Br. J. Pharmac. (1978), **63**, 577–585

POTENTIATION OF VASOCONSTRICTOR RESPONSES BY 3- AND 4-AMINOPYRIDINE

W. E. GLOVER

School of Physiology and Pharmacology, University of New South Wales, PO Box 1, Kensington N.S.W. 2033, Australia

1 In concentrations that are known to reduce potassium conductance in many excitable membranes, 3 and 4-aminopyridine (3-AP, 4-AP) potentiate vasoconstrictor responses of the isolated ear artery of the rabbit to noradrenaline and histamine.

2 3- and 4-AP have no effect on the responses of potassium-depolarized arteries to noradrenaline, histamine or calcium.

3 The results suggest that the aminopyridines have no direct effect on the contractile machinery or on pharmacomechanical coupling, but cause potentiation by influencing electrical events at the cell membrane.

4 4-AP causes a greater potentiation of the response to electrical stimulation than of the response to noradrenaline. This suggests that the aminopyridines may also cause an increase in the amount of noradrenaline released in response to sympathetic nerve stimulation.

Introduction

Tetraethylammonium (TEA) potentiates the contractile responses to a variety of agonists in isolated vascular preparations such as the carotid artery of the cat (Lum & Rashleigh, 1961), rabbit aorta (Kelkar, Gulati & Gokhale, 1964; Kalsner, 1973) and rabbit ear artery (Carroll & Morgans, 1978). Finding that the potentiation was reduced or abolished in a low-calcium medium or when calcium influx was inhibited, Kalsner (1973) concluded that TEA increases the utilization of extracellular and/or superficially bound calcium. TEA is well known to have a specific effect on potassium conductance in a number of excitable membranes (Hille, 1967; Volle, 1970), and Kalsner did not rule out the possibility that TEA had a primary effect on a potassium binding site that in turn altered calcium permeability. However, he referred to evidence which indicates that responses in the rabbit aorta are mediated not by action potentials but by non-electrical means (Waugh, 1962; Su, Bevan & Ursillo, 1964; Hinke, 1965; Hiraoka, Yamagishi & Sano, 1968; Somlyo & Somlyo 1968a,b; Axelsson, 1970), and pointed out that there is no evidence of a direct coupling of sodium or potassium to the contractile state and that potassium efflux does not appear to be an essential part of vascular smooth muscle contraction (Headings & Rondell, 1962; Hiraoka *et al.*, 1968; Somlyo & Somlyo, 1968b).

It has recently been shown that the aminopyridines, like TEA, also cause a selective block of potassium channels in a number of preparations including the axons of the cockroach (Pelhate & Pichon, 1974) and squid (Yeh, Oxford, Wu & Narahashi, 1976), myelinated nerve of the frog (Ulbricht & Wagner, 1976) and the presynaptic terminal of the squid giant synapse (Llinás, Walton & Bohr, 1976). These substances have now become indispensible tools for studying the behaviour of ionic channels in nerve and skeletal muscle. As yet there have been no reports of their effect on isolated preparations of vascular smooth muscle, although it has been shown that 2-, 3- and 4-aminopyridine (2-, 3- and 4-AP) have a pressor action in the cat and cause vasoconstriction in the perfused hindquarter of the rat (Fastier & McDowall, 1958), and 2-AP potentiates the vasoconstrictor action of adrenaline in the latter preparation (Fastier & Reid, 1948).

The present results show that the aminopyridines, like TEA, cause a potentiation of vasoconstrictor responses to electrical stimulation, noradrenaline and histamine in the ear artery of the rabbit. However, the aminopyridines have no effect on the responses of potassium-depolarized arteries to noradrenaline, histamine or calcium. The results are compatible with the hypothesis that the aminopyridines have no direct effect on the contractile machinery or on the pharmacomechanical pathway, but cause potentiation by in-

fluencing electrical events at the cell membrane. A brief account of some of the results has been published (Glover, 1977).

Methods

Crossbred New Zealand–Himalayan rabbits of either sex weighing 2.5 to 4.0 kg were killed by cervical dislocation. A short segment of the central artery of each ear was cannulated, and the paired segments were simultaneously perfused with Krebs bicarbonate solution of the following composition (mmol/l): NaCl 118, $NaHCO_3$ 25, NaH_2PO_4 1.33, KCl 4.7, $CaCl_2$ 3.7, $MgCl_2$ 1.44 and glucose 5.5, aerated with 5% CO_2 in O_2 at a pH of 7.4. In some experiments arteries were perfused with a 'high-K Krebs' solution in which K_2SO_4 99.5 and $KHCO_3$ 25 were substituted for NaCl and $NaHCO_3$, and in others, calcium-free solutions containing 1,2 bis, 2 aminoethoxyethane-NNN'N'-tetra-acetic acid (EGTA) 1 mmol/l were used.

