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Adrenoceptor blocking properties of atropine-like agents anisodamine and anisodine on brain and cardiovascular tissues of rats

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1 The cholinoceptor antagonists anisodamine and anisodine are widely used in the People's Republic of China for the management of acute circulatory shock but the mechanism of their beneficial effects is not fully known; we therefore investigated if these agents possessed adrenoceptor blocking properties.

2 The antagonistic effect of anisodamine and anisodine against the specific binding of the α_1-adrenoceptor ligand [^{3}H]-WB-4101 to cardiac and brain tissue membrane preparations and against the effects of phenylephrine on isolated aortic strips and left atria of rats were compared with classical muscarinic receptor and adrenoceptor blocking agents.

3 Both anisodamine and anisodine possessed α_1-adrenoceptor blocking properties; the order of potency of various agents in displacing the binding of [^{3}H]-WB-4101 to receptors and in antagonizing the effects of phenylephrine on aortic strips and left atria was: prazosin > atropine > anisodamine > scopolamine > anisodine.

4 It is concluded that both anisodamine and to a lesser extent anisodine possess α_1-adrenoceptor blocking properties; this antagonistic activity of anisodamine may contribute to its salutary effects on the microcirculation. However, it is unlikely that anisodine produces a significant adrenoceptor blockade in the clinically used dose-range.

Introduction

The cholinoceptor antagonists anisodamine and anisodine are widely used as cardiovascular agents in the People's Republic of China (Peking Friendship Hospital, 1975; Xiu *et al.*, 1982); anisodamine is used to maintain the competence of the microcirculation and anisodine is preferred to scopolamine for preanaesthetic medication in patients with circulatory shock. Anisodamine is related to atropine and possesses a hydroxyl group at position 6 of the tropane radical of atropine (Chinese Academy of Medical Sciences & Peking Friendship Hospital, 1975); anisodine possesses a hydroxyl attached to the assymetric carbon of scopolamine (Coordinating Group, 1976) (Figure 1).

The mechanism of the salutary effects of these agents on the microcirculation is not understood. Xiu *et al.* (1982) proposed that anisodamine improves microcirculation by inhibiting thromboxane synthesis and aggregating granulocytes and platelets. On the other hand, atropine-like agents have long been known to possess adrenergic antagonistic properties (Bussell, 1940; Burn & Dutta, 1948; Fleckenstein, 1952; Furchgott, 1955); recent studies have confirmed these observations and characterized these agents as specific α_1-adrenoceptor antagonists (Abraham *et al.*, 1981; Cantor *et al.*, 1983). α-Adrenoceptor antagonism can be beneficial in circulatory shock (Nickerson, 1962). However, whether or not anisodamine and anisodine possess adrenergic inhibiting activities is not known. In the present study we have compared the effects of anisodamine and anisodine with those of atropine, scopolamine and prazosin on the binding of α-adrenoceptor specific radioligands to brain and cardiac tissue membrane preparations, as well as on the responses of aortic strips and isolated left atria to phenylephrine in rats.

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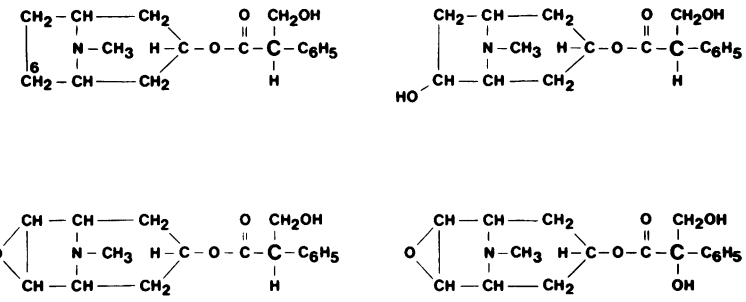

Figure 1 Chemical structures of atropine, anisodamine, scopolamine and anisodine.

Methods

Animals

Male Sprague-Dawley rats (Charles River, St. Constant, Quebec, Canada) weighing between 225–325 g were maintained on Purina rat diet and tap water *ad libitum*. Animals were decapitated and brains, hearts and thoracic aortae removed for the present studies.

