our pharmacological approach was to use

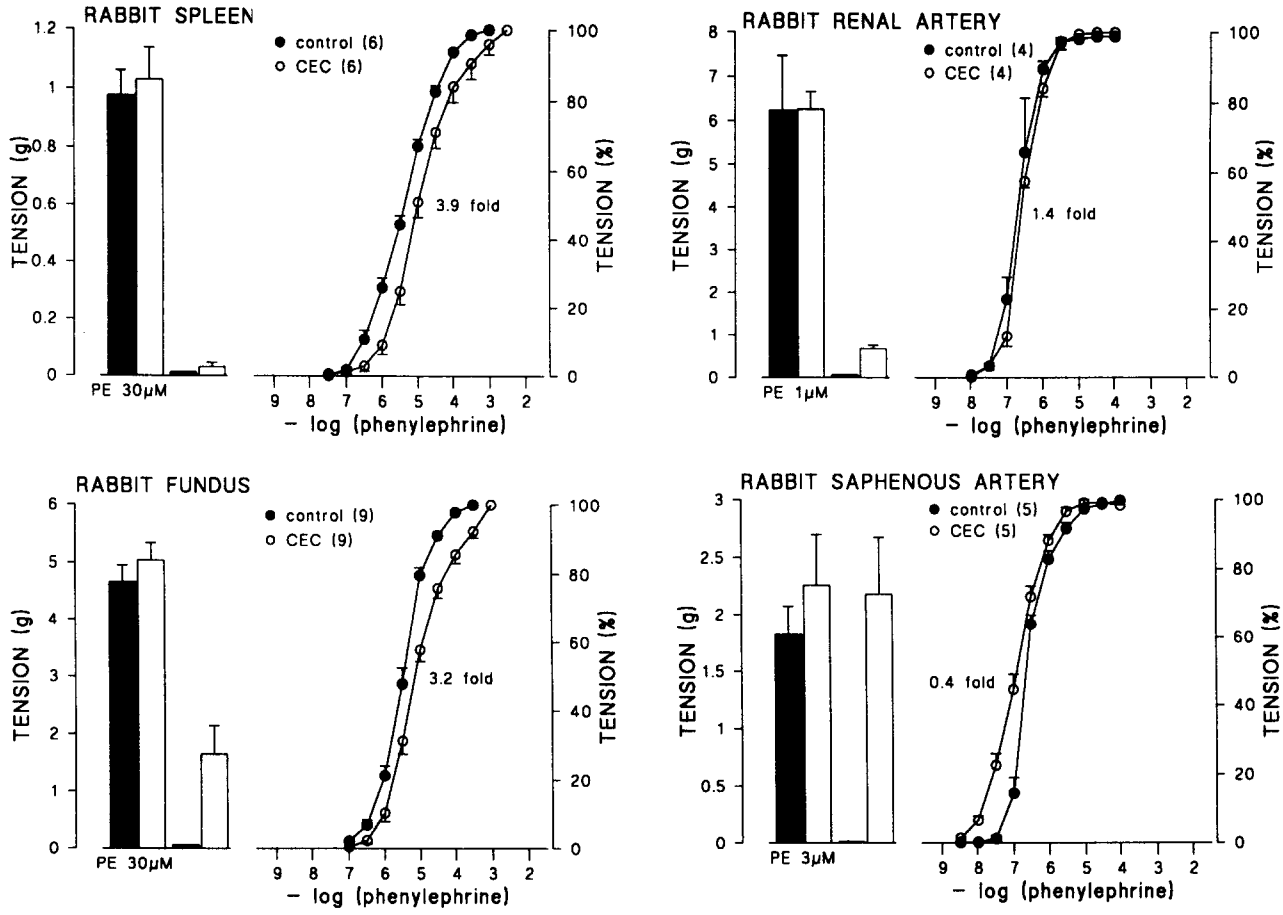

Fig. 6. Effect of chloroethylclonidine (CEC, 100 μM) on phenylephrine-induced contractile responses in rabbit spleen (upper left panel); fundus (lower left panel); renal artery (upper right panel) and saphenous artery (lower right panel). Histograms depict the maximal tension induced by the sensitizing concentration of phenylephrine for the two groups and the contractile response elicited by chloroethylclonidine in the treated group during the 30 min application period (see Methods). Each data point represents the mean and S.E.M., shown by vertical bars (n).

