Effect of subthreshold ouabain on the tone of guinea-pig aortic strips following repeated noradrenaline stimulation

¹Sergio Bova, *GianPaolo Rossi, Sisto Luciani, Patrizia Debetto,
*Achille C. Pessina & Gabriella Cargnelli

Department of Pharmacology, L.go Meneghetti, 2, 35131 Padova and *Institute of Clinical Medicine, Via Giustiniani, 2, University of Padova, 35100 Padova, Italy

1 The effect of ouabain at a concentration (0.8 μM) that does not induce contractile response in guinea-pig aortic strips has been studied on endothelium-denuded strips repeatedly stimulated with 1 μM noradrenaline or 60 mM K^+ applied for 5 min every 30 min.

2 The resting tone (i.e. the tone between one noradrenaline stimulation and the following) of the aortic strips exposed to ouabain increased progressively, whereas the control strips (no ouabain) completely relaxed on washout of the agonist. In the aortic strips stimulated by 60 mM K^+ , the resting tone did not increase.

3 The calcium antagonist, verapamil, did not affect the increase in tone, that was nevertheless strictly dependent on external calcium, since the contracted strips completely relaxed on calcium removal and promptly contracted again on calcium readdition. This finding indicates a mechanism independent of voltage-gated calcium channels.

4 Caffeine-induced contractions, taken as a measure of sarcoplasmic reticulum calcium content, were amplified by the presence of ouabain in aortic strips either stimulated by noradrenaline or unstimulated, with a larger increase in the former.

5 These results suggest that the repeated stimulation of guinea-pig aortic strips by noradrenaline in the presence of ouabain, by raising both intracellular Na^+ and Ca^{2+} , decreases the ouabain threshold concentration required for contraction, thus increasing the responsiveness of vascular smooth muscle to the glycoside.

Keywords: Vascular smooth muscle; hypertension; ouabain; Na^+ pump; $\text{Na}^+/\text{Ca}^{2+}$ exchange

Introduction

It has been proposed that in some forms of hypertension, circulating endogenous inhibitors of the plasmalemma Na^+ pump (Na^+-K^+ ATPase) could be responsible for the increased vascular smooth muscle tone (Blaustein, 1977; de Wardener & MacGregor, 1980; Hamlyn *et al.*, 1982). The recent identification (Hamlyn *et al.*, 1991) of an endogenous ouabain-like compound (OLC) identical to the digitalis glycoside, ouabain, in the blood of various mammals, including man and the observation that plasma levels of digitalis-like compounds increase in different forms of hypertension (Gruber *et al.*, 1980; 1982; Masugi *et al.*, 1986; Deray *et al.*, 1987; Moreth *et al.*, 1987) support the hypothesis that the inhibition of the Na^+ pump in vascular smooth muscle cells by endogenous factors could be involved in the pathophysiology of hypertension (Blaustein *et al.*, 1990; Bova *et al.*, 1990).

Cardiac glycosides induce a myogenic tonic contraction in isolated vascular smooth muscle preparations from animals and man (Moreland *et al.*, 1986; Bova *et al.*, 1988; Woolfson *et al.*, 1990) and long-term administration of ouabain produces hypertension in rats (Yuan *et al.*, 1993). However, since the OLC concentration (138 pmol l^{-1}) found in human plasma (Hamlyn *et al.*, 1991) is presumably not sufficient to induce an increase in vascular tone, mechanisms other than a direct contractile effect on vascular smooth muscle have been implicated, such as the inhibition of endothelium-induced vascular relaxation (Boulanger *et al.*, 1989; Woolfson & Poston, 1991) or the enhancement of endothelin secretion (Yamada *et al.*, 1990; Goligorsky *et al.*, 1991). Furthermore, OLC, at a concentration that does not stimulate a contractile response, increases the responsiveness to vasoconstrictor stimuli of isolated vascular smooth muscle preparations, possibly as a consequence of an increased calcium content of the sarco-

plasmic reticulum (Bova *et al.*, 1991). In addition, an increase in the responsiveness to vasoconstrictor stimuli has been demonstrated in man after short-term treatment with the cardiac glycoside, digoxin (Guthrie, 1984). There is evidence therefore that the inhibition of the Na^+ pump, even though not sufficient to induce contraction of vascular smooth muscle, amplifies the response to vasoconstrictor stimuli.

