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THE DIVERSE EFFECTS OF NORADRENALINE AND OTHER STIMULANTS ON ^{86}Rb AND ^{42}K EFFLUX IN RABBIT AND GUINEA-PIG ARTERIAL MUSCLE

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SUMMARY

1. The effects of noradrenaline and of raised external potassium ($[K^+]_o$) on the efflux of ^{86}Rb or ^{42}K and on tension were studied in preparations taken from eight different arteries under various conditions.

2. There was a 10-fold variation in the maximum ^{86}Rb efflux evoked by noradrenaline ($10^{-5} - 10^{-4}$ M) in the arteries studied, even though tension generated was comparable. Arterial contractions were either accompanied by large increases in ^{86}Rb efflux, e.g. rabbit ear artery and aorta, guinea-pig and rabbit pulmonary artery, or by small increases, e.g. rabbit and guinea-pig mesenteric artery, rabbit brachial artery and guinea-pig abdominal aorta.

3. Raising $[K^+]_o$ also had a diverse effect on ^{86}Rb and ^{42}K efflux: arteries giving small increases in efflux to noradrenaline also gave small increases in efflux to raised $[K^+]_o$. The maximum efflux evoked by raised $[K^+]_o$ was on average three times greater than the maximum efflux evoked by noradrenaline in the arteries studied. The heterogeneity of the efflux response could not be explained by the quantitative differences in the effects of noradrenaline or of raised $[K^+]_o$ on membrane potential or, in the case of noradrenaline, by differences in the α-receptors.

4. In arteries in which the noradrenaline-evoked ^{86}Rb efflux was small, histamine, 5-hydroxytryptamine, vasopressin and angiotensin also had little effect. Conversely, where noradrenaline produced a large increase in ^{86}Rb efflux these other stimulants had comparable effects.

5. Removal of extracellular calcium only slightly reduced the increment in ^{86}Rb efflux evoked by 66 mM-external K^+ in the rabbit aorta even though contractions were virtually abolished under these conditions. In the case of 10^{-5} M-noradrenaline, 40% of the contraction remained and its effect on efflux was significantly increased ($P < 0.05$) in calcium-free conditions. Essentially similar results were obtained using ^{42}K.

6. Tetraethylammonium (10–20 mM) produced a significant and substantial reduction ($P < 0.001$) in the ^{86}Rb efflux evoked by raised $[K^+]_o$ while only slightly affecting the noradrenaline-evoked efflux in the rabbit aorta.

7. It was concluded from these efflux experiments on vascular muscle that the channels through which potassium can escape, opened by depolarization and by

activation of α-receptors with noradrenaline, are from different populations, and that their properties vary from one artery to another. We have been unable to detect any substantial calcium-activated component in ^{42}K or ^{86}Rb efflux responses to raised $[K^+]_o$ or to noradrenaline.

INTRODUCTION

It is generally believed that exogenous noradrenaline causes contraction by a direct action on vascular smooth muscle via post-junctional α-adrenoreceptors which can be specifically blocked by α-adrenoreceptor antagonists (Starke, Endo & Tanbe, 1975; McGrath, 1981). The exact relationship between noradrenaline-evoked changes in tension and membrane potential is not clearly understood. It has been reported that α-adrenoreceptor activation by lower concentrations of noradrenaline in the rabbit main pulmonary and rabbit ear artery could produce contraction without changes in membrane potential, and it was concluded that membrane potential is not the predominant signal for tension generation (Su, Bevan & Ursillo, 1964; Bohr, 1973; Droogmans, Raeymaekers & Casteels, 1977). Somlyo & Somlyo (1968) proposed the term pharmacomechanical coupling to describe tension generated in the absence of depolarization. However, in contrast, Trapani, Matsuki, Abel & Hermsmeyer (1981) have supported the idea that tension produced by noradrenaline in the rabbit ear is closely regulated by changes in the membrane potential. Others have concluded that there is a good correlation between changes in the membrane potential and tension only at certain concentrations of noradrenaline (Casteels, Kitamura, Kuriyama & Suzuki, 1977*a*; Haeusler, 1978; Kuriyama & Suzuki, 1978). It has also been reported that noradrenaline can cause either an increase or a decrease in membrane resistance as measured by the change in amplitude of the electrotonic potential, and that this may be associated with an increase or decrease in potassium permeability (Casteels *et al.* 1977*a*, *b*; Droogmans *et al.* 1977; Suzuki, 1981; Karashima, 1981; Häusler, 1982).

The present experiments were designed to study the effect of noradrenaline on potassium permeability using ^{86}Rb as a marker for potassium, this being another method for studying the effects of noradrenaline on membrane events. It has previously been described how noradrenaline increased the efflux of ^{42}K in some arteries (Casteels *et al.* 1977*a*; Droogmans *et al.* 1977). In an attempt to study further the effects of α-adrenoreceptor activation and the effects of other smooth muscle stimulants we used a variety of arteries from both guinea-pig and rabbit. We were also interested to see whether the changes in potassium permeability produced by noradrenaline reflected the changes in membrane potential or membrane resistance reported in the literature. Some of these results were briefly described at a meeting of the Physiological Society (Bolton & Clapp, 1983).

METHODS

Tissue preparation

Adult rabbits (1·5–2·5 kg) or guinea-pigs (300–400 g) of either sex were killed by cervical dislocation. Arteries (diameters in parentheses) taken from rabbit were aorta (3·5–4·0 mm) (at aortic arch), main pulmonary (3·5 mm), branch of anterior mesenteric (1·0–1·5 mm), brachial (1·0–1·5 mm),

and ear (0·8–1·0 mm) and from guinea-pig, abdominal aorta (0·9 mm), main pulmonary (3·0 mm) and main anterior mesenteric (0·6–1·0 mm). They were excised and freed of connective tissue under a dissecting microscope. Rings or helical strips approximately 1 cm long were carefully cut to a width of 1·0–1·5 mm. Tissues prepared in this way were used for ion flux measurements and isometric tension recordings.

Measurement of ^{86}Rb efflux

Imaizumi & Watanabe (1981) have discussed the validity of using ^{86}Rb as a marker for ^{42}K and concluded that rubidium behaved in a similar manner to potassium in tracheal muscle. ^{86}Rb has also been widely used for investigations in other tissues, mainly because it has a longer half-life (19 days) compared to ^{42}K (12 h), making it more convenient and economical to use. Strips were loaded with ^{86}Rb (or ^{42}K) by incubating them for three or more hours in Krebs at 37 °C to which had been added ^{86}Rb (or ^{42}K) to a final concentration of between 0·1 and 0·3 mM (12 mM for ^{42}K) and final activity of 40 μCi ml^{-1} (6 μCi ml^{-1} for ^{42}K). All solutions were gassed with 95 % O_2 and 5 % CO_2, and the pH was 7·2. After the incubation period, each strip was washed to remove excess radioactivity and transferred to a perfusion chamber as described by Bolton & Clark (1981), and perfused with Krebs at 37 °C at a rate of 2·1 ml min^{-1}. Tension was measured isometrically. In order to facilitate comparison between large and small arteries, strips were subjected to a basal tension at which preliminary experiments showed that noradrenaline (10^{-6} M) produced maximum tension. The following basal tensions were used: rabbit aorta, 2·0 g; pulmonary, 1·8 g; ear, 1·1 g; brachial, 1·2 g; mesenteric, 1·0 g; guinea-pig pulmonary, 1·6 g; abdominal aorta, 1·4 g; mesenteric, 0·8 g. A period of 20 min was allowed before collection of the perfusate was begun at 1 min intervals. The amount of radioactivity in each sample was determined by gamma counting. Dose–response curves to noradrenaline or raised potassium for tension and efflux were obtained by exposing the tissue to increasing concentrations of the drug in Krebs for short periods of time: 10–15 min was allowed between drug applications. This method did not seem to reduce the maximum response obtainable (cf. Fig. 2*B* with Figs. 8 and 9; Fig. 6*B* with Figs. 8 and 9).

Solutions

A physiological solution of the following composition was used (mM): NaCl, 120; KCl, 5·9; $NaHCO_3$, 15·5; $MgCl_2$, 1·2; NaH_2PO_4, 1·2; $CaCl_2$, 2·5; glucose, 11·5. Solutions containing elevated concentrations of potassium were made by replacing an equivalent amount of NaCl with KCl. Dose–response curves to raised $[K^+]_o$ were performed in the presence of phentolamine (10^{-6} M) to prevent the action of any noradrenaline that might be released from nerve endings (Kirpekar & Wakade, 1968). Calcium-free solutions were prepared by omitting $CaCl_2$ from the Krebs solution and by adding 0·1–0·2 mM-EGTA.

