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SOME EFFECTS OF SODIUM NITROPRUSSIDE, METHOXYVERAPAMIL (D600) AND NIFEDIPINE ON RAT PORTAL VEIN

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1 The effects of methoxyverapamil (D600), nifedipine and sodium nitroprusside on noradrenaline-induced electrical and mechanical activity in rat portal vein have been examined.

2 D600 and nifedipine produced a concentration-dependent reduction in mechanical responses to noradrenaline whilst sodium nitroprusside had no effect. The effects of D600 and nifedipine were reversed by increasing the extracellular calcium concentration.

3 The mechano-inhibitory effects of D600 were accompanied by a marked reduction in electrical activity with some evidence of electromechanical uncoupling.

4 The mechano-inhibitory effects of nifedipine were associated with considerable electromechanical uncoupling.

5 It is concluded that in the concentrations used, D600 acts primarily by inhibiting calcium influx with some effect on electromechanical coupling whilst nifedipine interferes mainly with the coupling process. The inactivity of sodium nitroprusside suggests that the excitation-contraction coupling process in rat portal vein is relatively simple and further studies with this tissue seem indicated.

Introduction

The ability of verapamil and methoxyverapamil (D600) to suppress the mechanical activity of cardiac muscle without affecting the cardiac action potential has been described by Fleckenstein, Tritthart, Fleckenstein, Herbst & Grün (1969). These workers showed that the effects of verapamil and D600 could be reversed by increasing the extracellular calcium concentration and these drugs were thus described as *calcium* antagonists. Later work showed that their action was associated with an ability to reduce the inward calcium current which accompanied the cardiac action potential (Kohlhardt, Bauer, Krause & Fleckenstein, 1972). More recently, the effects of a further compound, nifedipine, have been described (Fleckenstein, Grün, Byon, Döring & Tritthart, 1975). In cardiac muscle, nifedipine also inhibits the inward calcium current and has been similarly classified as a calcium antagonist (Kohlhardt & Fleckenstein, 1977).

In smooth muscle containing tissues such as the gastric antrum and portal vein of the guinea-pig, verapamil, D600 and nifedipine partially inhibit agonist-induced mechanical activity with a reduction in electrical spike discharge (Golenhofen, 1979). However, in other tissues like rabbit aorta, the so-called calcium antagonists are relatively ineffective and drugs like sodium nitroprusside are potent inhibitors of tension development (Kreye, Baron, Luth & Schmidt-Gayk, 1975; Golenhofen, 1979).

A problem in the understanding of the mode of action of D600, nifedipine and sodium nitroprusside is that the mechanisms involved in the excitation-contraction coupling processes in the various types of smooth muscle are not fully understood. However, in rat portal vein, noradrenaline-induced mechanical changes seem to be associated with the influx of calcium ions. These seem to trigger the release of calcium from internal stores for the production of the mechanical response (Sigurdsson, Uvelius & Johansson, 1975; Weston, 1978).

In the present experiments the effects of D600, nifedipine and sodium nitroprusside on electrical and mechanical activity in rat portal vein have been examined. By use of this tissue it was hoped that some information could be obtained about their mode of action on excitation-contraction coupling processes in smooth muscle.

A preliminary account of some of these results has been given (Jetley & Weston, 1976).

Methods

Male Sprague–Dawley rats (250 to 350 g) were killed by stunning and bleeding and an approx 2 cm length of the portal-mesenteric vein was removed.

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Tissue bath experiments

Portal veins were set up in thermostatically controlled test baths at 37°C and in identical control baths. A bicarbonate-buffered physiological salt solution (PSS) was used. Tension changes in each vein were recorded isometrically at a resting tension of 0.5 g. The effects of increasing concentrations of noradrenaline were examined on both test and control veins after which the test tissues alone were exposed to either D600, nifedipine or sodium nitroprusside for 30 min. Responses to noradrenaline were then re-examined on both groups of tissues. The experiment was then repeated, exposing the test preparations to increasing concentrations of D600, nifedipine or sodium nitroprusside.

When the effects of increasing the extracellular calcium concentration were examined, the experiments were conducted as described but using a PSS containing 3-(N-morpholino)-propanesulphonic acid (MOPS) as buffer (MOPS-PSS) and containing either 2.5, 8, 25 or 80 mmol/l calcium chloride.

