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THE EFFECTS OF DRUGS ON THE RELATION BETWEEN THE ACTION POTENTIAL DISCHARGE AND TENSION IN A MAMMALIAN VEIN

BY

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There is some uncertainty whether contractions caused by drugs in vascular tissue are accompanied by an action potential discharge. Barr (1961) failed to record action potentials from the dog carotid artery treated with adrenaline, although he did so with direct electrical stimulation. Similarly Su, Bevan & Ursillo (1964) were unable to record action potentials from the pulmonary artery stimulated through its sympathetic nerve. On the other hand Funaki & Bohr (1964) were able to observe the influence of several drugs on the action potential discharge of the rat portal vein. We have observed that contractions in the rabbit anterior mesenteric vein due to noradrenaline are accompanied by action potentials only during the initial stage of drug action and feel that this may provide an explanation of these apparently conflicting reports. Electrical and mechanical activity from the rabbit anterior mesenteric vein has been recorded using the sucrose-gap electrode. This allowed us to study the action potential frequency over long periods during the onset and offset of drug action, a procedure not feasible with microelectrode recording. Because of the difficulties inherent in the use of the sucrose-gap with vascular tissue the amplitude of the action potentials was small compared with the values obtained with microelectrodes (Cuthbert, Matthews & Sutter, 1965). Consequently, in this paper, we have confined our remarks to the action potential frequency, realizing that changes occurred also in the size and configuration of the action potentials.

METHODS

Sucrose-gap technique

The anterior mesenteric veins of freshly killed rabbits were removed and divided longitudinally. Such longitudinal strips were mounted in a conventional sucrose-gap electrode (Burnstock & Straub, 1958) and a thread was attached to the free end in the active side of the apparatus for tension recording. The electrical activity was recorded from a pair of chlorided silver wires via the usual cathode follower stage to one beam of a dual-beam oscilloscope. The other beam was used to record isometric tension changes of the vein by means of an RCA 5734 mechano-electronic transducer. The active side of the apparatus was perfused with Krebs solution at 37 to 38° C and gassed with a mixture of 95% oxygen and 5% carbon dioxide. The reference side was perfused with isotonic potassium sulphate (17.8 g/l.) and the central compartment was perfused with 10% w/v sucrose solution. This latter solution was deionized before use by passing it through a column of

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Biodeminrolit Resin (Permutit). Drugs were given either by injection, in a small volume, into the flow of fluid perfusing the apparatus, or the solution was changed to Krebs solution containing dissolved drug. The Krebs solution had the following composition: (mM) NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgCl_2$, 1.2; $NaHCO_3$ 25; NaH_2PO_4, 1.1; and glucose, 5.6.

RESULTS

As reported previously (Cuthbert & Sutter, 1964; Cuthbert *et al.*, 1965) the rabbit anterior mesenteric vein is spontaneously active, action potentials occurring either singly, in pairs or in bursts. A strong rhythmic contraction followed by relaxation was usually associated with a burst of action potentials, whereas when the action potentials occurred singly, at regular intervals, the vein strip showed a maintained tone which changed when the action potential frequency changed. Both types of preparation have been considered in this analysis. The action potential frequency was found by dividing the records into intervals of 20 sec. The value for any particular interval was taken as the mean value of the sum for that interval and those on either side.

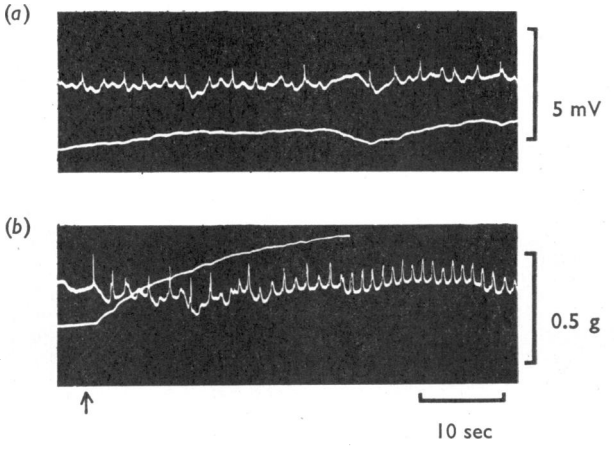

Fig. 1. Electrical activity (upper trace) and mechanical activity (lower trace) of a rabbit anterior mesenteric strip. (*a*) control record; (*b*) 0.2 μg of noradrenaline injected into the perfusing fluid at the arrow.

