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Some effects of D600, nifedipine and sodium nitroprusside on electrical and mechanical activity in rat portal vein

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In rat portal vein it has been proposed that contraction is associated with the release of superficially-bound Ca^{2+} and that this release is triggered by extracellular Ca^{2+} (Sigurdsson, Uvelius & Johansson, 1975). In the present experiments the effects of the so called calcium antagonist D600 [methoxyverapamil; 5-methyl-4-cyan-4-(3,4,5-trimethoxyphenyl)1-*N*-methyl-*N*-β-3,4-dimethoxy-phenylethyl)-aminohexane hydrochloride; Knoll], nifedipine [4-(2'-nitrophenyl)-2,-6-dimethyl-3,5-dicarbomethoxy-1,4-dihydropyridine; BAY a 1040, Bayer] and sodium nitroprusside on the electrical and mechanical activity of rat portal vein have been examined.

In normal physiological salt solution (PSS, containing 25 mM bicarbonate buffer, bubbled with 95%O_2/5%CO_2) both D600 (0.01–1 μM) and nifedipine (0.001–0.1 μM) shifted the noradrenaline dose-response curve to the right with a reduction in the maximum response. Sodium nitroprusside (0.1–10 μM) had no significant effect. Using a modified PSS (containing 10 mM MOPS [3-(*N*-morpholino) propanesulphonic acid; Calbiochem] buffer, bubbled with 100% O_2), the inhibitory effects of both D600 and nifedipine were antagonized by increasing the calcium concentration in the PSS (up to 80 mM).

The effects of the calcium antagonists on the mechanical and extracellularly-recorded electrical activity evoked by noradrenaline (1 μM; approximately an ED_{80}) were studied using a perfused capillary similar to that described by Golenhofen & v. Loh (1970). A Grass polygraph was used and the electrical and mechanical records were mathematically integrated to provide a quantitative measurement of drug responses. Sodium nitroprusside (0.1–10 μM) had no significant effect. D600 (0.01–1 μM) and nifedipine (0.001–0.1 μM) both reduced the mechanical activity evoked by noradrenaline (1 μM) to the same extent as observed in the tissue bath experiments. However, neither agent produced a reduction in electrical activity comparable with this reduction in mechanical activity. The degree of this electro-mechanical uncoupling was greater in the presence of nifedipine than in the presence of D600. When the electrical and mechanical responses produced by noradrenaline (1 μM) were examined in the presence of phentolamine (0.01–0.32 μM), both were similarly reduced. Higher concentrations of D600 (10 μM) and of nifedipine (1 μM) produced greater inhibition of spontaneous and noradrenaline-evoked electrical activity.

These results suggest that low-moderate concentrations of D600 and nifedipine prevent extracellular Ca^{2+} from triggering the release of Ca^{2+} from superficially-bound calcium stores. Higher concentrations, which reduce electrical activity to a greater extent are also able to antagonize transmembrane calcium flux. The inability of sodium nitroprusside to antagonize the phasic mechanical activity in rat portal vein is consistent with the work of Kreye, Baron, Lüth & Schmidt-Gayk (1975). These workers showed that sodium nitroprusside was most effective in antagonizing tonic mechanical responses in tissues where contraction was associated with a pool of calcium relatively independent of extracellular Ca^{2+}.

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A simple *in vitro* preparation of mammalian tissue exhibiting properties of slow tonic skeletal muscle

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Guinea-pig cremaster muscle can be set up as an isolated preparation (Ninomiya, 1975; Dale, Evinc & Vine, 1976). It gives dose-related contractures with acetylcholine which, on the basis of the affinity constants obtained with (+)-tubocurarine and atropine, involve nicotinic not muscarinic receptors (Dale *et al.*, 1976). Set up as a simple nerve-muscle preparation, with isometric recording, the muscle manifested twitches to single supra-maximal stimuli. Repetitive stimulation at 20 Hz resulted in fused tetanus, but maximum tension (40 G) only developed above 60 Hz. The ratio of twitch tension to maximum tetanic tension was approximately 0.1. The muscle was singularly resistant to fatigue, tetanic contraction at 20 Hz being maintained up to 10 minutes.

KCl (0.1 M) produced a sustained increase in tension, and depolarizing neuromuscular blocking agents (10^{-6}—5×10^{-5} M) evoked slow, dose-related contractures, up to 70% of the maximum response to KCl. When added during repetitive nerve stimulation, succinylcholine, at low concentrations, produced a sustained increase in tension but, initially, no inhibition of twitch; total neuromuscular block involving both twitch and tension increase, occurred at 4×10^{-5} M. (Figure 1).

Tetrodotoxin (10^{-7} M) modified the dose-response curve to acetylcholine and succinylcholine, but substantial responses to these agents could still be obtained. On the nerve-muscle preparation, tetrodotoxin eliminated the twitch leaving the tension increase virtually unaltered. With tubocurarine it could be shown that these tetrodotoxin-resistant responses involved nicotinic not muscarinic receptors. It thus appears that the tissue contains skeletal muscle

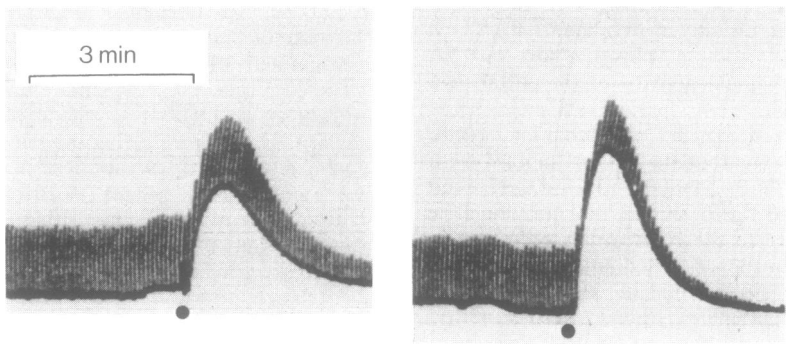

Figure 1 The response of the guinea-pig cremaster nerve/muscle preparation to succinylcholine. Bathing fluid: oxygenated Krebs-Henseleit solution at 32°C. Isometric recording with a Pye-Ether UFI transducer and George Washington Oscillograph 400 MD/2. Stimulation, using a Grass SD5 stimulator: 4 V, 0.2 Hz, 0.1 ms pulse duration.