In this context, we have hypothesized that vasoconstrictor stimuli can induce hyperresponsiveness of vascular smooth muscle to ouabain. In other words, the stimulation of vascular smooth muscle by vasoconstrictor stimuli could decrease the threshold for contraction to ouabain. To test this hypothesis, we have studied the effect of transient and repeated noradrenaline stimulation on guinea-pig aortic strips exposed to ouabain concentrations subthreshold for contraction. A preliminary account of these results was given to the American College of Cardiology (Bova *et al.*, 1992).

Methods

Aortae were excised from guinea-pigs (350–450 g) killed by a sharp blow to the neck. The thoracic part of the vessels was carefully cleaned of connective tissue, cut into helicoidal strips (10 $\times$ 1.5 mm) and the endothelium gently removed. The strips were then suspended vertically in 16 ml water-jacketed organ baths filled with physiological salt solution (PSS) aerated with 95% O_2 and 5% CO_2 and maintained at 35°C (pH 7.36–7.40). The composition of the solution was as follows (mM): NaCl 118, KCl 4.75, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 2.7, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.2, KH_2PO_4 1.2, NaHCO_3 25, glucose 11. One end of each strip was anchored to the bottom of the bath and the other connected to a force transducer (Battaglia-Rangoni TRB/200/2) coupled to a pen recorder (Battaglia-Rangoni KV 220) for monitoring isometric force generation. The tissues

¹ Author for correspondence.

were preloaded with approximately 1.8 g and allowed to equilibrate for at least 120 min before starting the experiment.

Ca²⁺-free solution was made by omitting CaCl₂ from, and adding 1 mM EGTA to the PSS. The calcium antagonist, verapamil (10 µM), was present in the PSS throughout the experiments performed with noradrenaline. Preliminary experiments showed that this concentration of verapamil inhibited the contraction induced by 1 µM noradrenaline by about 10%. The 60 mM K⁺ solution was made by adding the appropriate amount of KCl to the PSS, without substituting K⁺ for Na⁺, since lowering the Na⁺ gradient affects Na⁺/Ca²⁺ exchange activity. It has been reported that high K⁺ (up to 60 mM) does not have a hyperosmotic effect on vascular smooth muscle contractility (Ozaki *et al.*, 1978).

Experimental protocol

The aortic strips were repeatedly stimulated by 1 µM noradrenaline or 60 mM K⁺ applied for 5 min every 30 min. After the first reproducible response to noradrenaline or high K⁺ was obtained, the stimulation was continued in one set of strips in the absence, and in another set in the presence of 0.8 µM ouabain (subthreshold ouabain). The subthreshold ouabain concentration was previously determined in unstimulated strips, where it was found that ouabain up to 1 µM did not elicit a contractile response within a period of exposure of 240 min (Figure 1a). In all the experiments, a concentration of 0.8 µM ouabain was used. The subthreshold ouabain concentration was occasionally checked in unstimulated strips. The subthreshold concentration of K⁺ (0.5 mM) was determined as detailed in Results and aortic strips were repeatedly stimulated by 1 µM noradrenaline applied for 5 min every 30 min in the presence of 0.5 mM K⁺.

Statistical analysis

Data are expressed as means ± s.e.mean. Tests of significance were performed using Student's *t* test; a *P* < 0.05 was considered significant.

Drugs

The following drugs were used: noradrenaline bitartrate, ouabain, caffeine and amiloride (Sigma Chemical Co., St Louis, MO, U.S.A.); verapamil (Knoll, Ludwigshafen, Germany). All drugs, except caffeine, were made up as concentrated solutions in twice distilled water. Caffeine was dissolved in the PSS to obtain the desired concentration (10 mM).

