α1B-Adrenoceptor Subtype Mediating the Phenylephrine-Induced Contractile Response in Rabbit Corpus Cavernosum Penis

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Received February 13, 1996  Accepted May 23, 1996

ABSTRACT—The α1-adrenoceptor subtype mediating contraction to phenylephrine in rabbit corpus cavernosum penis (CCP) was investigated using selective α1-adrenoceptor subtype antagonists. WB4101 ((2-(2,6-dimethoxy-phenoxyethyl)-aminomethyl-1,4-benzodioxane) hydrochloride), 5-methylurapidil and tamsulosin concentration-dependently produced a parallel rightward shift of the concentration-response curve to phenylephrine, yielding pKB values of 8.05, 7.59 and 9.21, respectively. The slopes of the Schild plots were not different from unity. These antagonists did not affect the maximum response to phenylephrine. Oxymetazoline (1 μM), which initially caused a small contraction, produced a parallel rightward shift of the concentration-response curve to phenylephrine with an apparent pKB value of 6.99. However, oxymetazoline seemed to act as a non-surmountable antagonist to the phenylephrine-induced contraction, reducing the maximum response by 71.1%. Chloroethylclonidine (25 and 100 μM) produced a parallel rightward shift of the concentration-response curve to phenylephrine without altering the maximum response. These results show that the α1-adrenoceptor in rabbit CCP has a relatively low affinity for WB4101, 5-methylurapidil, tamsulosin and oxymetazoline and is sensitive to inactivation by chloroethylclonidine. It is suggested that the α1-adrenoceptor subtype mediating contraction to phenylephrine in rabbit CCP has the characteristics of the α1B-adrenoceptor subtype.

Keywords: Corpus cavernosum penis (rabbit), α1B-Adrenoceptor subtype, Phenylephrine, WB4101, 5-Methylurapidil

Clinically, some vasodilating drugs such as papaverine and prostaglandin E1, and also phentolamine, a non-selective α-adrenoceptor antagonist, have been used for the treatment of impotence (1). Phentolamine antagonizes adrenaline-, noradrenaline- and phenylephrine-induced contractions of corpus cavernosum penis (CCP) (2). Prazosin, a selective α1-adrenoceptor antagonist, also attenuates the noradrenaline- and electrical field stimulation-induced contractions of human and rabbit CCP (3); and a new selective α1A-adrenoceptor antagonist, tamsulosin (R-(−)-YM12617), exerts a more potent antagonistic activity than prazosin (4). These results indicate that contractions to catecholamines in the CCP are mediated via the α1-adrenoceptor subtype.

Recently, α1-adrenoceptors have been classified into three receptor subtypes. Morrow and Creese (5) reported that α1-adrenoceptors can be defined as α1A with a high affinity for WB4101 and α1B with low affinity for WB4101. Chloroethylclonidine inactivates the subtype with a low affinity for WB4101 (α1B) (6). Molecular biological studies have also identified α1-adrenoceptor subtypes, with three receptors being cloned and termed α1A (formerly known as α1c), α1B and α1D (formerly also known as α1a or α1a/d) (7–9). It appears that the pharmacological properties of the cloned α1B-receptor are identical to those of the native α1B-adrenoceptor. It has been suggested that the cloned α1A-(α1A)-receptor represents the native α1A-adrenoceptor (10–12), and the receptor in rat aorta represents a functional α1D-adrenoceptor (13). Both α1A- and α1B-adrenoceptor subtypes seem to be present in porcine CCP smooth muscle, and the α1A-adrenoceptor may be the predominant subtype (14). mRNAs for all three cloned receptor subtypes (α1B, α1C, α1D) of α1-adrenoceptor have been identified in human CCP tissues using in situ hybridization, and the α1C- and α1D-adrenoceptor subtypes seem to be predominant (15).

The aim of this study is to pharmacologically and functionally determine the predominant α1-adrenoceptor...
subtype mediating the responses of the rabbit CCP by comparing the effects of selective $\alpha_{1A}$-adrenoceptor antagonists (WB4101, 5-methylurapidil, tamsulosin), a partial $\alpha_1$-adrenoceptor agonist with $\alpha_{1A}$-adrenoceptor antagonistic activity (oxymetazoline) (16) and a relatively selective $\alpha_{1B}$-adrenoceptor antagonist (chloroethylclonidine) on the concentration-response curves to phenylephrine.