Fig. 1 shows part of a record obtained with noradrenaline. It shows the control activity, before the drug was given, and the activity recorded during drug application. A preliminary examination of the records obtained with noradrenaline showed there was an increased frequency of action potential discharge concomitant with the rise in tension caused by the drug. However, when the relation between action potential frequency and tension was plotted the correlation held only for the initial stage of the drug effect. The action potential frequency fell to the control value, or was even depressed, at a time when the tension remained elevated. The dissociation was more marked with the higher doses of noradrenaline (Fig. 2).

It might be argued that noradrenaline caused sufficient membrane depolarization to obliterate spike activity and that the sustained tension was associated with this depolarization. The doses of noradrenaline however caused little or no depolarization (1 to 2 mV) and furthermore the action potential discharge was not obliterated by depolarization with

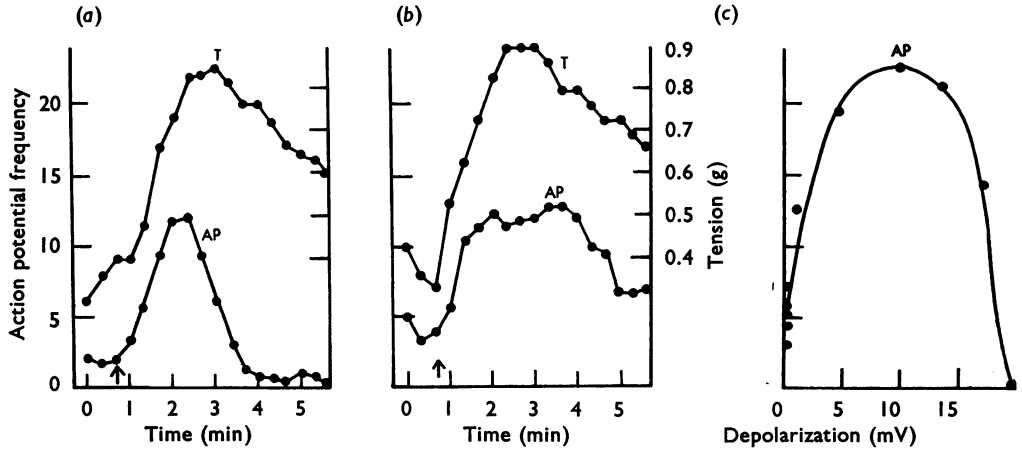

Fig. 2. (*a*) and (*b*). The relation between action potential frequency and tension in a rabbit anterior mesenteric vein. Ordinates: action potential frequency (expressed as number of action potentials occurring in 20 sec) and tension. At the arrows noradrenaline, in a volume of 0.05 ml., was injected into the perfusing fluid. In (*a*) 0.5 μg and in (*b*) 0.2 μg of noradrenaline was injected. Abscissa: minutes. In this and in subsequent figures the upper curve relates to tension and the lower curve to action potential frequency.

(*c*). The relation between action potential frequency and depolarization (in mV) caused by isotonic potassium sulphate in the same preparation as (*a*) and (*b*).

