British Journal of Pharmacology (2000) 131, 788–794

www.nature.com/bjp

Alpha-2 adrenoceptors are present in rat aorta smooth muscle cells, and their action is mediated by ATP-sensitive K^+ channels

[1]Grasiele Fauaz, [1]Teresa Feres, [1]Antonio C.R. Borges & *,[1]Therezinha B. Paiva

[1]Department of Biophysics, Escola Paulista de Medicina, Universidade Federal de São Paulo, 04023-062, São Paulo, SP, Brazil

1 The role of α_2-adrenoceptors in the response of aorta smooth muscle rings to the α_2-adrenoceptors agonists UK 14,304 and clonidine was studied.

2 Stimulation by 1–10 nM UK 14,304 caused dose-dependent relaxant responses in $BaCl_2$-contracted endothelium-denuded aorta rings, and hyperpolarization in rings with or without endothelium, which were inhibited by yohimbine and glibenclamide, but not affected by prazosin, propranolol, apamin or iberiotoxin. At higher concentrations (10 nM–10 μM) UK 14,304 also induced a depolarizing effect which was potentiated by yohimbine and inhibited by prazosin. These results indicate that UK 14,304 acts on α_2-adrenoceptors at lower concentrations and on both α_1- and α_2-adrenoceptors above 10 nM.

3 In rings, with or without endothelium, noradrenaline had a depolarizing effect which was inhibited by prazosin. Adrenaline did not affect the membrane potential but in the presence of prazosin caused hyperpolarization, which was inhibited by yohimbine and glibenclamide. These results indicate that noradrenaline is more selective for α_1-, whereas adrenaline has similar affinities for α_1- and α_2-adrenoceptors.

4 In aortae with endothelium, L-NNA caused a small depolarization but did not affect the hyperpolarization induced by UK 14,304, indicating that NO is not involved in that response.

5 Glibenclamide induced a small depolarization in aortae, with or without endothelium, indicating that ATP-sensitive K^+ channels may play a role in maintaining the smooth muscle's membrane potential.

6 Our results indicate that, in rat aorta, α_2-adrenoceptors are also present in the smooth muscle, and that these receptors act through small-conductance ATP-sensitive K^+ channels.
British Journal of Pharmacology (2000) **131,** 788–794

Keywords: Rat aorta; membrane potential; alpha-2 adrenoceptor; ATP-sensitive K^+ channel; UK 14,304

Abbreviations: Ad, adrenaline; Apa, apamin; ATP, adenosine triphosphate; CK, cromakalin; Clo, clonidine; EDRF, endothelium-derived hyperpolarizing factor; Gli, glibenclamide; ITX, iberiotoxin; L-NNA, N^{ω}-nitro-L-arginine; NA, noradrenaline; NO, nitric oxide; Prop, propranolol; Pz, prazosin; RMP, resting membrane potential; UK 14,304, 5-bromo-N-(4,5-dihydro-1H-imidazol-2-yl)-6-quinoxalinamine; Yo, yohimbine

Introduction

Although the presence of α_2-adrenoceptors was demonstrated in rat vascular smooth muscle cells by gene expression (Ping & Faber, 1993; Blaxall *et al*., 1994) the physiological role of extrajunctional α_2-adrenoceptors is not fully understood. Since these receptors are located some distance away from adrenergic nerve terminals, it has been suggested that they would not interact with neurally released noradrenaline but rather respond to circulating adrenaline, that would directly regulate vessel tone (Langer & Shepperson, 1982a, b).

According to Ruffolo *et al*. (1982), the α-adrenoceptor of rat aorta has properties of both α_1- and α_2-adrenoceptors, whereas according to Digges & Summers (1983) and Decker *et al*. (1984), rat aorta probably contains only α_1-adrenoceptors and therefore the contracting effect of clonidine results from its action on these receptors. In agreement with the latter authors, Silva *et al*. (1996) showed that the depolarization induced by non adrenergic agonists in rat mesenteric rings increase the agonistic effect of clonidine on α_1-adrenoceptors, which is inhibited by prazosin. These findings are also consistent with those related by Iwanaga *et al*. (1998) showing that α_1-adrenoceptors are the mediators of both the contraction and the relaxant responses induced by clonidine in rat aorta. On the other hand, Shimamura *et al*. (1995) exclude the involvement of α_1-adrenoceptors in the endothelium-dependent relaxation induced by clonidine and UK 14,304 in rat aorta.

Since we have previously shown that, in the rat mesenteric artery, clonidine acts on α_2, but not on α_1-adrenoceptors, causing membrane hyperpolarization (Silva *et al*., 1996), we have now used the same methodology and a more specific α_2-adrenoceptor agonist (UK 14,304) to better characterize this receptor in rat aorta rings. The aim of this work is to compare the pharmacological effects of α_2-adrenoceptors agonists (UK 14,304 and clonidine) with those on the vascular muscle cell membrane potential, both in the absence and in the presence of α_1- and α_2-adrenoceptors antagonists and to determine the role of the endothelium in these responses.

Methods

Animals

Measurements of mechanical responses and of membrane potential were made in aortic rings from females in estrus phase of the cycle normotensive Wistar rats from the Wistar

*Author for correspondence at: Department of Biophysics, Escola Paulista de Medicina, Rua Botucatu 862, 04023-062, São Paulo, SP, Brazil. E-mail: tbpaiva@biofis.epm.br

Institute, Philadelphia, PA, U.S.A., inbred at Escola Paulista de Medicina, SP, Brazil. The rats were 20–30 weeks old and weighed 200–220 g.