Results

Effect of subthreshold ouabain

As shown in Figure 1a, in guinea-pig aortic strips the exposure for 240 min to ouabain up to 1 µM did not affect the resting tone. The aortic strips responded with reproducible contractions to repeated stimulation by 1 µM noradrenaline, applied for 5 min every 30 min, and full relaxation on washout (Figure 1b). When stimulation by noradrenaline was carried out in the presence of a subthreshold concentration of ouabain (0.8 µM), relaxation was progressively impaired upon washout of the agonist and an increase of the vascular tone occurred, the magnitude of which increased with successive stimuli (Figure 1c and Table 1). The increase in tone was well maintained in the presence of ouabain, completely reversed when ouabain was removed (Figure 1c), and was not prevented by the Ca²⁺-entry blocker verapamil (10 µM) (Figure 2), indicating that voltage-operated Ca²⁺ channels were not involved. Furthermore, the maintenance of the increase in tone was strictly dependent on extracellular Ca²⁺, since the removal of Ca²⁺ from the bath induced a rapid relaxation of

the aortic strips, that responded with a verapamil-insensitive contraction upon reintroduction of external Ca²⁺ (Figure 3a). The contraction elicited by the reintroduction of Ca²⁺ was prevented by 0.5 mM amiloride (Figure 3b), indicating the involvement of Na⁺/Ca²⁺ exchange (Bova *et al.*, 1988).

In isolated vascular smooth muscle preparations, caffeine-induced Ca²⁺ release from sarcoplasmic reticulum (SR) elicits a phasic contraction that appears to be a measure of the SR calcium content (Ashida & Blaustein, 1987; van Breemen & Saida, 1989). In some experiments, contractions were induced by 10 mM caffeine before and after exposure to ouabain. As shown in Figure 4 and Table 2, in the presence of ouabain, caffeine-induced contractions were amplified in both unstimulated and noradrenaline-stimulated aortic strips, with a larger increase in the latter. The strips previously stimulated by noradrenaline in the presence of ouabain and then relaxed by Ca²⁺-free PSS did not respond to caffeine, indicating a low SR Ca²⁺ content.

When aortic strips exposed to subthreshold ouabain were stimulated by 60 mM K⁺, instead of by noradrenaline, no increase in tone was observed.

Effect of low K⁺-PSS

In isolated vascular smooth muscle preparations, a low K⁺-medium induces slowly developing, well maintained, contractile responses that have been ascribed to Ca²⁺ entry through Na⁺/Ca²⁺ exchange activated by the decrease of the Na⁺ gradient consequent on Na⁺ pump inhibition (Karaki *et al.*, 1978; Ozaki *et al.*, 1978; Bova *et al.*, 1988). The degree of inhibition is dependent on the extent of K⁺ lowering, since it has been shown that the activity of the Na⁺ pump is directly related to the extracellular K⁺ concentration (Lindenmayer & Schwartz, 1975; Moromizato *et al.*, 1992).

In our experiments, the exposure of aortic strips to external K⁺ concentrations lower than 0.5 mM induced a contractile response, while no response was observed at 0.5 mM external K⁺ or above (Figure 5a), so this concentration of K⁺ has been considered as subthreshold for the contractile response. In the aortic strips incubated in 0.5 mM K⁺-PSS, repeated stimulation by 1 µM noradrenaline for 5 min every 30 min elicited a progressive increase in the tone between each stimulus (Figure 5b and Table 1). This effect was not affected by 10 µM verapamil and was strictly dependent on extracellular Ca²⁺, since the contracted strips relaxed on Ca²⁺ removal and contracted again on Ca²⁺ readmission (Figure 5b and Table 1). This Ca²⁺-induced contraction was prevented by 0.5 mM amiloride (Figure 5c). The response to caffeine (10 mM) was increased both in the unstimulated and noradrenaline-stimulated aortic strips, with a greater increase in the latter (Figure 6 and Table 2). Therefore, the effects of subthreshold concentrations of ouabain and potassium on the tone of the aortic strips after noradrenaline stimulation were identical.

Discussion

The results of this study show that ouabain, at a concentration subthreshold for contraction, elicits an increase in tone between successive stimuli in guinea-pig aortic strips repeatedly stimulated by noradrenaline. This effect, the magnitude of which increases with the number of stimuli, is unaffected by the calcium antagonist, verapamil, indicating that voltage-operated calcium channels are not involved. Similar results are obtained when a low concentration of potassium is used instead of ouabain. In our experiments, the enhancement of agonist-induced contraction caused by ouabain (Ashida & Blaustein, 1987; Bova *et al.*, 1991) is not observed, presumably because a concentration of noradrenaline very close to that giving maximal contraction has been chosen to show better the effect of ouabain.