The flow rate through the arteries was kept at a constant value in the range 5 to 10 ml/min, giving a perfusion pressure of 20–30 mmHg. Care was taken to ensure that the cannula contributed negligible resistance. Perfusion pressure was measured with a Statham P23AC pressure transducer and recorded on a Grass Polygraph. Drugs were injected rapidly in a volume of 0.05 ml through a rubber connection close to the cannula or added directly to the perfusate. In the case of calcium, a 0.5 mol/l solution was used and injected in volumes of 0.05 to 0.2 ml, thus the doses injected were 25, 50 and 100 μmol. All drugs were dissolved in 0.9% w/v NaCl solution (saline) containing ascorbic acid 50 μg/ml and all experiments were carried out at 37°C.

Drugs

The following drugs were used: 3-aminopyridine and 4-aminopyridine (Sigma), guanethidine sulphate (Ciba-Geigy), histamine diphosphate (Fluka, AG), 5-hydroxytryptamine creatinine sulphate (NBC), noradrenaline acid tartrate (Winthrop), phentolamine mesylate (Ciba), and tetrodotoxin (Calbiochem). The amounts stated in the text refer to these compounds with the exception of noradrenaline where the amount stated refers to the base.

Electrical stimulation

The method used was that described by de la Lande & Rand (1965) as transmural stimulation. One platinum electrode was inserted into the rubber tubing close to the perfusion cannula and another was placed in the organ bath. A Grass SD9 stimulator was used to deliver trains of square wave pulses of supramaximal voltage (30 to 60 V) and 0.5 ms duration, each lasting 5 s. Graded responses were obtained by varying the frequency of stimulation (1 to 40 Hz).

Results

3-Aminopyridine

In 15 experiments, the addition of 3-AP (1 mmol/l) to the perfusate had no effect or caused a small increase in perfusion pressure (mean increase 1.5 mmHg ± 1.6 s.d.), but produced an immediate marked increase in the contractile response to injections of noradrenaline or histamine. Part of a typical record is shown in Figure 1. This shows the effect of 3-AP first added to one artery (at time 2 min) and later to the other artery (45 min) on the otherwise constant constrictor response to the injection of noradrenaline (2 ng). Allowing for the dead space in the perfusion system, potentiation was usually evident within 90 s, reached a maximum in about 3 min and was well sustained for periods of an hour or more.

In 10 experiments, dose-response relationships of the 2 arteries were established for noradrenaline before, during and after the addition of 3-AP to the solution perfusing one artery. Three doses of noradrenaline (4, 8 and 16 ng or, in the presence of 3-AP, 2, 4 and 8 ng) which were found to cause responses on the linear part of the log dose-response curve were used, and each dose was tested twice under each set of conditions in each artery. In these and in all subsequent experiments responses in the presence of aminopyridines were measured after at least 10 min exposure. The results were used to construct the mean log dose-response curves shown in Figure 2. The parallel shift to the left of the mean log dose-response curve of the experimental arteries corrected for the small shift to the left of the mean log dose-response curve of the control arteries indicates that 3-AP caused a 2.03 fold increase in sensitivity to noradrenaline. This figure also shows that the effect was reversible, and responses usually returned to control values within 5 min. The effect was reproducible, and in 5 experiments a second exposure to 3-AP resulted in a 1.7-fold increase in sensitivity.

Other concentrations of 3-AP were not studied systematically, but in 5 arteries 3-AP, 100 μmol/l, caused a small increase in the response to a constant dose of noradrenaline, and in 2 arteries there was no further potentiation on increasing the concentration from 1 to 2 mmol/l.

Qualitatively similar results were obtained in 5 experiments in which histamine was used as the constrictor agent.

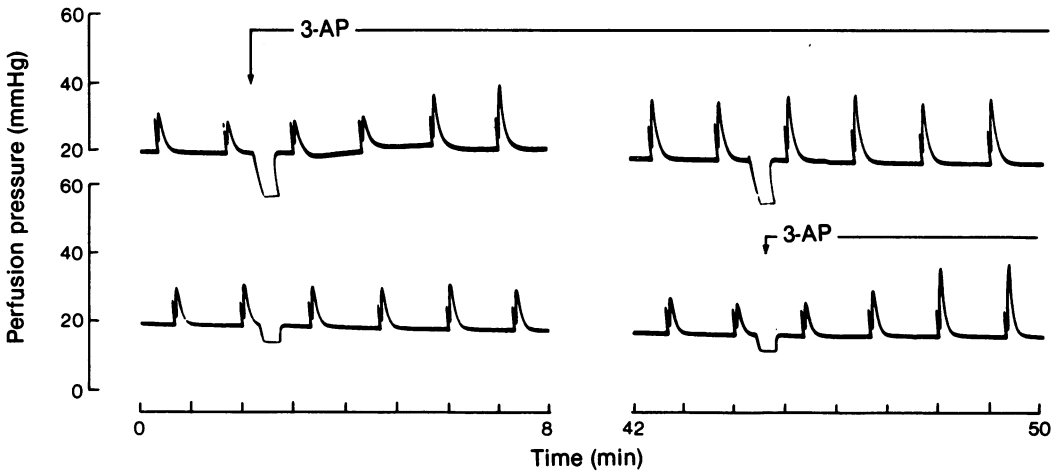

Figure 1 Effect of 3-aminopyridine (3-AP, 1 mmol/l) on responses of a pair of simultaneously perfused rabbit ear arteries to injections of noradrenaline (2 ng). Perfusion was stopped for the periods indicated by the brief falls in perfusion pressure. 3-AP was added to each perfusate at the time indicated ↓, otherwise perfusion continued with Krebs-bicarbonate solution.