MATERIALS AND METHODS

Tension recordings

Male New Zealand White rabbits (3.0–3.5 kg; Charles Rivers, Margate, UK) were killed by an overdose of sodium pentobarbital and exsanguinated. The penis was surgically removed, and the urethra and connective tissues were excised. Two preparations of the CCP were carefully dissected free from the tunica albuginea, and then each cut into three pieces (1.5 x 2 x 6 mm). The tissues were mounted in 5-ml organ baths filled with Krebs-bicarbonate solution of the following composition: 118.4 mM NaCl, 4.7 mM KCl, 1.9 mM CaCl$_2$, 25.0 mM NaHCO$_3$, 1.2 mM MgSO$_4$, 1.2 mM KH$_2$PO$_4$ and 11.7 mM glucose, at 37°C and oxygenated with 95% O$_2$ and 5% CO$_2$. Tissues were set up under 2-g resting tension and the tensions developed following the addition of phenylephrine were measured by means of isometric force transducers (UF1, 57 g sensitivity; Lectromed Ltd., Herts, UK) connected to a PCA-s1 computer (Tandon plc, Reddich, UK) through an analogue to digital converter (Cambridge Electronic Design Ltd., Cambridge, UK). Developed tension was recorded using “CHART” and analyzed using “SPIKE 2” software (Cambridge Electronic Design Ltd.).

Drug administration

Tissues were allowed equilibration periods of 2 hr with several changes of bathing solution. A control cumulative concentration-response curve to phenylephrine was first obtained, and then the tissues were washed with fresh solution several times and allowed to relax to the resting tension over the next 20–30 min. After washing, tissues were incubated with one concentration of WB4101, 5-methylurapidil, oxymetazoline or chloroethylclonidine for 30 min or of tamsulosin for 60 min, before the next phenylephrine administration. Similar procedures were repeated to obtain the concentration-response curves to phenylephrine in the presence of 3 different concentrations of antagonists. Control experiments were performed with identical washing and preincubation procedures but without the addition of $\alpha_1$-adrenoceptor antagonists, and these were used to correct for time-dependent changes in tissue sensitivity during the experiment. All concentration-response curves were obtained in the presence of cocaine (10 $\mu$M) and corticosterone (10 $\mu$M) to inhibit amine uptake and also propranolol (1 $\mu$M) to antagonize $\beta$-adrenoceptors.

Data analyses and statistics

Increases in developed tension to phenylephrine are plotted as a percentage of the maximum increase for each concentration-response curve and expressed, as means±S.E. Individual EC$_{50}$ values were determined, and geometric mean EC$_{50}$ values with 95% confidence limits calculated.

As a measure of antagonist affinity, Schild plots were constructed and pA$_2$ values determined from the intercept on the abscissa (17). If maximum responses were not altered by antagonists and the slopes of Schild plots were similar to unity, pK$_B$ values (−logarithm dissociation constant) were determined from the equation:

$$pK_B = \log (CR - 1) - \log [B]$$

where CR is the concentration-ratio (ratio of the EC$_{50}$ values in the absence and presence of the antagonist) obtained with a concentration [B] of antagonist.

Statistical significance was determined by the two-tailed Student’s t-test for paired data or one-way analysis of variance followed by Bonferroni’s test. A probability of less than 0.05 was considered to be significant.

Drugs

Chloroethylclonidine dihydrochloride, 5-methylurapidil and WB4101 ((2-(2,6-dimethoxy-phenoxyethyl)aminomethyl-1,4-benzodioxane) hydrochloride) were obtained from Research Biochemicals, Inc. (Natick, MA, USA). (−)-Tamsulosin hydrochloride (YM617) was a gift from Yamanouchi Europe B.V. (Leiderdorp, Netherlands). Cocaine hydrochloride, corticosterone 21-acetate, (−)-phenylephrine hydrochloride, oxymetazoline hydrochloride and (±)-propranolol hydrochloride were obtained from Sigma (Poole, UK). All other chemicals were of reagent grade. Corticosterone was dissolved in 50% ethanol. WB4101 was dissolved in distilled water and diluted in Krebs solution. All other drugs were made up in Krebs solution.