Mechanical responses

The animals were decapitated and bled, the thoracic aorta was removed and placed in Krebs-bicarbonate solution of the following composition (in mM): NaCl 122, KCl 5.9, $MgCl_2$ 1.25, $NaHCO_3$ 15, $C_6H_{12}O_6$ 11, $CaCl_2$ 1.25 (pH 7.4). Rings of 1-cm length were cut and mounted in chambers containing 5.0 ml of Krebs-bicarbonate solution under the optimum passive tension of 1 g (Gleason *et al*., 1985). The temperature was kept at $37 \pm 0.5^{\circ}C$ and the solution was bubbled with a gas mixture of 95% O_2 and 5% CO_2. After an equilibration period of 2 h, in which the medium was replaced every 10 min, the isometric contractile responses were recorded with a force-displacement transducer (F-60, Narco) and recorded in a physiograph (DMP-4B, Narco). For experiments with endothelium-denuded arteries, the endothelium was removed by rubbing with a thin plastic rod wrapped in cotton. The presence or absence of a functional endothelium was tested in all preparations by checking whether acetylcholine induced relaxation of the preparations, a response which is characteristic for vessels with an intact endothelium (Furchgott, 1981).

Cumulative concentration-response curves were obtained in the presence and in the absence of endothelium by stepwise increase of the concentration of adrenaline or UK 14,304. In endothelium-denuded rings, cumulative UK 14,304 concentration-response curves were also done in the presence of the α_1-adrenoceptor antagonist prazosin (1 nM), preincubated for 10 min.

For the measurement of relaxant effects, rings without endothelium were precontracted by a maximally effective concentration of $BaCl_2$ (1 mM) and cumulative concentration-response curves to the relaxant effect to UK (1–10 nM) were recorded. To study the effect of α_2-adrenoceptor blockade on the relaxation induced by an α_2-adrenoceptor agonist, yohimbine (100 nM) was applied to aortic rings 10 min before addition of UK 14,304.

Membrane potential

The aortic rings were placed in a 2 ml perfusion chamber and superfused at a rate of 3 ml min^{-1} with Krebs solution (pH 7.4, 37°C, aerated with 5% CO_2–95% O_2). Micropipettes (borosilicate glass capillaries 1B120F-6, World Precision Instruments, WPI) were made by means of a horizontal puller (Narishige model PN3) and filled with 2 M KCl (tip resistance 20–40 MΩ and tip potential <6 mV). The microelectrodes were mounted in Ag/AgCl half-cells on a micromanipulator (Leitz, Leica) and connected to an electrometer (Intra 767, WPI). The aortic rings, with and without endothelium, were initially equilibrated for 2 h under an optimal resting tension of 1.0 g, and the impalements of the smooth muscle cells were made from the adventitial side. The electrical signals were continuously monitored on an oscilloscope (54645A, Hewlett Packard) and recorded in a potentiometric chart recorder (2210, LKB-Produkter AB).

The successful implantation of the electrode was evidenced by a sharp drop in voltage upon entry into a cell, a stable potential (± 3 mV) for at least 1 min after impalement, a sharp return to zero upon exit, and minimal change ($<10\%$) in microelectrode resistance after impalement.

Membrane potential measurements were obtained in Krebs solution before and after stimulation of the vessels with noradrenaline (1 μM), adrenaline (1 μM). UK 14,304 (1 nM) and clonidine (1 nM) in the absence and in the presence of prazosin (1 or 100 nM), yohimbine (1 or 100 nM), propranolol (1 μM), glibenclamide (1 μM), apamin (100 nM), iberiotoxin (10 nM) and N^{ω}-nitro-L-arginine (L-NNA, 30 μM). These measurements were also obtained after stimulation by $BaCl_2$ or cromakalin in the absence or in the presence of UK 14,304 or yohimbine, respectively. The time of contact of the drugs with the preparations before the impalements was 10 min.

Drugs and solutions

Noradrenaline hydrochloride (NA), adrenaline bitartrate (Ad), prazosin (Pz), apamin (Apa), N^{ω}-nitro-L-arginine (L-NNA), yohimbine (Yo), glibenclamide (Gli), DL-propranolol HCl (Prop), clonidine (Clo), cromakalin (CK), $BaCl_2$ and iberiotoxin (ITX) were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. UK 14,304 (5-bromo-N-(4,5-dihydro-1H-imidazol-2-yl)-6-quinoxalinamine) was from Research Biochemicals International, Natick, MA, U.S.A. The inorganic salts were products of the highest analytical grade from Merck Darmstadt.

Stock solutions of (10 mM) yohimbine and (10 mM) UK 14,304 were prepared in saline (0.9% NaCl w v^{-1}) and dimethyl sulphoxide (DMSO), respectively. The maximum concentration of DMSO present in the medium was 0.001%, which did not affect the contractile responses elicited by NA or the hyperpolarizing responses of Ad in the presence of prazosin.

Statistical analysis

All data are expressed as means ± s.e.mean with the number of animals given in parentheses. Statistical comparisons between groups were made with the Student *t*-test. One-way analysis of variance (ANOVA) followed by the Newman-Keuls test was used for the comparisons between different conditions. A probability of $P<0.05$ was considered statistically significant. When more than one impalement was made on the same aortic ring from the same rat, the measurements were averaged and considered as $n=1$.

Results

Mechanical responses

The α_2-adrenoceptor agonist UK 14,304 did not elicit a contractile or relaxant response in aortic rings with endothelium. In endothelium-denuded rings, however, small contractile responses were observed upon stimulation by high concentrations (1 μM) of UK 14,304. These responses were inhibited by the α_1-adrenoceptors antagonists prazosin (1 nM) (Figure 1A).