Ligand-receptor binding

[^{3}H]-WB-4101 and [^{3}H]-clonidine were used as radioligands for α_1- and α_2-adrenoceptors respectively. Membrane preparations for the receptor assay of the heart were made as follows (Benfey *et al.*, 1983): ventricles were homogenized with a Polytron homogenizer in 15–20 ml ice-cold 10 mM Tris-HCl-buffer (pH 7.8); the homogenate diluted with an equal volume of 1 M KCl, left on ice for 10 min and then filtered through 8 layers of cheese cloth and centrifuged at 48,000 *g* for 10 min; the pellet was suspended in 40 ml of 50 mM Tris-HCl buffer (pH 7.8), recentrifuged at 48,000 *g* and this pellet suspended in sufficient buffer to yield membrane preparations from 1 g tissue in 80 ml suspension. Membrane preparations from brain were made after the removal of cerebella (U'Prichard *et al.*, 1979). Briefly, the tissue was homogenized in 20 ml of ice-cold 50 mM Tris-HCl buffer (pH 7.8) with a Polytron homogenizer, centrifuged twice at 48,000 *g* for 10 min with an intermediate rehomogenization and the final pellet suspended in 4.9 volumes of 50 mM Tris-HCl buffer (pH 7.8).

In the initial studies, the binding of [^{3}H]-WB-4101 to heart and brain membranes, in the absence and in the presence of 10 μM phentolamine, and the binding of [^{3}H]-clonidine to brain membrane preparations, in the absence and the presence of 10 μM noradrenaline, were determined at increasing concentrations of radioligands; the specific binding was the difference between the binding in the absence and the presence of the competitors. Effects of various test agents on the specific binding were determined at 0.2 nM [^{3}H]-WB-4101 and 1 nM [^{3}H]-clonidine. Briefly, 0.8 ml heart membrane preparation (0.55 ± 0.02 mg protein) was incubated at 25°C with 0.2 nM [^{3}H]-WB-4101 in a total volume of 1 ml Tris-HCl buffer (pH 7.8) for 20 min; 1 ml of brain membrane preparation (1.3 ± 0.04 mg protein) was incubated at 25°C with 0.2 nM [^{3}H]-WB-4101 or 1 nM [^{3}H]-clonidine in 2 ml buffer for 20 or 30 min, respectively (Abraham *et al.*, 1981). These studies were done in the absence or the presence of 5–6 fold increasing concentrations of atropine, anisodamine, scopolamine, anisodine, prazosin, phentolamine and yohimbine. Each assay was done in triplicate. Reactions were terminated by vacuum filtration through Whatman glass fibre filters (GF/C); filters were rinsed twice with 4 ml Tris-HCl buffer and then placed inside counting vials containing 10 ml Scintiverse (Fisher) for the counting of radioactivity. Proteins were measured according to Lowry *et al.* (1951) with bovine serum albumin as the standard.

IC_{50} (concentration causing 50% inhibition of the specific binding) values were determined from the linear regression of the log probability plots of the displacement curves for each competitor. Apparent K_i values were calculated according to the formula (Cheng & Prusoff, 1973): $K_i = IC_{50}/[1+(C/K_D)]$, where C was the concentration of the ligand; K_D value for [^{3}H]-WB-4101 was 0.4 ± 0.03 nM ($n = 5$) in heart and 0.6 ± 0.02 nM ($n = 5$) in brain membrane preparations; the K_D value for [^{3}H]-clonidine in brain membrane preparations was 7.3 ± 0.31 nM ($n = 5$).