different agonists and antagonists and to examine the correlation between their potency and their affinity for three different cloned α_1-adrenoceptor subtypes (a, b and c). Finally, we used the alkylating derivative of clonidine, chloroethylclonidine, which inactivates totally the α_{1B}-adrenoceptor and partially the α_{1c}-adrenoceptor, whereas it is ineffective against the α_{1A}-adrenoceptor (Han et al., 1987; Minneman et al., 1988; Schwinn et al., 1990).

As already reported for rabbit aorta (Ruffolo and Waddell, 1982; Awad et al., 1983) and urethra (Honda et al., 1985; Yoshida et al., 1991) clonidine, an α_2-adrenoceptor agonist, acts as partial agonist, whereas norepinephrine and phenylephrine, an α_1-adrenoceptor agonist, are full agonists. Yohimbine comparably antagonized phenylephrine-induced contraction in the two preparations and had a potency similar to that obtained in urethra by Honda et al. (1985), using noradrenaline, phenylephrine and clonidine as agonists. This potency was about two log units lower than the values expected for an effect of yohimbine on α_2-adrenoceptors and reflects the interaction of yohimbine with α_1-adrenoceptors. Thus, the results confirm that adrenergic contractions are mainly mediated by α_1-adrenoceptors in these two tissues. Regarding the affinity of different agonists for the three cloned receptors in transfected cells, it has been reported that methoxamine possesses the same affinity for the α_{1a}-adrenoceptor and α_{1c}-adrenoceptor, but it is about 10-fold less sensitive for the α_{1b}-adrenoceptor (Schwinn and Lomasney, 1992). The present study shows that methoxamine has the same potency in rabbit aorta as in urethra. In addition, phenylephrine is about 4- and 80-fold more sensitive than methoxamine on α_{1c}- and on α_{1a}-adrenoceptors, respectively (Schwinn and Lomasney, 1992). At the same time, methoxamine and phenylephrine had comparable sensitivity in urethra, but phenylephrine was about 10 times more potent than methoxamine in aorta. Thus, these results indicate that agonists act on different α_1-adrenoceptors in aorta and in urethra, suggesting that contraction is mediated mainly through the α_{1a}-adrenoceptor and α_{1c}-adrenoceptor, respectively.

A lack of significant correlation between the po-

tency of different α_1-adrenoceptor antagonists tested against the phenylephrine-induced contraction in aorta and in urethra strengthened the suggestion that different α_1-adrenoceptor subtypes mediated the contractile response of the two preparations. Functional studies of antagonists globally indicate that, except REC15\2739, all antagonists tested are more potent in aorta than in urethra. In this respect, abanoquil is the most selective antagonist studied, being 100-fold more potent in aorta than in urethra. Interestingly, in [^{3}H]prazosin binding experiments in cloned cell lines, abanoquil is a 100-fold more potent inhibitor of α_{1a}-adrenoceptor than of α_{1c}- or α_{1b}-adrenoceptors (Marshall et al., 1992). However, such a selectivity for abanoquil was not observed in [^{3}H]prazosin binding experiments in cloned human α_1-adrenoceptor subtypes (Forray et al., 1994). Nevertheless, the potency of different α_1-adrenoceptor antagonists tested in rabbit urethra was significantly correlated with their affinity for the cloned human α_{1c}-, but not α_{1a}- or α_{1b}-, adrenoceptor subtype, using the values of two independent studies. Such a clear correlation with the potency of different α_1-adrenoceptor antagonists tested in rabbit aorta and their affinity for one subtype of cloned human α_1-adrenoceptor was not found. The potency to inhibit contraction of the aorta was significantly correlated with both α_{1a}- and α_{1c}-adrenoceptors in one case (Forray et al., 1994) or with any human α_1-adrenoceptor subtype in the other (Weinberg et al., 1994). Thus our results strengthen the possible mediation of contraction through α_{1c}-adrenoceptors in rabbit urethra as in human prostate (Forray et al., 1994). Determination of the α_1-adrenoceptor subtypes which mediate contraction in rabbit aorta is still unclear. It is possible that there is not sufficient homology between the vascular α_1-adrenoceptor of rabbit aorta and the human α_1-adrenoceptor subtypes to permit a close correlation or that vascular contraction is due to activation of another α_1-adrenoceptor subtype (e.g. α_{1L}-adrenoceptor). Alternatively, the contraction induced by phenylephrine in rabbit aorta may be the result of activation of several α_1-adrenoceptor subtypes. In this respect, two types of receptors, α_{1A}- and α_{1B}-adrenoceptors, have been observed in rabbit aorta, however contraction seems to be mainly due to α_{1A}-adrenoceptors (Suzuki et al., 1990; Torres-Márquez et al., 1991).