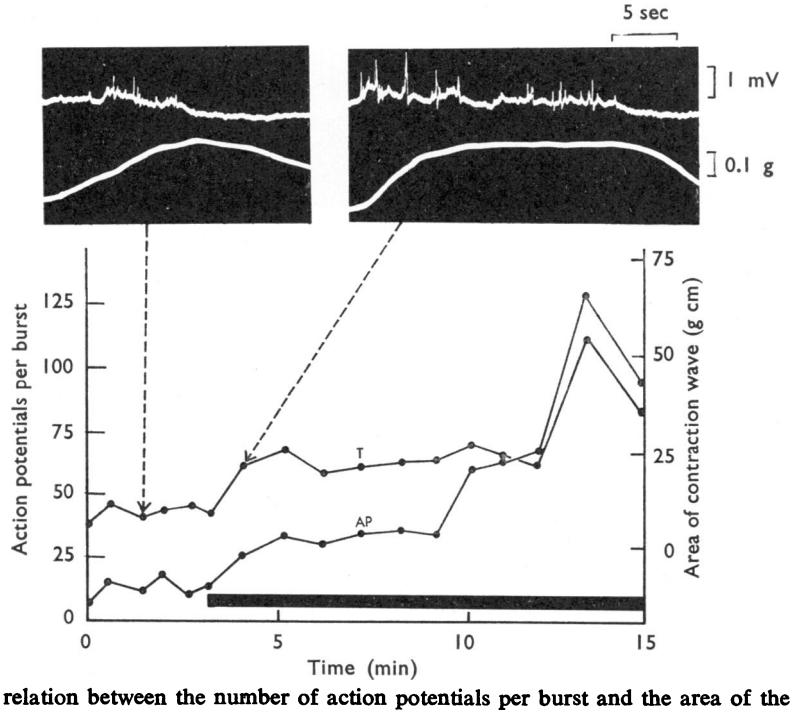

Fig. 3. The relation between the number of action potentials per burst and the area of the contraction wave (in g cm) before and during perfusion with Krebs solution containing three times the normal potassium concentration (14.1 mequiv). The potassium-rich solution was present during the time indicated by the bar. Sample recordings are shown at the top of the figure.

potassium-rich solutions. Fig. 2,*c* shows the depolarization produced by isotonic potassium sulphate solution in the same preparation as illustrated in Fig. 2,*a* and *b*. A substantial action potential discharge was still present when 10 to 15 mV of depolarization had occurred.

In the foregoing experiment, however, the potassium concentration was not static and the effect of exposure to constant concentrations of potassium ions was investigated in other preparations. Two types of condition were chosen, first a concentration of potassium causing 1 to 2 mV of depolarization, that is, similar in extent to that produced by noradrenaline, and second a concentration of potassium causing considerably more depolarization than produced by the drug. Fig. 3 illustrates the results obtained with a threefold (4.7 to 14.1 mequiv) increase in potassium concentration. This concentration produced a 2 mV depolarization. In this experiment the increase in activity caused by the excess potassium not only increased the amplitude of the contractions but also their duration. Consequently the area of the contraction waves (expressed as g cm) and the number of action potentials during each contraction wave have been plotted against time. It is seen that no dissociation of electrical and mechanical activity occurs as with noradrenaline. The correlation of action potential frequency with tension still held for a 5.4-fold (4.7 to 25.4 mequiv) increase in potassium concentration (Fig. 4). This concentration of potassium produced a 6 mV depolarization, far greater than that observed with noradrenaline. The increase in action potential amplitude seen with this concentration of potassium was probably due to a greater synchronization of the action potential discharge.