4-Aminopyridine

Noradrenaline. Qualitatively similar results were obtained with 4-AP, but in 5 experiments potentiation of responses to noradrenaline was seen at a concentration of 10 μmol/l, and the optimum concentration was 100 μmol/l. In 4 experiments, 4-AP (100 μmol/l) had a direct vasoconstrictor action on the preparation and caused an increase in perfusion pressure of 12 to 30 mmHg. However, in another 10 arteries, the effect of this concentration on perfusion pressure was comparable to that of 3-AP (1 mmol/l), producing a mean increase of 2.2 mmHg ± 2.0 s.d. In these 10 arteries, this concentration of 4-AP caused a 2.38 fold increase in sensitivity to noradrenaline. As before, this was calculated from the parallel shift of the mean log dose-response curve corrected for any non-specific change in sensitivity by reference to the control artery. In 2 arteries, doubling the concentration of 4-AP to 200 μmol/l did not produce any additional potentiation, while increasing it further to 500 μmol/l caused a slight shift to the right of the log dose-response curves.

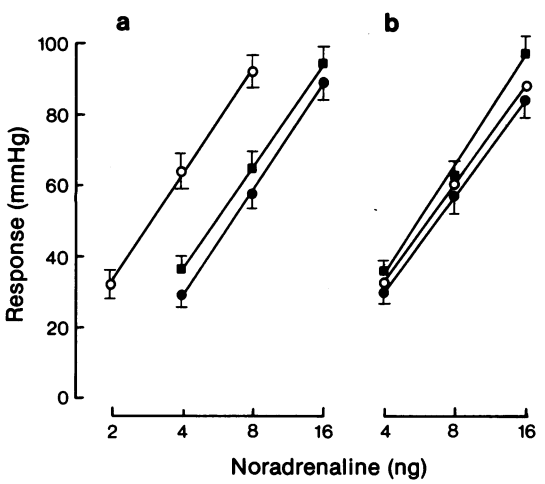

Figure 2 Effect of 3-aminopyridine (3-AP, 1 mmol/l) on the responses of rabbit ear arteries to noradrenaline. Each point is the mean of 2 observations on each of 10 arteries and vertical bars indicate s.e. mean. (a) (●) Before, (○) during, (■) after addition of 3-AP (1 mmol/l) to perfusate; (b) simultaneous observations on paired control arteries.

Histamine. 4-AP (100 μmol/l) also caused marked potentiation of the responses to histamine. As with 3-AP, the onset of potentiation was rapid, and on return to perfusion with Krebs bicarbonate solution responses quickly returned to near the initial level. The results of 5 experiments are summarized in Figure 3. The mean log dose-response curve for histamine was slightly but not significantly steeper in the presence of 4-AP and the increase in sensitivity calculated from the shift to the left of the curve was ×4.2 to 5.25.

Electrical stimulation. In 6 arteries, 4-AP (100 μmol/l) potentiated the responses to electrical stimulation. In each experiment the voltage was supramaximal (30 to 60 V), pulse duration (0.5 ms)

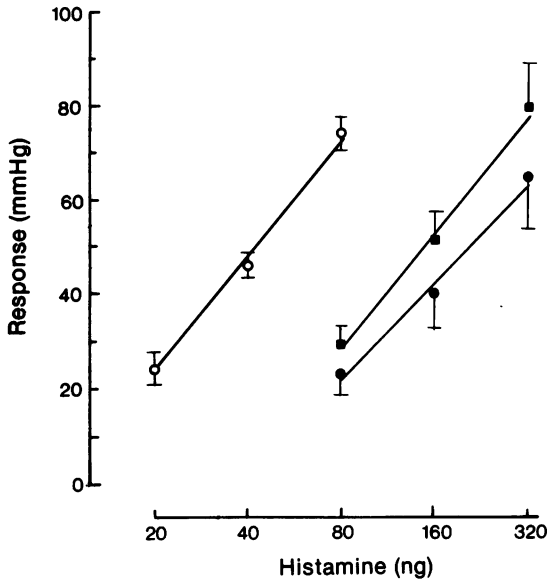

Figure 3 Effect of 4-aminopyridine (4-AP) on the responses of rabbit ear arteries to histamine. Each point is the mean of 2 observations on each of 10 arteries and vertical bars indicate s.e. mean. (●) Before, (○) during, (■) after addition of 4-AP (100 μmol/l) to perfusate.

and train length (5 s) were kept constant, and graded responses obtained by varying the frequency of stimulation, using 3 frequencies in the range 1 to 40 Hz. The arteries varied in their sensitivity to electrical stimulation and it was not possible to construct a mean log frequency-response curve, hence the individual results are summarized in Figure 4.

