J. Pharm. Pharmacol. 1993, 45: 922-924
Communicated December 11, 1992

Characterization of the α_1-adrenoceptors of the rat prostate gland

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Abstract—The α_1-adrenoceptor-mediated responses of the rat prostate to phenylephrine have been examined in-vitro. Phenylephrine induced concentration-dependent contractions of the isolated prostate gland which were antagonized by WB4101 (1–30 nM). Schild plot analysis of the antagonism yielded a straight line with a slope not significantly different from unity. The pK_B value of 9·2 was similar to that obtained for WB4101 on the rat vas deferens (9·4) but was greater than that obtained on the rat spleen (8·4). Chloroethylclonidine depressed responses to phenylephrine of the rat spleen but not the prostate or the vas deferens. These results indicate that the rat prostate gland possesses a typical α_{1A}-adrenoceptor similar to that found in the vas deferens.

Although generally thought of as a glandular tissue, the prostate is mainly composed of smooth muscle which receives a dense adrenergic innervation (Bartsch et al 1979; Gosling 1986). Pharmacological analysis of the rabbit prostate gland indicates that the contractile responses of this tissue to catecholamines are mediated solely via a population of α_1-adrenoceptors (Honda et al 1985). In recent years however, it has become clear that α_1-adrenoceptors are not a homogeneous group and can be subclassified into α_{1A} with a high affinity for WB4101 and α_{1B} with a low affinity for WB4101 (Morrow & Creese 1986). These two receptor subtypes have different transduction mechanisms (Han et al 1987a) and are differentially inactivated by the alkylating agent chloroethylclonidine which is selective for the α_{1B}-receptor subtype (Han et al 1987b; Minneman et al 1988). A third α_1-adrenoceptor (α_{1C}) has also been cloned which has a high affinity for WB4101 and is inactivated by chloroethylclonidine (Schwinn et al 1990).

The present study was designed to characterize the α_1-adrenoceptor subtypes mediating contraction of the rat prostate gland, by comparing the effects of WB4101 and chloroethylclonidine on this tissue with those obtained on the rat vas deferens and spleen, tissues in which responses are mediated via α_{1A}- and α_{1B}-adrenoceptors, respectively (Han et al 1987a).

Materials and methods

Male Wistar rats, 200–250 g, were killed by a blow to the head and exsanguinated. Whole prostate glands, vasa deferentia and hemi-spleen (cut longitudinally) were isolated and set up in a Krebs-bicarbonate solution of composition (mM): NaCl 118·4; KCl 4·7; $CaCl_2$ 1·9; $NaHCO_3$ 25·0; $MgSO_4$ 1·2; KH_2PO_4 1·2; glucose 11·7 gassed with 5% CO_2 in O_2 and maintained at 37°C. Tissues were set up under 1 g resting tension and the tension developed by all tissues following the addition of phenylephrine was measured by means of isometric force transducers (Lectromed UF1, 57 g sensitivity) and recorded on a Devices M19 chart recorder.

Drug administration. Tissues were equilibrated for 60 min with several changes of bathing medium. Concentration-response curves to phenylephrine were then obtained in the presence of cocaine (10 μM), corticosterone (10 μM) and propranolol (1 μM).

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Cumulative concentration-response curves were obtained on prostate and spleen; responses to single concentrations (washing between each administration) were obtained on the vas deferens.

Calculation of pA_2 values. Phenylephrine concentration-response curves were obtained in the absence of antagonist and in the presence of WB4101 (1–300 nM). Control experiments were performed with identical washing procedures but without the addition of antagonist, and these were used to correct for time-dependent changes in tissue sensitivity during the course of the experiment.

Incubation with chloroethylclonidine. Tissues were equilibrated for 30 min and a cumulative concentration-response curve obtained to phenylephrine. After washing, tissues were incubated with chloroethylclonidine (25 μM) for 45 min followed by a wash-out every 10 min for 60 min, before repeating the phenylephrine administrations. Due to difficulties in repeating phenylephrine concentration-response curves on the spleen, only one curve was obtained on each tissue.

Data analysis. Increases in developed tension to phenylephrine were plotted as a percentage of the maximum increase for each concentration-response curve. Individual EC50 values were determined and geometric mean EC50 values with 95% confidence limits calculated. Differences in mean EC50 values were analysed using Student's *t*-test applied to individual logarithmic EC50 values.

As a measure of antagonist affinity, pK_B values (−log dissociation constant) were determined from the equation:

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

where CR is the concentration-ratio (ratio of the EC50 values in the presence and absence of the antagonist) obtained with a concentration B of antagonist. Schild plots were also constructed and pA_2 values determined from the intercept on the abscissa (Arunlakshana & Schild 1959). pK_B values for WB4101 in the various tissues were compared using Student's *t*-test.