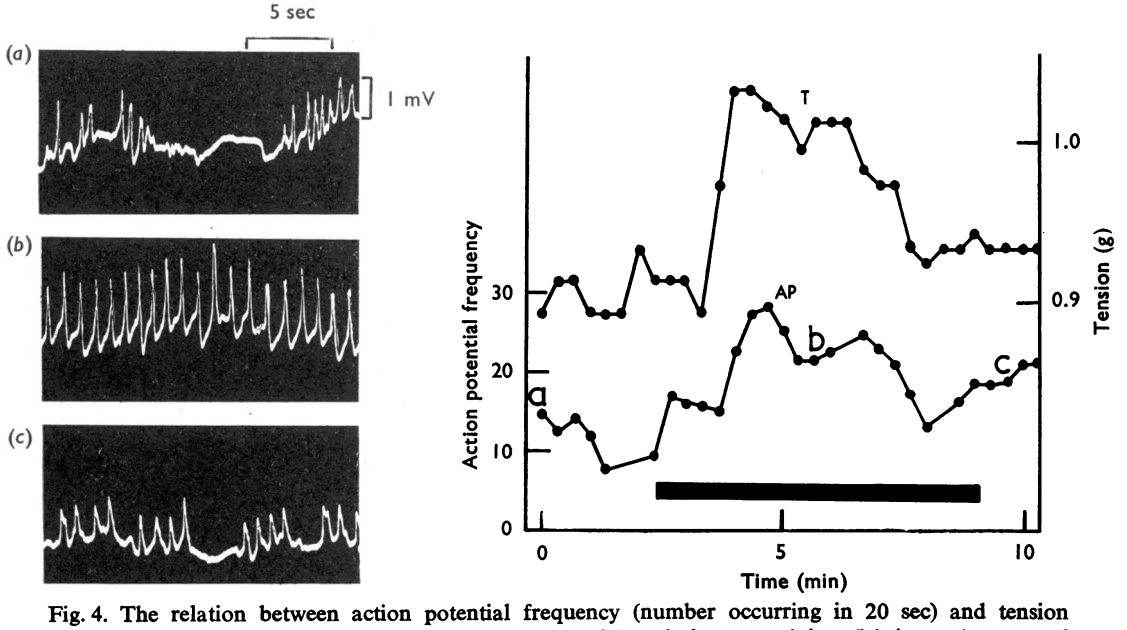

Fig. 4. The relation between action potential frequency (number occurring in 20 sec) and tension before and during perfusion with a potassium-rich solution containing 5.4-times the normal potassium concentration (25.4 mequiv). The potassium-rich solution was present during the time indicated by the bar. Sample records are shown on the left and the parts of the graph to which they refer are indicated by letters.

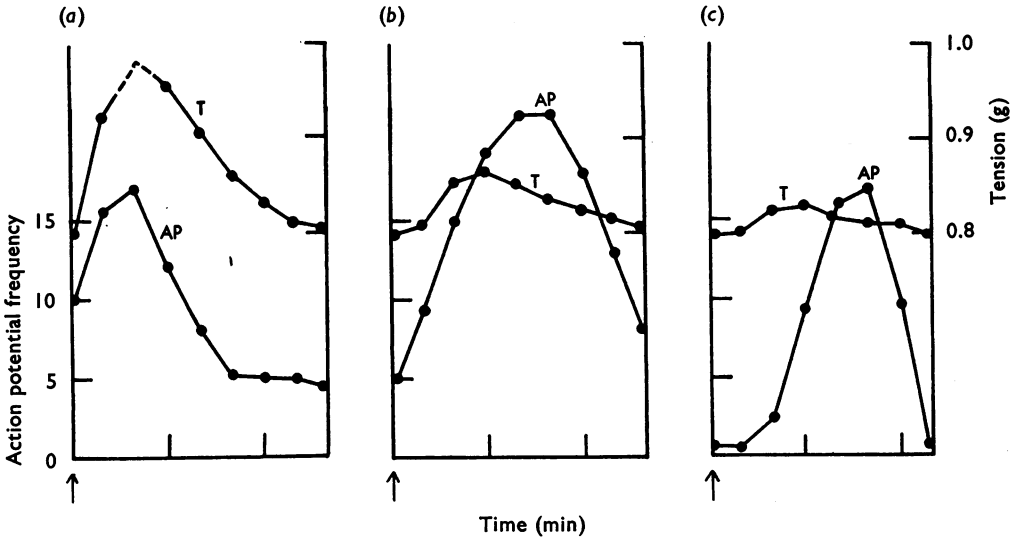

Fig. 5. The relation between action potential frequency (number of action potentials occurring in 20 sec) and tension in a vein. At the arrows 0.5 μg of noradrenaline in 0.05 ml. of calcium-free Krebs solution was injected into the fluid perfusing the vein. The perfusing solution was (*a*) Krebs solution, (*b*) calcium-free solution after 8 min, and (*c*) calcium-free solution after 13 min.

A second argument against our interpretation of the results with noradrenaline is that, with this method of recording, electrical activity is recorded from only a small region of tissue near the recording electrode, whereas the tension response is from the whole vein strip. Thus it is possible that other parts of the tissue showed electrical activity at a time when that part near the recording electrode was quiescent. The maintained tension in the absence of an action potential discharge was, however, a consistent feature of the noradrenaline response and it is unlikely that that part of the preparation close to the recording electrode was always quiescent during the later stages of drug action.

The relations between electrical and mechanical activity usually seen with noradrenaline could be altered by removing calcium from the perfusing medium (Fig. 5). Initially, in this preparation, noradrenaline produced its usual effect in which the action potential discharge was suppressed at a time when the tension remained elevated. After removing calcium the increase in the action potential discharge to the same dose of noradrenaline was greater and prolonged, whereas the tension response was reduced. Even after 13 min in calcium-free solution noradrenaline still caused a considerable membrane response but now the tension response was negligible.