The irreversible α-adrenoceptor ligand chloroethylclonidine is often used to determine the classification of receptors. Chloroethylclonidine inactivates the α_{1B}-adrenoceptor, and to a lesser extent the α_{1c}-adrenoceptor, but is ineffective on the α_{1A}-adrenoceptor (Han et al., 1987; Minneman et al., 1988; Schwinn et al., 1990). However, in our tissue bath study, we were unable to demonstrate a clear dissociation between the effects of chloroethylclonidine in rabbit aorta and urethra. In fact, chloroethylclonidine (100 μM) effectively shifted to the right the α_1-adrenoceptor agonist concentration-effect curve in rat aorta, as already reported (Han et al., 1990; Piascik et al., 1990; Oriowo and Ruffolo, 1992), but had a weaker effect on rabbit aorta (present study; Muramatsu et al., 1990; Takayanagi et al., 1991; Oriowo and Ruffolo, 1992) and on rabbit urethra (present study; Yoshida et al., 1991; Testa et al., 1993). The rightward shift of the concentration-response curve for phenylephrine in rabbit aorta and urethra, 5.6- and 2-fold, respectively, does not permit any dissociation of α_1-adrenoceptor subtypes between the two tissues; chloroethylclonidine did not cause any rightward shift of the concentration-response curve for phenylephrine in renal artery, as reported previously (Oriowo et al., 1992). It is however relatively ineffective in mesenteric, carotid, ear and ovarian arteries (Muramatsu et al., 1990; Oriowo et al., 1992). In rabbit non-vascular tissues such as rabbit spleen, which express α_{1b}-adrenoceptor subtypes (Schwinn et al., 1991) and gastric fundus, chloroethylclonidine, without having a significant contractile effect, caused a weak rightward shift of the phenylephrine concentration-response curve by 3.9- and 3.2-fold, respectively. Finally, in rabbit saphenous artery, chloroethylclonidine induced an efficacious contraction and caused a significant leftward shift of the concentration-response curve for phenylephrine. Taken together, the results reported here indicate that the contraction induced by α-adrenoceptor agonists in different rabbit tissues is relatively insensitive to the effect of chloroethylclonidine, even in tissues which express the α_{1b}-adrenoceptor subtype, such as the spleen. Thus, the effectiveness of chloroethylclonidine on α_1-adrenoceptor subtypes may be species dependent. Interestingly, Hoo et al. (1994) recently demonstrated the inability of chloroethylclonidine to inactivate α_{1B}-adrenoceptors in canine vascular membrane under various in vitro conditions.

In conclusion, this study reveals that significant heterogeneity exists in α_1-adrenoceptor subtypes in rabbit aorta and urethra. In rabbit urethra, contraction is mainly mediated by a subtype corresponding to the human α_{1c}-adrenoceptor. However, the use of chloroethylclonidine to determine the α_1-adrenoceptor subtype classification, although effective in rat tissues such as aorta, does not seem to be a suitable tool in rabbit tissues.

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