Electrophysiological experiments

Intracellular recording The method used has been described by Small & Weston (1977; 1979). Noradrenaline was injected into the flow of PSS perfusing the recording bath to achieve the desired concentration. Antagonists were added to the reservoirs of PSS 30 min before re-exposure to noradrenaline in the continuing presence of the antagonist.

Extracellular recording The perfused capillary method of Golenhofen & v. Loh (1970) was used in conjunction with a Grass Polygraph. The time constant of the preamplifier used to record the extracellular electrical changes was 0.03 ms. Addition of noradrenaline to a calibrated reservoir containing the PSS perfusing the tissue was used to achieve the desired concentration. Antagonists were also added to the reservoirs and a 30 min equilibration period allowed before responses to noradrenaline were re-examined. The electrical and mechanical signals were each integrated to provide a quantitative estimate of any changes. The effects of noradrenaline were measured by subtracting the integrated values obtained in the 2 min before exposure to noradrenaline from those values obtained during the first 2 min of exposure to noradrenaline.

Drugs and solutions

The following drugs were used: D600 (Knoll); MOPS (3-(N-morpholino)-propanesulphonic acid, Calbiochem); nifedipine (BAY a1040, Bayer); noradrenaline hydrochloride (BDH); phentolamine hydrochloride (Ciba); sodium nitroprusside (BDH). Experiments with nifedipine were illuminated solely with a sodium lamp.

The bicarbonate-PSS had the following composition (mmol/l): NaCl 117.9, KCl 4.74, $CaCl_2$ 2.54, $MgCl_2$ 1.19, $NaHCO_3$ 25 and glucose 11.1. It was bubbled with 95% O_2 and 5% CO_2.

The MOPS-PSS had the following composition (mmol/l): NaCl 129.7, KCl 5.9, $CaCl_2$ 2.54, $MgCl_2$ 1.19, MOPS 10 and glucose 11.1. The pH of the solution was adjusted to 7.4 with NaOH and it was isosmotic with the bicarbonate-PSS. The calcium concentration of this solution was increased by adding $CaCl_2$ to give a final solution containing 8, 25 or 80 mmol/l Ca^{2+}.

Results

Tissue bath experiments

In bicarbonate-PSS, both D600 (0.01 to 1 μmol/l) and nifedipine (0.001 to 0.1 μmol/l) produced a concentration-dependent reduction in the responses to noradrenaline whilst sodium nitroprusside (0.1 to 10 μmol/l) had no effect (Figure 1). Identical results were obtained with MOPS-PSS. In the concurrent control experiments there were no significant changes in the responses to noradrenaline.

When the calcium concentration was increased from 2.5 mmol/l (normal) to 8, 25 and 80 mmol/l the ability of D600 and nifedipine to increase the noradrenaline EC_{50} and to reduce the noradrenaline maximum response was partially overcome. An analysis of variance carried out on the data obtained from the 2.5, 8 and 25 mmol/l Ca^{2+} experiments showed that these effects of calcium were significant (noradrenaline EC_{50}: D600, $P = 0.03$; noradrenaline maximum: D600, $P = 0.01$; noradrenaline EC_{50}: nifedipine, $P = 0.01$; noradrenaline maximum: nifedipine, $P = 0.008$). The data obtained from the 80 mmol/l Ca^{2+} experiments were excluded from the analysis because the control responses to noradrenaline were significantly reduced probably due to osmotic effects. However, in the presence of 80 mmol/l Ca^{2+}, a significant reduction of responses to noradrenaline was only seen after exposure to the highest concentrations of either D600 or nifedipine. To illustrate some of the results obtained from these experiments, the interaction between D600 and calcium on the noradrenaline maximum response is shown in Figure 2. Very similar results were obtained in the nifedipine:calcium experiments.

