Role of Na^+/K^+ ATPase on the Relaxation of Rabbit Ear and Femoral Arteries

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Abstract

The role of Na^+/K^+ ATPase in vascular relaxation has been studied by determining its inhibitory effects on 2-mm segments from rabbit central ear and femoral arteries, mounted for isometric tension recording.

Acetylcholine (10^{-8}–10^{-4} M), the nitric oxide donor sodium nitroprusside (10^{-8}–3×10^{-4} M), the potassium channel agonist cromakalim (10^{-8}–3×10^{-5} M), histamine (10^{-8}–10^{-4} M) in the presence of the H_1 antagonist chlorpheniramine (10^{-5} M), and papaverine (10^{-6}–3×10^{-4} M) all produced arterial relaxation in ear and femoral arteries precontracted with endothelin 1. Addition of potassium (6×10^{-3}–$1·2 \times 10^{-2}$ M) caused relaxation of the same arteries preincubated in potassium-free medium.

Ouabain (10^{-5} M) an inhibitor of Na^+/K^+ ATPase, reduced the relaxation of ear arteries, but not of femoral arteries, in response to acetylcholine; it also reduced the response to sodium nitroprusside, cromakalim or histamine, and abolished the relaxation to potassium, but did not modify the response to papaverine, in both types of artery.

These results suggest that Na^+/K^+ ATPase might play a role in the relaxation of ear and femoral arteries to nitrovasodilators, to potassium channel openers and to activation of histamine receptors, and that Na^+/K^+ ATPase might play a role in the cholinergic relaxation of ear, but not femoral arteries, suggesting that the mechanism of cholinergic relaxation might differ in each type of artery.

Sodium-potassium-adenosinetriphosphatase (Na^+/K^+ ATPase) plays an important role in several cell functions by controlling intracellular ionic concentrations and membrane potential (Kaplan 1985). One of these functions might be the regulation of vascular tone, because vasoactive substances can modulate the activity of this ionic pump (Brock et al 1982; Gupta et al 1994). The role of Na^+/K^+ ATPase on vascular relaxation is, however, at present unsettled. It has been proposed that activation of Na^+/K^+ ATPase is involved in arterial relaxation to cholinergic stimulation, because this response is reduced by the Na^+/K^+ ATPase inhibitor ouabain in the canine femoral artery (DeMey & Vanhoutte 1980), the rabbit renal artery (Kitagawa et al 1994) and the rat mesenteric artery (Adeagbo & Malik 1990). Ouabain did not, however, modify the cholinergic relaxation of the canine coronary artery (Chen et al 1989) and the rat pulmonary (Archer & Cowan 1991) and muscular (Vicaut et al 1994) arteries. In bioassay experiments it has been observed that ouabain inhibits the release of relaxing factor from the endothelium of canine femoral arteries, but not the relaxation produced by this factor in the smooth muscle of canine coronary arteries (Feletou & Vanhoutte 1988). These conflicting reports suggest that species or regional differences, or both, might affect the participation of Na^+/K^+ ATPase in the cholinergic vascular relaxation.

Acetylcholine could produce endothelium-dependent relaxation by two different mechanisms: by releasing nitric oxide (Palmer et al 1988), which stimulates cyclic GMP production (Ignarro & Kadowitz 1985), and by releasing a hyperpolarizing factor (Taylor & Weston 1988), which acti-

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vates potassium channels (Chen & Suzuki 1989). The objective of the present study was to analyse the role of Na^+/K^+ ATPase in cholinergic vascular relaxation, by studying the effects of ouabain on the relaxation of rabbit ear and femoral arteries to acetylcholine, and by comparing these effects with those on the relaxation of these arteries by the nitric oxide donor sodium nitroprusside and by the potassium channel opener cromakalim. Although the mechanisms of action of nitric oxide and hyperpolarizing factor are not completely clear, they might be related to the effects of sodium nitroprusside and cromakalim, in the sense that they act on the smooth muscle by increasing cyclic GMP and by opening potassium channels, respectively. These results were, moreover, also compared with the effects of ouabain on the relaxation of these arteries to histamine H_2 receptor activation, and to papaverine, which are unrelated to the mechanisms of cholinergic relaxation (Needleman et al 1985; Fernández et al 1994).