Antagonism against phenylephrine

Two spirally-cut strips (approximately 2 mm wide and 25 mm long) from each thoracic aorta were set up at approximately 2 g tension in 50 ml tissue organ baths filled with Krebs buffer of the following composition (mM): NaCl 117, $NaHCO_3$ 25, KCl 4.7, $CaCl_2$ 1.8, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 10, disodium edetate

0.03. The Krebs buffer was equilibrated with 5% CO_2 plus 95% O_2 and maintained at 37°C. The preparation was washed every 10 to 15 min with fresh buffer for 2 h before the addition of drugs. Cumulative dose-response curves to phenylephrine were determined by increasing its concentration in the bath by a factor of approximately 3; the next higher concentration was added after the response to the previous concentration had reached a plateau (approximately 5 min). Contractions were recorded isometrically by means of Grass force-displacement transducers (FT 03C) on a Grass polygraph. One aortic strip served as the control; to the other strip the antagonist was added 20 min before starting the construction of the dose-response curves. It was established in preliminary experiments that the responses of the two aortic strips from the same animal were similar and that 3–4 dose-response curves could be determined with each pair of strips. A minimum of 60 min elapsed between the construction of any two dose-response curves, during which time preparations were washed every 5–10 min.

In order to determine the effects of various antagonists on the inotropic activity of phenylephrine, the left atrium was excised and divided into two identical strips. Each strip was mounted in a tissue organ bath as described above but at 32°C and at a resting tension of approximately 1 g. The preparation was stimulated at 0.5 Hz, 0.3–1 ms pulse duration and 1.5 times the threshold voltage; 300 nM propranolol was added to both strips in order to minimize effects due to β-adrenoceptor stimulation. The construction of cumulative dose-response curves to phenylephrine was started 30 min after the addition of propranolol and 20 min after the addition of anisodamine, atropine or prazosin to one atrial strip. Only one dose-response curve was determined on each atrial preparation. The next higher (approximately 3 fold) concentration of phenylephrine was added after the response to the previous concentration had reached a plateau (10–20 min).

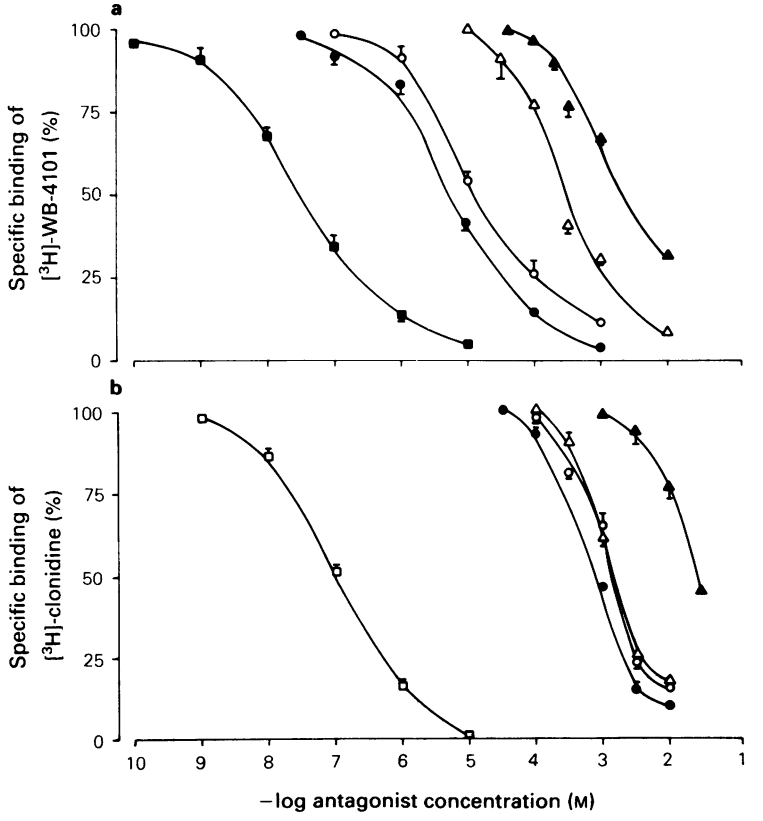

Figure 2 Inhibition of the specific binding of 0.2 nM [^{3}H]-WB-4101 to rat cardiac membrane preparations (a) and of 1 nM [^{3}H]-clonidine to brain membrane preparations (b) by various inhibitors: prazosin (■); atropine (●); anisodamine (○); scopolamine (△); anisodine (▲); yohimbine (□). Each data point is the mean, with vertical lines showing s.e., of 4–5 experiments in triplicate.