Electrophysiological experiments

Extracellular recordings The effects of D600, nifedipine and sodium nitroprusside on the electrical and

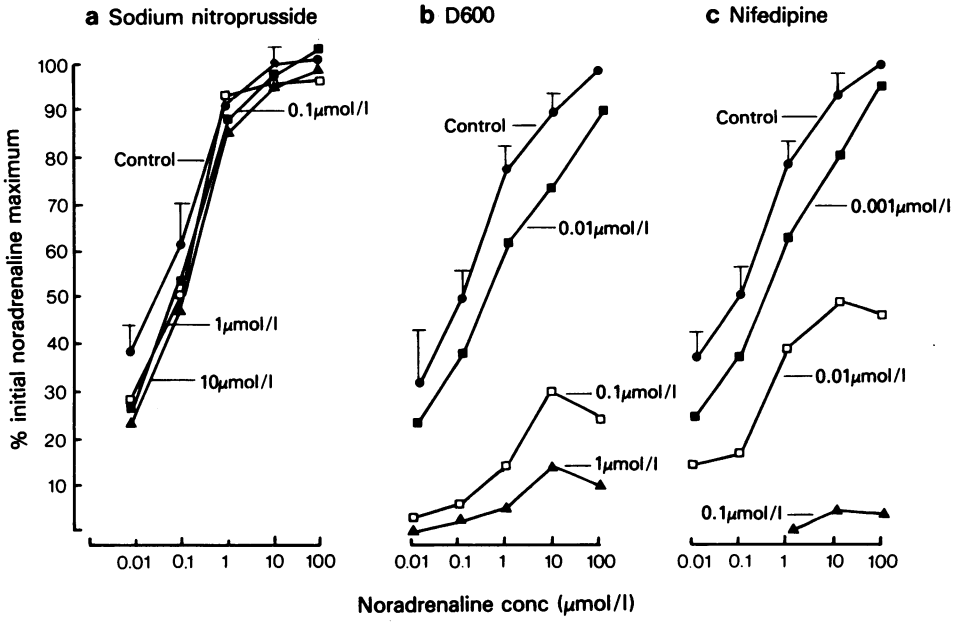

Figure 1 Effects of (a) sodium nitroprusside, (b) D600, (c) nifedipine on mechanical responses to noradrenaline in rat portal vein in bicarbonate-PSS: (●) control responses; (■), (□), (▲) responses in the presence of increasing concentrations of antagonist as indicated. Ordinate scale, % of the initial noradrenaline maximum response, means of six experiments; vertical lines show s.e. mean.

mechanical responses produced by noradrenaline 1 μmol/l (≙EC_{80}) were examined. Sodium nitroprusside (0.1 to 10 μmol/l) had no effect (Figure 3). Both D600 (0.01 to 1 μmol/l) and nifedipine (0.001 to 0.1 μmol/l) produced a reduction in electrical and mechanical activity (Figures 4 and 5) but when the integrated responses were examined, neither agent produced a reduction in electrical activity comparable with that in mechanical activity (Figure 6).

These results suggested that D600 and nifedipine

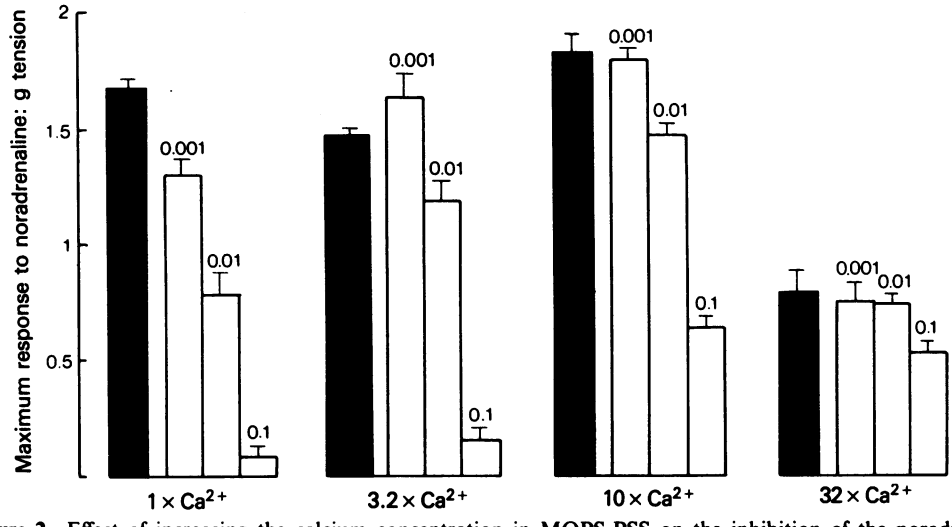

Figure 2 Effect of increasing the calcium concentration in MOPS-PSS on the inhibition of the noradrenaline maximum response by nifedipine in rat portal vein. Solid columns: noradrenaline maximum responses; open columns: noradrenaline maximum responses in the presence of increasing concentrations of nifedipine (μmol/l) as indicated. All responses are means of six experiments and are expressed in absolute units of tension; vertical lines show s.e. mean. The interaction between D600 and calcium was similar.