In endothelium denuded aortic rings pre-contracted by 1 mM $BaCl_2$, UK 14,304 induced concentration-dependent relaxant responses in the range from 1 to 10 nM (Figure 1B), which were inhibited by yohimbine (100 nM) (Figure 1C) or by glibenclamide (1 μM) (not shown).

Membrane potential

The resting membrane potential measured from the adventitial side of aortic rings in the presence of endothelium averaged -52.6 ± 0.5 mV ($n=49$). Similar resting membrane potentials

(−53.9±2.3 mV; $n=12$) were observed in endothelium-denuded rings.

UK 14,304 induced a concentration-dependent hyperpolarizing response (Figures 2A and 3A,B) which was in the same concentration range (0.1 to 3 nM) used to induce relaxant responses in $BaCl_2$ pre-contacted aortic rings (Figure 1B). The hyperpolarization induced by UK 14,304 was also able to revert the depolarizing effect induced by $BaCl_2$ (Figure 2B).

Pre-incubation with the α_2-adrenoceptor antagonist yohimbine (1 nM) significantly reduced the hyperpolarizing response, which was not affected by the α_1-adrenoceptor antagonist prazosin (1 nM) or by the β-adrenoceptor antagonist propranolol (1 μM) (Figure 2C).

However, by increasing the UK 14,304 concentration from 10 nM to 10 μM a concentration-dependent depolarization was observed (Figure 3A). This behaviour resulted from activation of both α_1- and α_2-adrenoceptors since α_2 inhibition by yohimbine increased the depolarizing response, whereas the hyperpolarizing response was enhanced by inhibiting the α_1-adrenoceptors by prazosin (Figure 3A).

To investigate the mechanism involved in the hyperpolarizing responses coupled to α_2-adrenoceptors, K^+ channel blockers were tested. Addition of the Ca^{2+}-dependent K^+ channel blockers apamin (small conductance channel blocker) or iberiotoxin (blocker of large conductance K^+ channels) did

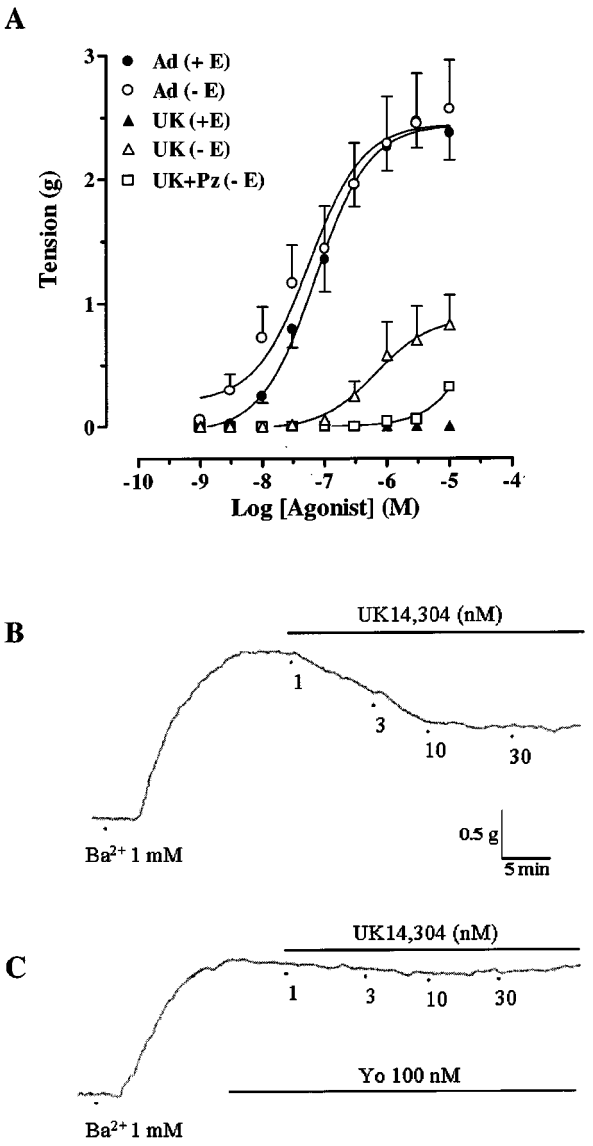

Figure 1 (A) Cumulative concentration-response curves for adrenaline and UK 14,304 (UK) in aorta rings, with (+E) or without (−E) endothelium. The curves for UK (−E) were also obtained in the presence of 1 nM prazosin (Pz). Symbols represent mean increase in tension±s.e.mean ($n=4-10$). (B, C) Representative recordings ($n=6$) showing the concentration-dependent relaxation response elicited by UK 14,304 in endothelium denuded aortic rings pre-contracted by 1 mM $BaCl_2$ in the absence (B) and in the presence (C) of 100 nM yohimbine (Yo).