Materials. WB4101 was obtained as the hydrochloride salt from Research Biochemicals Inc. (Natick, MA). All other drugs were obtained commercially as the hydrochloride salts from Sigma (Poole, Dorset) and were prepared fresh in Krebs-bicarbonate solution. All reagents were of Analar grade.

Results

Antagonism by WB4101. Concentration-response curves to phenylephrine were shifted to the right in all tissues by WB4101 (Fig. 1), whilst maximum responses were not significantly altered. The competitive antagonist, however, had a significantly greater affinity for the receptors of the vas deferens and prostate than of the spleen, as evidenced by the greater shifts in concentration-response curves and hence the greater pK_B values obtained for WB4101 on these tissues (Table 1). Schild plots for all three tissues had slopes not significantly different from unity and the pA_2 values obtained for WB4101 were greater on the vas deferens and prostate than on the spleen (Fig. 2).

Table 1. pA_2 and pK_B values for WB4101 on rat vas deferens, spleen and prostate using phenylephrine as agonist. The mean of n values ± s.e.m. is given and also the slopes of the Schild plots.

Tissue	pK_B	n	pA_2	Slope
Prostate	9·20 ± 0·15	20	9·71	0·95 ± 0·33
Vas deferens	9·44 ± 0·13	25	9·41	1·12 ± 0·33
Spleen	8·40 ± 0·08*	16	8·51	0·91 ± 0·14

* $P < 0·05$ compared with vas deferens or prostate.

Effects of chloroethylclonidine. Incubation with chloroethylclonidine (25 μM) for 45 min followed by 60-min wash-out did not affect the contractile responses of the rat prostate to phenylephrine (Fig. 3). Identical incubation conditions shifted phenylephrine concentration-response curves of the spleen to the right, significantly ($P < 0·001$) increasing EC50 values from 4·2 (1·8–9·7) to 696·2 (357·5–1355·8) μM without altering maximum responses (controls = 0·27 ± 0·03 g, chloroethylclonidine = 0·23 ± 0·03 g). On the vas deferens, identical incubations with chloroethylclonidine had no significant effect on responses to phenylephrine, EC50 values and maximum responses being similar in control (14·8 (6·1–35·9) μM, 1·57 ± 0·18 g) and chloroethylclonidine-treated tissues (19·4 (6·4–58·9) μM, 1·23 ± 0·28 g).

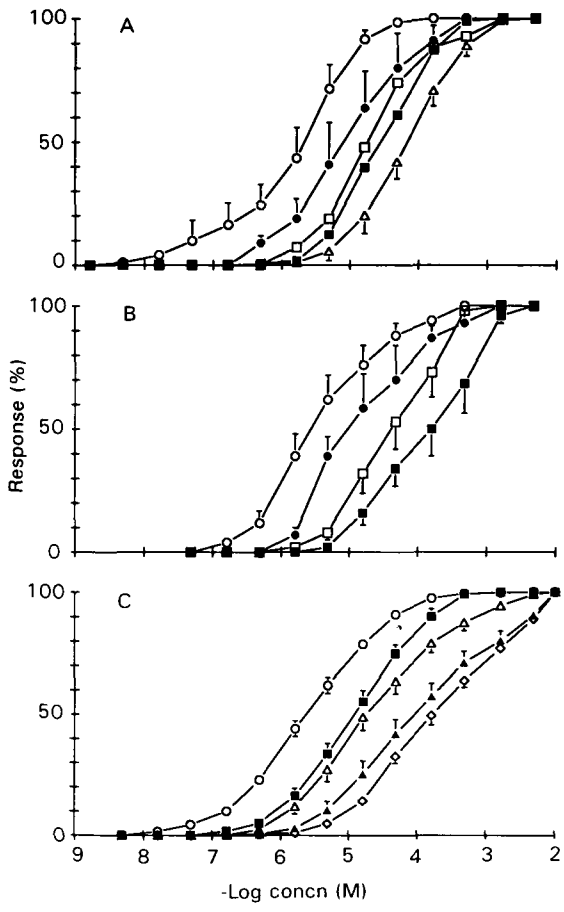

FIG. 1. Concentration-response curves to phenylephrine of: A rat prostate, B vas deferens and C spleen in the absence of antagonist (○) and in the presence of WB4101 at concentrations of 1 nM (●), 3 nM (□), 10 nM (■), 30 nM (△), 100 nM (▲) and 300 nM (◇).

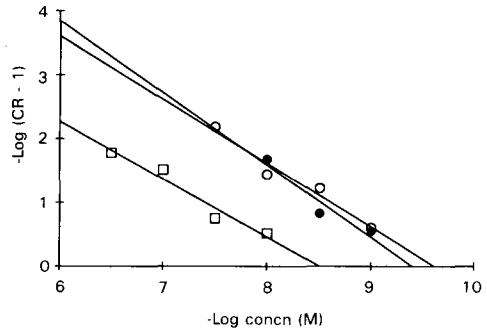

FIG. 2. Schild plots for the antagonism of responses to phenylephrine by WB4101 in rat prostate (●), vas deferens (○) and spleen (□). The slopes of all the lines were similar to unity.