Dose-response curves on aortic strips were used for the calculation of pA_2 values (negative log molar concentration of the antagonist causing a two fold shift of the dose-response curve; Arunlakshana & Schild, 1959); the antagonist potency on the atrial tissue was based on the dissociation constant (K_B; Besse & Furchgott, 1976), where K_B equalled the molar concentration of the antagonist divided by the dose-ratio minus 1. Because of the low antagonist potency of anisodine on the aorta and the low potency as well as depressant effects of scopolamine and anisodine on atria, the pA_2 and K_B values of these agents could not be derived.

Statistics

Group means were compared by one-way analysis of variance followed by comparisons of each pair in the group (Bonferroni); a probability of less than 0.05 was assumed to denote a significant difference. Throughout this paper, means ± s.e. are presented.

Chemicals

The following agents were received as gifts: anisodamine and anisodine (Beijing Drug Company, Beijing, the People's Republic of China), phentolamine mesylate (Ciba-Geigy, Dorval, Quebec, Canada), prazosin (Pfizer, Kirkland, Quebec, Canada). The following agents were purchased: [^{3}H]-WB-4101 ([2,6-dimethoxyethyl] aminomethyl-1,4-benzodioxane) (19.8 Ci mmol^{-1}) and [^{3}H]-clonidine (28.3 Ci mmol^{-1}) from New England Nuclear, Boston, Massachusetts, U.S.A.; atropine from BDH, Montreal, Quebec, Canada; (−)-noradrenaline bitartrate dihydrate from Calbiochem, San Diego, California, U.S.A.; phenylephrine hydrochloride from K&K Laboratories, Plainview, New York, U.S.A.; yohimbine hydrochloride from Aldrich Chemicals, Milwaukee, Wisconsin, U.S.A.; (±)-propranolol from Sigma Chemical Company, St. Louis, Missouri, U.S.A.

Results

Displacement of α-adrenoceptor ligands

At 0.2 nM, the specific binding of [^{3}H]-WB-4101 (fmol mg^{-1} protein) was 13.2 ± 1.2 to cardiac and 10 ± 1.3 to brain membrane preparations; the binding of 1 nM [^{3}H]-clonidine to brain membrane preparations was 8.3 ± 0.7 fmol mg^{-1} protein. Displacement curves of [^{3}H]-WB 4101 binding by various agents (Figure 2a) yielded the following order of potency (Table 1): prazosin > phentolamine > atropine > anisodamine > scopolamine > anisodine; these agents were much more effective in displacing the binding of the α_1-adrenoceptor ligand WB-4101 (Figure 2a) than of the α_2-adrenoceptor ligand clonidine (Figure 2b).

Antagonism of the effects of phenylephrine on aortic strips

Various antagonists caused a parallel shift to the right of the dose-response curves to phenylephrine. The

Table 1 Inhibition of [^{3}H]-WB-4101 (0-.2 nM) and [^{3}H]-clonidine (1 nM) binding to rat cardiac and brain membrane preparations by muscarinic receptor and adrenoceptor blocking agents

Agents	*[^{3}H]-WB-4101 binding*		*[^{3}H]-clonidine binding*
	Heart	*Brain*	*Brain*
	*pK$_i$**		
Atropine	5.40 ± 0.05	5.46 ± 0.08	3.87 ± 0.11
Anisodamine	5.06 ± 0.07	5.06 ± 0.11	3.02 ± 0.06
Scopolamine	3.88 ± 0.07	3.56 ± 0.09	2.92 ± 0.16
Anisodine	2.85 ± 0.22	2.63 ± 0.04	1.61 ± 0.30
Prazosin	9.10 ± 0.18	8.64 ± 0.06	5.20 ± 0.31
Phentolamine	7.47 ± 0.23	Not done	Not done
Yohimbine	Not done	5.89 ± 0.12	7.26 ± 0.36

Data show mean ± s.e., $n = 4-5$.