Taking into account that ouabain and low potassium elicit myogenic, slow developing contractile responses in guinea-pig aortic strips and human subcutaneous resistance vessels (Ozaki *et al.*, 1978; Moreland *et al.*, 1986; Bova *et al.*, 1988; Woolfson *et al.*, 1990), our results indicate that noradrenaline stimulation apparently triggers the contractile effect of either ouabain or low potassium. The contractile response of vascular smooth muscle to ouabain or low potassium has been ascribed to Ca^{2+} entry through the $\text{Na}^+/\text{Ca}^{2+}$ exchange system as a consequence of Na^+ pump inhibition (Ozaki *et al.*, 1978; Bova *et al.*, 1988; Woolfson *et al.*, 1990). $\text{Na}^+/\text{Ca}^{2+}$

exchange was first recognized as one of the two mechanisms, together with the ATP-dependent Ca^{2+} pump, that extrude calcium from the cytosol through the plasmalemma of vascular smooth muscle cells (Schatzmann, 1985). More recent evidence indicates that the exchanger is a bidirectional transport system operating in the Ca^{2+} entry mode when the Na^+ gradient across the plasma membrane decreases (Ashida & Blaustein, 1987; Nabel *et al.*, 1988; Battle *et al.*, 1991).

In our experiments, the exposure of aortic strips to sub-threshold ouabain increases the contractile response to caffeine in either unstimulated or noradrenaline-stimulated strips,

Figure 1 Traces of the contractile responses to 1 µM noradrenaline of three separate guinea-pig aortic strips. (a) Trace of the first reproducible response to noradrenaline and resting tone of the strip exposed to 1 µM ouabain for over 240 min. (b) Trace of the response of one strip to successive noradrenaline stimulations in the absence of ouabain. (c) Trace of the responses of one aortic strip to noradrenaline in the presence of 0.8 µM ouabain (left) and after removal of ouabain (right).

Table 1 Resting tone between successive contractions induced by 1 µM noradrenaline in the presence of ouabain or low K^+ in guinea-pig aortic strips

Number of contractions	Resting tone (mg)			
	I	II	III	IV
Ouabain (0.8 µM)	0	103 ± 13	213 ± 20	300 ± 32
K^+ (0.5 mM)	137 ± 41	258 ± 26	323 ± 19	398 ± 24

Resting tone is the tone maintained by the aortic strips before each noradrenaline addition. Noradrenaline-induced contractions are numbered after the beginning of the exposure to 0.8 µM ouabain or 0.5 mM K^+ . Data are mean values ± s.e. mean of at least nine experiments performed as shown in Figures 1 and 5b.

with a higher increase in the latter. This indicates that the glycoside increases the Ca^{2+} concentration in SR of vascular smooth muscle cells and that the stimulation by noradrenaline amplifies this effect. Taking into account that ouabain promotes Ca^{2+} entry via the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (Ozaki *et al.*, 1978; Bova *et al.*, 1988; Woolfson *et al.*, 1990), the lack of effect of the concentration of ouabain used here on the resting tone of the unstimulated strips may be explained by a partial inhibition of the Na^+ pump that does not increase the intracellular Na^+ concentration to a sufficient extent to transport enough Ca^{2+} for contraction. However, Ca^{2+} transported

into the cells, although not sufficient to elicit a contraction, enhances the size of the SR Ca^{2+} store, as shown also by Ashida & Blaustein (1987). The increase in tone in noradrenaline-stimulated strips may be accounted for by the further enhancement of the SR Ca^{2+} concentration (as shown by higher caffeine-induced contraction in stimulated versus unstimulated strips), that could be explained by noradrenaline-induced Ca^{2+} influx through receptor-operated calcium channels in cells where $\text{Na}^+/\text{Ca}^{2+}$ exchange is functionally excluded as a Ca^{2+} extrusion mechanism and operates as a Ca^{2+} influx mechanism.