In experiments 1 to 3 the log frequency-response curves in the range covered were straight and 4-AP produced nearly parallel shifts to the left indicating respective increases in sensitivity of ×4.0, 2.8 and 5.2. The increase in sensitivity in experiments 4 and 5 was approximately ×4 to 5, while in experiment 6 it was in the range ×1.8 to 4.4.

In 4 experiments the effect of 4-AP (100 μmol/l) on the response to electrical stimulation was compared with its effect on the response to noradrenaline. In each case 4-AP caused a greater potentiation of the responses to electrical stimulation than of the responses to noradrenaline. The mean increase in sensitivity to electrical stimulation calculated from the shift of the log frequency-response curves was ×4.5 ± 0.26 while the mean increase in sensitivity to noradrenaline was ×2.5 ± 0.13 ($P < 0.001$).

In all experiments the response to electrical stimulation was subsequently shown to be abolished by tetrodotoxin (1 μg/ml), guanethidine (1 μg/ml) or phentolamine (1 μg/ml).

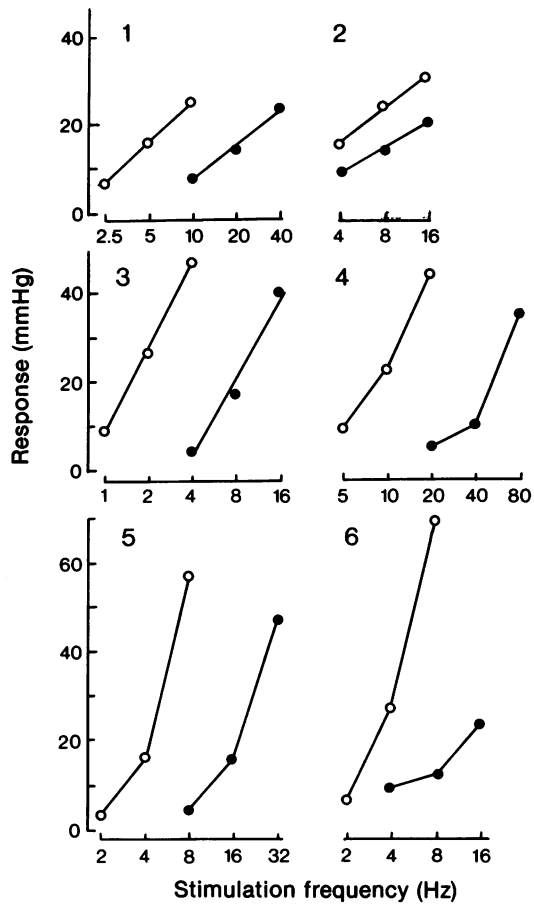

Figure 4 Effect of 4-aminopyridine (4-AP, 100 μmol/l) on the responses of 6 rabbit ear arteries to electrical stimulation. Each point is the mean of 2–3 observations. Pulse duration and voltage were kept constant in each experiment and graded responses (ordinate scales: increase in perfusion pressure, mmHg) were obtained by varying the frequency (abscissa scales: Hz). (●) Control; (○) in presence of 4-AP.

'Depolarized' arteries

Noradrenaline. In 6 experiments, after an initial period of perfusion with the normal Krebs-bicarbonate solution, the arteries were perfused with a high-K Krebs solution, that is a solution in which the sodium chloride and sodium bicarbonate were replaced iso-osmotically with potassium sulphate and potassium bicarbonate. This produced an immediate increase in perfusion pressure of more than 200 mmHg, but after several hours the perfusion pressure progressively fell. Perfusion was continued for 5 to 6 h and the arteries stored overnight at 4°C in