*pK$_i$ is the negative molar concentration of K_i, where $K_i = IC_{50}/[1 + (C/K_D)]$, C is the concentration of the radioligand, and K_D the dissociation constant; K_D values for [^{3}H]-WB-4101 were 0.4 ± 0.3 nM ($n = 5$) in cardiac and 0.6 ± 0.02 nM ($n = 5$) in brain tissue and K_D value for [^{3}H]-clonidine was 7.3 ± 0.31 nM ($n = 5$) in brain tissue. Significant ($P < 0.05$) differences in mean pK$_i$ values are as follows: each mean in column 2 differs from all other means in that column (WB-4101 binding to heart); each mean in column 3 differs from all other means in that column; each mean in column 4 differs from all other means in that column; pK$_i$ values in columns 2 and 3 for none of the agents differ from each other; for each agent, its pK$_i$ in column 4 differs from its pK$_i$ values in columns 2 and 3.

Table 2 Antagonism of the effects of phenylephrine on rat aortic strips and left atria *in vitro*

Antagonists	*Aortic strips* (pA_2)	*Left atria* (pK_B)
Atropine	5.4 ± 0.06	5.2 ± 0.09
Anisodamine	4.7 ± 0.13	4.7 ± 0.07
Scopolamine	3.5 ± 0.06	Not done
Prazosin	9.6 ± 0.32	8.5 ± 0.10

Data show mean ± s.e.; $n = 4-5$.
pA_2 is the negative molar concentration of the antagonist that caused a two fold shift of the dose-response curve; pK_B is the negative log of K_B (the concentration of the antagonist divided by the dose-ratio minus 1). Each pA_2 value in column 2 significantly ($P < 0.05$) differs from all other pA_2 values; each pK_B value in column 3 significantly ($P < 0.05$) differs from all other pK_B values.

order of the antagonistic potencies based on pA_2 values (Table 2) derived from Schild plots (Figure 3) was as follows: prazosin > atropine > anisodamine > scopolamine; because of the low potency of anisodine, the pA_2 for this agent could not be calculated; the slope of the Schild plot for prazosin was 0.96 but for other agents it was 0.86–0.88.

Antagonism of the effects of phenylephrine on left atria

The maximal inotropic effect of phenylephrine was only 60–70% of that produced by noradrenaline. Prazosin, atropine and anisodamine at concentrations approximately 10 times their IC_{50} values (concentrations inhibiting the binding by 50%) produced comparable shifts of the dose-response curves to phenylephrine (Figure 4); K_B values yielded the following order of potency: prazosin > atropine > anisodamine. Because of the low antagonistic activities and myocardial depressant effects of scopolamine and anisodine at millimolar concentrations, K_B values for these agents could not be calculated.

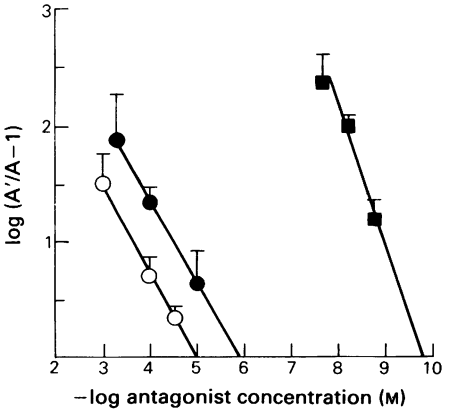

Figure 3 Schild plots of data from rat aortic strips. Determinations of dose-response curves to phenylephrine were started 20 min following the addition of anisodamine (○), atropine (●) and prazosin (■). Each data point is the mean, with vertical lines showing s.e., of 4–5 experiments.

Relationship between displacement of binding and antagonism of the effects of phenylephrine

A linear relationship was found to exist between the antagonistic potencies of these agents against phenylephrine on aortic strips (Figure 5a) and left atria (Figure 5b) and their potencies as competitors for the specific binding of [^{3}H]-WB-4101 to cardiac membrane preparations (Table 1).