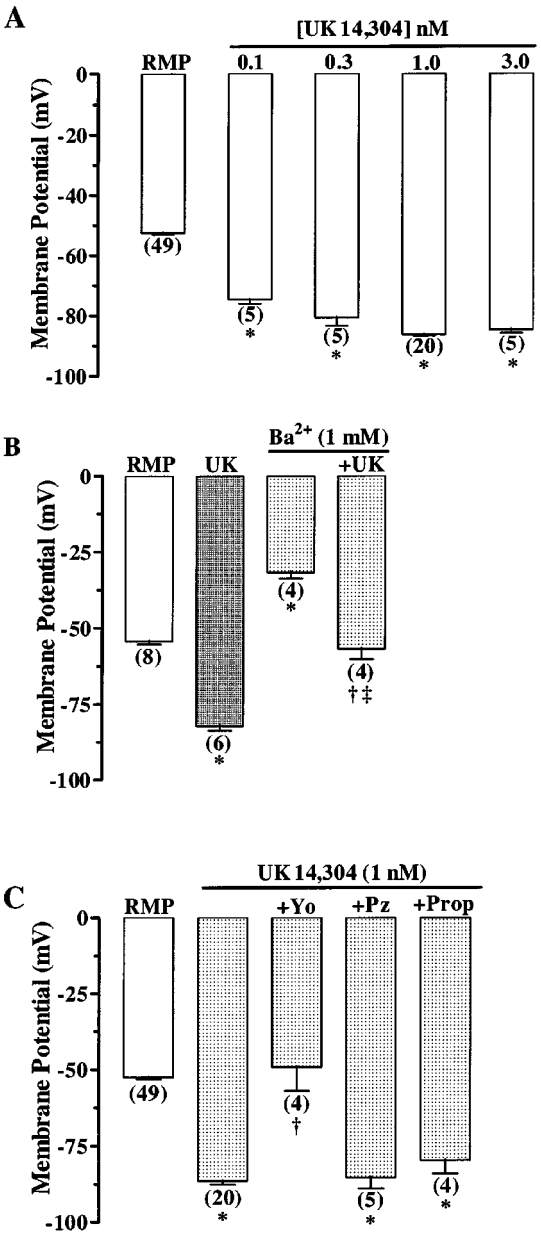

Figure 2 (A) Membrane potential measured in intact rings of aortae (with endothelium), in the absence (RMP), and in the presence of different UK 14,304 concentrations. (B) Membrane potential measured in rings without endothelium, in the absence (RMP) and in the presence of 1 nM UK 14,304 (UK), 1 mM $BaCl_2$ and Ba^{2+} plus 1 nM UK 14,304. (C) Membrane potentials measured in intact rings (with endothelium), in the absence (RMP) and in the presence of 1 nM UK 14,304 (UK) and UK plus 1 nM yohimbine (Yo) or 1 nM prazosin (Pz) or 1 μM propranolol (Prop). For each aortic ring obtained from individual rats (the number of which is shown in parentheses below the bars), 5 to 12 cells were impaled, and the averages of the respective measurements were used to obtain the mean±s.e.mean. *$P<0.05$ versus respective RMP (Newman-Keuls test). †$P<0.05$ versus the membrane potential measured in the presence of UK (Newman-Keuls test). ‡$P<0.05$ versus the membrane potential measured in the presence of $BaCl_2$ (Newman-Keuls test).

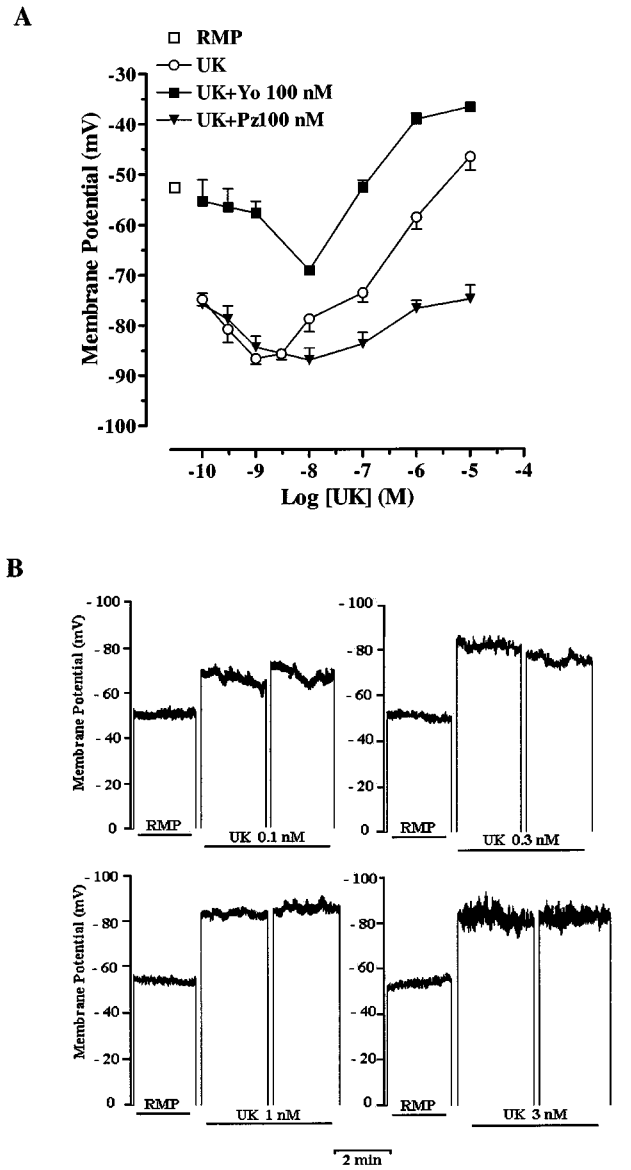

Figure 3 (A) Concentration-response curves for the hyperpolarization induced by UK 14,304 (UK) on intact aortic rings from NWR in the absence (RMP) and in the presence of 100 nM yohimbine or 100 nM prazosin. Values are means ± s.e.mean; $n = 5$ in each group. (B) Representative experimental trace of the record of smooth muscle membrane potential from rat aortic rings showing the resting membrane potential (RMP) and the hyperpolarization induced by different UK concentrations indicated by the horizontal bars.