Other drugs causing a contraction of vein strips were used to see if they evoked patterns of activity similar to that produced by noradrenaline. With histamine (10 μg/ml. applied for 60 sec) a response similar to that with noradrenaline was obtained, but the dissociation of the membrane and tension effects was less marked (Fig. 6). The effect was least marked with angiotensin (Fig. 7). In this instance the action potential discharge and the tension decayed in an almost parallel manner even though the drug was allowed to remain in contact with the tissue.

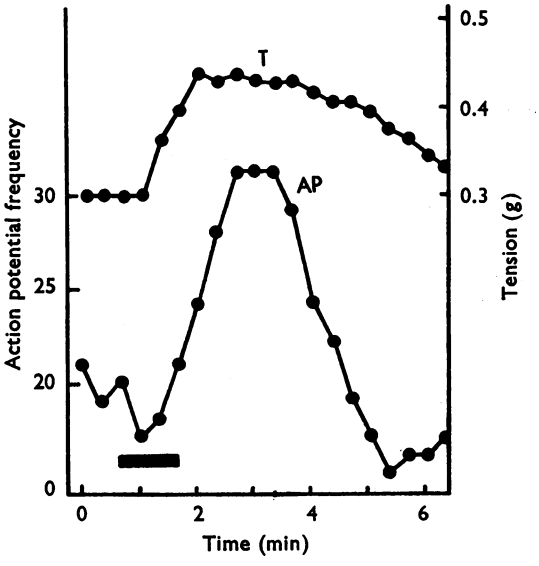

Fig. 6. The relation between action potential frequency (number occurring in 20 sec) and tension in a rabbit anterior mesenteric vein strip. Histamine (10 μg/ml.) was present for the time indicated by the bar.

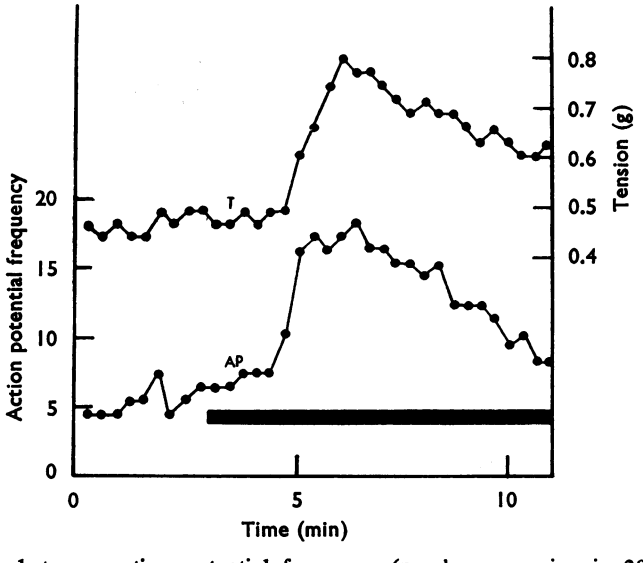

Fig. 7. The relation between action potential frequency (number occurring in 20 sec) and tension during exposure to angiotensin (1 μg/ml.). The drug was in contact with the tissue during the period shown by the bar.