Material and Methods

Ouabain octahydrate, acetylcholine chloride, sodium nitroprusside, cromakalim, histamine dihydrochloride, papaverine hydrochloride and N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME) were from Sigma. Endothelin 1 (human and porcine) was from Peninsula Laboratories Europe, and D-chlorpheniramine maleate was from Polaramine, Schering-Plough.

Thirty five male New Zealand White rabbits, 2–2·5 kg, were killed by intravenous injection of sodium pentobarbital, 100 mg kg^{-1}. Central ear and femoral arteries were dissected free and cut into 2-mm long cylindrical segments. Each seg-

ment was prepared for isometric tension recording in a 6-mL organ bath containing modified Krebs–Henseleit solution with the composition (mM): NaCl, 115; KCl, 4·6; KH_2PO_4, 1·2; $MgSO_4$, 1·2; $CaCl_2$, 2·5; $NaHCO_3$, 25; glucose, 11·1. The solution was equilibrated with 95% oxygen and 5% carbon dioxide to give a pH of 7·3–7·4, and maintained at 37°C. Briefly, the method consists of passing two fine, stainless steel pins, 150 μm in diameter, through the lumen of the vascular segment. One pin is fixed to the organ bath wall, the other is connected to a strain gauge for isometric tension recording, thus enabling the application of passive tension in a plane perpendicular to the long axis of the vascular cylinder. The recording system included a Universal Transducing Cell UC3 (Statham Instruments), a Statham Microscale Accessory UL5 (Statham Instruments) and a Beckman type RS recorder (model R-411, Beckman Instruments).

A previously determined resting passive tension of 0·5 g was applied to the vascular segments; they were then left to equilibrate for 60–90 min before any drug was added. For central ear and femoral arteries precontracted with endothelin 1 ($10^{-8}–10^{-7}$ M) the relaxation was studied by obtaining cumulative concentration-response curves to acetylcholine ($10^{-8}–10^{-4}$ M), sodium nitroprusside ($10^{-8}–10^{-4}$ M), cromakalim ($10^{-8}–3 \times 10^{-5}$ M), histamine ($10^{-8}–10^{-4}$ M), or papaverine ($10^{-6}–3 \times 10^{-4}$ M), experiments being performed in the absence and in the presence of the Na^+/K^+ ATPase inhibitor ouabain (10^{-5} M). The response to croma-kalim was also studied in the presence of the inhibitor of nitric oxide synthesis N^G-nitro-L-arginine methyl ester (L-NAME; 10^{-4} M), and in segments without endothelium in order to assess whether the response to cromakalim was mediated by nitric oxide or by the vascular endothelium. The endothelium was removed by gentle rubbing of the vascular lumen with a steel rod, the adequacy of endothelium removal being tested by the abolition of the relaxation to acetylcholine (10^{-6} M) in precontracted arterial segments. The relaxation to histamine was studied, in every case, in arterial segments pretreated with the H_1 antagonist chlorpheniramine (10^{-5} M) because block-ade of H_1 receptors was necessary to evidence the relaxation to histamine (Fernández et al 1994). Ouabain, L-NAME or chlorpheniramine were added to the bath 30 min before beginning the experiments.

The selectively of ouabain was assessed against potassium-induced relaxation (Webb & Bohr 1978). Ear and femoral arteries were incubated for 1 h in potassium-free solution, prepared by equimolecular substitution in the Krebs solution of KCl by NaCl, and of KH_2PO_4 by NaH_2PO_4; after pre-contraction with endothelin 1 ($10^{-8}–10^{-7}$ M), potassium chloride $6 \times 10^{-3}–1·2 \times 10^{-2}$ M was added, both in the absence and in the presence of ouabain (10^{-5} M).