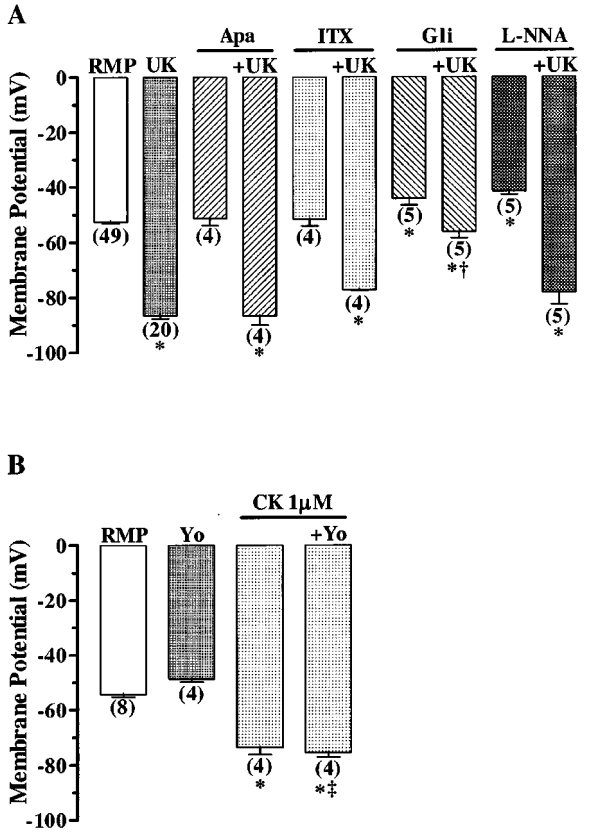

Figure 4 (A) Membrane potential measured in intact rings of aortae (with endothelium), in the absence (RMP) and in the presence of 1 nM UK 14,304 (UK); UK plus 100 nM Apamin (Apa), UK plus 10 nM Iberiotoxin (ITX), UK plus 1 μM glibenclamide (Gli) and UK plus 30 μM L-NNA. (B) Effect of 1 nM yohimbine (Yo) on the hyperpolarization induced by 1 μM cromakalin (CK). For each aortic ring obtained from individual rats (the number of which is shown in parentheses below the bars), 5 to 12 cells were impaled, and the averages of the respective measurements were used to obtain the mean ± s.e.mean. $^*P < 0.05$ versus respective RMP (Newman-Keuls test). †$P < 0.05$ versus the membrane potential measured in the presence of UK (Newman-Keuls test). ‡$P < 0.05$ versus the membrane potential measured in the presence of yohimbine (Newman-Keuls test).

not affect the resting membrane potential (Figure 4A). On the other hand, glibenclamide, a blocker of ATP-sensitive K^+ channels, depolarized the smooth muscle cells in rings with or without endothelium (Figure 4A, Table 1).

The hyperpolarizing responses induced by 1 nM UK 14,304 (-86.6 ± 1.1 mV; $n = 20$) were totally blocked by 1 μM glibenclamide, whereas neither apamin nor iberiotoxin inhibited these responses (Figure 4A).

To evaluate the participation of endothelium dependent relaxing factor (EDRF) in the hyperpolarizing response induced by UK 14,304, endothelium intact aortic rings were pretreated (for 10 min) with the NO synthase inhibitor L-NNA (30 μM). This treatment caused a reduction of the resting membrane potential, but the responses to UK 14,304 were not affected (Figure 4A).

To verify the yohimbine inhibitory effect specificity on α_2-adrenoceptors or on ATP-sensitive K^+ channels, the hyperpolarizing response induced by cromakalin, an opener of ATP-sensitive K^+ channels was tested. Figure 4B shows that rings pre-incubated with yohimbine did not inhibit the hyperpolarizing response induced by cromakalin.

To reinforce our study, another α_2-adrenoceptor agonist, clonidine, was also tested. This agonist induced concentration-dependent hyperpolarization (from 0.1 to 3 nM), which was also antagonized by yohimbine and glibenclamide (Figure 5A).

Noradrenaline had a depolarizing effect (from -52.6 ± 0.5 to -39.31 ± 1.4 mV), which was abolished in the presence of the α_1-adrenoceptor antagonist prazosin (1 nM, -54.3 ± 1.7 mV). On the other hand, adrenaline, which does not have selectivity to α_1- or α_2-adrenoceptors, did not cause significant change in the resting membrane potential (-55.5 ± 1.8 mV), but a hyperpolarizing response (-65.4 ± 2.7 mV) was observed when α_1-adrenoceptor stimulation was excluded by the presence of prazosin (Figure 5B). This hyperpolarizing response was also inhibited by yohimbine and glibenclamide (Figure 5B).

Table 1 Effect of UK 14,304, glibenclamide (Gli), apamin (Apa) and L-NNA on the membrane potential of aorta rings with and without endothelium

	Membrane potential (mV)			
	With endothelium	n	*Without endothelium*	n
RMP	−52.6±0.5	49	−53.9±2.3	12
UK 14,304 (1 nM)	−86.6±1.1*	20	−82.3±0.5*	12
Gli (1 μM)	−44.3±2.3*	05	−45.1±2.9*	04
UK + Gli	−56.3±2.3†	05	−63.0±2.8*†	04
Apa (100 nM)	−51.5±2.5	04	−51.1±1.9	04
UK + Apa	−86.7±3.2*	04	−83.0±2.7*	04
L-NNA (30 μM)	−41.6±1.3*	05	−54.6±1.4	04
UK + L-NNA	−78.4±4.3*	05	−78.8±2.2*	04

Values are given as mean±s.e.mean. RMP, resting membrane potential. *n*, number of animals. *$P<0.05$ versus RMP (Newman-Keuls test). †$P<0.05$ versus UK (Newman-Keuls test).