RESULTS

Control experiments

Phenylephrine (1 mM) caused a long-lasting contraction, and the phenylephrine-induced contraction was concentration-dependent (Fig. 1). Repeating concentration-response curves to phenylephrine did not result in any significant change in either EC$_{50}$ values or maximum responses (Fig. 2). In control experiments (n=7), the geometric mean EC$_{50}$ value for the first concentration-
response curve [7.4 (95% confidence limits = 4.4 – 12.6) 
μM] to phenylephrine was almost identical to those of 
subsequent curves, the EC₅₀ value being 10.3 (6.4 – 16.6) 
μM for the fourth control concentration-response curve. 
Similarly, the maximum responses were also unchanged 
(first curve = 3.64 ± 0.83 g, fourth curve = 4.00 ± 0.58 g).

**Competitive antagonists**

WB4101 (30 – 300 nM), 5-methylurapidil (0.1 – 1 μM) 
and tamsulosin (1 – 10 nM) behaved as competitive an-
tagonists, shifting the concentration-response curve to 
phenylephrine to the right without altering maximum 
responses (Fig. 3, Table 1). Schild plots for all three an-
tagonists (Fig. 4) had slopes not significant from unity 
(Table 2) and had X-axis intercepts of 8.05, 7.78 and 9.34 
for WB4101, 5-methylurapidil and tamsulosin, respec-
tively. The shifts of the concentration-response curves 
obtained in individual experiments were also used to cal-

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**Fig. 1.** Typical tracings of phenylephrine-induced contractile response in rabbit corpus cavernosum penis. A: single concent-
tration response, B: cumulative concentration response.

**Fig. 2.** Contractile response to phenylephrine in rabbit corpus 
cavernosum penis obtained from control experiments without any 
antagonists. Each curve shows the first (○), second (●), third (▲) 
and fourth (●) response in the same tissue. Each symbol with a 
vertical bar represents the mean with S.E. of 7 experiments.
calculate mean pK_B values, and the mean values are shown in Table 2.

**Non-competitive antagonists**

Oxymetazoline (1 μM) caused a small contraction that returned to resting tension within 30 min and then behaved as an antagonist of phenylephrine-induced contractions. Oxymetazoline shifted the concentration-response curve to phenylephrine to the right but also reduced the maximum response (Fig. 5A, Table 1). The apparent pK_B value calculated using this one concentration of antagonist was 6.99.

Chloroethylclonidine also produced significant rightward shifts of the concentration-response curves to phenylephrine, causing 6- and 32-fold shifts at 25 and

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>Maximum response (g)</th>
<th>Absence</th>
<th>Presence</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB4101, 30 nM</td>
<td>4.46±1.13</td>
<td>4.19±0.96</td>
<td></td>
</tr>
<tr>
<td>5-Methylurapidil, 1 μM</td>
<td>3.79±0.72</td>
<td>3.39±0.52</td>
<td></td>
</tr>
<tr>
<td>Tamsulosin, 10 nM</td>
<td>3.82±1.02</td>
<td>4.66±0.58</td>
<td></td>
</tr>
<tr>
<td>Oxymetazoline, 1 μM</td>
<td>6.09±0.84</td>
<td>1.76±0.12**</td>
<td></td>
</tr>
<tr>
<td>Chloroethylclonidine, 100 μM</td>
<td>4.82±1.31</td>
<td>4.20±1.14</td>
<td></td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E. of 7 experiments. **P<0.01 vs absence of antagonist.
100 μM, respectively (Fig. 5B). Maximum responses to phenylephrine were not significantly affected by chloroethylclonidine (Table 1).