Discussion

The main purpose of this study was to find out if the muscarinic receptor blocking agents anisodamine and anisodine, which are acclaimed in the People's Republic of China as beneficial in patients with circulatory shock (Peking Friendship Hospital, 1975; Coordinating Group, 1976; Xiu *et al.*, 1982) possess adrenoceptor antagonistic properties like atropine and scopolamine (Cantor *et al.*, 1983).

We used established techniques for a quantitation of the interaction between atropine-like agents and α-adrenoceptor radioligands (Abraham *et al.*, 1981; Cantor *et al.*, 1983) and for estimating the antagonism against phenylephrine on isolated rat aortic strips (Ruffolo *et al.*, 1982; Digges & Summers, 1983) and left atria (Benfey *et al.*, 1979). The K_i values for the displacement of [^{3}H]-WB-4101 binding by atropine and scopolamine determined in the present study are in close agreement with values obtained by others (Cantor *et al.*, 1983). Although rat aorta does contain both α_1- and α_2-adrenoceptors (Ruffolo *et al.*, 1982), the effects of phenylephrine are almost entirely due to a stimulation of α_1-adrenoceptors (Digges & Summers, 1983). Likewise, phenylephrine has been used as the agonist of choice for a study of α_1-adrenoceptor-mediated effects on the left atria of rats (Benfey *et al.*, 1979). Thus we have confirmed the α_1-adrenoceptor blocking properties of atropine and scopolamine (Cantor *et al*, 1983) and have shown this to be true for anisodamine and anisodine. The conclusion that the adrenergic inhibitory activities of these agents are caused by a relatively specific blockade of α_1-adrenoceptors is supported by the observation that anisodamine caused a significantly greater dis-

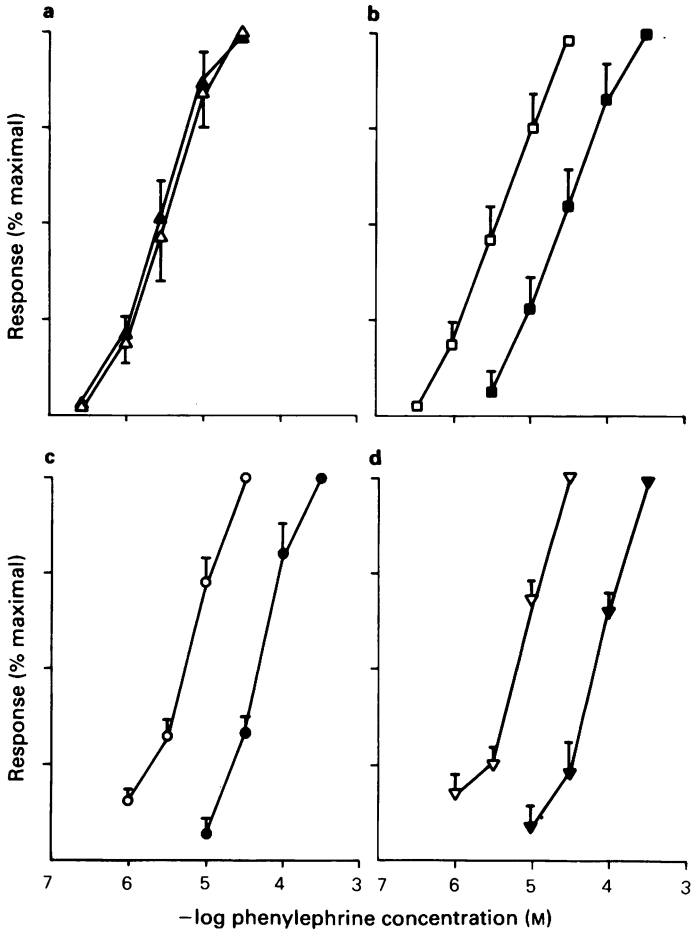

Figure 4 Effect of various antagonists on the dose-response curve to phenylephrine on isolated left atria of rats. (a) Responses of the two strips from the same atrium; (b) control (□) and in the presence of 2×10^{-8} M prazosin (■); (c) control (○) and in the presence of 1.5×10^{-4} M anisodamine (●); (d) control (▽) and in the presence of 6×10^{-5} M atropine (▼). Each curve is derived from 4–5 separate experiments and data points are the means with vertical lines showing s.e.

placement of the α_1-adrenoceptor specific radioligand [^{3}H]-WB-4101 than of the α_2-adrenoceptor specific radioligand [^{3}H]-clonidine (U' Prichard *et al.*, 1979; Abraham *et al.*, 1981).