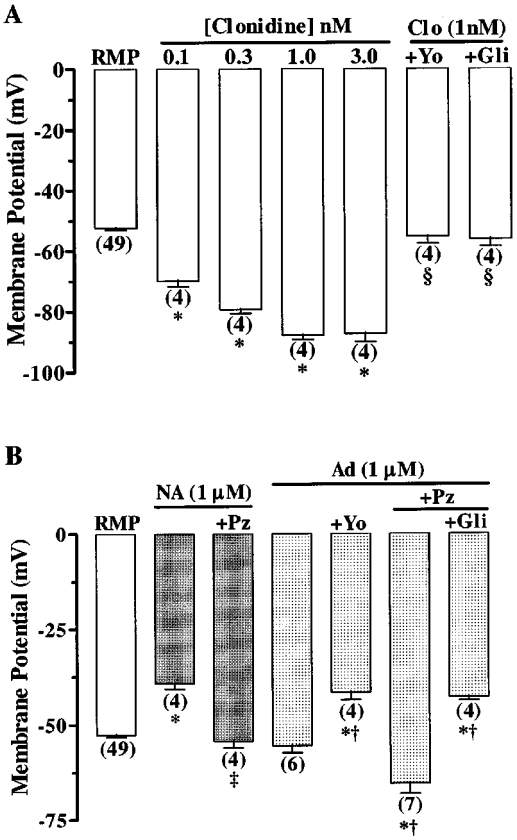

Figure 5 (A) Measurements of membrane potential obtained in intact rings of aortae, stimulated by stepwise increase of the clonidine concentration. The effects of 1 nM yohimbine (Yo) and 1 μM glibenclamide (Gli) on the hyperpolarization elicited by 1 nM clonidine (Clo) are also shown. (B) Membrane potential measured in intact aortae rings (with endothelium) in the absence (RMP) and in the presence of 1 μM noradrenaline (NA); NA plus 1 nM prazosin (Pz); 1 μM adrenaline (Ad); Ad plus 1 nM yohimbine (Yo); 1 nM Pz or Pz plus 1 μM (Gli). For each aortic ring obtained from individual rats (the number of which is shown in parentheses below the bars), 5 to 12 cells were impaled, and the averages of the respective measurements were used to obtain the mean±s.e.mean. *$P<0.05$ versus respective RMP (Newman-Keuls test). †$P<0.05$ versus the membrane potential measured in the presence of Ad (Newman-Keuls test). ‡$P<0.05$ versus the membrane potential measured in the presence of NA (Newman-Keuls test). †$P<0.05$ versus the membrane potential measured in the presence of 1 nM Clo (Newman-Keuls test).

Similar results were also obtained in endothelium-denuded aortic rings (Table 1), indicating the presence of α_2-adrenoceptors also in the smooth muscle.

Discussion

The present study demonstrates that UK 14,304 at low concentration (below 3 nM) is highly selective for α_2-adrenoceptors, whereas at high concentration (above 10 nM) it activates both α_1- and α_2-adrenoceptors.

In endothelium denuded, but not in endothelium intact aortic rings, UK 14,304 induced small concentration-dependent contractions which were observed above 100 nM and were inhibited by prazosin (Figure 1A), indicating that these responses are mediated by α_1-adrenoceptors. In endothelium intact aortic rings, where α_2-adrenoceptors were characterized by Bockman *et al.* (1996), the contractile responses are probably blunted by the presence of these receptors.

To better visualize the relaxant response to UK 14,304, aortic rings were pre-contracted by $BaCl_2$, since most vasoconstrictor agonists inhibit ATP-sensitive K^+ channels (Quayle *et al.*, 1997) blunting the relaxant response due to activation of these channels.

In precontracted endothelium denuded aortic rings, concentration-dependent relaxant responses induced by UK 14,304 were observed which were inhibited by yohimbine (Figure 1B, C) or glibenclamide.

The same concentration range of UK 14,304 that relaxed precontracted isolated rings induced concentration-dependent hyperpolarizing responses (Figures 2A and 3B), which were able to revert the depolarization induced by $BaCl_2$ (Figure 2B).

The hyperpolarization induced by UK 14,304 were probably due to α_2-adrenoceptors stimulation, since they were completely inhibited by yohimbine and were not affected by prazosin or propranolol (Figure 2C). In contrast, at concentrations above 10 nM UK 14,304 had a depolarizing effect, which was potentiated by yohimbine and inhibited by prazosin (Figure 3). These results lead us to conclude that UK 14,304, besides its high affinity for α_2-adrenoceptors, also is able to interact with α_1-adrenoceptors when in higher concentrations.

To characterize the signal transduction pathway coupled to α_2-adrenoceptors, we examined the changes in membrane potential induced by UK 14,304 in the presence of K^+ channel blockers, since in mesenteric artery rings the hyperpolarizing responses induced by this agonist was shown to be due to opening of small-conductance Ca^{2+}-dependent K^+ channels (Silva *et al.*, 1996).

Our results show that small or higher conductance Ca^{2+}-dependent K^+ channels are not involved in mediating the hyperpolarizing responses induced by α_2-adrenergic agonists, since these responses were not affected by apamin or iberiotoxin respectively (Figure 4A). On the other hand,

glibenclamide, which selectively inhibits ATP-sensitive K^+ channels (Kovacs & Nelson, 1991), prevented the hyperpolarization due to stimulation of α_2-adrenoceptors.