Figure 2 Traces of the contractile responses to $1 \mu\text{M}$ noradrenaline of two separate guinea-pig aortic strips exposed to $10 \mu\text{M}$ verapamil and repeatedly stimulated in the absence (a) and presence (b) of $0.8 \mu\text{M}$ ouabain.

Figure 3 Effect of Ca^{2+} removal and readmission in the absence (a) and presence (b) of 0.5 mM amiloride in two separate guinea-pig aortic strips previously stimulated by $1 \mu\text{M}$ noradrenaline in the presence of ouabain.

Figure 4 Traces of the contractile responses of three separate aortic strips to 10 mM caffeine applied both before and after repeated stimulation by $1 \mu\text{M}$ noradrenaline (a), exposure to $0.8 \mu\text{M}$ ouabain (b), and repeated stimulation by $1 \mu\text{M}$ noradrenaline in the presence of ouabain (c).

Table 2 Caffeine-induced contractions in guinea-pig aortic strips

	Peak developed tension (mg)	
	Unstimulated	Stimulated
Control	92 ± 18	92 ± 18
Ouabain ($0.8 \mu\text{M}$)	$211 \pm 30^*$	$362 \pm 55^{**}$
$\text{K}^+(0.5 \text{ mM})$	$240 \pm 30^*$	$380 \pm 20^{**}$

Caffeine (10 mM) was applied before and after the exposure of either unstimulated or noradrenaline-stimulated aortic strips to ouabain or low K^+ . The values of peak developed tension for the stimulated strips refer to caffeine-induced contractions measured from the maintained tension to the peak contraction. The unstimulated strips were exposed to ouabain or low K^+ -PSS for the same period of time as the stimulated strips (about 150 min). Data are mean values $\pm$ s.e. mean of at least five experiments performed as shown in Figures 4 and 6. $^*P < 0.05$ vs control in unstimulated strips (Student's *t* test for paired data); $^{**}P < 0.05$ stimulated vs unstimulated strips (Student's *t* test for unpaired data).

Figure 5 Traces of the contractile responses to 1 μ M noradrenaline of three separate aortic strips. (a) Trace of the first reproducible response to noradrenaline and resting tone of the strip exposed to low K⁺-PSS for over 240 min. (b) Trace of the responses of strip repeatedly stimulated by 1 μ M noradrenaline in low K⁺-PSS and in the presence of 10 μ M verapamil, followed by Ca²⁺ removal and readmission. (c) Effect of 0.5 mM amiloride on Ca²⁺-induced contraction.

However, the stimulation of ouabain-exposed strips by noradrenaline should have produced some other modification in addition to the increase in stored Ca²⁺. In fact, strips previously stimulated by noradrenaline and then relaxed by Ca²⁺-free PSS respond with a verapamil-insensitive, amiloride-inhibited, tonic contraction when Ca²⁺ is readmitted. This in spite of the low SR Ca²⁺ content, as shown by the lack of response to caffeine. Since it has been reported that in vascular smooth muscle cells several agonists induce a dose-dependent increase in Na⁺ influx following the increase in intracellular Ca²⁺ (Smith & Brock, 1983; Aaronson & Jones, 1988; Vallega *et al.*, 1988; Okada *et al.*, 1991), it can be assumed that Na⁺ entry at each noradrenaline stimulation results in a progressive intracellular Na⁺ accumulation because the Na⁺ pump is inhibited by ouabain. The enhancement of intracellular Na⁺ concentration increases the driving force for Ca²⁺ influx through Na⁺/Ca²⁺ exchange. The inhibition by amiloride of the contraction induced by Ca²⁺ in Ca²⁺-free PSS-relaxed aortic strips supports this explanation, since it has been shown that amiloride prevents contractile responses of guinea-pig aortic strips induced by Ca²⁺ entry through Na⁺/Ca²⁺ exchange (Bova *et al.*, 1988). Therefore, it can be proposed that repeated noradrenaline stimulation decreases the threshold of ouabain for contraction of aortic strips not only by contributing to Ca²⁺ entry (via receptor-operated calcium channels) but also by inducing influx of Na⁺. A further mechanism may be postulated: since intracellular Na⁺ activates the turnover rate of the Na⁺ pump

Figure 6 Traces of the contractile responses of three separate aortic strips to 10 mM caffeine applied both before and after repeated stimulation by 1 μ M noradrenaline (a), exposure to low K⁺-PSS (b), and repeated stimulation by 1 μ M noradrenaline in the presence of low K⁺-PSS (c).