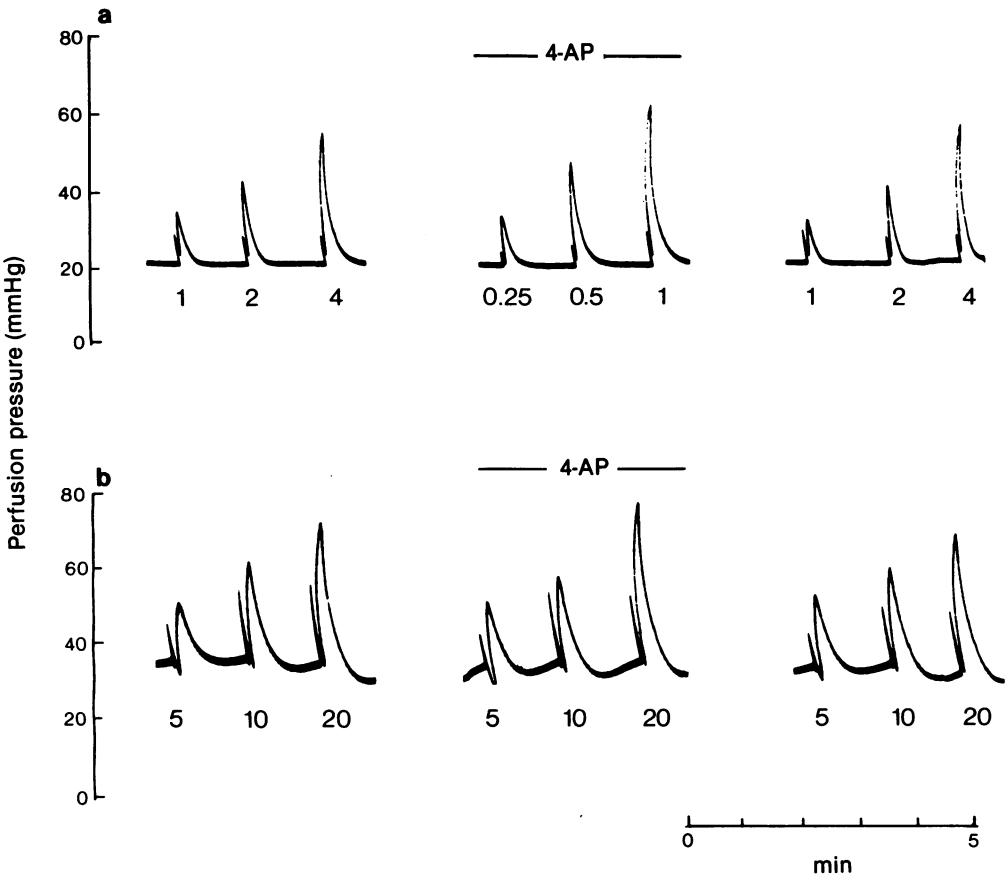

Figure 5 Effect of 4-aminopyridine (4-AP, 100 μmol/l) on the responses of a rabbit ear artery to noradrenaline (dose shown in ng) before (a) and during (b) depolarization by perfusion with high-K Krebs. Each panel shows typical responses to graded doses before, during and after the addition of 4-AP to the perfusate.

a solution of the same composition. Perfusion was resumed the next day with high-K Krebs, and after 1 to 2 h perfusion pressure was in the range 40 to 60 mmHg and the arteries responded well to injections of noradrenaline. However in each of the 6 experiments the addition of 4-AP (100 μmol/l) to the perfusate at this time had no effect on perfusion pressure or on the responses to noradrenaline.

Figure 5 illustrates a typical experiment. When the artery was initially perfused with normal Krebs, 4-AP produced the usual reversible increase in sensitivity to noradrenaline; in this case it was ×4. After the artery had been perfused with or exposed to high-K Krebs for approximately 24 h and was presumably depolarized, constrictor responses comparable to those seen initially could be obtained by increasing the dose of noradrenaline 5-fold, but 4-AP had no effect on these responses. In the 2 experiments in which it was tested, the potentiating effect of 4-AP was restored when perfusion was again resumed with normal Krebs solution.

In 2 further experiments 3-AP (1 mmol/l) had no effect on the responses of depolarized arteries to noradrenaline.

Histamine. In 2 experiments 4-AP (100 μmol/l) and in a further 2 experiments 3-AP (1 mmol/l) had no effect on the responses of depolarized arteries to histamine.

Calcium. Injections of calcium chloride had no effect on perfusion pressure when the arteries were perfused with normal Krebs. In 6 experiments arteries were perfused for 30 to 60 min with calcium-free Krebs and then with calcium-free high-K Krebs. In the absence of calcium, the high-K Krebs had no effect on per-