Drugs and isotopes

The following drugs were used: noradrenaline hydrochloride (Sigma), phentolamine mesylate (BDH), 5-hydroxytryptamine (5-HT, Sigma), histamine dihydrochloride (Koch-Light), tetraethylammonium chloride (TEA, BDH), prazosin hydrochloride (Pfizer), ethylene glycol bis (2-aminoethyl) tetra-acetic acid (EGTA, Fisons), vasopressin (Parke Davis), angiotensin II amide (gift from CIBA), prostaglandin $F_{2\alpha}$ (Upjohn). $^{86}RbCl$ and isotonic ^{42}KCl (Amersham) were obtained as solutions containing 1 and 0·25 mCi ml^{-1} respectively.

Treatment of results

The ^{86}Rb efflux rate constant was calculated by dividing the number of counts in the perfusate collected over 1 min by the averaged number of counts in the tissue at that time. The efflux rate constant was plotted against time using a computer program. No drug-induced response was elicited until 30 min had elapsed after setting up the strip, at which time the rate of loss of ^{86}Rb (or ^{42}K) was steady and is presumed to reflect the rate of loss across the smooth muscle cell membranes (Casteels, 1970). No systematic attempt was made to remove endothelium but its contribution must be small. The increment in efflux rate was calculated by subtracting the basal efflux rate determined by averaging the five samples obtained before applying the drug from the peak efflux rate produced upon drug application. In an attempt to quantify the tension generated by both large and small arteries, tension was expressed as the force generated per unit of cross-sectional area. This was calculated by measuring the length of each muscle strip under tension and its weight (the weighing

being done in saturated air and the reading taken when the weight remained constant) and taking the density of arterial muscle to be 1·07 g cm^{-3}. Then:

$$\text{cross-sectional area } (cm^2) = \frac{\text{weight of strip (g)}}{1{\cdot}07 \text{ g } cm^{-3} \times \text{length of strip (cm)}}.$$

The apparent equilibrium constant for prazosin against noradrenaline was calculated assuming the Gaddum–Schild equation applies: dose ratio $= 1 + B/K_D$ where B = concentration of antagonist (M) and K_D = equilibrium constant (M).

Statistical methods

The results obtained were expressed as the mean value ± standard error of the mean, and the statistical significance assessed by the Student's t test. P values less than 0·05 were considered as significant.

RESULTS

After the initial 20 min perfusion period, the rate of loss of both ^{42}K and ^{86}Rb remained relatively constant, suggesting that they are both lost from a single compartment. The basal efflux rates of ^{42}K and ^{86}Rb were not significantly different although the rate of ^{86}Rb efflux tended to be slower than that of ^{42}K. Since ^{86}Rb is a more convenient isotope to use, this was generally used in preference to ^{42}K.

The effect of noradrenaline on ^{86}Rb loss in arterial smooth muscle

The effect of noradrenaline on ^{86}Rb efflux rate and tension was studied in a range of arteries. Noradrenaline was applied for 2 (at high doses) or 3 min (at low doses) which was generally long enough for the rise in efflux rate to reach its peak. In rabbit aorta, pulmonary artery, ear artery, and guinea-pig pulmonary artery application of increasing doses of noradrenaline caused a dose-dependent increase in both tension and ^{86}Rb efflux rate. Similar large increases in ^{42}K efflux have been reported by Briggs & Melvin (1961; rabbit aorta), Casteels *et al.* (1977*a*; rabbit pulmonary) and by Droogmans *et al.* (1977; rabbit ear) in response to noradrenaline, indicating that ^{86}Rb can be used as a tracer for potassium ions. These arteries are referred to as 'high-fluxing' arteries in the text. Fig. 1*A* shows an example of a typical experiment taken from rabbit aorta. The threshold for contraction and an increase in the basal efflux was 5×10^{-8} M-noradrenaline in this particular artery; however, the threshold varied according to the species and artery. At noradrenaline concentrations greater than 10^{-6} M there was a substantial increase in ^{86}Rb efflux, approximately four times that of resting basal efflux. In a series of experiments on the rabbit aorta, the average basal efflux was $0{\cdot}48 \pm 0{\cdot}04 \times 10^{-2}$ min^{-1} (mean ± S.E. of the mean, $n = 25$) and the maximal increase in ^{86}Rb efflux which occurred at 10^{-4} M-noradrenaline was $1{\cdot}62 \pm 0{\cdot}15 \times 10^{-2}$ min^{-1} ($n = 5$).

In contrast, application of noradrenaline to the guinea-pig mesenteric artery and abdominal aorta, and rabbit mesenteric and brachial arteries, produced only small increases in ^{86}Rb efflux. Fig. 1*B* shows guinea-pig mesenteric artery as an example of a 'low-fluxing' artery where noradrenaline, even at high concentrations ($> 10^{-5}$ M), produced only a 25 % increase in ^{86}Rb efflux above basal rate. The average basal efflux in the guinea-pig mesenteric arteries was $1{\cdot}09 \pm 0{\cdot}09 \times 10^{-2}$ min^{-1} ($n = 30$), and this was roughly twice the mean resting basal efflux seen in rabbit arteries. The average maximum increase in efflux obtained with 10^{-5} M-noradrenaline was

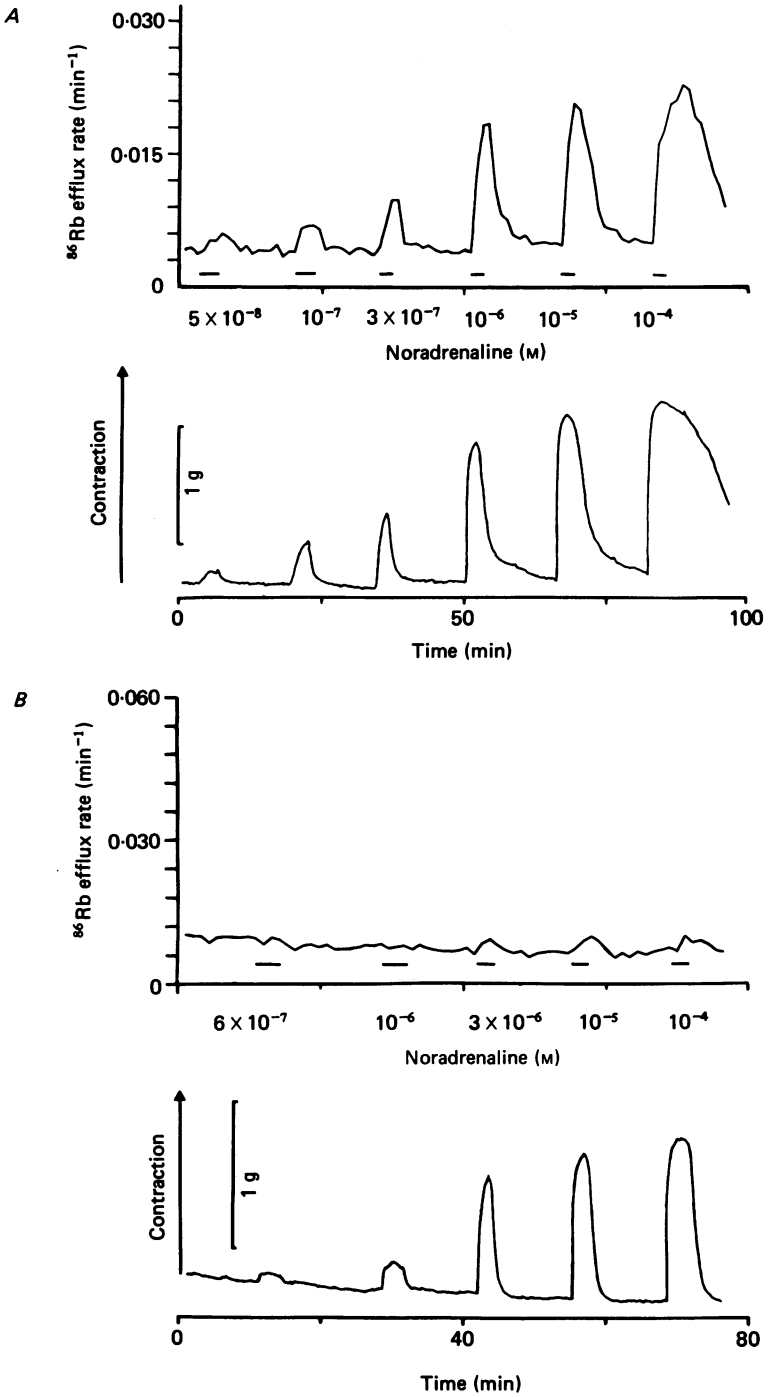


Fig. 1. *A*, effect of noradrenaline on ^{86}Rb efflux rate and tension in the rabbit aorta. Changes in efflux and tension were recorded simultaneously from the same tissue. Noradrenaline applied for 2 or 3 min caused a dose-dependent increase in tension and ^{86}Rb efflux. *B*, effect of noradrenaline on ^{86}Rb efflux and tension in the guinea-pig mesenteric artery. Here application of noradrenaline produced only a small increase in efflux.

