Br. J. Pharmac. (1984), **83**, 187–193

Potassium induces relaxation and hyperpolarization of circular muscles but contraction of longitudinal muscles of pig duodenum

I. Kimura, M. Kimura & M. Kimura

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Toyama Medical & Pharmaceutical University, 2630 Sugitani, Toyama 930–01, Japan

1 The mechanisms by which K^+ relaxes circular muscles of pig duodenum were investigated, and compared with the response of the longitudinal muscles to K^+.

2 Circular muscles were concentration-dependently relaxed by 8.3–23.6 mM K^+, but contracted by 47.2–143.4 mM K^+.

3 Longitudinal muscles were contracted by 11.8–94.4 mM K^+.

4 The relaxation of circular muscles was correlated with hyperpolarization (4 mV), but evoked Ca^{2+} spikes were not suppressed.

5 Neither ouabain (0.14 μM) nor phentolamine (10 μM) blocked the relaxation, but tetrodotoxin (TTX, 0.63 μM) blocked both the relaxation and hyperpolarization. Mesaconitine (0.16 μM) increased the relaxation. Inhibitory junction potentials and concomitant relaxations were also blocked by TTX.

6 The results suggest that K^+-induced relaxation is caused by the release of a non-adrenergic inhibitory transmitter.

Introduction

In a previous paper on pig bile duct ampulla (Kimura *et al.*, 1983), we showed that CCK (cholecystokinin)-C-terminal peptides relax circular muscle segments by an action other than direct suppression of calcium influx, but arrest the spontaneous contraction of longitudinal muscle segments by suppressing the calcium influx. In duodenum taken about 3 cm from the bile duct ampulla, both muscle segments respond similarly to those in ampulla and can be more easily separated anatomically than in the ampulla.

Potassium-induced relaxation has been well documented in arterial muscles (Chen *et al.*, 1972; Toda 1974; 1976; Haddy, 1975; 1978; Bonaccorsi *et al.*, 1977; Webb & Bohr, 1978; Reiner, 1978; Hagen *et al.*, 1982, Harder, 1982), and in the anococcygeus muscle (Ishii & Shimo, 1980). The 'relaxation' referred to in these publications was an inhibition of noradrenaline, 5-hydroxytryptamine, acetylcholine, prostaglandin $F_{2\alpha}$ or K^+-free solution-induced contraction. In the present paper the relaxation in duodenal circular muscles is a change in resting tone, as documented in guinea-pig taenia coli (Ishii & Shimo, 1979). We investigated the mechanism by which K^+ induces relaxation in pig duodenum, intending to confirm the effect in both longitudinal and circular muscles by using electrophysiological and mechanical methods of recording. However, we found that the K^+-induced relaxation of the latter muscle was distinctly different from K^+-induced response of the former.

Methods

Pigs of either sex weighing about 90 kg were killed by electric shock in a slaughter house.

The duodenum situated about 2–5 cm from the bile duct ampulla was isolated. After the serous and mucous membranes surrounding the segments were cut away, longitudinal and circular muscles were separated and cut into 2 × 10 mm sections for mechanical recording and 5 × 15 mm sections for simultaneous electrical and mechanical recording. A modified Krebs solution of the following composition (mM) was used; NaCl 122, KCl 5.9, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 15.5 and glucose 11.5. The bath solu-

tion was maintained at 37 °C, and bubbled with 95% O_2 and 5% CO_2. By replacing the NaCl with KCl, we prepared a solution containing 8.3, 11.8, 23.6, 47.2 and 94.4 mM K^+. Ca^{2+}-free solutions with or without 2mM EGTA were used.

Mechanical measurements

The longitudinal or circular smooth muscles were suspended in an organ bath containing 5 ml of Krebs solution. Isotonic contractions were recorded on a technicorder F (Type 3051, Yokogawa) with a DISP transducer coupled to a Biophysiograph (Type 180–2, San-ei). The resting tone was adjusted to 500 mg. K^+-relaxations (or contractions) were obtained by exchanging the normal Krebs solution in a stepwise manner with solutions containing increasing concentrations of K^+.