(Eisner, 1986), the increase in intracellular Na⁺ induced by noradrenaline could result in an increased affinity of ouabain for the pump, thus explaining the response to the subthreshold concentration of the glycoside. However, the observation that the rise in tone is induced also by subthreshold K⁺-PSS indicates that an increased binding of ouabain is not required to account for our results.

The increase in intracellular Na⁺ induced by noradrenaline could also explain why repeated stimulation by high K⁺ of aortic strips in the presence of subthreshold ouabain does not induce an increase in tone, since Na⁺ influx associated with high K⁺-mediated contraction is smaller than that induced by noradrenaline stimulation (Aaronson & Jones, 1988). Furthermore, the mutually antagonistic actions of potassium and digitalis glycosides on the Na⁺ pump (Schwartz *et al.*, 1975) could contribute to the ineffectiveness of high K⁺ stimulation.

In conclusion, the repeated stimulation by noradrenaline of guinea-pig vascular smooth muscle *in vitro* lowers the threshold concentration of ouabain required for contraction. If vasoactive stimuli have similar effects in man *in vivo*, OLC could contribute to enhance vascular tone in hypertension not only by amplifying vasoconstrictor-evoked contractions (Bova *et al.*, 1991), but also by a direct contractile action. A physiological role and pathophysiological implications of the presence of OLC in human plasma have indeed been suggested (Hamlyn *et al.*, 1991). Even though the concentration of ouabain used in this study is much higher than the plasma level of OLC found in man, it should be taken into account that, at least for the positive inotropic effect of digitalis, the *in vitro* sensitivity of the guinea-pig to ouabain is roughly ten

fold lower than that in man (Brown *et al.*, 1986). Furthermore, the ratio between active concentrations of ouabain on human myocardium *in vitro* and therapeutic plasma levels of cardiac glycosides *in vivo* is about 50.