**DISCUSSION**

At the α₁-adrenoceptors of the rabbit CCP, WB4101, 5-methylurapidil and tamsulosin acted as competitive antagonists with a high affinity for the receptor. The affinity value (pKₐ) of 8.05 for WB4101, however, is significantly lower than those reported at the α₁A-adrenoceptors of rat vas deferens (9.01–9.58) (18–22) and human vas deferens (9.2) (16), but is similar to those obtained for the α₁B-adrenoceptors of the rat spleen (8.4) (19) and guinea pig aorta (8.4; R. Chess-Williams et al., unpublished data), using identical experimental conditions. Similar results are obtained for the radioligand binding studies of 5-methylurapidil; and the values obtained for the α₁B-adrenoceptors of the rat liver and spleen (pKi = 7.56–7.63) (10, 18, 23, 24) are similar to the affinity of 5-methylurapidil in the rabbit CCP (pKₐ = 7.59).

Affinity values (pKₐ) for 5-methylurapidil at functional α₁B-adrenoceptors are significantly higher (8.43–9.10) (18, 20–22) than that obtained from rabbit CCP. Therefore, the results of WB4101 and 5-methylurapidil suggest that there may be a functionally predominant α₁B-adrenoceptor population present in the rabbit CCP.

Tamsulosin is an α₁-adrenoceptor antagonist that distinguishes between α₁-adrenoceptor subtypes, being selective for the α₁A-adrenoceptor subtype (pKi = 10.64) over the α₁B subtype (pKi = 9.06) in the radioligand binding study on the cloned receptor (25). The affinity of tamsulosin for the α₁-adrenoceptor of the rabbit CCP (pKₐ = 9.21) is similar to the value for the α₁B-adrenoceptor in the radioligand binding study on the cloned receptor and is similar to the value of 9.0 (pKₐ) obtained for the functional α₁B-adrenoceptors of the rat spleen (26). In functional studies, tamsulosin has been shown to exert non-competitive effects at the α₁A-adrenoceptors of the rat and human vas deferens (27), reducing maximum responses to phenylephrine and producing Schödl plots with slopes greater than unity. No such non-competitive effects were observed on the rabbit CCP. The results of tamsulosin in the rabbit CCP also support the suggestion of a functionally predominant α₁B-adrenoceptor population in the rabbit CCP.

Oxymetazoline caused a small contraction and acted as an antagonist of phenylephrine-induced contractions in the rabbit CCP. Oxymetazoline acts as a partial agonist at α₁-adrenoceptors with low relative efficacy as compared to noradrenaline (28) and does not cause any agonistic effect in guinea pig spleen (21). In human vas deferens, oxymetazoline does not cause any agonistic effect and acts as an antagonist of phenylephrine-induced contraction (16). Therefore, oxymetazoline may have partial agonistic activity followed by an antagonistic one in rabbit CCP. The affinity of oxymetazoline in rabbit CCP (apparent pKₐ = 6.99) is lower than those reported for the α₁A-adrenoceptors in radioligand binding studies in rat salivary gland (pKi = 8.06, 8.49) and similar to those values reported for α₁B-adrenoceptors in rat liver (pKi = 6.63, 6.68) (10, 29). From the data using competitive antagonists and oxymetazoline, it appears that the responses to phenylephrine in rabbit CCP are mediated predominantly via a population of α₁B-adrenoceptors, and this conclusion is supported in experiments performed with chloroethylclonidine.

Chloroethylclonidine caused a parallel shift without altering the maximum response in rabbit CCP. Early studies with chloroethylclonidine suggested that this compound was an irreversible antagonist that selectively inactivated the α₁B- but not the α₁A-adrenoceptor subtype (6). Recent studies, however, have demonstrated the ability of chloroethylclonidine to inactivate all the α₁-adrenoceptor
subtypes to some extent (30). The \(\alpha_{1B}\) and \(\alpha_{1D}\)-adrenoceptor subtypes appear to be equally sensitive to chloroethylclonidine, whilst higher concentrations or longer incubation periods are required to inactivate the \(\alpha_{1A}\) subtype. We have previously shown that the same concentration (25 \(\mu\)M) of chloroethylclonidine results in an antagonism of functional \(\alpha_{1B}\) but not \(\alpha_{1A}\)-adrenoceptor-mediated responses (19). The effect observed with chloroethylclonidine is, therefore, indicative of the presence of either an \(\alpha_{1B}\) or \(\alpha_{1D}\)-adrenoceptor in rabbit CCP, but the presence of an \(\alpha_{1D}\)-adrenoceptor in rabbit CCP can be ruled out by the low affinity values obtained for WB4101 and tamsulosin. The \(pK_{B}\) values of 8.05 and 9.21 obtained for WB4101 and tamsulosin in the present study are similar to the values of 8.43 and 9.06 obtained for the cloned \(\alpha_{1}\)-adrenoceptor, but they are significantly lower than the values of 9.37 and 10.06 reported for the cloned \(\alpha_{1}\)-adrenoceptor (10, 25). The apparent \(pK_{B}\) value (6.99) obtained for oxymetazoline also supports the conclusion that responses in the rabbit CCP are mediated via an \(\alpha_{1B}\)-adrenoceptor, since the cloned \(\alpha_{1D}\)-adrenoceptor has a tenfold lower affinity for this drug (\(pK_{B}=6.02\)) (10). The data obtained with the competitive antagonists and oxymetazoline can, therefore, only be explained by the presence of a functional \(\alpha_{1B}\)-adrenoceptor in this tissue.