Relaxation was expressed as a percentage of the contractile tone achieved with endothelin 1. Concentrations of drugs causing 50% of the maximum relaxation (EC50) were calculated from each individual concentration–response curve and in each case pD_2 was calculated as the negative logarithm of the EC50. The data for percentage relaxation and pD_2 values are expressed as means ± s.e.m., and were evaluated by analysis of variance applied to each group of data, followed by the Dunnett t-test to compare each experimental condition with its control. A probability value of less than 0·05 was considered significant.

Results

Ear arteries

Application of endothelin 1 to ear arteries induced contractile tone that was similar in the absence (2·4 ± 0·09 g) or presence (2·5 ± 0·07 g) of ouabain.

Figs 1 and 2 and Table 1 show the relaxing response of precontracted ear arteries to the agonists studied in control
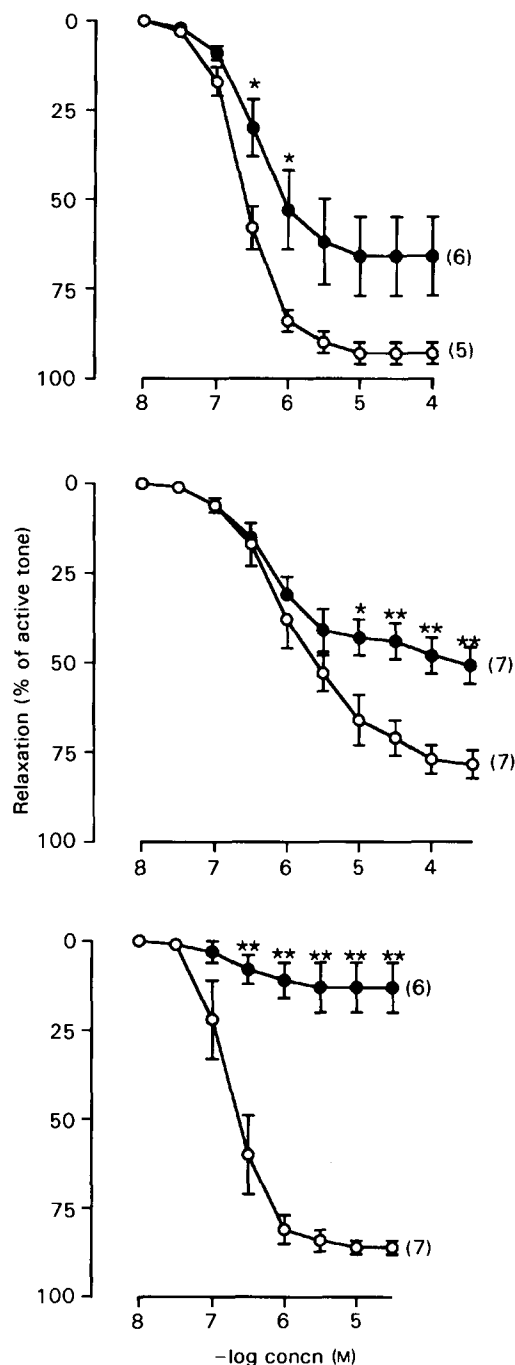