References

- AARONSON, P.I. & JONES, A.W. (1988). Ca dependence of Na influx during treatment of rabbit aorta with NE and high K solutions. *Am. J. Physiol.*, **254**, C75–C83.
- ASHIDA, T. & BLAUSTEIN, M.P. (1987). Regulation of cell calcium and contractility in mammalian arterial smooth muscle. The role of sodium-calcium exchange. *J. Physiol.*, **392**, 617–635.
- BATTLE, D.C., GODINICH, M., LAPOINTE, M.S., MUNOZ, E., CARONE, F. & MEHERING, N. (1991). Extracellular Na⁺ dependency of free cytosolic Ca²⁺ regulation in aortic vascular smooth muscle cells. *Am. J. Physiol.*, **261**, C845–C856.
- BLAUSTEIN, M.P. (1977). Sodium ions, calcium ions, blood pressure regulation and hypertension: a reassessment and a hypothesis. *Am. J. Physiol.*, **232**, C165–C173.
- BLAUSTEIN, M.P., BOVA, S., YUAN, X.-J. & GOLDMAN, W.F. (1990). The role of sodium/calcium exchange in the regulation of vascular contractility. In *Calcium Transport and Intracellular Calcium Homeostasis*. ed. Datsu, T. & Bronner, F. pp. 123–132. Berlin: Springer-Verlag.
- BOULANGER, C., HENDRICKSON, H., LORENZ, R.R. & VANHOUTTE, P.M. (1989). Release of different relaxing factors by cultured porcine endothelial cells. *Circ. Res.*, **64**, 1070–1078.
- BOVA, S., BLAUSTEIN, M.P., HARRIS, D.W., LUDENS, J.H., DUCHARME, D.W. & HAMLYN, J.M. (1991). Effects of an endogenous ouabainlike compound on heart and aorta. *Hypertension*, **17**, 944–950.
- BOVA, S., CARGNELLI, G. & LUCIANI, S. (1988). Na/Ca exchange and tension development in vascular smooth muscle: effect of amiloride. *Br. J. Pharmacol.*, **93**, 601–608.
- BOVA, S., CARGNELLI, G., ROSSI, G.P. & PESSINA, A.C. (1992). Subthreshold ouabain concentrations affect resting tone of aortic strips contracted by noradrenaline (Ne) and histamine (H) (abstract). *J. Am. Coll. Cardiol.*, **19**, 75A.
- BOVA, S., GOLDMAN, W.F., YUAN, X.-J. & BLAUSTEIN, M.P. (1990). Influence of the Na⁺ gradient on Ca²⁺ transients and contraction in vascular smooth muscle. *Am. J. Physiol.*, **259**, H409–H423.
- BROWN, L., LORENZ, B. & ERDMAN, E. (1986). Additive and non-additive positive inotropic effects in human and guinea-pig myocardium. In *Cardiac Glycosides 1785–1985*. ed. Erdman, E., Greef, K. & Skou, J.C. pp. 195–205. New York: Springer-Verlag.
- DE WARDENER, H.E. & MACGREGOR, G.A. (1980). Dahl's hypothesis that a saluretic substance may be responsible for a sustained rise in arterial pressure: its possible role in essential hypertension. *Kidney Int.*, **18**, 1–9.
- DERAY, G., RIEU, M., DEVYNCK, M.A., PERNOLLET, M.G., CHANSON, P., LUTON, J.P. & MEYER, P. (1987). Evidence for an endogenous digitalis-like factor in the plasma of patients with acromegaly. *New Engl. J. Med.*, **316**, 575–580.
- EISNER, D.A. (1986). The Na-K pump in cardiac muscle. In *The Heart and Cardiovascular System*. ed. Fozzard, H.A., Haber, E., Jennings, R.B., Katz, A.M. & Morgan, H.E. pp. 489–507. New York: Raven Press.
- GOLIGORSKY, M.S., IJIMA, K., MORGAN, M., YANAGISAWA, M., MASAKI, T., LIN, L., NASIILETTI, A., KASKEL, F., FRAZER, M. & BADR, K.F. (1991). Role of endothelium in the development of Dahl hypertension. *J. Cardiovasc. Pharmacol.*, **17** (suppl 7), S84–S91.
- GRUBER, K.A., RUDEL, L.L. & BULLOCK, B.C. (1982). Increased circulating levels of an endogenous digoxin-like factor in hypertensive monkeys. *Hypertension*, **4**, 348–354.
- GRUBER, K.A., WHITAKER, J.M. & BUCKALEW, V.H. (1980). Endogenous digitalis-like substance in plasma volume-expanded dogs. *Nature*, **287**, 743–745.
- GUTHRIE, T.P.Jr. (1984). Effects of digoxin on responsiveness to the pressor actions of angiotensin and noradrenaline. *J. Clin. Endocrinol. Metab.*, **58**, 76–80.
- HAMLYN, J.M., BLAUSTEIN, M.P., BOVA, S., DUCHARME, D.W., HARRIS, D.W., MANDEL, F., MATHEWS, W.R. & LUDENS, J.H. (1991). Identification and characterization of a ouabain-like compound from human plasma. *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 6259–6263.