It is of interest that the order of α_1-adrenoceptor antagonistic potencies of various agents determined in the present study corresponds to their muscarinic receptor blocking potencies, namely, atropine > anisodamine > scopolamine > anisodine (Chinese Academy of Sciences & Peking Friendship Hospital, 1975; Coordinating Group, 1976; Cantor *et al.*, 1983). However, all these agents are far less potent antagonists of adrenoceptors than of muscarinic receptors, which has been previously demonstrated for several atropine-like agents (Cantor *et al.*, 1983). The existence of a positive correlation between the antagonistic potencies of various muscarinic receptor blocking agents, against receptor-ligand binding and against phenylephrine effects, suggests that a blockade of pharmacological effects is primarily caused by an antagonism of α_1-adrenoceptors, as has been found for benzodioxane derivatives (Kapur *et al.*, 1979). It should, however, be pointed out that the slopes of Schild plots for atropine, anisodamine (Figure 2) and scopolamine ranged between 0.86 to 0.88 and were not unity as required for a rigorous application of this

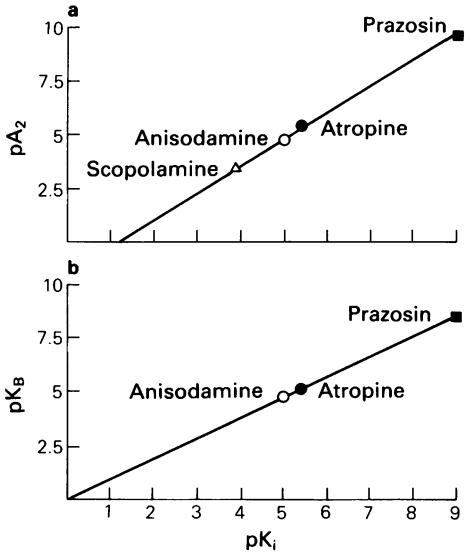

Figure 5 The relationship between the antagonism by prazosin, atropine, anisodamine and scopolamine of the effects of phenylephrine on aortic strips (a) and left atria (b) of rats and their effects on the specific binding of [^{3}H]-WB-4101 to rat cardiac membrane preparations.

technique; this could be due to some non-specific or uncharacterized effects of the antagonists, but the pA_2 values determined by us may still serve as a fair approximation of their relative antagonistic potencies on aortic strips.

Although the present studies demonstrate that anisodamine and to a lesser extent anisodine possess α_1-adrenoceptor blocking properties, they by no means prove that their salutary effects in circulatory shock are the result of this property. Xiu *et al.* (1982) attributed the beneficial effects of anisodamine in shock to its ability to inhibit the metabolism of arachidonic acid. However, these effects were observed only at millimolar concentrations, which may not be easily achieved in clinical practice even following excessively high doses of anisodamine (Peking Frienship Hospital, 1975). Using rat pleural polymorphonuclear leukocytes as a model (Yue *et al.*, 1983), we found that inhibition of arachidonic acid metabolism was observed only when atropine, anisodamine and scopolamine were used at millimolar concentrations and the incubation period was extended to 30 min (unpublished data). In comparison, α_1-adrenoceptor blockade was exerted by micromolar concentrations of anisodamine, which can be clinically achieved and, like other adrenoceptor antagonists (Nickerson, 1962), be effective in maintaining the competence of microcirculation. On the other hand, anisodine is far too weak as an adrenoceptor antagonist for it to be likely that this property makes an important contribution to its clinical usefulness in shock.

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