Electrical measurements

A 300 mg load was applied to the tissue. The partition stimulating method of Abe & Tomita (1968) was used for stimulation and recording. One end of the muscle was passed through holes in two Ag-AgCl plates 10 mm apart and connected to an isometric transducer (SB-1T-H, Nihon Kohden) for recording tension. A current (10 μA; 10, 20, 30 or 700 ms; 0.033 Hz) was applied between these two plates. The other end of the preparation was pinned to a rubber plate. An intracellular glass electrode (tip resistance, 20–80 MΩ, filled with 3 M KCl), was inserted into the pinned area under a stereotaxic microscope.

Reagents

Acetylcholine chloride (Daiichi), ouabain 8–9 H_2O (Merk), phentolamine mesylate (Ciba), tetrodotoxin (TTX, Sankyo) and EGTA (Nakarai) were used. Mesaconitine (a gift from H. Hikino, Dept. of Pharmacognosy, Tohoku University) was dissolved in 0.1 N HCl solution before dilution.

Results

Concentration-response curves of circular and longitudinal muscles of pig duodenum to potassium

The Na^+ in Krebs solution was replaced with K^+ to prepare 8.3, 11.8, 23.6, 47.2, 94.4 and 143.4 mM K^+

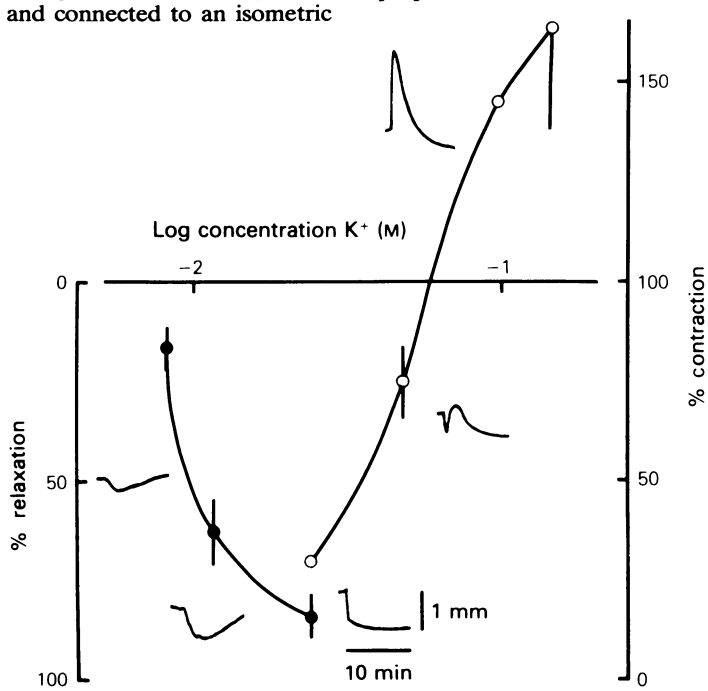

Figure 1 Log concentration-response curves for the isotonic relaxation (●) and contraction (○) of the circular muscles of pig duodenum induced by increasing concentrations of K^+, added cumulatively. The relaxation is expressed as a percentage of maximal response to Ca^{2+}-free Krebs solution containing 2mM EGTA and the contractile response was measured from the point of maximal relaxation. Each point represents the mean (n = 2–4) and the vertical lines show s.e.mean. The traces show typical responses (changes in length) to 8.3, 11.8, 23.6, 47.2 and 94.4 mM K^+.