$0{\cdot}26 \pm 0{\cdot}02 \times 10^{-2}$ min^{-1} ($n = 6$), this increase being some six times less than that seen in the rabbit aorta.

Noradrenaline produced a range of increases in ^{86}Rb efflux rate in eight arteries studied and the results are summarized in Fig. 2. Fig. 2*A* shows the increases in tension and Fig. 2*B* shows the increases in efflux both plotted against the noradrenaline concentration ($10^{-9} - 10^{-4}$ M). The threshold for contraction and increases in ^{86}Rb efflux varied some 600-fold between different arteries and there was also a 10-fold range in the maximum increase in efflux evoked by noradrenaline (Fig. 3*A*). If the effects of noradrenaline on tension and efflux are expressed as a percentage of the maximum obtained, it was found that concentrations of noradrenaline producing 50% of maximum efflux (EC_{50} efflux) and 50% maximum contraction (EC_{50} contraction) were not significantly different in five arteries, (e.g. Fig. 4) but were significantly different ($P < 0{\cdot}05$) in the rabbit ear and guinea-pig pulmonary arteries.

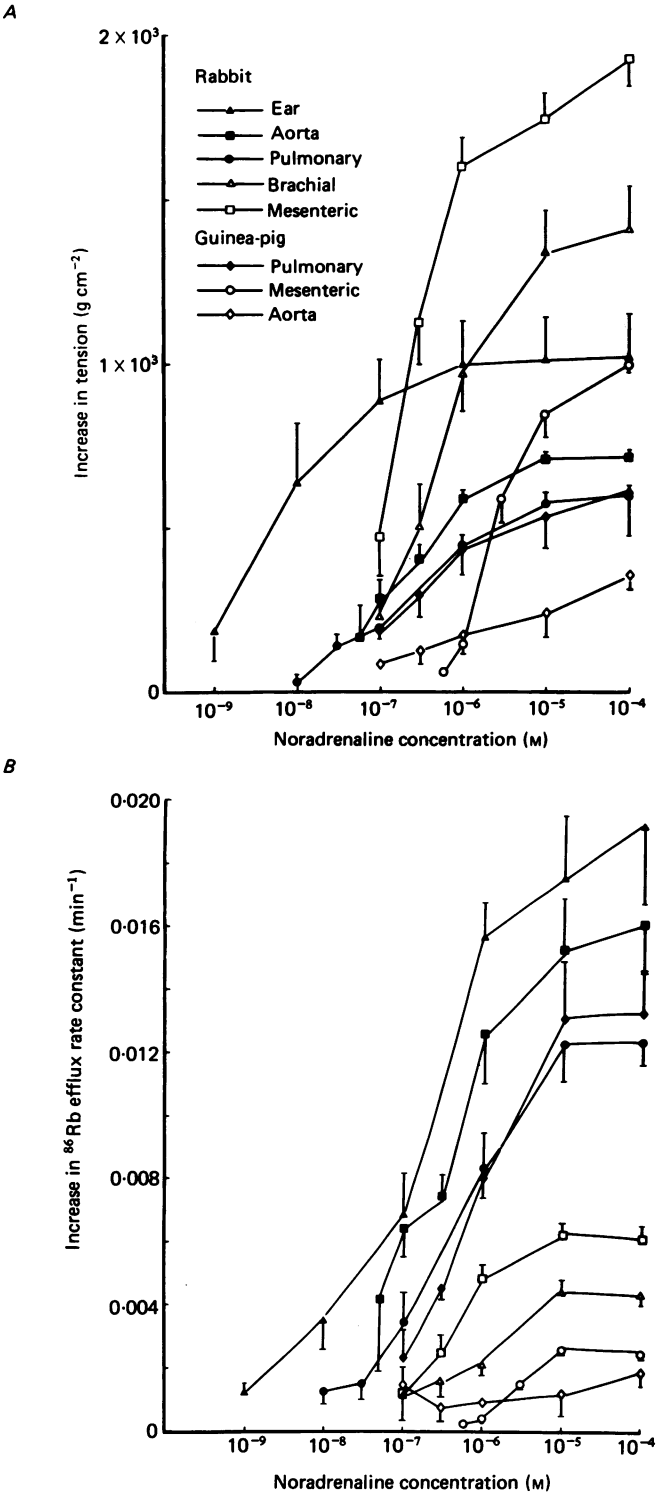
The small maximal increases in efflux seen with noradrenaline in some arteries could not be attributed to the fact that they generate less maximal tension. If the maximal tension generated by noradrenaline was plotted against the maximum ^{86}Rb efflux evoked by noradrenaline (Fig. 3*A*) it is obvious that there was no correlation between them. For example, the maximum tension generated by the guinea-pig mesenteric artery at 10^{-4} M-noradrenaline was $10{\cdot}00 \pm 0{\cdot}71 \times 10^2$ g cm^{-2} ($n = 6$) whereas the rabbit aorta, at the same concentration, generated $7{\cdot}22 \pm 0{\cdot}26 \times 10^2$ g cm^{-2} ($n = 5$). Neither was the ability of an artery to produce flux dependent on size, for example the large rabbit aorta and much smaller rabbit ear are both high-fluxing arteries.

The increase in ^{42}K efflux with noradrenaline in low- and high-fluxing arteries differed only inasmuch as ^{42}K always produced a bigger efflux response than ^{86}Rb, showing that using rubidium does not give rise to the heterogeneity of the efflux responses.

The effects of prazosin on efflux and tension

We looked at the effect of prazosin, a specific α_1-adrenoreceptor antagonist (Cambridge, Daney & Massingham, 1977) on efflux and tension in the rabbit aorta and mesenteric artery to see whether the α-adrenoreceptor properties differed in low-and high-fluxing arteries, since there have been reports that vascular smooth muscle possesses both α_1- and α_2-adrenoreceptors (Drew & Whiting, 1979; Timmermans, Kwa & Van Zwieten, 1979). Presumably noradrenaline acts on both subtypes. Doses of noradrenaline were applied (for 3 min at low and 2 min at high doses) that would give approximately 30% and 70% of the maximal response before and after the addition of prazosin (10^{-7} M), which produced roughly a 10-fold shift in the dose–response curve to noradrenaline in both arteries. Prazosin reduced the noradrenaline-evoked ^{86}Rb efflux and contraction to a similar extent in both the rabbit mesenteric artery and aorta. Table 1 shows the apparent equilibrium constants

Fig. 2. *A*, summary of the effects of noradrenaline (10^{-9}–10^{-4} M) on tension in eight arteries. The threshold for contraction seen with noradrenaline varies some 600-fold (10^{-9}–6×10^{-7} M). The results shown for each drug dose are the mean values taken from five or six strips; vertical lines show S.E. of the mean. *B*, summary of the effects of noradrenaline (10^{-9}–10^{-4} M) on increases in ^{86}Rb efflux rate.



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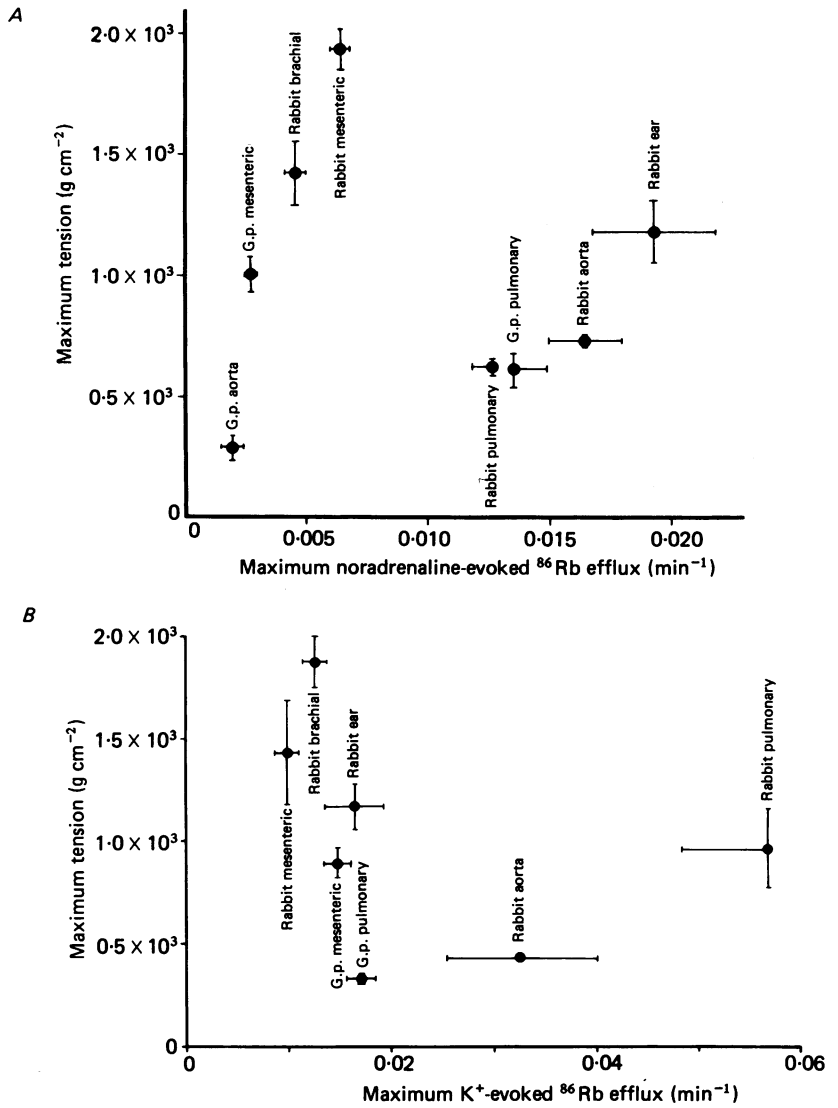