- HAMLYN, J.M., RINGEL, R., SCHAEFFER, J., LEVINSON, P.D., HAMILTON, B.P., KOWARSKI, A.A. & BLAUSTEIN, M.P. (1982). A circulating inhibitor of (Na-K)-ATPase associated with essential hypertension. *Nature*, **300**, 650–652.
- KARAKI, H., OZAKI, H. & URAKAWA, N. (1978). Effects of ouabain and potassium-free solution on the contraction of isolated blood vessels. *Eur. J. Pharmacol.*, **48**, 439–443.
- LINDENMAYER, G.E. & SCHWARTZ, A. (1975). A kinetic characterization of calcium on (Na⁺/K⁺)-ATPase and its potential role as a link between extracellular and intracellular events: hypothesis for digitalis-induced inotropism. *J. Mol. Cell. Cardiol.*, **7**, 591–612.
- MASUGI, F., OGIHARA, T., HASEGAWA, T., TOMII, A., NAGANO, M., HIGASHIMORI, K., KUMAHARA, K. & TERANO, Y. (1986). Circulating factor with ouabain-like immunoreactivity in patients with primary aldosteronism. *Biochem. Biophys. Res. Commun.*, **135**, 41–45.
- MORELAND, R.S., TERRY, C.M. & WEBB, R.C. (1986). Contractile responses to ouabain and K⁺-free solution in aorta from hypertensive rats. *Am. J. Physiol.*, **250**, H612–H619.
- MORETH, K., RENNER, D. & SCHONER, W. (1987). A quantitative receptor assay for 'digitalis-like' compounds in serum: demonstration of raised concentrations in essential hypertension and correlation with arterial blood pressure. *Klin. Wochenschr.*, **65**, 179–184.
- MOROMIZATO, H., MIYAGI, H., NAKAYAMA, M., HIGUCHI, M. & SAKANASHI, M. (1992). Effects of ouabain on contractile response to noradrenaline in isolated rat aorta. *Gen. Pharmacol.*, **23**, 279–283.
- NABEL, E.G., BERK, B.C., BROCK, T.A. & SMITH, T.W. (1988). Na⁺-Ca²⁺-exchange in cultured vascular smooth muscle cells. *Circ. Res.*, **62**, 486–493.
- OKADA, K., ISHIKAWA, S. & SAITO, T. (1991). Mechanisms of vasopressin-induced increase in intracellular Na⁺ in vascular smooth muscle cells. *Am. J. Physiol.*, **261**, F1007–F1012.
- OZAKI, H., KARAKI, H. & URAKAWA, N. (1978). Possible role of Na-Ca exchange mechanism in the contractions induced in guinea-pig aorta by potassium free solution and ouabain. *Arch. Pharmacol.*, **304**, 203–209.
- SCHATZMANN, H.J. (1985). Calcium extrusion across the plasma membrane by the calcium-pump and the Ca²⁺-Na⁺ exchange system. In *Calcium and Cell Physiology*. ed. Marmé, D. pp. 18–52. Berlin: Springer-Verlag.
- SCHWARTZ, A., LINDENMAYER, G.E. & ALLEN, J.C. (1975). The sodium-potassium adenosine triphosphatase: pharmacological, physiological and biochemical aspects. *Pharmacol. Rev.*, **27**, 4–134.
- SMITH, J.B. & BROCK, T.A. (1983). Analysis of angiotensin-stimulated sodium transport in cultured smooth muscle cells from rat aorta. *J. Cell Physiol.*, **114**, 284–290.
- VALLEGA, G.A., CANESSA, M.L., BERCK, B.C., BROCK, T.A. & ALEXANDER, R.W. (1988). Vascular smooth muscle Na⁺-H⁺ exchanger kinetics and its activation by angiotensin II. *Am. J. Physiol.*, **254**, C751–C758.
- VAN BREEMAN, C. & SAIDA, K. (1989). Cellular mechanisms regulating [Ca²⁺]_i smooth muscle. *Annu. Rev. Physiol.*, **51**, 315–329.
- WOOLFSON, R.G., HILTON, P.J. & POSTON, L. (1990). Effects of ouabain and low sodium on contractility of human subcutaneous resistance arteries. *Hypertension*, **15**, 583–590.
- WOOLFSON, R.G. & POSTON, L. (1991). Effect of ouabain on endothelium-dependent relaxation of human resistance arteries. *Hypertension*, **17**, 619–625.
- YAMADA, H., GOTO, A., HUI, C. & SUGIMOTO, T. (1990). Endogenous digitalis-like factor as a stimulator of endothelin secretion from endothelial cells. *Biochem. Biophys. Res. Commun.*, **172**, 178–183.
- YUAN, C.M., MANUNTA, P., HAMLYN, J.M., CHEN, S., BOHEN, E., YEUN, J., HADDY, F.J. & PAMNANI, M.B. (1993). Long-term ouabain administration produces hypertension in rats. *Hypertension*, **22**, 178–187.

(Received November 8, 1993
Revised December 3, 1993
Accepted December 12, 1993)