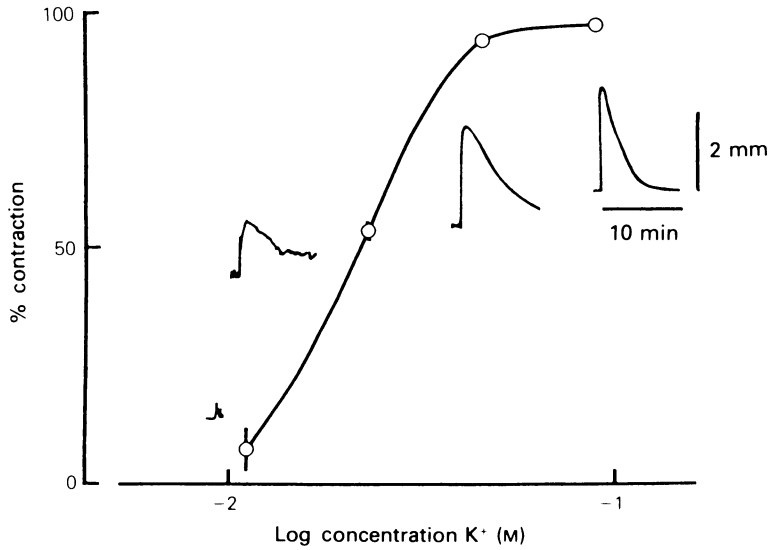

Figure 2 Log concentration-response curve for the contraction of the longitudinal muscles induced by increasing concentrations K^+ added cumulatively. Isotonic contractions are expressed as a percentage of the maximal contraction induced by 143.4 mM K^+ solution and each point represents the mean ($n = 3$) with vertical lines showing s.e.mean. Each trace shows a typical response to 11.8, 23.6, 47.2 or 94.4 mM K^+.

solutions. In circular muscles K^+ produced an immediate and concentration-dependent relaxation at the first three concentrations. The 23.6 mM K^+-induced relaxation was estimated to be 80 % of the maximal one in Ca^{2+}-free Krebs solution containing 2 mM EGTA. The 47.2 mM K^+ solution initially relaxed the muscle and thereafter immediately contracted it. Contraction was measured from the point of maximal relaxation. At the last three concentrations, K^+ contracted the circular muscle in a concentration-dependent manner, as shown in Figure 1, where % contraction was represented on the same scale as above. The contraction induced by 143.4 mM K^+ was maximal.

In longitudinal muscles 11.8, 23.6, 47.2 and 94.4 mM K^+ produced only contraction, as shown in Figure 2, where the response to 143.4 mM K^+ was maximal. Note that 47.2 mM K^+ also induced maximal contractions.

Thus, it appears that K^+-induced relaxation is a distinctive property of circular muscles.

Potassium-induced resting membrane potential changes in circular muscles

The relation between log concentration of K^+ and membrane potential is plotted in Figure 3. The representative traces show first a depolarization, secondly a hyperpolarization (Figure 3a), and thirdly a depolarization (Figure 3b) on exposure to 23.6 mM K^+. The relaxation induced by 23.6 mM K^+ was observed simultaneously with the hyperpolarization. The graph shows the second and third potential changes plotted against K^+ concentration. Over the range of concentrations of 11.8 to 29.5 mM K^+ -1.93 to -1.53 on a log scale), the membrane hyperpolarized to 4 mV in a concentration-dependent manner; however, at 35.4 mM phase (a) was no longer discernible. Depolarization during phase (b) was enhanced gradually, and at 35.4 mM K^+ it was steeply increased. In some tissues which were slowly relaxed by 23.6 mM K^+ hyperpolarizing fluctuations of resting membrane potential were observed before the relaxation.

These results indicate that relaxation was caused by the K^+-induced hyperpolarization; the fluctuating hyperpolarization may reflect endogenous (neurogenic) release of an inhibitory transmitter.

The effects of phentolamine, ouabain and tetrodotoxin on potassium-induced relaxation in circular muscles

In vascular smooth muscles K^+-induced inhibition of contractions to acetylcholine (ACh) was termed a 'relaxation'. In circular muscles of pig duodenum K^+-induced inhibition of ACh (0.3 mM)-elicited contractions was not readily measurable, as phasic rather than tonic contractions were provoked (Figure 4). Thus, the K^+-induced relaxation was simpler to in-