On the rabbit CCP, chloroethylclonidine produced a significant rightward shift of the concentration-response curves to phenylephrine without the reduction of maximum responses. Chloroethylclonidine reduces the maximum responses to noradrenaline in the rat spleen (20, 31), in which the \(\alpha_{1}\)-adrenoceptor subtype is functionally the \(\alpha_{1B}\)-subtype. Therefore, the competitive antagonism of chloroethylclonidine to the phenylephrine-induced contraction suggests that a significant receptor reserve may exist in the rabbit CCP when using phenylephrine.

While the present study shows the \(\alpha_{1}\)-adrenoceptor subtype to predominate in the rabbit CCP, the fact that \(\alpha_{1A}\)-adrenoceptor seems to be the predominant receptor subtype in porcine corpus cavernosum smooth muscle (14) demonstrates that there are species differences in predominance of \(\alpha_{1}\)-adrenoceptor subtypes. In humans, mRNA levels suggest that the \(\alpha_{1A}\) and \(\alpha_{1D}\)-adrenoceptor subtypes are predominantly expressed in corpus cavernosum smooth muscle (15), but studies identifying the pharmacological characteristics of the functional receptor have yet to be performed.

Muramatsu et al. (1990) proposed another subclassification of \(\alpha_{1}\)-adrenoceptor subtypes (\(\alpha_{1H}\): high sensitive, \(\alpha_{1L}\): low sensitive, \(\alpha_{1N}\): neither \(\alpha_{1H}\) nor \(\alpha_{1L}\)) based on the sensitivity to prazosin (32). It is difficult to directly discuss the \(\alpha_{1H}\), \(\alpha_{1L}\) and \(\alpha_{1N}\)-adrenoceptor subtypes in rabbit CCP from the viewpoint of prazosin which we did not use in this study. However, the \(\alpha_{1}\)-adrenoceptor is sensitive, and the \(\alpha_{1L}\) or \(\alpha_{1N}\)-adrenoceptor is insensitive to chloroethylclonidine (32), whereas the \(\alpha_{1}\)-adrenoceptor subtype in rabbit CCP was sensitive to chloroethylclonidine. Furthermore, \(\alpha_{1}\)-adrenoceptors can be subdivided into five subtypes (\(\alpha_{1A}, \alpha_{1B}, \alpha_{1D}, \alpha_{1L}, \alpha_{1N}\)); and the \(\alpha_{1A}, \alpha_{1B}\) and \(\alpha_{1D}\)-adrenoceptor subtypes are categorized into the \(\alpha_{1H}\) group (33). Consequently, it is suggested that the \(\alpha_{1}\)-adrenoceptor in rabbit CCP belongs to the \(\alpha_{1H}\)-subtype consisting of \(\alpha_{1A}, \alpha_{1B}\) and \(\alpha_{1D}\)-adrenoceptors.

In conclusion, our results indicate that the contractions to phenylephrine of rabbit CCP are mediated by the \(\alpha_{1}\)-adrenoceptor subtype. The rightward shift of the concentration-response curve to phenylephrine caused by chloroethylclonidine without altering the maximum response suggests that there is a significant receptor reserve for responses induced by phenylephrine.

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