FIG. 1. Relaxation of rabbit central ear arteries precontracted with endothelin 1 ($10^{-8}–10^{-7}$ M) in response to acetylcholine (top), sodium nitroprusside (middle) and cromakalim (bottom), under control conditions (○) and in the presence of ouabain (10^{-5} M, ●). The relaxation is expressed as a percentage of the achieved active tone. Values are means ± s.e.m. $*P < 0·05$, $**P < 0·01$ compared with control. The number of vascular segments is given in parentheses.
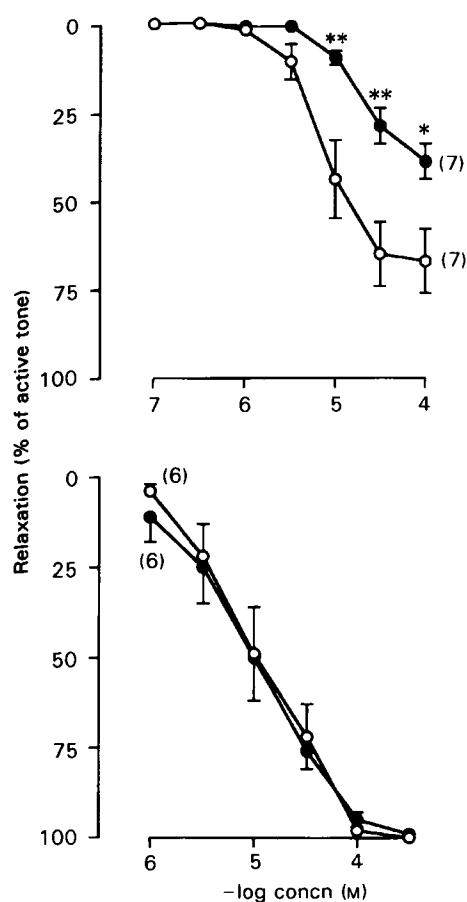
FIG. 2. Relaxation of rabbit central ear arteries precontracted with endothelin 1 (10^{-8}–10^{-7} M) in response to histamine in the presence of chlorpheniramine (10^{-5} M) (top) or papaverine (bottom), under control conditions (O) and in the presence of ouabain (10^{-5} M, ●). The relaxation is expressed as a percentage of the achieved active tone. Values are means ± s.e.m. *$P < 0.05$, **$P < 0.01$ compared with control. The number of vascular segments is given in parentheses.

conditions and in the presence of ouabain. Acetylcholine produced concentration-dependent relaxation, and in the presence of ouabain the relaxation to acetylcholine was reduced ($P < 0.0001$ by analysis of variance), although the reductions in maximum relaxation or in the EC50, each one analysed separately, were not statistically significant by Dunnett test. The relaxation of ear arteries induced by low concentrations

(10^{-8}–3×10^{-6} M) of sodium nitroprusside was not modified by ouabain, but that resulting from higher concentrations (10^{-5}–3×10^{-4} M) of sodium nitroprusside was significantly reduced by ouabain ($P < 0.0001$ by analysis of variance). Cromakalim induced concentration-dependent relaxation of ear arteries and this relaxation was not modified by endothelium removal (5 segments) or L-NAME treatment (6 segments) (data not shown), but it was nearly abolished by ouabain ($P < 0.0001$ by analysis of variance). Histamine, in the presence of the H$_1$ inhibitor chlorpheniramine, produced relaxation which was reduced by ouabain ($P < 0.0001$ by analysis of variance). Papaverine produced relaxation of the arteries, but this effect was not modified by ouabain ($P > 0.05$).

In the arteries incubated in potassium-free solution and precontracted with endothelin 1, addition of potassium 6×10^{-3} M under control conditions produced a relaxation of 90–100% of the active tone (4 animals), whereas in the presence of ouabain addition of potassium (6×10^{-3} M or 1.2×10^{-2} M) did not produce significant relaxation (4 animals).

Femoral arteries

The contractile tone induced by endothelin 1 on rabbit femoral arteries was greater than that on ear arteries, and was similar under control conditions (3.1 ± 0.11 g) and in the presence of ouabain (2.8 ± 0.12 g).

The relaxation of femoral arteries to the vasodilators studied under control conditions and in the presence of ouabain is shown in Figs 3 and 4 and Table 2. The relaxation of femoral arteries to acetylcholine was not significantly modified by ouabain ($P > 0.05$). The relaxation of femoral arteries to sodium nitroprusside was, however, reduced by ouabain ($P < 0.0001$ by analysis of variance). Cromakalim induced relaxation which was not modified by endothelium removal (5 segments) or by treatment with L-NAME (6 segments). Ouabain, however, reduced ($P < 0.0001$ by analysis of variance) the sensitivity (EC50) of femoral arteries to cromakalim, without significantly modifying the maximum relaxation. Ouabain reduced ($P < 0.0001$ by analysis of variance) the sensitivity and the maximum relaxation of femoral arteries to histamine in the presence of chlorpheniramine. The relaxation of femoral arteries to papaverine was not modified in the presence of ouabain ($P > 0.05$).