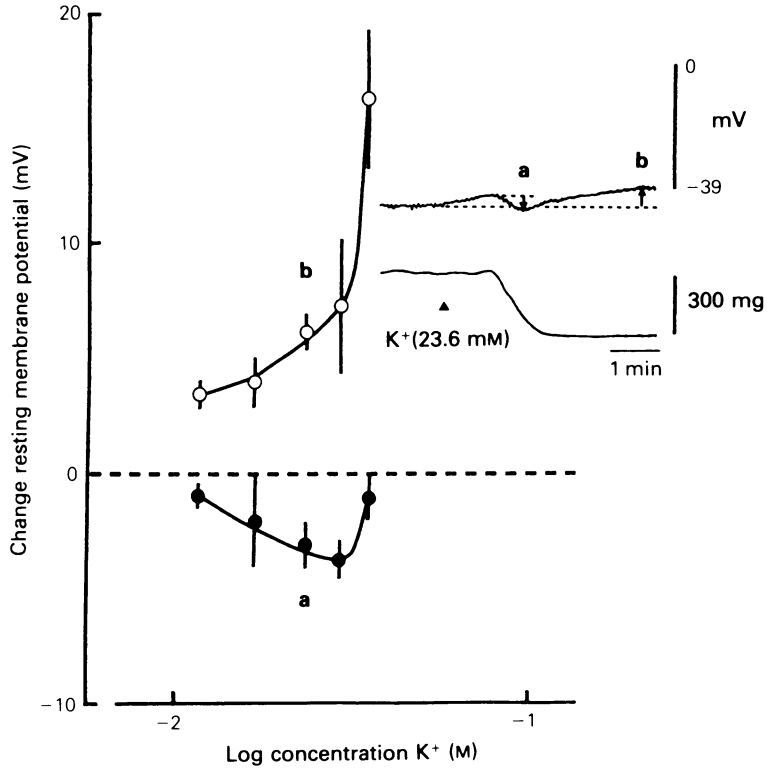

Figure 3 Change in resting membrane potential of circular muscles to increasing concentrations of K^+ (added cumulatively) shown as log concentration-effect curves. Each point represents the mean ($n = 3-7$) and vertical lines show s.e.mean. Typical records show K^+-induced relaxation (lower trace), and hyperpolarization (a, 4 mV upper trace) after initial short-term depolarization, followed by depolarization (b, 5 mV). The 0 membrane potential was taken to be the plateau of the first depolarization induced by K^+ in (a), and the resting membrane potential before K^+ addition in (b).

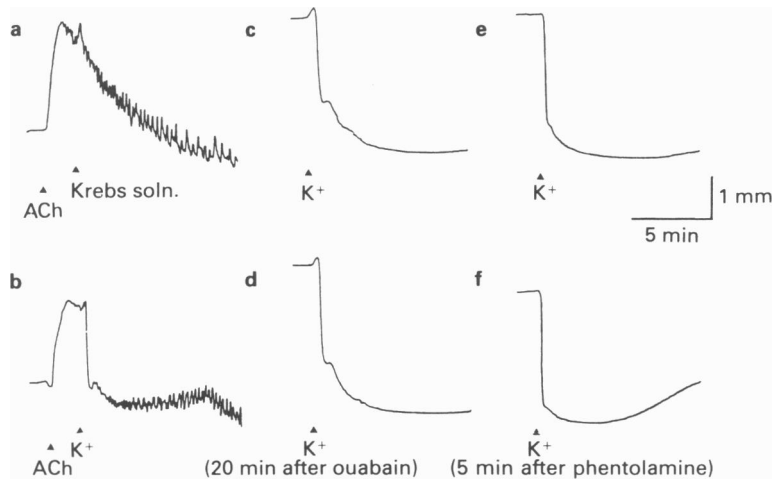

Figure 4 Typical inhibitory responses of the circular muscle induced by K^+ (23.6 mM) under different conditions. (a) The effect of Krebs solution on the contractile response to acetylcholine (ACh, 0.3 mM) and (b) the effect of K^+ on this response. (c and e) show control response of the muscle to K^+ (i.e. no pretreatment), (d) K^+-induced relaxation after ouabain pretreatment (0.136 μM for 20 min) and (e) shows a similar response to K^+ after pretreatment with phentolamine (10μM for 5 min). These concentrations of ouabain and phentolamine did not change the K^+-induced relaxation of normal tone. The effects were observed consistently in 2–3 experiments.