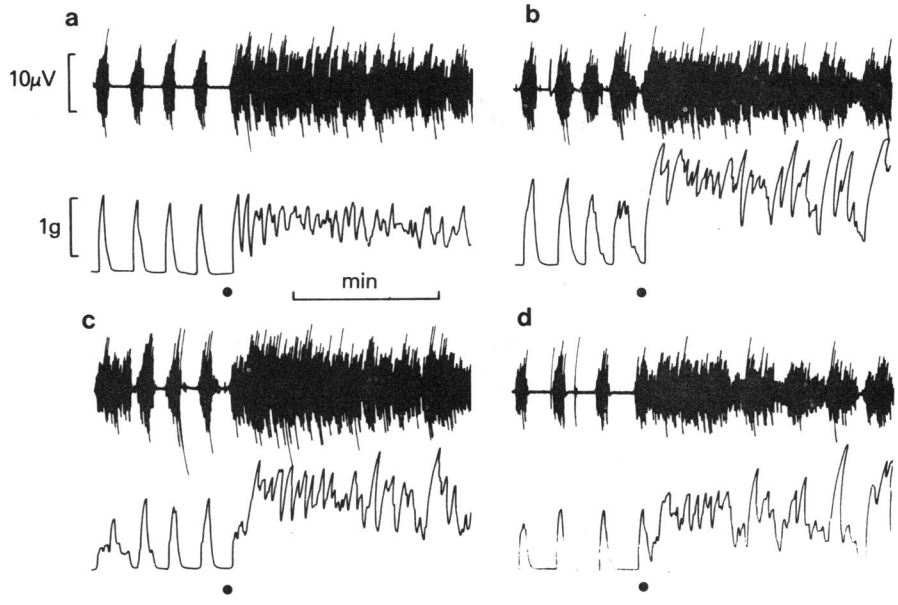

Figure 3 Effect of sodium nitroprusside on extracellular electrical activity (upper traces) and mechanical activity (lower traces) in a single experiment in rat portal vein. Responses to noradrenaline 1 μmol/l (at dots) are shown in (a) control and after 30 min exposure to sodium nitroprusside (b) 0.1 μmol/l, (c) 1 μmol/l, and (d) 10 μmol/l.

could have acted in part as electro-mechanical uncoupling agents. However, since only a fraction of the electrical changes was recorded using the capillary, it could not be assumed that a reduction in mechanical activity would necessarily be accompanied by a comparable reduction in recorded electrical activity. To test this possibility, the effects of noradrenaline were examined in the presence of increasing concentrations