In femoral arteries incubated in potassium-free solution and precontracted with endothelin 1, addition of 6×10^{-3} M potassium did not produce any effect (4 animals), but addition of 1.2×10^{-2} M potassium produced relaxation of 90–100%

Table 1. Maximum relaxation (E$_{max}$, percent of active tone) and the negative logarithm of the EC50 (pD$_2$) of relaxation to acetylcholine, sodium nitroprusside, cromakalim and histamine in the presence of chlorpheniramine or papaverine, and contractile tone (g) in rabbit central ear arteries in the absence and in the presence of ouabain (10^{-5} M).

	Control			Ouabain		
	E$_{max}$	pD$_2$	Tone	E$_{max}$	pD$_2$	Tone
Acetylcholine	93 ± 3	6.6 ± 0.07	2.1 ± 0.3 (5)	66 ± 11	6.3 ± 0.14	2.5 ± 0.2 (6)
Sodium nitroprusside	79 ± 3	5.9 ± 0.17	2.1 ± 0.2 (7)	53 ± 5**	6.0 ± 0.12	2.3 ± 0.1 (7)
Cromakalim	86 ± 2	6.7 ± 0.13	2.5 ± 0.1 (7)	13 ± 7**	–	2.0 ± 0.2 (6)
Histamine	67 ± 9	5.1 ± 0.07	2.5 ± 0.2 (7)	39 ± 5*	4.7 ± 0.03**	2.3 ± 0.2 (7)
Papaverine	100 ± 1	5.0 ± 0.17	2.3 ± 0.2 (6)	99 ± 1	5.3 ± 0.23	2.4 ± 0.1 (6)

Values are means ± s.e.m. *$P < 0.05$; **$P < 0.01$ compared with control. The number of vascular segments is given in parentheses.
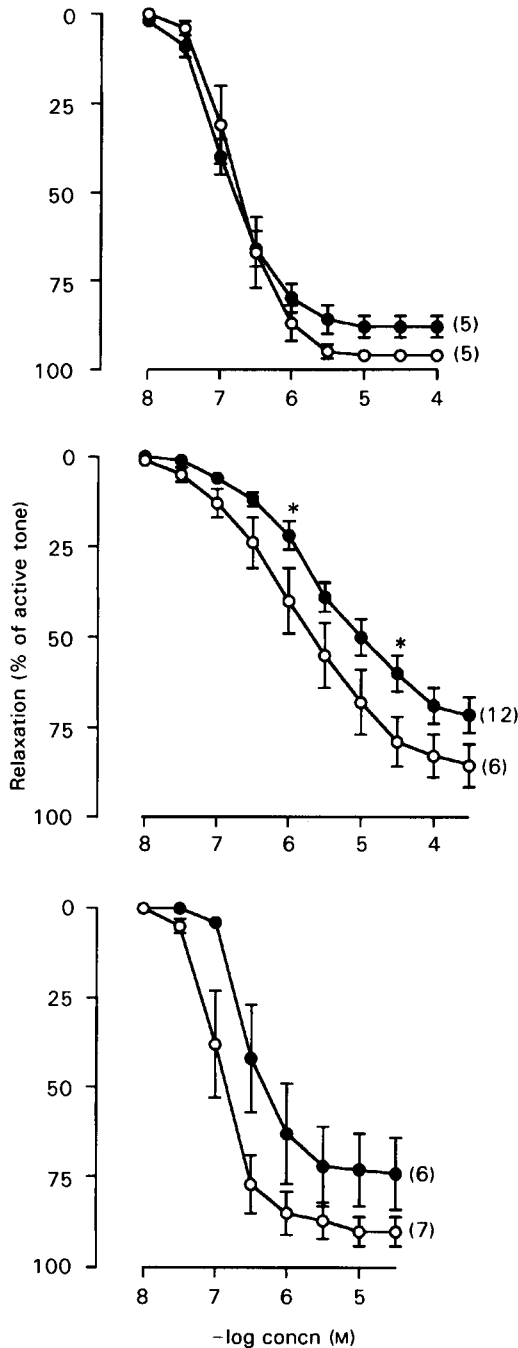
FIG. 3. Relaxation of rabbit femoral arteries precontracted with endothelin 1 (10^{-8}–10^{-7} M) in response to acetylcholine (top), sodium nitroprusside (middle) and cromakalim (bottom), under control conditions (O) and in the presence of ouabain (10^{-5} M, ●). The relaxation is expressed as a percentage of the achieved active tone. Values are means ± s.e.m. *$P < 0.05$, compared with control. The number of vascular segments is given in parentheses.