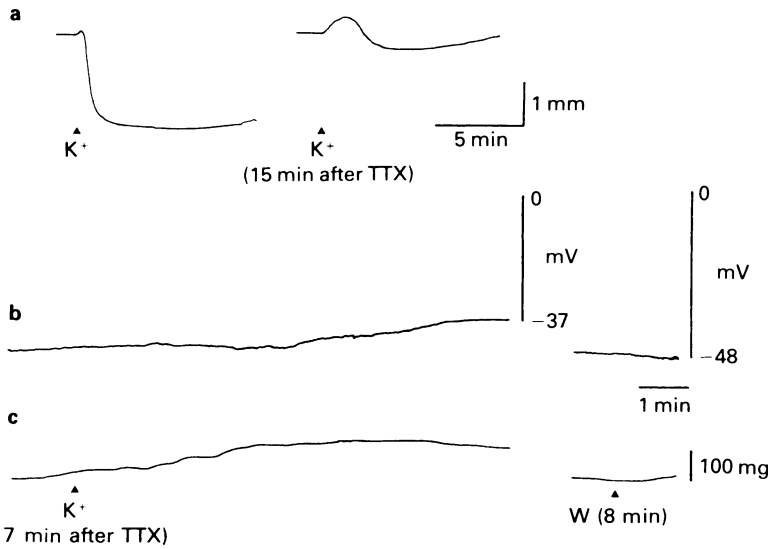

Figure 5 The reduction or suppression of K^+-induced relaxation and hyperpolarization of circular muscles by pretreatment with tetrodotoxin (TTX, 0.626 μM). The tissue was treated with TTX for 15–17 min before the addition of a solution containing K^+(23.6 mM) plus TTX. The effects were observed consistently in 5 experiments. (a) Isotonic tension changes induced by K^+ before an after pretreatment with TTX. (b) Membrane potential changes and (c) alterations in isometric tension. W; washing out.

vestigate than the K^+-induced inhibition of contracted muscles.

In order to elucidate the mechanism of K^+-induced relaxation, the circular muscles were treated for 5–20 min with phentolamine (10 μM), ouabain (0.136 μM) and tetrodotoxin (0.626 μM) before inducing a relaxation response by addition of K^+ 23.6 mM. The K^+-induced relaxation was hardly affected by ouabain, and was only slightly effected by phentolamine as shown in Figure 4.

In longitudinal muscles the contraction induced by K^+ 23.6 mM was not suppressed by tetrodotoxin, and was increased 40% by ouabain (data not shown). On the other hand, in circular muscles the K^+-induced relaxation was greatly reduced by tetrodotoxin (Figure 5), while the K^+-induced contraction was enhanced. After treatment with tetrodotoxin, 23.6 mM K^+ only depolarized the membrane.

When circular muscles were pretreated for 6.5 min with mesaconitine (0.16 μM), an agent which increases sodium permeability (Catterall *et al.*, 1981), the K^+-induced relaxation (11.8 mM) was remarkably increased (data not shown), in contrast to the effect of tetrodotoxin. This suggests that the K^+-induced relaxation is closely related to the neurogenic release of inhibitory substances.

Evoked spikes during potassium-induced relaxation and inhibitory junction potentials

Transmural stimulation (700 ms, 0.05 V) of circular muscles evoked spikes, which were considered Ca

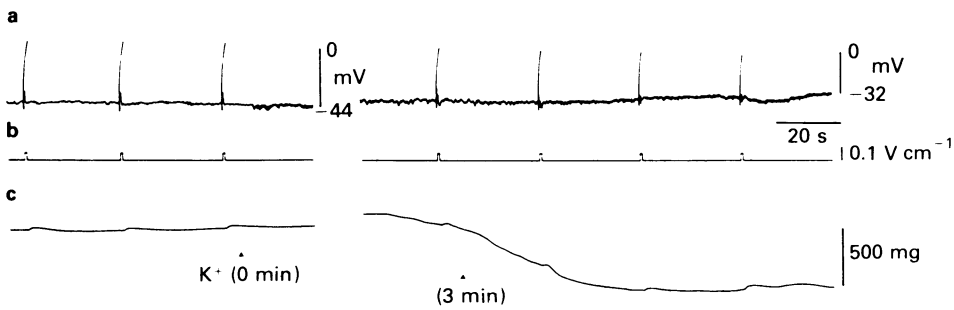

Figure 6 Records from one of three experiments showing evoked spikes (0.03 Hz, 700 ms) during K^+ (23.6 mM)-induced relaxation in circular muscles. Note that evoked spikes were not suppressed during relaxation. (a) Shows membrane potential (b) current monitor and (c) tension changes.