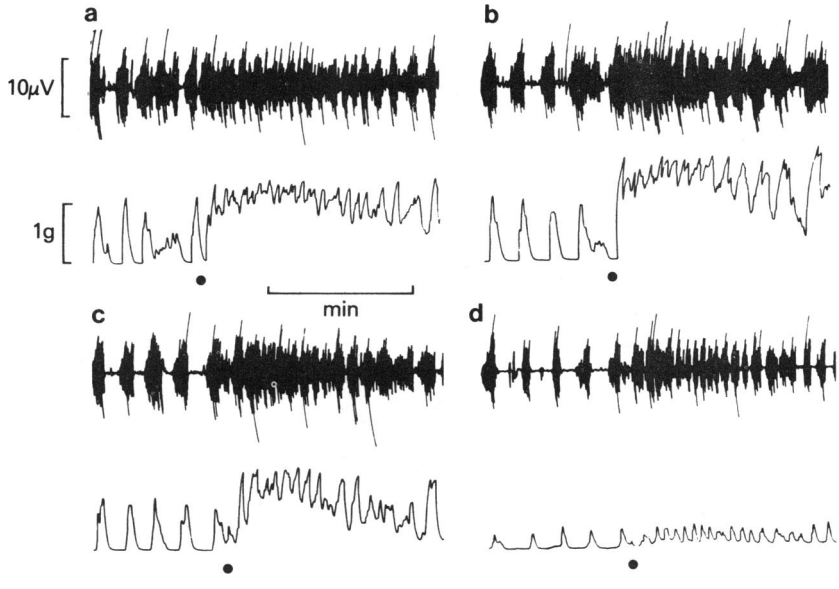

Figure 4 Effect of D600 on extracellular electrical activity (upper traces) and mechanical activity (lower traces) in a single experiment in rat portal vein. Responses to noradrenaline 1 μmol/l (at dots) are shown in (a) control and after 30 min exposure to D600 (b) 0.01 μmol/l, (c) 0.1 μmol/l and (d) 1 μmol/l.

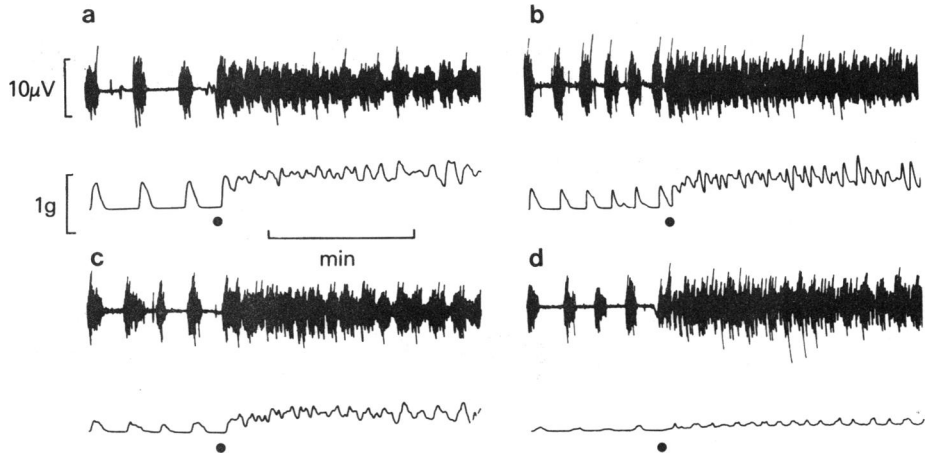

Figure 5 Effect of nifedipine on extracellular electrical activity (upper traces) and mechanical activity (lower traces) in a single experiment in rat portal vein. Responses to noradrenaline 1 μmol/l (at dots) are shown in (a) control and after 30 min exposure to nifedipine (b) 0.001 μmol/l, (c) 0.01 μmol/l and (d) 0.1 μmol/l.

of phentolamine, which was assumed to act by α-adrenoceptor blockade and to involve no electromechanical uncoupling component. The results of these experiments showed that there was a parallel reduction in both electrical and mechanical activity as the concentration of phentolamine was increased (Figure 7).

When the levels of integrated electrical activity produced by noradrenaline in the presence of either D600 or nifedipine were compared with those measured in the presence of phentolamine at similar levels of mechanical activity (Figure 8), significant differences were found (*t* test; D600, 1 μmol/l, $P = 0.01$; nifedipine, 0.01 and 0.1 μmol/l, $P < 0.001$ and $P < 0.001$ respectively).

Intracellular recordings The effects of D600 (μmol/l) and nifedipine (0.1 μmol/l) on responses to noradrenaline were investigated since, at these concentrations, the greatest degree of apparent electromechanical uncoupling had been measured in the extracellular recordings.

Noradrenaline (1μmol/l) alone produced a depolarization and marked increase in spike discharges.