Fig. 3. *A*, plot of the maximal tension generated by noradrenaline (10^{-5}–10^{-4} M) against the maximal increases in ^{86}Rb efflux in a range of arteries. Notice that there is no correlation between maximum tension generated and maximum efflux evoked by noradrenaline. *B*, plot of the maximal tension generated by raised $[K^+]_o$ against the maximal increases in ^{86}Rb efflux. Again there is no correlation between maximum tension generated and maximum efflux evoked by raised $[K^+]_o$. G.p. = guinea-pig.

for prazosin measured from the efflux and tension responses. These values do not significantly differ on the same tissue or between arteries. Also the values calculated from these experiments were not significantly different from that ($6{\cdot}2 \times 10^{-9}$ M) calculated in the rat anococcygeus for the α_1-adrenoreceptor (Doxey, Smith & Walker, 1977). The results suggest that α_2-adrenoreceptors if present do not contribute significantly to the observed responses (cf. Vanhoutte, 1982) and that noradrenaline activates a similar population of α-adrenoreceptors in both cases.

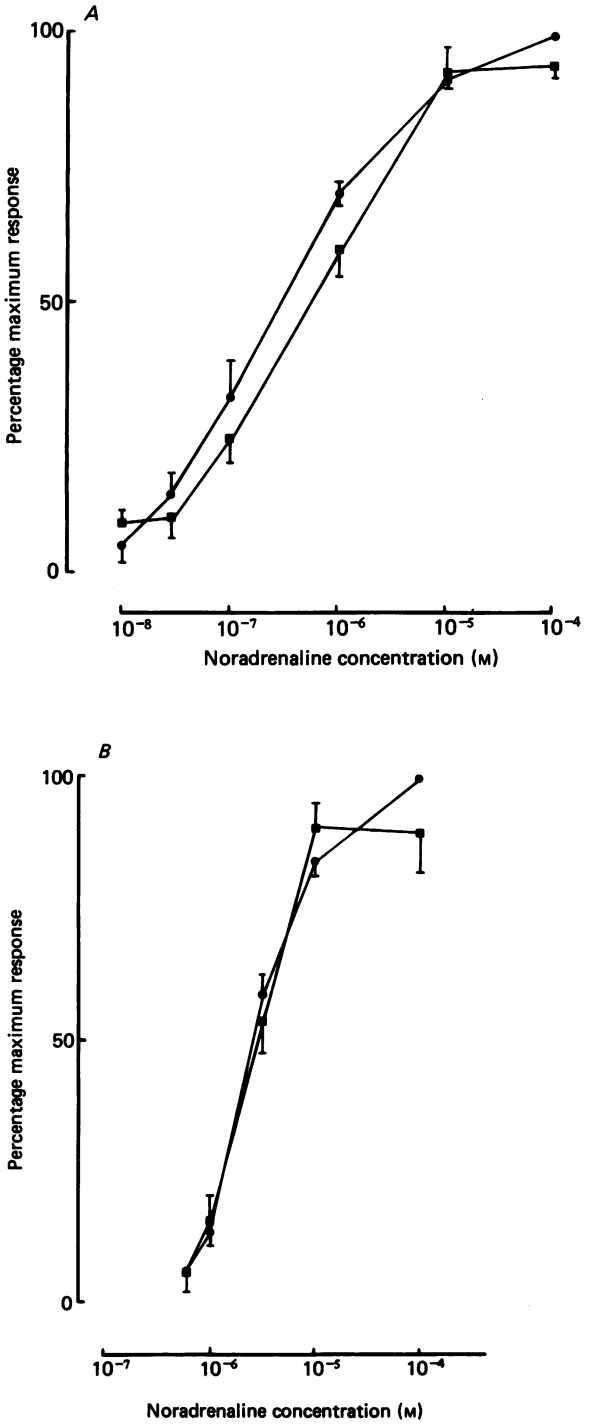

Fig. 4. Effect of noradrenaline on ^{86}Rb efflux (—■—) and tension (—●—), in *A*, rabbit pulmonary and *B*, guinea-pig mesenteric artery, each point plotted as the percentage of the maximum response obtained averaged over five experiments (means ± S.E. of mean). There is a similarity in the dose–response curves for efflux and tension in both cases.

Effect of calcium-free conditions on noradrenaline-evoked contractions

The response to noradrenaline has been suggested to depend on both the influx of extracellular calcium and release of intracellular calcium (e.g. Bolton, 1979). We have therefore studied the effect of noradrenaline (10^{-5} M) on tension under calcium-free conditions in the presence of 10^{-4} M-EGTA to ascertain whether low- or high-fluxing arteries utilized calcium in different ways. Arterial strips were left to equilibrate for 45 min and reproducible responses to noradrenaline were obtained in normal Krebs. Noradrenaline was added (for 2 min) 5, 15 and 35 min after incubation in calcium-free Krebs. Application of 10^{-5} M-noradrenaline produced smaller contractions in calcium-free solution, the amplitude of which declined with longer incubation times. Results showed that there was no relationship between the magnitude of the efflux response

TABLE 1. The apparent equilibrium constant of prazosin against noradrenaline in rabbit arterial smooth muscle

	Contraction (M)	^{86}Rb efflux (M)
Aorta	$9{\cdot}6 \pm 3{\cdot}2 \times 10^{-9}$ ($n = 5$)	$5{\cdot}3 \pm 1{\cdot}2 \times 10^{-9}$ ($n = 5$)
Mesenteric artery	$8{\cdot}9 \pm 2{\cdot}4 \times 10^{-9}$ ($n = 5$)	$4{\cdot}6 \pm 1{\cdot}8 \times 10^{-9}$ ($n = 5$)

and their calcium sensitivity. In the rabbit ear (a high-fluxing artery), a small, non-sustained contraction (33 % of control) was produced with noradrenaline after 5 min in calcium-free conditions, which disappeared on the second application of noradrenaline. In contrast, the rabbit aorta (also a high-fluxing artery) was relatively insensitive to calcium-free conditions, 60 % of the contraction still remaining after 35 min in calcium-free solution. On the other hand the guinea-pig mesenteric artery was very sensitive to the removal of extracellular calcium, only 13 % of the control response remaining after 5 min in calcium-free conditions, whereas in the rabbit mesenteric artery (also a low-fluxing artery) 67 % of the contraction to noradrenaline remained after 5 min in calcium-free conditions.