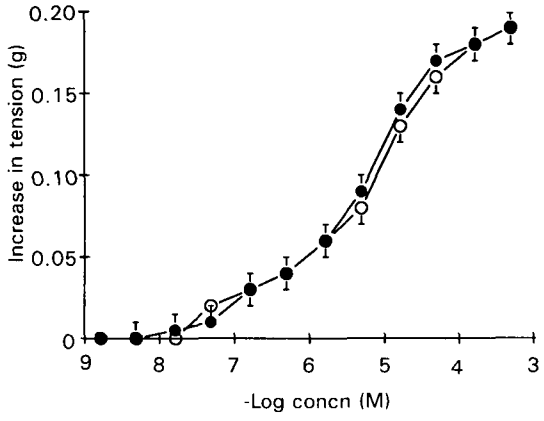

FIG. 3. Concentration-response curves to phenylephrine of rat prostate following control incubation (○) or following incubation with chloroethylclonidine (25 μM) for 45 min (●).

Discussion

The affinity of WB4101, whether expressed as pK_B or pA_2 values, was significantly greater for the vas deferens than for the rat spleen, indicating that the responses of these tissues are mediated via α_{1A}- and α_{1B}-adrenoceptors, respectively. It has been shown in radioligand binding studies that the rat spleen possesses a homogeneous population of α_{1B}-adrenoceptors (Han et al 1987b). The rat vas deferens, however, possesses a mixed α_1-adrenoceptor population, but the responses of this tissue appear to be mediated via the population of α_{1A}-receptors (Han et al 1987a) a conclusion supported in the present study since WB4101 had a high affinity and Schild slopes were similar to unity in this tissue.

The affinity of WB4101 obtained for the rat prostate was similar to that obtained for the vas deferens and significantly higher than that obtained for the spleen, indicating that the responses to phenylephrine in the prostate are mediated predominantly via a population of α_{1A}-adrenoceptors. This was also supported by the experiments with chloroethylclonidine, which irreversibly and selectively inactivates the α_{1B}-adrenoceptor subtype and is an important and widely used tool employed in the study and classification of α_1-adrenoceptor subtypes (Han et al 1987b; Minneman et al 1988). In the present study, chloroethylclonidine depressed contractile responses of the rat spleen, but not the prostate or the vas deferens, again indicating that the prostate and vas deferens possess typical α_{1A}-adrenoceptors which differ from the α_{1B}-adrenoceptors found in the spleen.

The experimental conditions used in this study were those suggested by Furchgott (1977) for the classification of adrenoceptors. Experiments were performed on tissues from animals pretreated with reserpine to prevent any effects from neuronally-released catecholamines and responses were obtained in the presence of inhibitors of amine uptake. Although phenylephrine is a relatively selective agonist at α_1-adrenoceptors, it does have a weak action at β-adrenoceptors in some tissues with a high β-adrenoceptor density (Chess-Williams et al 1990) and responses were therefore obtained in the presence of propranolol. The finding that Schild plots on all tissues gave straight lines with slopes similar to unity confirms that phenylephrine was acting via a single adrenoceptor in these experiments.

Recently, radioligand-binding experiments have been carried out on bovine prostate gland and the majority (90%) of α_1-adrenoceptors found to have a low affinity for WB4101 (Maruyama et al 1992). This suggests that the predominant receptor in the bovine prostate (α_{1B}) differs from that of the rat prostate (α_{1A}). Furthermore, radioligand-binding and isolated tissue experiments have shown the human prostate gland to contain an α_1-adrenoceptor, which appears to be the recently cloned α_{1C}-adrenoceptor (Marshall et al 1992). This receptor had a high affinity for WB4101 ($pA_2 = 9{\cdot}0$) but was inactivated by chloroethylclonidine, unlike the receptor we have identified in the rat prostate. Species differences in α_1-adrenoceptor subtype populations have also been identified in vascular tissues (Tian et al 1990).

Currently, α_1-adrenoceptor antagonists (prazosin, phenoxybenzamine) which do not distiguish between the α_1-adrenoceptor subtypes are used in the treatment of bladder outlet obstruction associated with benign prostate hyperplasia (Hedlund et al 1983; Cain 1986) but the demonstration of various receptor subtypes may allow the development of α_1-adrenoceptor antagonists selective for the prostate gland.

The present study therefore demonstrates that the contractile responses of the rat prostate gland to phenylephrine are mediated via a typical α_{1A}-adrenoceptor which is similar to that found in the vas deferens.

Helen O'Brien and Andrea Jackson are in receipt of studentships from the Medical Research Council and the British Heart Foundation, respectively.

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