of the active tone, which was abolished in the presence of ouabain (4 animals).

Discussion

We have found that ouabain inhibits the relaxation of rabbit ear and femoral arteries to some vasoactive stimuli. Ouabain, at
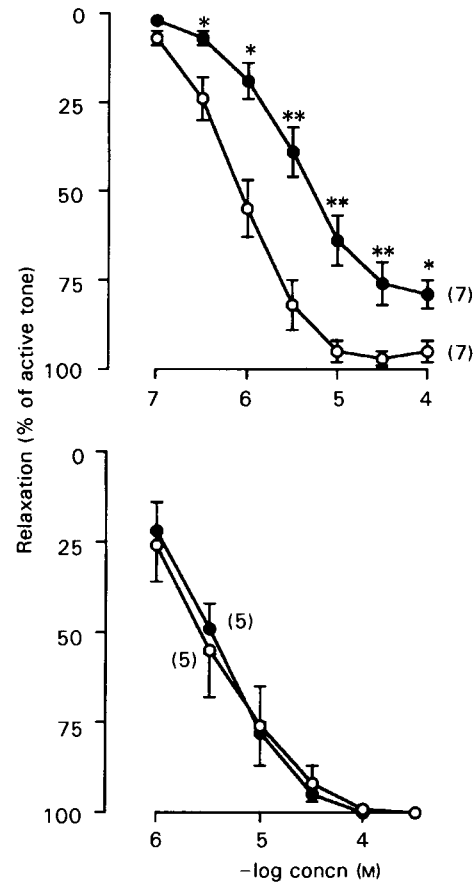

FIG. 4. Relaxation of rabbit femoral arteries precontracted with endothelin 1 (10^{-8}–10^{-7} M) in response to histamine in the presence of chlorpheniramine (10^{-5} M) (top) or papaverine (bottom), under control conditions (O) and in the presence of ouabain (10^{-5} M, ●). The relaxation is expressed as a percentage of the achieved active tone. Values are means ± s.e.m. *$P < 0.05$, **$P < 0.01$ compared with control. The number of vascular segments is given in parentheses.

the concentration used in this study (10^{-5} M), is often used for inhibiting Na^+/K^+ ATPase (Fleming 1980) and this concentration, moreover, inhibited potassium-induced relaxation of ear and femoral arteries, which suggests that it is indeed inhibiting Na^+/K^+ ATPase under the conditions of our study (Webb & Bohr 1978). Although it cannot be excluded that ouabain might have other effects in addition to Na^+/K^+ ATPase inhibition, it has been reported that this concentration produces very little change in permeability to potassium in the rabbit ear artery (Hendrickx & Casteels 1974), which suggests that it does not have an important direct effect on potassium channels. In addition, in our study ouabain did not modify the relaxation to the non-specific smooth muscle relaxant papaverine, suggesting that it does not have a general inhibitory effect on relaxation. These results therefore suggest that Na^+/K^+ ATPase might play a role in the relaxation of rabbit ear and femoral arteries, but that this role might vary depending of the relaxing stimulus and of the vascular bed involved.