Effect of raised $[K^+]_o$ *on* ^{86}*Rb efflux*

Since noradrenaline had such a diverse effect on efflux, we examined other smooth muscle stimulants. Raising $[K^+]_o$ contracts these arteries and presumably depolarizes them to a similar extent whereas noradrenaline may not. The $[K^+]_o$ was raised above normal (5·9 mM) up to 142 mM for 2–5 min periods (Fig. 5*A* and *B*) and dose–response curves constructed (Fig. 6*A* and *B*). The maximum efflux evoked by raised $[K^+]_o$ was larger than the efflux seen with 10^{-5} M-noradrenaline (Fig. 5*A* and *B*). The maximum increase in ^{86}Rb efflux seen with 126 mM-K^+ was $3{\cdot}25 \pm 0{\cdot}71 \times 10^{-2}$ min^{-1} ($n = 5$) in the rabbit aorta and $0{\cdot}98 \pm 0{\cdot}10 \times 10^{-2}$ min^{-1} ($n = 6$) in the rabbit mesenteric artery compared to the maximum efflux seen with 10^{-5}–10^{-4} M-noradrenaline in the rabbit aorta of $1{\cdot}62 \pm 0{\cdot}15 \times 10^{-2}$ min^{-1} ($n = 5$) and $0{\cdot}63 \pm 0{\cdot}04 \times 10^{-2}$ min^{-1} ($n = 6$) in rabbit mesenteric artery. We studied the effect of raised $[K^+]_o$ on ^{86}Rb loss in seven arteries and found that raised $[K^+]_o$ also had diverse effects on efflux (Fig. 6*A*). However, low-fluxing arteries to noradrenaline generally produced lower efflux responses to raised $[K^+]_o$. The average maximum effect of raised $[K^+]_o$ on efflux in all these arteries was three times greater than the maximum effect of noradrenaline on efflux in

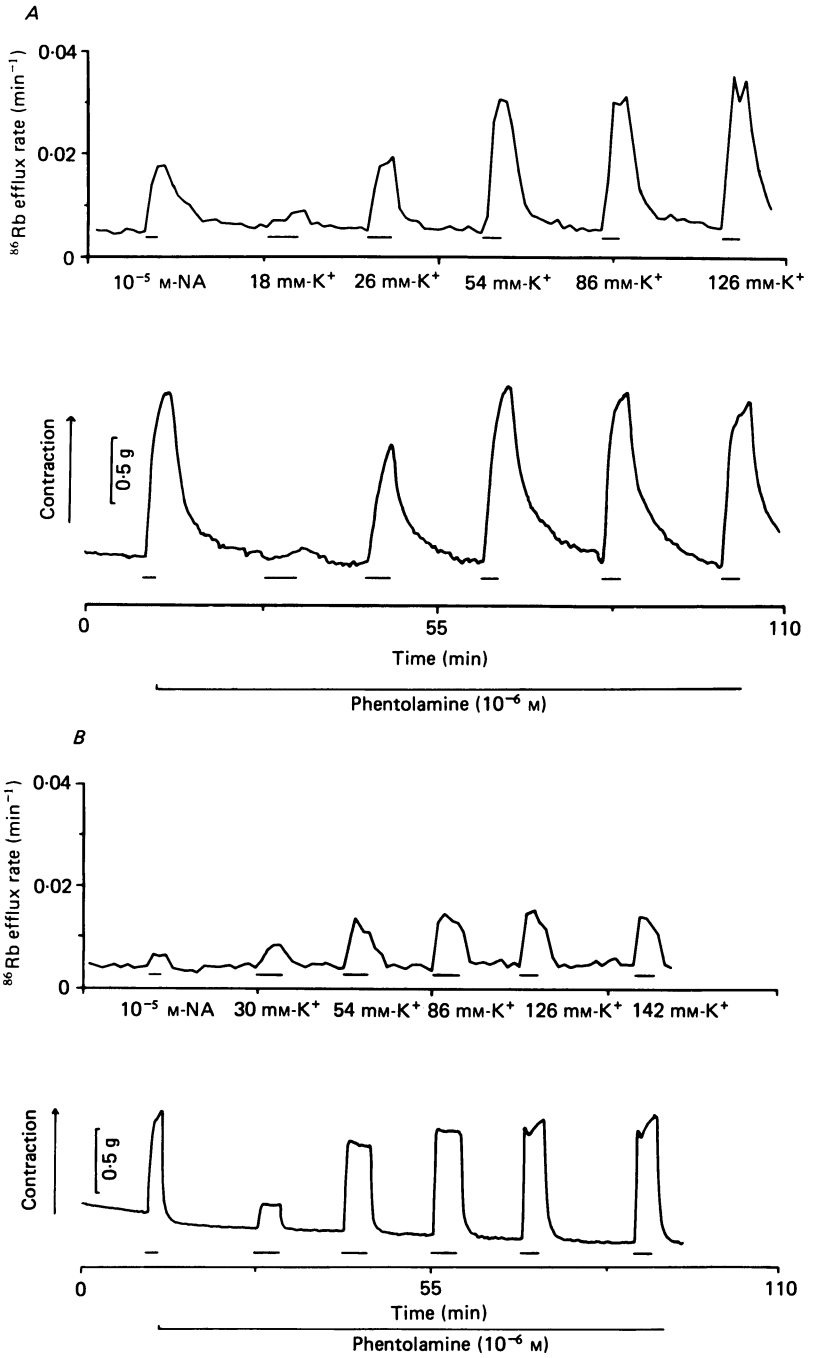

Fig. 5. *A*, effect of raised $[K^+]_o$ on ^{86}Rb efflux rate and tension, in the presence of 10^{-6} M-phentolamine, in rabbit aorta. Low doses of raised $[K^+]_o$ were applied for 4–5 min and high doses for 3 min. For comparison, 10^{-5} M-noradrenaline (NA) was applied at the beginning of the experiment. *B*, the effect of raised $[K^+]_o$ and ^{86}Rb efflux rate and tension in rabbit mesenteric artery. Raising $[K^+]_o$ has a larger maximal effect than noradrenaline in both arteries.

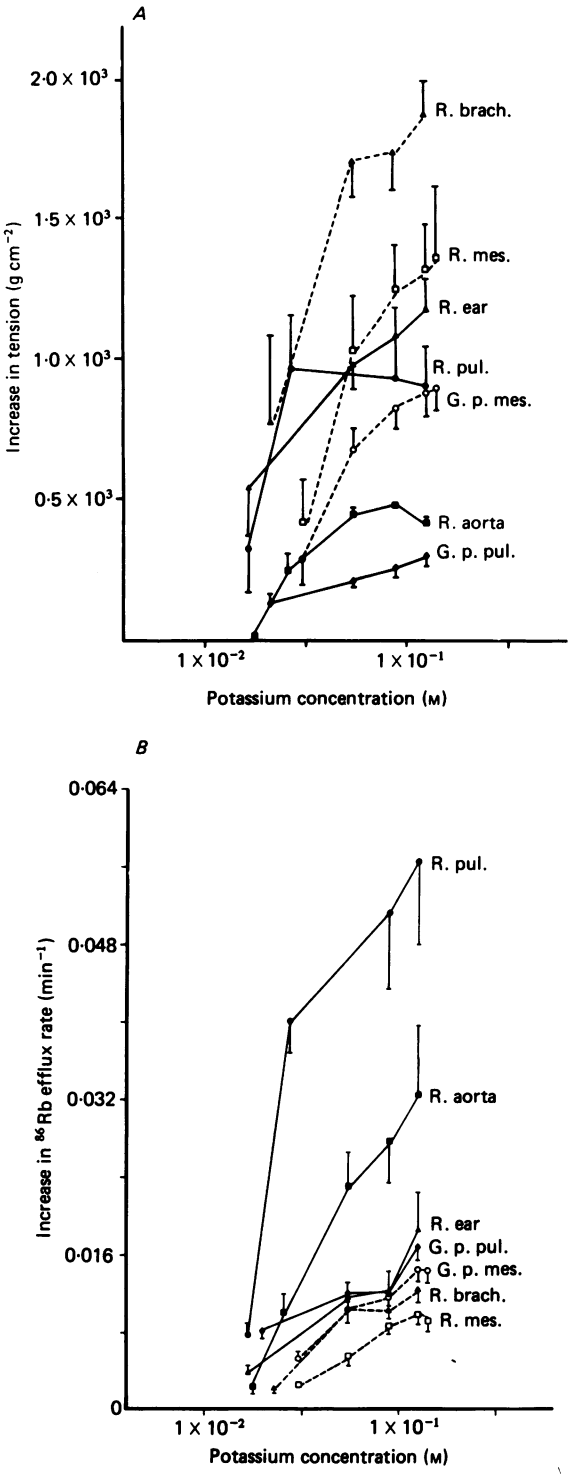

Fig. 6. For legend see opposite page.

previous experiments (Fig. 3*A* and *B*). As with noradrenaline, arteries producing lower efflux responses to raised $[K^+]_o$ did not generate less maximum tension (Fig. 3*B*). The rabbit ear was an exception and showed a lower maximum efflux to raised $[K^+]_o$ than to noradrenaline. Clearly depolarization alone is not the only factor governing the loss of ^{86}Rb, and this is demonstrated by the observation that different arteries have different thresholds for both increases in tension and efflux and hence presumably different thresholds for the opening of potential-sensitive calcium and potassium channels; in the rabbit pulmonary artery the approximate threshold was 17 mM-K^+ compared to 30 mM-K^+ in the guinea-pig mesenteric artery. The maximum tension generated by raised $[K^+]_o$ is comparable to the maximum tension obtained with noradrenaline. These experiments show that raised $[K^+]_o$ is more effective at increasing ^{86}Rb efflux than noradrenaline, perhaps partly because it depolarizes vascular smooth muscle to a greater extent.