ATP-sensitive K^+ channels are thought to play a minor role in maintaining the membrane potential under basal conditions, since it is believed that these channels are closed at normal intracellular ATP concentrations (Mishra & Aaronson, 1999). However, our results show that glibenclamide caused significant depolarization of the vascular smooth muscle membrane, indicating that ATP-sensitive K^+ channels are constitutively active, thus contributing to the resting membrane potential, as also proposed by Mishra & Aaronson (1999). This assumption is also in agreement with the proposal by Quayle *et al*. (1997) that ATP-sensitive K^+ channels, even in the absence of endogenous activators, may contribute to the regulation of the basal blood flow in a number of vascular beds.

Removal of the endothelium did not affect resting membrane potential (Table 1), indicating that EDRF was not involved in the maintenance of the resting membrane potential (RMP). However, in the presence of the NO synthesis inhibitor L-NNA, a reduction of the RMP was observed, which did not affect the responses to the α_2-adrenoceptors agonist (Figure 4A), indicating that NO is not involved in these responses. These results are different from those of Zimmermann *et al*. (1997) who found that removal of endothelium or addition of L-NNA decreased the sensitivity to ATP-sensitive K^+ channel openers and depolarized rat cerebral arteries. However, the electrophysiological and pharmacological properties of vascular smooth muscles are dependent on the artery type and segments (Zhang & Bolton, 1996), and the comparison with our results is difficult since the responses of cerebral arteries to physiological stimuli are very different from those of conductance arteries.

Since yohimbine and glibenclamide were the only agents able to inhibit the hyperpolarizing response to UK 14,304, it is relevant to verify the specificity of the yohimbine inhibitory action towards α_2-adrenoceptors or to ATP-sensitive K^+ channels. Figure 4B shows that yohimbine did not affect the hyperpolarizing response induced by cromakalin, a ATP-sensitive K^+ channel opener, indicating that yohimbine inhibitory action is probably on α_2-adrenoceptors.

To further examine our assumption, the effect of clonidine, another α_2-adrenoceptor agonist, was studied utilizing the same protocol as for UK 14,304. Similar results were obtained: concentration-dependent hyperpolarization responses that were inhibited by yohimbine and by glibenclamide (Figure 5A).

It is interesting to note that noradrenaline evoked a significant depolarization that was totally inhibited by prazosin, whereas adrenaline did not alter the resting membrane potential but had a significant hyperpolarizing effect in the presence of prazosin (Figure 5B). These results also indicate that noradrenaline is more selective for α_1-adrenoceptors whereas adrenaline has similar affinities for α_1- and α_2-adrenoceptors.

Catecholamines are considered important factors in the regulation of vascular tone and their vasoconstrictor effect being due to stimulation of smooth muscle α_1-adrenoceptors. It has been shown that α_2-adrenoceptors are located away from adrenergic nerve terminals and close to circulating adrenaline (Langer & Shepperson, 1982a), and our results show that adrenaline interacts with smooth muscle α_2-adrenoceptors inducing hyperpolarization and relaxation by opening K^+ channels. It is probable that α_2-adrenoreceptors are also important for the control of muscular tone, since impairment of K^+ channels contribute to the maintenance of the hypertensive state of spontaneously hypertensive rats (Feres *et al*., 1998) and the correction of that impairment leads to normalization of the blood pressure in these animals (Borges *et al*., 1999).

In conclusion, the controversy about the physiological role of α_2-adrenoceptors could probably be attributed to the fact that the agonists used are not selective and most of the experiments were performed on noradrenaline precontracted rings (Nomura *et al*., 1995; Lembo *et al*., 1997; Shimamura *et al*., 1995; Bockman *et al*., 1996). In those preparations the responses to α_2-adrenoceptors agonists are impaired because noradrenaline is known to inhibit ATP-sensitive K^+ channels (Quayle *et al*. 1997; Inokuchi *et al*., 1992) which are mediators of α_2-adrenoceptor responses.

Although Bockman *et al*. (1996) have shown that α_2-adrenoceptors agonists induce their vascular relaxant responses by acting on α_2-adrenoceptors located in the endothelium, our results indicate that in rat aorta these receptors are also present in the smooth muscle, since the responses were observed in aortic ring preparations with or without endothelium. Our results are in agreement with those of Silva *et al*. (1996) in rat mesenteric arteries, and emphasize the importance of the electrophysiological methodology for the identification of the role of α_2-adrenoceptors in vascular smooth muscle.

This work was supported by grants and fellowships from the Brazilian National Research Council (CNPq) and by the São Paulo State Research Foundation (FAPESP). The technical assistance of Nelson Alves Mora is gratefully acknowledged.

References

BLAXALL, H.S., HASS, N.A. & BYLUND, D.B. (1994). Expression of alpha-2 adrenergic receptor genes in rat tissues. *Receptor,* **4,** 191 – 199.

BOCKMAN, C.S., GONZALEZ-CABRERA, I. & ABEL, P.W. (1996). Alpha-2 adrenoceptor subtype causing nitric oxide-mediated vascular relaxation in rats. *J. Pharmacol. Exp. Ther.,* **278,** 1235 – 1243.

BORGES, A.C.R., FERES, T., VIANNA, L.M. & PAIVA, T.B. (1999). Recovery of impaired K^+ channels in mesenteric arteries from spontaneously hypertensive rats by prolonged treatment with cholecalciferol. *Br. J. Pharmacol.,* **127,** 772 – 778.