The relaxation to acetylcholine was differently affected by ouabain in ear and femoral arteries. In ear arteries, but not in femoral arteries, ouabain reduced the response to acetylcholine, suggesting that Na^+/K^+ ATPase is involved in the cho-

Table 2. Maximum relaxation (E$_{max}$, percent of active tone) and the negative logarithm of the EC50 (pD$_2$) of relaxation to acetylcholine, sodium nitroprusside, cromakalim and histamine in the presence of chlorpheniramine or papaverine, and contractile tone (g) in rabbit femoral arteries in the absence and in the presence of ouabain (10^{-5} M).

	Control			Ouabain		
	E$_{max}$	pD$_2$	Tone	E$_{max}$	pD$_2$	Tone
Acetylcholine	96 ± 1	6·8 ± 0·14	3·0 ± 0·1 (5)	88 ± 3	6·9 ± 0·07	2·3 ± 0·2 (5)
Sodium nitroprusside	85 ± 5	5·9 ± 0·21	2·8 ± 0·3 (6)	72 ± 5	5·6 ± 0·12	2·5 ± 0·2 (12)
Cromakalim	90 ± 4	6·9 ± 0·11	3·7 ± 0·2 (7)	74 ± 10	6·4 ± 0·13*	3·2 ± 0·5 (6)
Histamine	97 ± 2	6·1 ± 0·12	2·6 ± 0·2 (7)	79 ± 4**	5·4 ± 0·14**	3·0 ± 0·3 (7)
Papaverine	100 ± 1	5·5 ± 0·22	2·9 ± 0·2 (5)	100 ± 1	5·5 ± 0·11	2·9 ± 0·2 (5)

Values are means ± s.e.m. *$P < 0.05$; **$P < 0.01$ compared with control. The number of vascular segments is given in parentheses.

linergic relaxation of ear, but not of femoral arteries. It might, therefore, be hypothesized that the mechanisms of cholinergic relaxation are partially different in both types of artery. These results might be compared with previous studies made in our laboratory on the mechanisms of cholinergic relaxation in these arteries (Monge et al 1993; García-Villalón et al 1995). In these studies it was found that the relaxation of ear and femoral arteries to acetylcholine was endothelium-dependent (Monge et al 1993) and might in part be mediated by release of nitric oxide and in part by stimulation of potassium channels (García-Villalón et al 1995). The present results enable us to make some proposals about the role of Na$^+$/K$^+$ ATPase in these mechanisms. In this study it has been found that ouabain reduces the relaxation of ear and femoral arteries both to sodium nitroprusside, which is a nitric oxide donor, and to cromakalim, which is a potassium channel opener. Na$^+$/K$^+$ ATPase might, therefore, be involved in the relaxation to nitric oxide and in the relaxation produced by activation of potassium channels.

The reduction of the vascular relaxation to nitrovasodilators, such as sodium nitroprusside, by inhibition of Na$^+$/K$^+$ ATPase has been described before (Foley 1984). Although nitric oxide and nitrovasodilators produce relaxation by increasing cyclic GMP (Ignarro & Kadowitz 1985), stimulation of Na$^+$/K$^+$ ATPase by sodium nitroprusside has been described in rabbit aorta smooth muscle (Gupta et al 1994), and Na$^+$/K$^+$ ATPase stimulation would result in smooth muscle hyperpolarization and relaxation (Fleming 1980). It is possible, therefore, that relaxation to nitric oxide or nitrovasodilators might be mediated in part by cyclic GMP production, and in part by hyperpolarization owing to Na$^+$/K$^+$ ATPase stimulation. This participation of Na$^+$/K$^+$ ATPase might not be present in every type of artery, however, as relaxation of cat cerebral arteries to nitric oxide (Alonso et al 1992) or that of human subcutaneous arteries to sodium nitroprusside (Woolfson & Poston 1991) was not modified by ouabain. Our results suggest that in rabbit ear and femoral arteries, Na$^+$/K$^+$ ATPase might be involved in the relaxation to nitrovasodilators.