Other agonists

We also examined a number of other smooth muscle stimulants: namely prostaglandin $F_{2\alpha}$, 5-hydroxytryptamine, histamine, vasopressin and angiotensin. We found that these stimulants were as potent or were less potent than noradrenaline in evoking ^{86}Rb efflux in both low- (rabbit brachial, mesenteric and guinea-pig mesenteric) and high-fluxing (rabbit ear) arteries (Fig. 7*A* and *B*) suggesting that it is the properties of the ion channels through which ^{86}Rb and hence potassium are lost which are different in different arteries.

The effect of calcium-free, EGTA solution on efflux and tension in the rabbit aorta

It has been suggested that in vascular muscle a rise in intracellular calcium, which occurs during noradrenaline-evoked contractions, activates potassium channels (Haeusler & Thorens, 1980; Häusler, 1982). For this reason, we studied the effect of calcium-free solution, containing 0·1–0·2 mM-EGTA, on efflux and tension.

Single applications of noradrenaline (10^{-5} M) or of 66 mM-K^+ (a concentration chosen to roughly match the effect of noradrenaline on efflux) were made for 2 or 3 min respectively to the same tissue. Tissues were perfused with calcium-free Krebs solution (containing EGTA) for 20 min before drug application, the protocol for the control experiments being similar. The average increase in ^{86}Rb efflux produced by 66 mM-K^+ was significantly ($P < 0.05$) but only slightly reduced from $1.99 \pm 0.11 \times 10^{-2}$ min^{-1} ($n = 5$) to $1.59 \pm 0.11 \times 10^{-2}$ min^{-1} ($n = 5$) in calcium-free solution (Fig. 8*A*). However, the average basal efflux after 20 min in calcium-free solution was $0.88 \pm 0.07 \times 10^{-2}$ min^{-1} while in the presence of calcium in the same tissues it was $0.49 \pm 0.04 \times 10^{-2}$ min^{-1}. Peak (basal efflux plus increment) efflux to 66 mM-K^+ was not significantly affected by calcium-free solution. In contrast, noradrenaline produced a substantial contraction in calcium-free solution. The

Fig. 6. The increases in tension (*A*) and in ^{86}Rb efflux (*B*) plotted against $[K^+]_o$. Dotted lines represent 'low-fluxing' arteries to noradrenaline which are: rabbit brachial (R. brach.), rabbit mesenteric (R. mes.) and guinea-pig mesenteric (G.p. mes.). Continuous lines represent 'high-fluxing' arteries which are: rabbit ear (R. ear), rabbit pulmonary (R. pul.), rabbit aorta (R. aorta) and guinea-pig pulmonary (G.p. pul.). It can be seen that low-fluxing arteries to noradrenaline are also low-fluxing arteries to raised $[K^+]_o$.

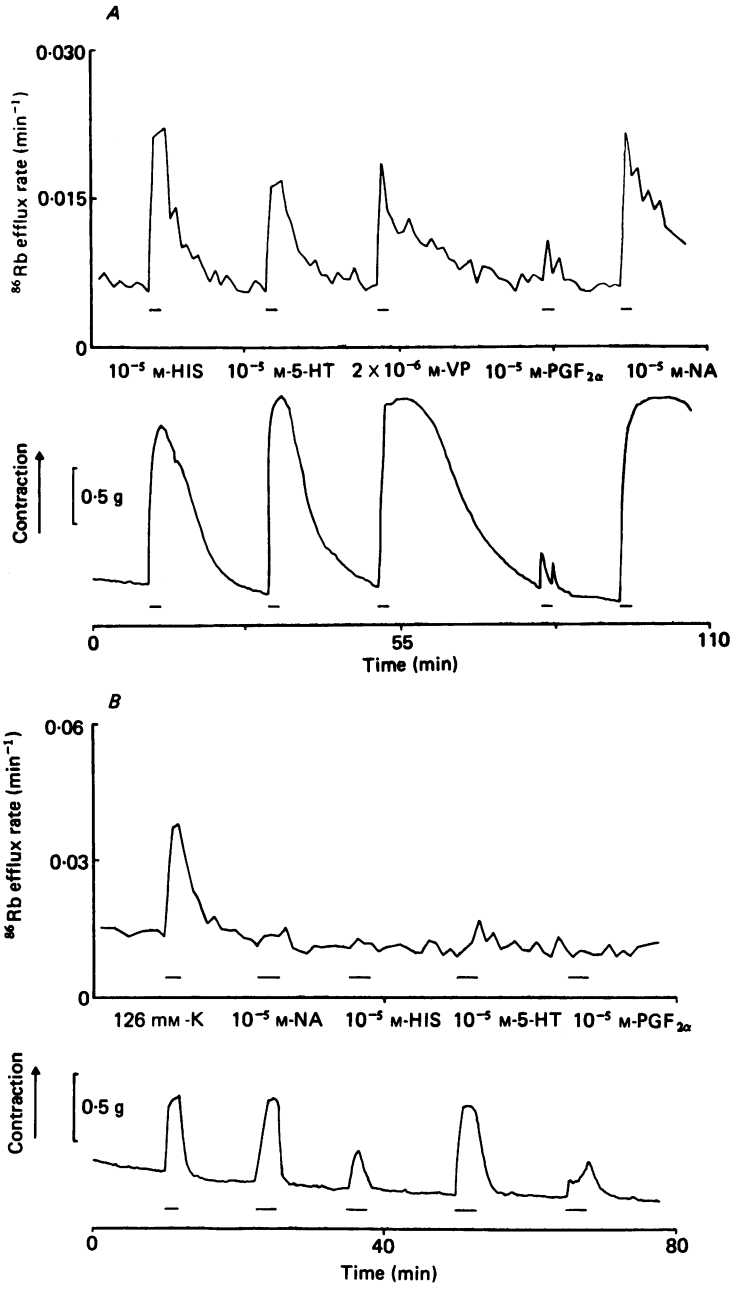

Fig. 7. *A*, the effects of various agonists on the rate of loss of ^{86}Rb and tension in the rabbit ear artery. Histamine (HIS), 5-hydroxytryptamine (5-HT), vasopressin (VP), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and noradrenaline (NA) were applied for 2 min. Agonists were either as potent or less potent than noradrenaline at increasing efflux. *B*, the effects of other agonists on ^{86}Rb efflux and tension in the guinea-pig mesenteric artery, a low-fluxing artery to noradrenaline. Histamine, 5-HT and prostaglandin $F_{2\alpha}$ are also weak agonists for increasing ^{86}Rb efflux compared to raised $[K^+]_o$ (126 mM) which produces significant increases.