DECKER, N., EHRHARDT, J.D., LECLERC, G. & SCHARTZ, J. (1984). Postjunctional α-adrenoceptors, α_1 and α_2 subtypes in rat vasculature in vitro and in vivo. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **326,** 1 – 6.

DIGGES, K.G. & SUMMERS, R.J. (1983). Characterization of postsynaptic α-adrenoceptors in rat aortic strips and portal veins. *Br. J. Pharmacol.,* **79,** 655 – 665.

FERES, T., BORGES, A.C.R., SILVA, E.G., PAIVA, A.C.M. & PAIVA, T.B. (1998). Impaired function of alpha-2 adrenoceptors in smooth muscle mesenteric arteries from spontaneously hypertensive rats. *Br. J. Pharmacol.,* **125,** 1144 – 1149.

FURCHGOTT, R.F. (1981). The requirement for endothelial cells in relaxation of arteries by acetylcholine and some other vasodilators. *Trends Pharmacol. Sci.,* **2,** 173 – 176.

GLEASON, M.M., RATZ, P.H. & FLAIN, S.F. (1985). Measurements of calcium flux in tethered rings of rabbit aorta under tension. *Am. J. Physiol.,* **249,** H470 – H476.

INOKUCHI, H., YOSHIMURA, M., POLOSA, C. & NISHI, S. (1992). Adrenergic receptors (α_1 and α_2) modulate different potassium conductances in sympathetic preganglionic neurons. *Can. J. Physiol. Pharmacol.,* **70,** S92 – S97.

IWANAGA, S., SHIBATA, O., TSUDA, A., HASHIMOTO, S., MAKITA, T., CHO, S. & SUMIKAWA, K. (1998). The role of α_1-adrenoceptors in the clonidine-induced contraction and relaxation of rat aorta. *Res. Commun. Mol. Pathol. Pharmacol.,* **102,** 137 – 147.

KOVACS, R.J. & NELSON, M.T. (1991). ATP-sensitive K^+ channels from aortic smooth muscle incorporated into planar lipid bilayers. *Am. J. Physiol.,* **261,** H604 – H609.

LANGER, S.Z. & SHEPPERSON, N.B. (1982a). Postjunctional α_1- and α_2-adrenoceptors: Preferential innervation of α_1-adrenoceptors and the role of neuronal uptake. *J. Cardiovasc. Pharmacol.,* **4,** S8 – S13.

LANGER, S.Z. & SHEPPERSON, N.B. (1982b). Recent development in vascular smooth muscle pharmacology: the post-synaptic α_2-adrenoceptor. *Trends Pharmacol. Sci.,* **3,** 400 – 444.

LEMBO, G., IACCARINO, G., VECCHIONE, C., BARBATO, E., MORISCO, C., MONTI, F., PARRELLA, L. & TRIMARCO, B. (1997). Insulin enhances endothelial alpha2-adrenergic vasorelaxation by a pertussis toxin mechanism. *Hypertension,* **30,** 1128 – 1134.

MISHRA, S.K. & AARONSON, P.I. (1999). A role for a glibenclamide-sensitive, relatively ATP-insensitive K^+ current in regulating membrane potential and current in rat aorta. *Cardiovasc. Res.,* **44,** 429 – 435.

NOMURA, S., SUNAGANE, N., LEE, T.J., URUNO, T. & KUBOTA, K. (1995). Inhibition of alpha1-adrenoceptor-mediated contractions in isolated tail arteries and aorta of the rat by alpha2-adrenoceptor agonists. *Res. Commun. Mol. Pathol. Pharmacol.,* **90,** 307 – 320.

PING, P. & FABER, J.E. (1993). Characterization of alpha-adrenoceptor gene expression in arterial and venous smooth muscle. *Am. J. Physiol.,* **265,** H1501 – H1509.

QUAYLE, J.M., NELSON, M.T. & STANDEN, N.B. (1997). ATP-sensitive and inwardly rectifying potassium channels in smooth muscle. *Physiol. Rev.,* **77,** 1165 – 1232.

RUFFOLO, JR., R.R., WADDELL, J.E. & YADEN, E.Y. (1982). Heterogeneity of postsynaptic alpha-adrenergic receptors in mammalian aortas. *J. Pharmacol. Exp. Ther.,* **221,** 309 – 314.

SHIMAMURA, K., MATSUDA, K., YAMAMOTO, K. & SUNANO, S. (1995). Alteration in the release of endothelium-derived relaxing factors by α-adrenoceptor stimulation in the aorta of stroke-prone spontaneously hypertensive rats. *Clin. Exp. Pharmacol. Physiol. Suppl.,* **1,** S144 – S145.

SILVA, E.G., FERES, T., VIANNA, L.M., OKUYAMA, P. & PAIVA, T.B. (1996). Dual effect of clonidine on mesenteric artery adrenoceptors: agonistic (alpha-2) and antagonistic (alpha-1). *J. Pharmacol. Exp. Ther.,* **277,** 872 – 876.

ZHANG, H.-L. & BOLTON, T.B. (1996). Two types of ATP-sensitive potassium channels in rat portal vein smooth muscle cells. *Br. J. Pharmacol.,* **118,** 105 – 144.

ZIMMERMANN, P.A., KNOT, H.J., STEVENSON, A.S. & NELSON, M.T. (1997). Increased myogenic tone and diminished responsiveness to ATP-sensitive K^+ channel openers in cerebral arteries from diabetic rats. *Circ. Res.,* **81,** 996 – 1004.

(Received January 19, 2000
Revised July 4, 2000
Accepted July 28, 2000)