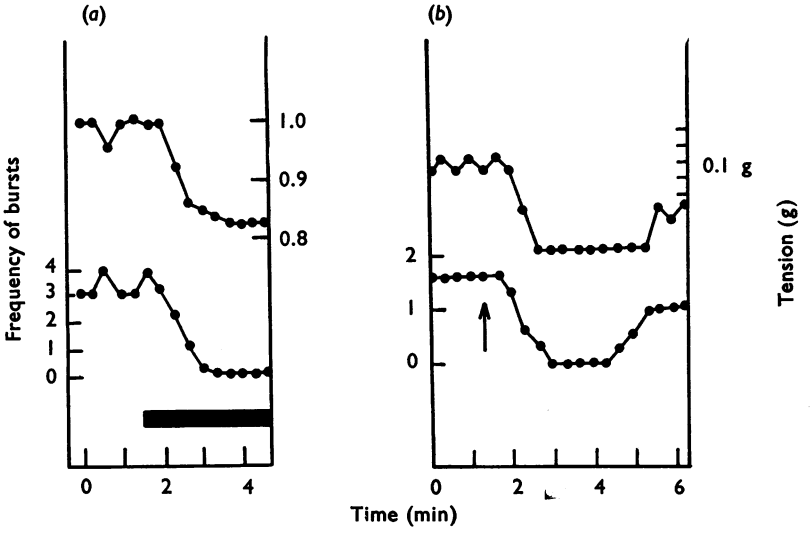

Fig. 8. (*a*) The relation between the frequency of action potential bursts (number occurring in 20 sec) and tension before and during exposure of theiphylline (1 mg/ml.). 8he drug was present during the time shown by the bar.

(*b*) The relation between the frequency of action potential bursts (number occurring in 20 sec) and tension and the effects of isoprenaline. At the arrow isoprenaline (1 μg), in a volume of 0.1 ml., was injected into the perfusing fluid.

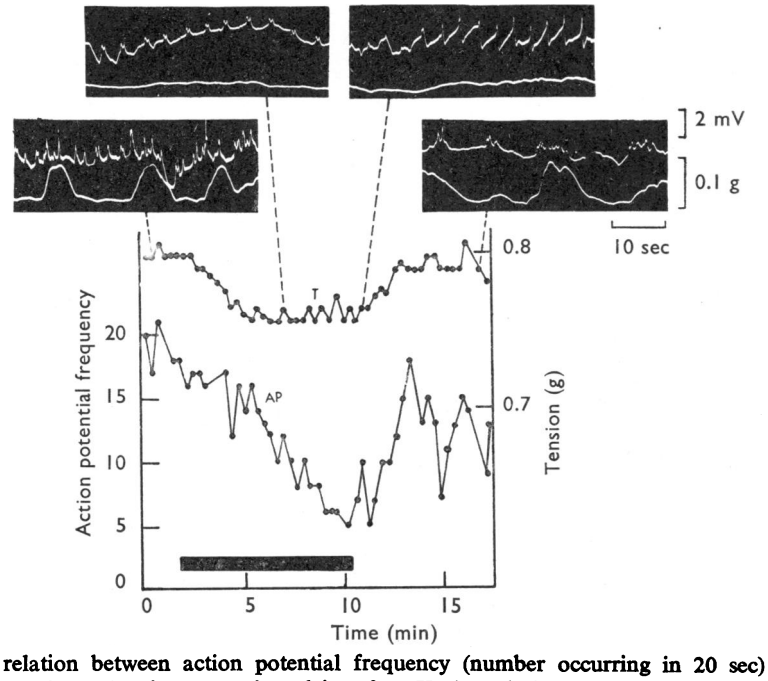

Fig. 9. The relation between action potential frequency (number occurring in 20 sec) and tension in a vein strip. The tissue was in calcium-free Krebs solution for the period indicated by the bar. Sample electrical and mechanical records are shown above.

A few experiments were performed in which isolated vein strips were suspended in " depolarizing Ringer solution " in an organ-bath. In this solution the sodium chloride and sodium bicarbonate were replaced by potassium sulphate and potassium bicarbonate respectively. Under these conditions an action potential discharge was impossible. The responses to both noradrenaline and angiotensin were reduced compared with those obtained in Krebs solution ; however, those to angiotensin were proportionately smaller, especially with successive doses. Thus some response to angiotensin could still be obtained under conditions where membrane excitation was ruled out. However, this drug appeared to depend more on membrane excitation to produce its effect than did noradrenaline. This finding is consistent with the earlier results reported in this paper.

In the case of drugs causing a relaxation of spontaneously active anterior mesenteric vein strips the correlation between action potential frequency and tension was good. Such was the case with theophylline and isoprenaline as illustrated in Fig. 8. The effects of these two drugs were reversible and the duration of action depended on the drug concentration and the length of exposure to the drug. The results with theophylline and isoprenaline are to be contrasted with those of calcium removal (Fig. 9). This procedure reduced, and finally abolished, both the action potential discharge and the tension responses. However the onset of the decrease in spike activity lagged behind the decrease in tension. Thus for a period of 5 to 6 min these two activities were uncoupled, that is the tension did not reflect the activity of the membrane.