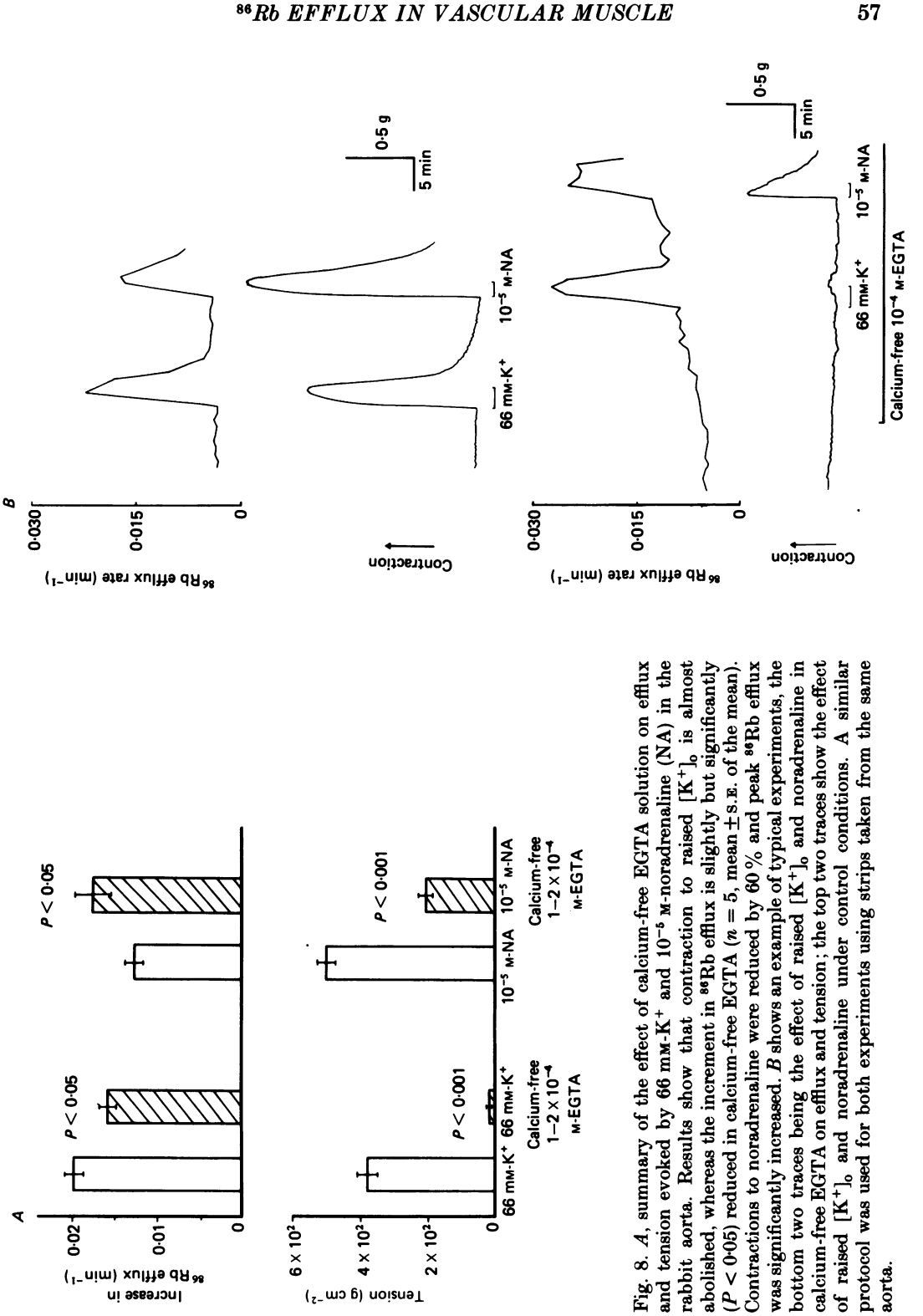

Fig. 8. *A*, summary of the effect of calcium-free EGTA solution on efflux and tension evoked by 66 mM-K^+ and 10^{-5} M-noradrenaline (NA) in the rabbit aorta. Results show that contraction to raised $[K^+]_o$ is almost abolished, whereas the increment in ^{86}Rb efflux is slightly but significantly ($P < 0{\cdot}05$) reduced in calcium-free EGTA ($n = 5$, mean ± S.E. of the mean). Contractions to noradrenaline were reduced by 60% and peak ^{86}Rb efflux was significantly increased. *B* shows an example of typical experiments, the bottom two traces being the effect of raised $[K^+]_o$ and noradrenaline in calcium-free EGTA on efflux and tension; the top two traces show the effect of raised $[K^+]_o$ and noradrenaline under control conditions. A similar protocol was used for both experiments using strips taken from the same aorta.

increment in ^{86}Rb efflux produced by noradrenaline was signficantly ($P < 0.05$) increased in calcium-free solution from $1.27 \pm 0.11 \times 10^{-2}$ min^{-1} ($n = 5$) to $1.79 \pm 0.18 \times 10^{-2}$ min^{-1} ($n = 5$).

Since it has been recently suggested that it may not always be possible to assess the membrane potassium permeability using ^{86}Rb (Petersen & Maruyama, 1984) we decided to repeat similar experiments to the above but using ^{42}K and ^{86}Rb in a double isotope experiment. This would determine whether ^{86}Rb acted essentially in the same way as ^{42}K. Tissues were loaded with ^{42}K and ^{86}Rb in normal physiological solution containing the isotopes at a final concentration of 3 μCi ml^{-1} and 20 μCi ml^{-1} respectively. ^{42}K and ^{86}Rb were counted simultaneously and the counts were corrected for scatter of radioactivity into their respective counting windows. The results obtained showed that ^{86}Rb and ^{42}K behaved similarly. In calcium-free solution the increase in ^{42}K efflux to 60 mM-K^+ was significantly ($P < 0.05$) reduced from $3.44 \pm 0.35 \times 10^{-2}$ min^{-1} ($n = 5$) to $2.54 \pm 0.11 \times 10^{-2}$ min^{-1} ($n = 5$). In the same experiment ^{86}Rb efflux was significantly ($P < 0.05$) reduced from $2.81 \pm 0.37 \times 10^{-2}$ min^{-1} ($n = 5$) to $1.80 \pm 0.08 \times 10^{-2}$ min^{-1} ($n = 5$). Peak (basal plus increment) ^{86}Rb and ^{42}K effluxes to 60 mM-K^+ were not significantly changed in calcium-free solution. The results with noradrenaline (10^{-5} M) showed that in calcium-free solution, the increments in ^{42}K and ^{86}Rb effluxes were significantly increased (both $P < 0.001$). In calcium-free solution the resting fluxes were increased. It was also found that the increases in effluxes evoked by 60 mM-K^+ or noradrenaline were significantly greater ($P < 0.05$ and $P < 0.001$ respectively) using ^{42}K compared to ^{86}Rb, suggesting potassium to be more permeable than rubidium.

Substantial increases in ^{42}K efflux were also observed in calcium-free (no EGTA) high-magnesium (12 mM-Mg^{2+}) solution to both 66 mM-K^+ and 10^{-5} M-noradrenaline. Basal ^{42}K efflux did not increase in this calcium-free solution. These results show that a substantial increase in ^{86}Rb or ^{42}K efflux is produced by raised $[K^+]_o$ or noradrenaline under calcium-free conditions.

Effect of TEA on efflux evoked by raised $[K^+]_o$ and noradrenaline

Similar experiments to the above were carried out using rabbit aorta either in the presence or absence of 10 or 20 mM-TEA (a potassium channel blocker). TEA was applied for 12 min before drug application. Results showed that TEA had no effect on tension generated by 66 mM-K^+ or 10^{-5} M-noradrenaline. However, TEA significantly ($P < 0.001$) reduced the increment in efflux evoked by raised $[K^+]_o$ by 66% but did not significantly reduce the efflux evoked by 10^{-5} M-noradrenaline (Fig. 9). TEA produced a significantly ($P < 0.001$) greater reduction in the ^{86}Rb efflux response to 66 mM-K^+ than to noradrenaline (10^{-5} M) when comparison of responses obtained on the same tissues was made. TEA was also found to increase basal efflux by 24% (cf. the rabbit pulmonary and rabbit ear arteries, Casteels *et al.* 1977*a*; Droogmans *et al.* 1977). Reversing the order of application of raised $[K^+]_o$ and noradrenaline gave a similar result. Thus, TEA seems to differentiate between the channels opened by raised $[K^+]_o$ and noradrenaline, presumably the former being potential activated and sensitive to TEA, the latter being receptor operated and relatively insensitive to blockade by TEA.