Our results also suggest that Na$^+$/K$^+$ ATPase is involved in the relaxation produced by activation of potassium channels in vascular smooth muscle. The potassium channel agonist cromakalim produced relaxation of ear and femoral arteries, and this relaxation was not modified by removal of the endothelium or by inhibition of nitric oxide synthesis by L-NAME, suggesting that this drug acts directly on the smooth muscle.

The relaxation caused by cromakalim in ear and femoral arteries was, however, reduced by ouabain, which agrees with observations in human and canine mesenteric arteries (Hong et al 1993) and suggests that cromakalim produces activation of Na$^+$/K$^+$ ATPase. The mechanism of this activation is not known, but it has been suggested that the opening of potassium channels activates Na$^+$/K$^+$ ATPase indirectly by altering intracellular ionic concentrations (Hong et al 1993).

In the present study we have observed, therefore, that ouabain reduced the relaxation of ear arteries to acetylcholine, as well as to the nitric oxide donor nitroprusside and to the potassium channel opener cromakalim. It might be suggested that ouabain inhibits the cholinergic relaxation of ear arteries by acting on both the nitric oxide-dependent and the potassium channel-dependent mechanisms of cholinergic relaxation. In femoral arteries, however, ouabain did not modify the relaxation to acetylcholine, suggesting that in these arteries Na$^+$/K$^+$ ATPase plays a minor role in the cholinergic response. This might seem surprising, because ouabain did reduce the response of femoral arteries to sodium nitroprusside and to cromakalim. In a previous study (García-Villalón et al 1995) it was found that nitric oxide and potassium channels also played a role in cholinergic relaxation of femoral arteries; a marked relaxation was, however, still observed in these arteries after inhibition of both nitric oxide synthesis and potassium-channel activation. These results (García-Villalón et al 1995) and the present ones suggest that a mechanism of relaxation that is resistant to ouabain might be involved in the cholinergic response of femoral, but not ear, arteries. In rat pulmonary (Archer & Cowan 1991) and muscular (Vicaut et al 1994) arteries it has been observed that relaxation to acetylcholine is mediated by an endothelial factor different from nitric oxide which is not affected by ouabain. A similar factor could be present in the cholinergic response of rabbit femoral arteries. It is also possible that the potassium channel involved in relaxation to acetylcholine in femoral arteries is of a type different from that activated by cromakalim, and might be differently affected by ouabain.

For comparison, we also studied the effects of ouabain on vascular relaxation to stimuli not related to cholinergic mechanisms, i.e. papaverine and histamine. The relaxation to papaverine was not affected by ouabain in ear and femoral arteries, suggesting that ouabain probably does not have a non-specific effect on vascular relaxation. On the other hand, the relaxation to histamine, in the presence of an H$_1$ inhibitor, was reduced by ouabain in ear and femoral arteries. Little infor-

mation is available in literature on the role of Na^+/K^+ ATPase in the response to histamine. Adeagbo & Malik (1990) found that relaxation to histamine was reduced by ouabain, but the receptors involved were not analysed. Previous studies (Fernández et al 1994) indicate that under these conditions the relaxation of ear and femoral arteries to histamine is a result of stimulation of H_2 receptors located in the smooth muscle, and the present results suggest, therefore, that Na^+/K^+ ATPase might be involved in the relaxation to activation of histaminergic H_2 receptors. As the results with papaverine argue against non-specific involvement of Na^+/K^+ ATPase in smooth muscle relaxation, it is possible that it intervenes in receptor coupling or in second messenger mechanisms of the H_2 histaminergic receptor.

In summary, these results suggest that Na^+/K^+ ATPase might be involved in vascular relaxation and that this involvement differs depending on the relaxing stimulus. Na^+/K^+ ATPase might play a greater role in the relaxation of ear arteries to cholinergic stimulation than it does for femoral arteries; this could be related to regional differences in cholinergic relaxation mechanisms.

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