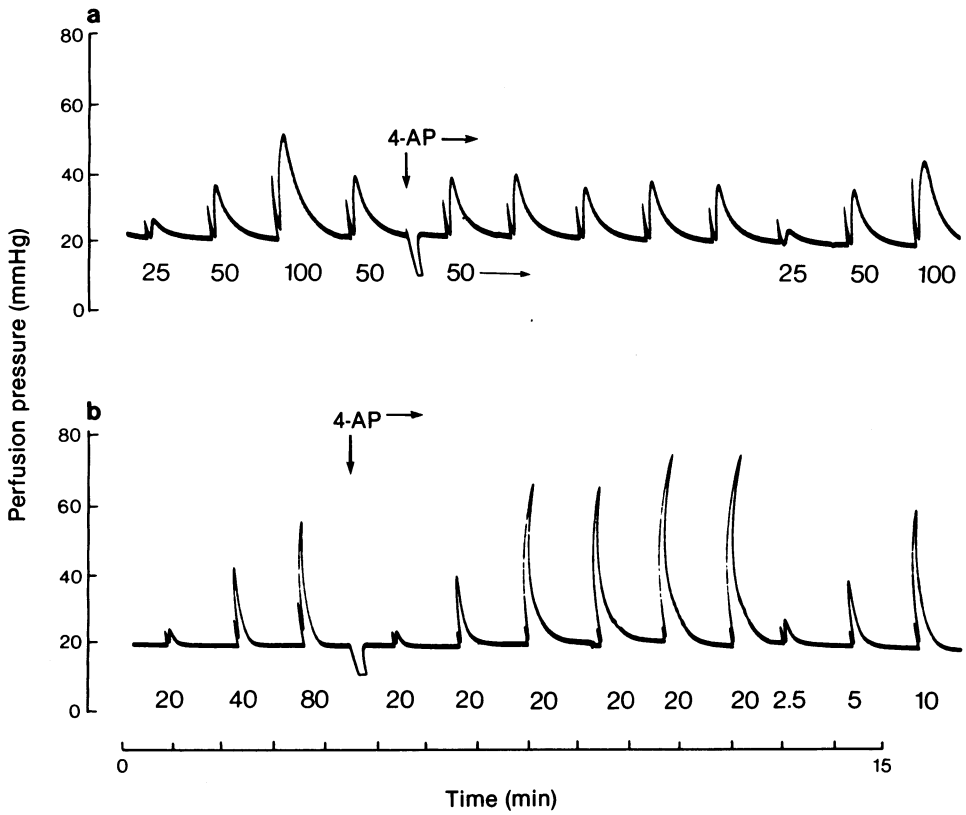

Figure 6 Effect of 4-aminopyridine (4-AP, 100 μmol/l) on the responses of a rabbit ear artery to calcium (a) and noradrenaline (b). (a) Artery perfused with calcium-free high-K Krebs; calcium doses shown in μmol; (b) 15 min after return to normal Krebs; noradrenaline doses shown in ng.

fusion pressure, but the arteries now gave graded responses to injections of calcium. However, under these conditions 4-AP (100 μmol/l) had no effect on perfusion pressure and did not have any effect on the responses to calcium. Part of a typical experiment is illustrated in Figure 6. In this experiment the responses to noradrenaline which were abolished when the artery was perfused with calcium-free high-K Krebs showed only a partial recovery when perfusion was resumed with normal Krebs. However, it can be seen that the addition of 4-AP now caused a greater than usual (approximately 8 fold) increase in sensitivity to noradrenaline.

In a further 2 experiments 3-AP (1 mmol/l) had no effect on the responses of depolarized arteries to calcium.

Discussion

The results show that 3- and 4-AP potentiated vasoconstrictor responses to both noradrenaline and histamine in the isolated ear artery of the rabbit. The potentiation occurred rapidly, it lasted for as long as the aminopyridine was present in the perfusate and was readily reversible. 4-AP was approximately ten times more potent than 3-AP in that potentiation occurred at a threshold concentration of about 10 μmol/l with 4-AP compared with 100 μmol/l for 3-AP, and concentrations of 100 μmol/l and 1 mmol/l respectively caused comparable shifts of the log dose–response curves for noradrenaline. The concentrations used are in the range which have been shown to cause a selective block of potassium channels in a number of excitable membranes (Pelhate & Pichon, 1974; Llinás *et al.*, 1976; Ulbricht & Wagner, 1976; Yeh *et al.*, 1976).

TEA is also known to cause potentiation of vasoconstrictor responses in isolated arterial preparations in concentrations that reduce current through potassium channels in other excitable tissues (Kalsner, 1973), and in similar experiments to the present using the ear artery of the rabbit, Carroll & Morgans (1978) showed that TEA potentiated the constrictor response

to histamine. It therefore seems possible that since the aminopyridines and TEA have similar potentiating effects, the mechanism whereby they produce potentiation may be related to their well-known actions on potassium conductance in other tissues.

However, before concluding that the aminopyridines cause potentiation by influencing electrical events at the cell membrane it is necessary to consider other possibilities.

The aminopyridines have a weak vasoconstrictor action, with again 4-AP being more potent than 3-AP, but in many of the present experiments both drugs caused potentiation without having any effect on perfusion pressure. The potentiation was non-specific since responses to both noradrenaline and histamine were potentiated, and it seems unlikely that it was due to interference with the inactivation of noradrenaline or histamine.

It is generally accepted that the contraction of vascular smooth muscle is initiated by a rise in the concentration of intracellular free or activator calcium, and that the resting plasma membrane is impermeable to calcium. However, vascular smooth muscle incubated in a calcium-free high-potassium solution contracts on exposure to calcium, presumably because calcium passes through the membrane which has been made highly permeable by the potassium-induced depolarization (Bohr, 1973). In the present experiments, 4-AP had no effect on the responses of such a 'depolarized' preparation to calcium. This suggests that the aminopyridines have no effect on the contractile machinery itself, nor do they have any effect on calcium permeability or on the subsequent sequestration or extrusion of calcium in at least a depolarized artery. Rather, this result suggests that the aminopyridines may cause potentiation by an effect on the chain of events which follows stimulation of the α-adrenoceptor or histamine receptor and leads to an increase in the activator calcium, that is, the excitation-contraction pathway.