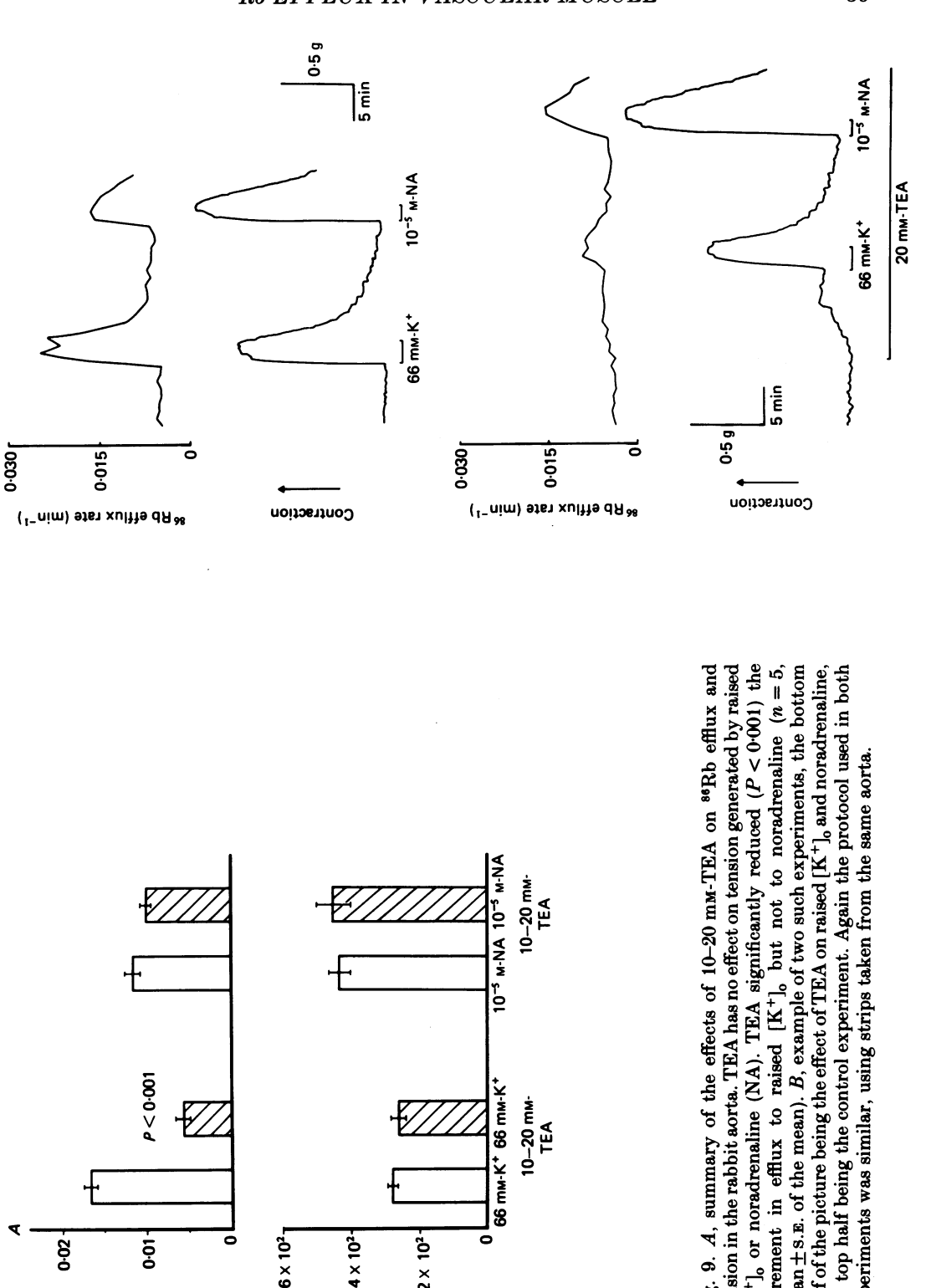

Fig. 9. *A*, summary of the effects of 10–20 mM-TEA on ^{86}Rb efflux and tension in the rabbit aorta. TEA has no effect on tension generated by raised $[K^+]_o$ or noradrenaline (NA). TEA significantly reduced ($P < 0·001$) the increment in efflux to raised $[K^+]_o$ but not to noradrenaline ($n = 5$, mean ± S.E. of the mean). *B*, example of two such experiments, the bottom half of the picture being the effect of TEA on raised $[K^+]_o$ and noradrenaline, the top half being the control experiment. Again the protocol used in both experiments was similar, using strips taken from the same aorta.

DISCUSSION

The results described in this paper suggest that potassium channels of different vascular smooth muscles exhibit considerable heterogeneity in their properties. Raising $[K^+]_o$ produces at most only small quantitative differences in effects on membrane potential in the various arteries: to take specific examples, in guinea-pig mesenteric artery the relationship between membrane potential and $[K^+]_o$ as measured by Bolton, Lang & Takewaki (1984) is much the same as that described by Casteels *et al.* (1977*b*) in rabbit pulmonary artery, yet the maximum increase in ^{86}Rb efflux was about four times greater in the latter. Such results indicate that it is not quantitative differences in the effect of raising $[K^+]_o$ on membrane potential which explain the heterogeneous efflux responses. While differing degrees of depolarization cannot account for the heterogeneity of efflux responses to raised $[K^+]_o$, it may still be suggested to contribute to the heterogeneity of efflux responses to noradrenaline. However, again this seems not to be the explanation. Comparing the low-fluxing guinea-pig mesenteric and high-fluxing rabbit pulmonary arteries, in the former 10^{-4} M-noradrenaline depolarizes by about 13 mV to near −50 mV (Bolton *et al.* 1984), while according to Casteels *et al.* (1977*b*) and Haeusler & Thorens (1980) the depolarization in rabbit pulmonary artery is maximal at 10–13 mV, carrying the membrane potential to about −47 mV with 5×10^{-6} M-noradrenaline or more. Thus differences in the depolarizing effects of noradrenaline in these two arteries, if they exist, seem too small to explain the five times larger effect of noradrenaline on ^{86}Rb efflux in pulmonary artery; nor by the use of prazosin could we detect differences in the α-adrenoreceptor, activation of which causes the contractile and efflux responses. It appears that, as with raising $[K^+]_o$, the properties of the channels through which ^{86}Rb or ^{42}K escapes upon α-adrenoreceptor activation differ in the various arteries.

We can also dispose of a number of possible but trivial causes of this heterogeneity. The results cannot be explained by variations in the ease with which ^{86}Rb permeates potassium channels in various arteries, since essentially similar results were obtained using ^{42}K. The effects of raising $[K^+]_o$ on efflux were studied in the presence of sufficient concentrations of phentolamine, an α-adrenoreceptor blocker, to preclude noradrenaline released from nerve endings acting to increase ^{86}Rb efflux. The tension responses of high-fluxing and low-fluxing arteries were comparable, arguing against the latter being more damaged by preparative procedures. Differences in the thickness of arterial strips may somewhat alter the rate of escape of tracer ions from them, and so the time course of responses of different arteries may vary – since we measured the peak increment in efflux this might be suggested to give rise to differences. However, there was no correlation between the thickness of an artery and its ability to produce flux. We are left, then, with the likelihood that the potassium channels opened by raising $[K^+]_o$ or by noradrenaline in different vascular smooth muscle cells differ in their numbers and/or conductance.

Nevertheless, it is notable that raised $[K^+]_o$ seems to produce a larger increment in ^{86}Rb efflux than noradrenaline. The explanation is probably that raised $[K^+]_o$, unlike noradrenaline, increases single potassium channel conductance (C. D. Benham, T. B. Bolton & R. J. Lang, unpublished observations) and that the maximal depolar-

ization produced by raised $[K^+]_o$ in these arteries was considerably more than the maximum depolarization by noradrenaline (Mekata, 1979). The maximal effect of raised $[K^+]_o$ on ^{86}Rb efflux was on average about three times greater than the maximal effect of noradrenaline in both high-fluxing and low-fluxing arteries, and no other receptor stimulant was appreciably more effective than noradrenaline.

TEA produced a substantial reduction in ^{86}Rb efflux evoked by raised $[K^+]_o$ while at most only slightly affecting the noradrenaline-evoked ^{86}Rb efflux. This implies that α-adrenoreceptor activation with noradrenaline can open channels through which ^{86}Rb or ^{42}K escape which cannot be opened by raising $[K^+]_o$ (see also Bolton *et al.* 1984), i.e. noradrenaline opens a population of channels associated with the receptor ('receptor-operated channels'; Bolton, 1979) which are not opened simply by depolarization of the membrane. These receptor-operated channels presumably also pass sodium, chloride, or calcium ions to cause the observed depolarization. The approximate 50 % reduction of ^{86}Rb efflux to raised $[K^+]_o$ by 20 mM-TEA is consistent with other results in smooth muscle (Imaizumi & Watanabe, 1981; Benham, Bolton & Lang, 1983).

The experiments described here provide evidence that ^{86}Rb efflux evoked by raised $[K^+]_o$ occurs mostly through potassium channels which are not calcium activated. A substantial component of efflux still remained in the absence of extracellular calcium and in the presence of EGTA, even though the contraction was virtually abolished under these conditions, which implies that a significant rise in internal calcium does not occur. As peak efflux was unchanged, the decrease in the efflux response to raised $[K^+]_o$ in calcium-free solution can be explained by the increase in basal efflux brought about probably by depolarization of the membrane (cf. Keatinge, 1972; Casteels *et al.* 1977*a*, *b*), which presumably opens some of the same channels opened by raised $[K^+]_o$. In contrast, the increase in noradrenaline efflux in calcium-free solution may be due to enhanced electrical activity. In the case of raised $[K^+]_o$ the loss of a small calcium-activated component of ^{86}Rb efflux in calcium-free solution might be difficult to detect. Indeed, calcium-activated potassium channels have been observed in single-channel recordings (although not all potassium channels are sensitive to calcium) made by the patch-clamp technique from isolated smooth muscle cells from small (100 μm diameter) guinea-pig mesenteric arteries (Benham, Bolton, Lang & Takewaki, 1984). The explanation of this discrepancy is uncertain. It seems unlikely that in calcium-free EGTA solution the concentration of calcium inside the cell was insufficient to trigger contraction but still great enough to trigger opening of calcium-activated channels which are activated in the range 10^{-8}–10^{-6} M-internal Ca^{2+} (Benham *et al.* 1984).

The main implications of these experiments are that both the potassium channels opened by depolarization of the membrane of the vascular smooth muscle cell, and those channels through which potassium can escape opened by activation of α- or other receptors, vary in their properties from one artery to another. In both cases we have been unable to detect an important calcium-activated component in potassium efflux, and the channels through which potassium escapes following noradrenaline application include some which are not opened by depolarization of the membrane or blocked by TEA.

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