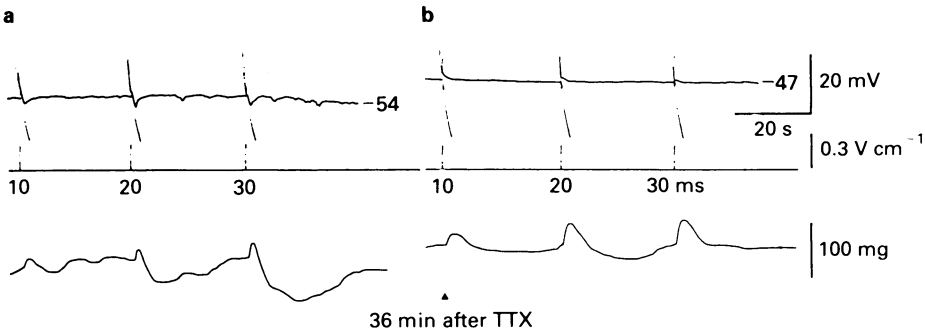

Figure 7 Inhibitory junction potentials and concomitant relaxation caused by transmural stimulation (0.033 Hz, 10–30 ms) before (a) or after (b) pretreatment with TTX (0.626 μM, 36 min). Top, membrane potential; middle, current monitor; bottom, tension changes.

spikes (Kimura *et al.*, 1983). During K^+-induced relaxation the spikes were not suppressed, indicating that the relaxation was not caused by suppression of Ca^{2+} influx during action potentials (Figure 6).

In two experiments, short stimulation pulses (0.23 V; 10, 20 or 30 ms), were used to evoke inhibitory junction potentials and simultaneous relaxation. After the circular muscles were pretreated with tetrodotoxin (0.626 μM) for 36 min neither relaxation nor inhibitory junction potentials were observed (Figure 7), suggesting that transmural stimulation causes neural release of an inhibitory transmitter. That the same concentration of tetrodotoxin suppressed both K^+-induced relaxation and that accompanying inhibitory junction potentials is consistent with this notion.

Discussion

The mechanism by which K^+ induces a relaxation response is puzzling as the Nernst equation predicts a membrane depolarization. Increased Na^+ pump activity and the consequent hyperpolarization have been proposed as a possible mechanism. Harder (1982) speculated that K^+ might increase the Ca^{2+}-sensitive outward K^+ current and/or decrease the inward Ca^{2+} current. However, the K^+-induced inhibition of the contractile responses elicited by stimulant drugs seems to involve more complex mechanisms. The present paper investigated the K^+-induced relaxation from resting tone which was not inhibited by ouabain. During relaxation the evoked calcium spikes were not suppressed. The relaxation was, therefore, not considered to be produced by direct suppression of calcium influx during action potentials. Another possibility is that the response to K^+ is due to the release of inhibitory transmitters. Inhibitory junctional transmission has been found to be non-cholinergic and non-adrenergic in the guinea-pig duodenum (Ookawa, 1984), mouse anococcygeus muscle (Gibson & Tucker, 1982; Gibson & Yu, 1983), and guinea-pig ileum (Holman & Weinrich, 1975; Bauer & Kuriyama, 1982 a,b). These inhibitory junction potentials were suppressed by tetrodotoxin, as was also found in this paper.

We have previously demonstrated that cholecystokinin-C-terminal peptides induce relaxation of circular muscles without suppressing calcium influx but do not have this action on longitudinal muscles. The present results demonstrate that circular and longitudinal muscles of pig duodenum exhibit quite different responses to K^+. In longitudinal muscles simple contraction was seen, while the relaxation in circular muscles was followed by K^+-induced contraction. Vascular smooth muscles shown to be relaxed by K^+ were frequently prepared in helical strips, suggesting that the responses observed reflect those of circular muscles.

Recently acetylcholine-induced relaxation has been explained by the release of an inhibitory substance (prostacyclin) from rabbit aorta endothelium (Beetens *et al.*, 1983). Therefore, the mechanism by which K^+ induces relaxation in pig circular muscles might involve the release of inhibitory transmitters from nerve terminals or endothelial cells.

References

ABE, Y. & TOMITA, T. (1968). Cable properties of smooth muscle. *J. Physiol.*, **196**, 87–100.

BAUER, V. & KURIYAMA, H. (1982a). Evidence for non-cholinergic, non-adrenergic transmission in the guinea-pig ileum. *J. Physiol.* **330**, 95–110.

BAUER, V. & KURIYAMA, H. (1982b). The nature of non-

cholinergic, non-adrenergic transmission in longitudinal and circular muscles of the guinea-pig ileum. *J. Physiol.* **323,** 375–391.