DISCUSSION

The most unexpected finding in this work is the absence of spike activity while the tension remained elevated after the application of noradrenaline to vein strips. Objections to the validity of this finding have been dealt with in Results. Other instances, albeit under very different conditions, are known where a mechanical response to drugs is unaccompanied by membrane potential changes. Evans, Schild & Thesleff (1958) found that various smooth muscles responded to drugs in potassium-rich solutions in which the membrane was completely depolarized. Their work was extended, for example by Edman & Schild (1962), who concluded that in potassium-rich solutions excitant drugs caused both an increased calcium influx into the cell and the liberation of calcium from some bound form from a superficial part of the cell, probably the cell membrane. There is no reason to suppose that drugs affect calcium movement differently in normal physiological solutions.

Results presented here have shown that calcium is necessary for tension development triggered by action potential discharges occurring spontaneously or induced by drugs. It is suggested that, in the rabbit anterior mesenteric vein, noradrenaline liberates calcium from some cellular binding site, so causing tension development without necessarily exciting the cell membrane. In this connexion it is noteworthy that Bohr (1963) has divided the contraction produced by noradrenaline in artery strips into a fast and slow component. The fast component he ascribes to membrane excitation whereas the second slow component he attributes to " the availability of calcium for the coupling process." The slow process might correspond to the phase of drug action during which the membrane was inactive, as reported by us.

The increased response of the cell membrane to noradrenaline after the removal of calcium can be attributed to increased excitability of the cell membrane, as was found in other smooth muscles (Holman, 1958). Also the reduced tension response to noradrenaline in these circumstances is attributable to a failure of the coupling mechanism (Robertson, 1960), as is the rapid failure of the tension response during calcium deprivation (Axelsson, 1961).

If our hypothesis concerning the action of noradrenaline is correct then it must be concluded that histamine is less effective and angiotensin is almost ineffective in releasing calcium from the cell membrane. An increase in potassium concentration was found to affect membrane activity and tension in a parallel manner which would argue against potassium being able to release membrane calcium. Results by others have been interpreted in ways similar to the views expressed in this paper. Hinke, Wilson & Burnham (1964) found that removal of calcium abolished the response of arterial smooth muscle to noradrenaline and potassium. On adding a small amount of calcium they found that the response to noradrenaline was almost maximal, whereas the response to potassium depended on the external calcium concentration. They suggested that potassium was only able to increase the calcium influx, whereas noradrenaline could displace bound calcium from some site which was fully repleted on exposure to low calcium concentrations. Hinke and Wilson (1962) found that the response of arterial smooth muscle to angiotensin depended more on the external sodium concentration than did the response to noradrenaline. This again suggests that the former drug depends upon membrane excitation for its effects, whereas noradrenaline can cause a contraction without membrane depolarization.

The proposed hypothesis may, we feel, explain why some workers have failed to record action potentials in vascular smooth muscle in response to drugs or nerve stimulation. First the dissociation of the action potential discharge and tension may be more marked in other tissues, even to the complete absence of electrical changes Secondly, high concentrations of drug or prolonged exposure to drugs would reduce the chance of seeing the electrical discharge. In our experiments noradrenaline was injected, in a small volume, into the perfusing fluid, so that the drug contact time was very short, 1 to 2 sec assuming no dilution occurred. This, together with the tissue used, may have provided optimal conditions for recording membrane responses to the application of drugs.

SUMMARY

1. Simultaneous records of electrical and mechanical activity have been obtained from longitudinal strips of rabbit anterior mesenteric vein.

2. In general, excitant drugs increased the action potential frequency at the same time as the tension increased. Relaxant drugs produced the converse effect.

3. After noradrenaline the action potential discharge subsided or disappeared at a time when the tension remained elevated. This effect was less obvious with histamine, and almost absent with angiotensin. Action potential frequency and tension were correlated during exposure to solutions containing up to five times the normal potassium concentration.

4. It is suggested that noradrenaline causes contractions in this vein by directly liberating bound calcium, possibly from the cell membrane, as well as by the more usual processes associated with an action potential discharge. This suggestion is believed to account for the dissociation of the electrical and mechanical events prominent during the later stages of noradrenaline action.

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