Two types of excitation-contraction coupling have been described, electromechanical and pharmacomechanical (Somlyo & Somlyo, 1968a). In the present experiments, the artery contracted in response to noradrenaline and histamine even when it was perfused with a high-potassium solution and was presumably completely depolarized. However, under these conditions the aminopyridines did not cause any potentiation, and it seems reasonable to conclude that they have no effect on the pharmacomechanical pathway which is responsible for excitation-contraction coupling in the potassium-depolarized artery. There is no reason to suppose that the pathway for such non-electrical activation is any different when the cells are polarized, hence the results suggest that the aminopyridines cause potentiation by influencing electrical events.

Casteels & Droogmans (1976) have shown that the doses of noradrenaline used in this work do not cause depolarization of the smooth muscle cell but TEA does. If TEA achieves this by suppressing the rectifying properties of the cell by blocking potassium conductance then the aminopyridines could have a similar effect. The following hypotheses concerning the mechanism of the potentiating action of the aminopyridines and TEA are therefore consistent with the present results and the previous electrophysiological observations. The cell membrane of the smooth-muscle cell in the isolated artery is highly rectified and has a stable membrane potential. Noradrenaline does not cause depolarization, but brings about an increase in activator calcium by means of the pharmacomechanical pathway only. In the presence of an aminopyridine or TEA, the potassium conductance of the membrane is reduced and the rectifying properties are suppressed. Noradrenaline still activates the pharmacomechanical pathway but, in addition, causes depolarization or may even produce action potentials. Activator calcium is thus provided by the electrical as well as the non-electrical pathway and hence the contractile response is enhanced.

Alternatively, the amount of activator calcium made available by the pharmacomechanical pathway might be dependent on the membrane potential. TEA in high concentrations (10 mmol/l) certainly causes depolarization in the rabbit ear artery and induces action potentials which are accompanied by phasic contractions (Casteels & Droogmans, 1976). We have no information on the effect of the aminopyridines on membrane potential, but they have a weak vasoconstrictor action, and it seems likely that the aminopyridines also cause depolarization. Activator calcium may come from either intracellular or extracellular sources, and it is well known that membrane permeability to calcium is increased as the membrane potential is decreased. It is therefore possible that the aminopyridines depolarize the cell membrane to an extent that may or may not be sufficient to produce a contraction, but which in either case enhances an increase in calcium permeability brought about by the pharmacomechanical action of noradrenaline or histamine. The aminopyridines had no effect on the contractile responses of the depolarized arteries to calcium, but presumably these arteries were completely depolarized and hence calcium permeability may have reached its maximum.

The conclusion therefore is that the aminopyridines and TEA have a common effect on the membrane of the vascular smooth muscle cell which is related to their known action on potassium conductance in other tissues. Whether or not they act by enabling noradrenaline to cause depolarization or by altering the resting membrane potential requires an electrophysiological investigation.

Finally, the present results show that 4-AP potentiated the responses of the rabbit ear artery to electrical stimulation. The pulse duration was short (0.5 ms) and the responses were abolished by tetrodotoxin, guanethidine or phentolamine indicating that they were mediated by release of the sympathetic transmitter and not by direct electrical stimulation of the smooth muscle. It is therefore not suprising that these responses were also potentiated by 4-AP. However, in each of 4 experiments 4-AP caused a greater potentiation of the responses to electrical stimulation than of the responses to noradrenaline. It has been proposed that the aminopyridines facilitate the release of acetylcholine in response to nerve stimulation in a number of preparations including the chick biventer cervicis (Bowman, Harvey & Marshall, 1977) and also facilitate adrenergic transmission in the rabbit vas deferens (Johns, Golko, Lauzon & Paton, 1976). The results therefore suggest that the aminopyridines cause an increase in the amount of noradrenaline released in response to nerve stimulation in the rabbit ear artery.

This work was supported by a grant from the National Health and Medical Research Council of Australia. I am grateful to Miss R. Diprose and Mrs M. de Detrich for technical assistance and to Professor P. W. Gage for helpful suggestions.

References

AXELSSON, J. (1970). Mechanical properties of smooth muscle, and the relationship between mechanical and electrical activity. In *Smooth muscle*, ed Bülbring, E., Brading, A., Jones, A. & Tomita, T. pp 289–315. London: Edward Arnold.

BOHR, D.F. (1973) Vascular smooth muscle updated. *Circulation Res.*, **32,** 665–672.

BOWMAN, W.C., HARVEY, A.L. & MARSHALL, I.G. (1977). The actions of aminopyridines on avian muscle. *Naunyn-Schmiedebergs Arch. Pharmac.*, **297,** 99–103.