BEETENS, J.R., VAN HOVE, C., RAMPART, M. & HERMAN, A.G. (1983). Acetylcholine stimulates the release of prostacyclin by rabbit aorta endothelium. *J. Pharm. Pharmac.*, **35,** 251–252.

BONACCORSI, A., HERMSMEYER, K., APRIGLIANO, O., SMITH, C.B., & BOHR, D.F. (1977). Mechanism of potassium relaxation of arterial muscle. *Blood Vessels* **14,** 261–276.

CATTERALL, W.A., HARTSHORNE, R.P. & TAMKUN, M.M. (1981). Molecular pharmacology of voltage-sensitive sodium channels from mammalian brain. In *Topics in Molecular Pharmacology, Vol. 1* ed. Burgen, A.S.V. & Roberts, G.C.K. pp. 222–245.

CHEN, W.T., BRACE, R.A., SCOTT, J.B., ANDERSON, D.K. & HADDY, F.J. (1972). The mechanism of the vasodilator action of potassium. *Proc. Soc. exp. Biol. Med.* **140,** 820–824.

GIBSON, A. & TUCKER, J.F. (1982). The effects of vasoactive intestinal polypeptide and of adenosinse 5′-triphosphate on the isolated anococcygeus muscle of the mouse. *Br. J. Pharmac.*, **77,** 97–103.

GIBSON, A. & YU, O. (1983). Biphasic non-adrenergic, non-cholinergic relaxation of the mouse anococcygeus muscle. *Br. J. Pharmac.*, **79,** 611–615.

HADDY, F.J. (1975). Potassium and blood vessels. *Life Sci.*, **16,** 1489–1498.

HADDY, F.J. (1978). The mechanism of potassium vasodilation. In *Mechanisms of vasodilation. Satellite Symp. Int. Cong. Physiol. Sci.*, **27,** 200–205.

HAGEN, E.C., JOHNSON, J.C. & WEBB, R.C. (1982). Ouabain binding and potassium relaxation in aortas from renal hypertensive rabbits. *Am. J. Physiol.*, **243,** H896-H902.

HARDER, D.R. (1982). Membrane electrical activation of arterial smooth muscle. In *Vascular smooth muscle: metabolic ionic and contractile mechanisms,* ed. Crass, M.F., III & Barnes, C.D., pp. 71–97, New York: Academic Press.

HOLMAN, M.E. & WEINRICH, J.P. (1975). The effects of calcium and magnesium on inhibitory junctional transmission in smooth muscle of guinea-pig small intestine. *Pflügers Arch.*, **360,** 109–119.

ISHII, T. & SHIMO, Y. (1979). Potassium-induced relaxation of guinea-pig taenia coli. *Arch. int. Pharmacodyn.*, **239,** 36–44.

ISHII, T. & SHIMO, Y. (1980). Potassium-induced relaxation of the rat anococcygeus muscle. *Arch. int. Pharmacodyn.*, **243,** 27–36.

KIMURA, I., KIMURA, M., NAKAYAMA, N. & KIMURA, M. (1983). Relaxation and Ca-spike suppression in circular and longitudinal muscles of hog bile duct ampulla by CCK-C-terminal peptides. *Arch. int. Pharmacodyn,* **265,** 320–333.

OOKAWA, H. (1984). Effects of ATP, its related nucleotides and VIP on the inhibitory potentials in the smooth muscle cells of the guinea-pig duodenum. *Tohoku J. exp. Med.*, **142,** (in press).

TODA, N. (1974). Responsiveness to potassium and calcium ions of isolated cerebral arteries. *Am. J. Physiol.*, **227,** 1206–1211.

TODA, N. (1976). Potassium-induced relaxation in isolated cerebral arteries contracted with prostaglandin $F_{2\alpha}$. *Pflügers Arch.*, **364,** 235–242.

REINER, O. (1978). The role of the electrogenic sodium pump in the potassium relaxation of the rabbit ear artery. *Naunyn-Schmiedebergs Arch. Pharmac.*, **303,** 213–220.

WEBB, R.C. & BOHR, D.F. (1978). Potassium-induced relaxation as an indicator of Na^+-K^+ ATPase activity in vascular smooth muscle. *Blood Vessels*, **15,** 198–207.

(Received January 26, 1984
Revised April 11, 1984)