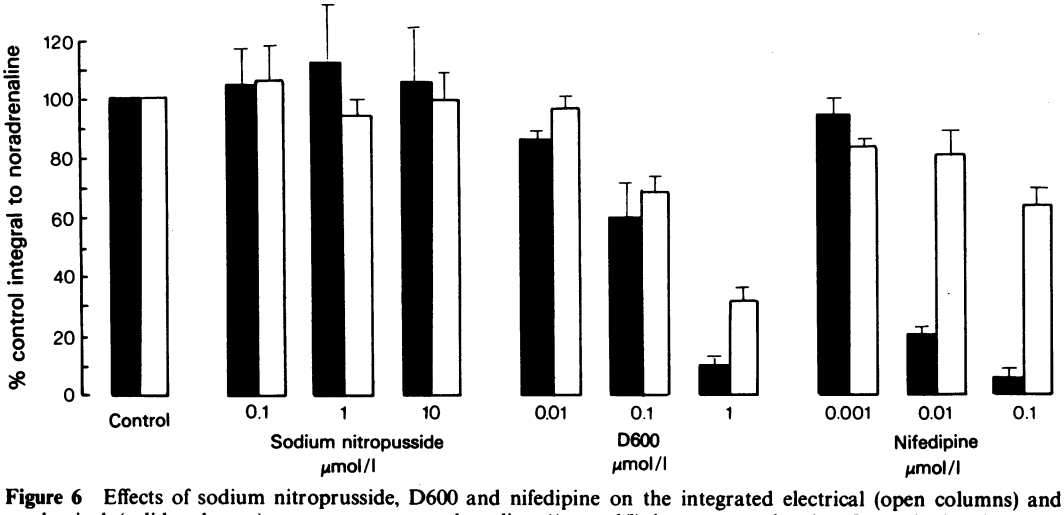

Figure 6 Effects of sodium nitroprusside, D600 and nifedipine on the integrated electrical (open columns) and mechanical (solid columns) responses to noradrenaline (1 μmol/l) in rat portal vein. Control electrical and mechanical responses to noradrenaline (1 μmol/l) are each expressed arbitrarily as 100%. All responses shown are means of six experiments; vertical lines show s.e. mean.

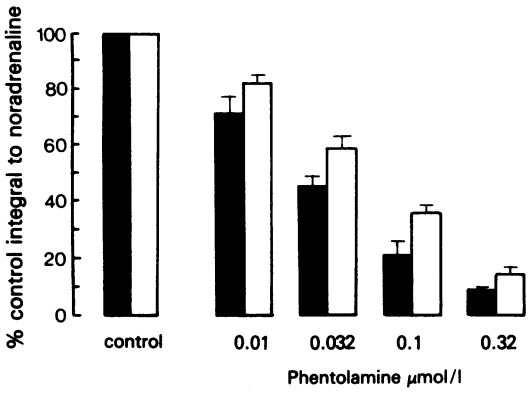

Figure 7 Effect of increasing concentrations of phentolamine on the integrated electrical (open columns) and mechanical (solid columns) responses to noradrenaline (1 μmol/l) in rat portal vein. Control electrical and mechanical responses are each arbitrarily expressed as 100%. All responses shown are means of six experiments; vertical lines show s.e. mean.

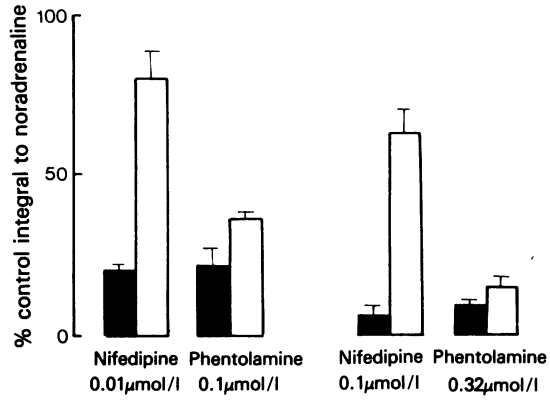

Figure 8 Comparison of the effects of approximately equal mechano-inhibitory concentrations of nifedipine (0.01 and 0.1 μmol/l) and phentolamine (0.1 and 0.32 μmol/l). The histograms show the integrated electrical (open columns) and mechanical (solid columns) responses to noradrenaline (1 μmol/l) in the presence of the concentrations of nifedipine and phentolamine indicated. Data taken from Figures 6 and 7. All responses shown are means of six experiments; vertical lines show s.e. mean.

Exposure to D600 (1 μmol/l, $n = 5$) had no effect on the resting membrane potential. However, within a few minutes, spontaneous spike discharges and mechanical activity were virtually abolished and, in the presence of D600, noradrenaline produced an essentially spike-free depolarization with a small rise in tension (Figure 9).