CARROLL, P.R. & MORGANS, D. (1978). Blockade by phentolamine of the potentiating action of tetraethylammonium in the rabbit ear artery. *Clin. exp. Pharmac. Physiol.*, (in press).

CASTEELS, R. & DROOGMANS, G. (1976). Membrane potential and excitation-contraction coupling in the smooth muscle cells of the rabbit ear artery. *J. Physiol.*, **263,** 163–164P.

DE LA LANDE, I.S. & RAND, M.J. (1965). A simple isolated nerve-blood vessel preparation. *Aust. J. exp. Biol. med. Sci.*, **43,** 639–656.

FASTIER, F.M. & McDOWALL, M.A. (1958). A comparison of the pharmacological properties of the three isomeric aminopyridines. *Aust. J. exp. Biol. med. Sci.*, **36,** 365–372.

FASTIER, F.N. & REID, C.S.W. (1948) Circulatory properties of amidine derivaties. II. Potentiation of the vasoconstrictor action of adrenaline. *Br. J. Pharmac.*, **3,** 205–210.

GLOVER, W.E. (1977) Effect of 3- and 4-aminopyridine on vascular smooth muscle. *Proc. Aust. Physiol. Pharmac. Soc.*, **8,** 7P.

HEADINGS, V.E. & RONDELL, P.A. (1962) Arterial muscle contraction and potassium movements *in vitro*. *Am. J. Physiol.*, **202,** 17–20.

HILLE, B. (1967). The selective inhibition of delayed potassium currents in nerve by tetraethylammonium ion. *J. gen. Physiol.*, **50,** 1287–1302.

HINKE, J.A.M. (1965). Calcium requirements for noradrenaline and high potassium ion contraction in arterial smooth muscle. In *Muscle*, ed. Paul, W.M., Daniel, E.E., Kay, C.M. & Monckton, G. pp 269–285. New York: Pergamon Press.

HIRAOKA, M., YAMAGISHI, S. & SANO, T. (1968). Role of calcium ions in the contraction of vascular smooth muscle. *Am. J. Physiol.*, **214,** 1084–1089.

JOHNS, A., GOLKO, D.S., LAUZON, P.A. & PATON, D.M. (1976). The potentiating effects of 4 aminopyridine on adrenergic transmission in the rabbit vas deferens. *Eur. J. Pharmac.*, **38,** 71–78.

KALSNER, S. (1973). Mechanism of potentiation of vascular responses by tetraethylammonium: a novel form of sensitization. *Can. J. Physiol. Pharmac.*, **51,** 451–457.

KELKAR, V.V., GULATI, O.D. & GOKHALE, S.D. (1964). Effect of ganglion blocking drugs on the responses of the rabbit aortic strip to adrenaline, noradrenaline and other vasoactive substances. *Arch. int. Pharmacodyn.*, **149,** 209–222.

LLINÁS, R., WALTON, K. & BOHR, V. (1976). Synaptic transmission in squid giant synapse after potassium conductance blockage with external 3- and 4-aminopyridine. *Biophys. J.*, **16,** 83–86.

LUM, B.K.B. & RASHLEIGH, P.L. (1961). Potentiation of vasoactive drugs by ganglionic blocking agents. *J. Pharmac. exp. Ther.*, **132,** 13–18.

PELHATE, M. & PICHON, Y. (1974). Selective inhibition of potassium current in the giant axon of the cockroach. *J. Physiol.*, **242,** 90–91P.

SOMLYO, A.V. & SOMLYO, A.P. (1968a). Electromechanical and pharmacomechanical coupling in vascular smooth muscle. *J. Pharmac. exp. Ther.*, **159,** 129–145.

SOMLYO, A.V. & SOMLYO, A.P. (1968b). Vascular smooth muscle. I. Normal structure, pathology, biochemistry, and biophysics. *Pharmac. Rev.*, **20,** 197–272.

SU, C., BEVAN, J.A. & URSILLO, R.C. (1964). Electrical quiescence of pulmonary artery smooth muscle during sympathomimetic stimulation. *Circulation Res.*, **15,** 20–27.

ULBRICHT, W. & WAGNER, H.-H. (1976). Block of potassium channels of the nodal membrane by 4-aminopyridine and its partial removal on depolarization. *Pflügers*

Arch., **367,** 77–87.

VOLLE, R.L. (1970). The actions of tetraethylammonium ions on potassium fluxes in frog sartorius muscles. *J. Pharmac. exp. Ther.*, **172,** 230–238.

WAUGH, W.H. (1962). Role of calcium in contractile excitation of vascular smooth muscle by epinephrine and potassium. *Circulation Res.*, **11,** 927–940.

YEH, J.Z., OXFORD, G.S., WU, C.H. & NARAHASHI, T. (1976). Dynamics of aminopyridine block of potassium channels in squid axon membrane. *J. gen. Physiol.*, **68,** 519–535.

(*Received May* 5, 1977.
Revised February 24, 1978.)