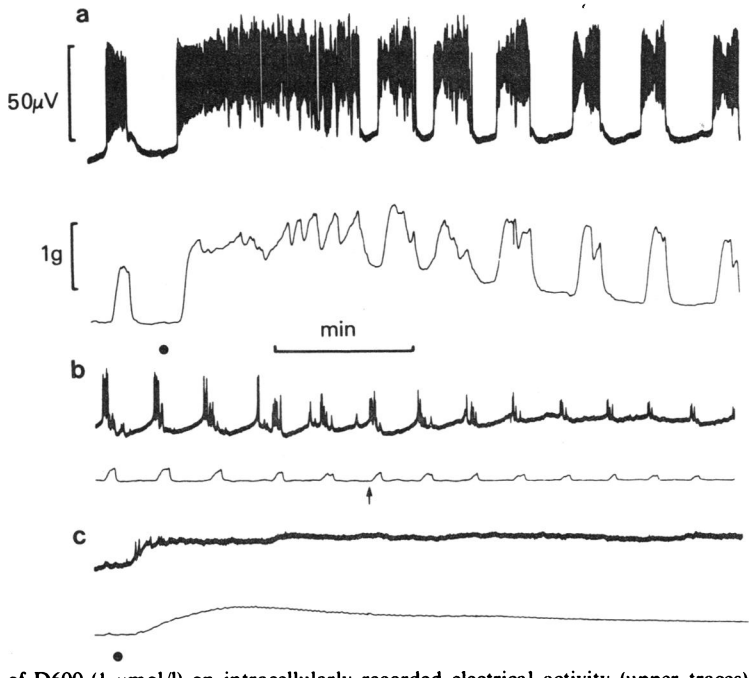

Figure 9 Effect of D600 (1 μmol/l) on intracellularly recorded electrical activity (upper traces) and mechanical activity (lower traces) in rat portal vein. (a) Control response to noradrenaline 1 μmol/l (at dots); (b) effect of D600 1 μmol/l on spontaneous spike discharges, arrow marks the 5 min exposure point; (c) response to noradrenaline 1 μmol/l after 30 min exposure to D600 1 μmol/l. Continuous intracellular recordings from the same cell.

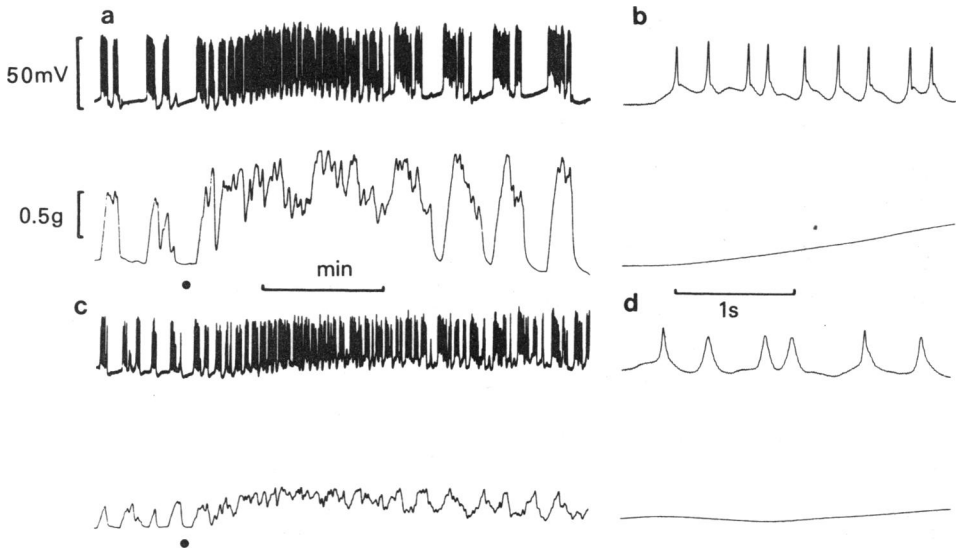

Figure 10 Effect of nifedipine 0.1 μmol/l on intracellularly recorded electrical activity (upper traces) and mechanical activity (lower traces) in rat portal vein. (a) Control responses to noradrenaline 1 μmol/l (at dots); (b) spike shape in a multispike complex immediately before exposure to noradrenaline in (a); (c) response to noradrenaline 1 μmol/l after 30 min exposure to nifedipine 0.1 μmol/l; (d) spike shape immediately before noradrenaline exposure in (c). Continuous intracellular recordings from the same cell.

Exposure to nifedipine (0.1 μmol/l, $n = 5$) had no effect on membrane potential and spike discharges were not abolished. Instead, the size of spikes and their rates of rise and fall were reduced. In the presence of nifedipine, noradrenaline produced a depolarization together with an increase in the frequency of the modified spikes and a reduced mechanical response (Figure 10).

Discussion

In vascular smooth muscle current experimental evidence suggests that contraction results from an increase in the intracellular calcium concentration although in most tissues details of the excitation-contraction (E-C) coupling process have not yet been determined (Kuriyama, Ito & Suzuki, 1977; Weiss, 1977).

In rat portal vein a noradrenaline-induced contraction is accompanied by an increase in the lanthanum-resistant calcium fraction (Weston, 1978). Although this may only be one of several such intracellular fractions (Freeman & Daniel, 1973), its measurement provides an estimate of the transmembrane calcium flux associated with the excitatory action of agonists like noradrenaline (Godfraind, 1976). The effect of the calcium ions entering the cells during excitation is probably enhanced by calcium release from stores within the tissue (Sigurdsson *et al.*, 1975). Thus there seem to be close parallels between E-C coupling processes in vascular and intestinal smooth muscle (Bolton & Bülbring, 1978).

The present experiments were conducted against a background of information which suggested that the mechanism of action of D600 and nifedipine was similar. In cardiac muscle the major action of both drugs seems to be interference with the slow calcium channel (Kohlhardt *et al.*, 1972; Bayer, Rodenkirchen, Kaufmann, Lee & Hennekes, 1977; Kohlhardt & Fleckenstein, 1977). In smooth muscle, both D600 and nifedipine inhibit calcium-induced contractions in potassium-depolarized rabbit blood vessels (Schümann, Görlitz & Wagner, 1975). In addition there are apparent similarities in the ability of the two drugs to inhibit a variety of agonist-induced contractions in many types of smooth muscle (Golenhofen, 1979).

However, in rat portal vein, D600 greatly reduced the noradrenaline-stimulated increase in the lanthanum-resistant calcium fraction, whereas nifedipine had little effect (Weston, 1978). The electrophysiological data obtained in the present study strongly support the view that, in the concentrations employed, the primary action of D600 is inhibition of calcium influx, whilst nifedipine interferes with the process of E-C coupling. The observation that increasing the extracellular calcium concentration could reverse to some extent the inhibitory action of D600 and nifedipine suggests that the coupling process as well as the

electrical activity of the tissue are dependent on extracellular calcium.

Concentrations of nifedipine greater than 10^{-7} mol/l produce a marked reduction of spike activity in rat portal vein (Weston, unpublished observations). It is clear therefore that the uncoupling action of nifedipine occurs over a fairly narrow concentration range in this tissue.

D600 and nifedipine produced a complete inhibition of noradrenaline-induced mechanical changes in the portal vein whereas in aorta (Schümann, Görlitz & Wagner, 1975; Golenhofen & Weston, 1976) and mesenteric artery (Schümann *et al.*, 1975) only a partial inhibition of responses to noradrenaline is observed. Such differences are probably a reflection of the relative importance of extracellular calcium in the changes mediated by noradrenaline in these tissues. In larger arteries at least, internal calcium stores which are relatively independent of extracellular calcium are of great importance (Bohr, 1973).

The inability of sodium nitroprusside to modify responses to noradrenaline in rat portal vein is of some interest. In most vascular tissues, sodium nitroprusside produces a partial inhibition of noreadrenaline-induced contractions and often the residual mechanical component can be inhibited with D600 or nifedipine (Kreye *et al.*, 1975; Golenhofen & Weston, 1976; Golenhofen, 1979). The drug does not prevent calcium influx into rabbit mesenteric artery or vein (Zsotér, Henein & Wolchinsky, 1977), findings which are consistent with the results of the present study.

For some time, the apparent association between calcium and the characteristic inhibitory actions of D600, nifedipine and sodium nitroprusside has been used to distinguish between different E-C coupling mechanisms in a variety of smooth muscles (Golenhofen, 1976; 1979). As improvements are made in techniques for measuring calcium fluxes and electrical changes, the basis for such a differentiation will be better understood. At present, it seems important to select for further study a tissue with a simple E-C coupling mechanism. The results of the current experiments suggest that the rat portal vein is such a tissue and its future use may simplify the understanding of these complex processes in smooth muscle.

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