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Effect of verapamil on rhythmic contractions in isolated rat vasa deferentia

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Both extracellular and intracellular sources of calcium have been shown to be involved in the contractile process in smooth muscle (Hurwitz & Suria, 1971). We have studied excitation-contraction coupling in the vas deferens using verapamil which is known to block selectively transmembrane calcium ion influx, causing electro-mechanical decoupling and inhibiting contraction (Haeusler, 1971; 1972).

Vasa deferentia from Wistar rats (250–450 g body weight) were suspended in Krebs-Henseleit solution (Na^+ = 143, K^+ = 5.6, HCO_3^- = 25, glucose = 11.1, Mg^{2+} = 1.2, $H_2PO_4^-$ = 1.2, Ca^{2+} = 2.5, SO_4^{2-} = 1.2 and Cl^- = 135.8 mM) at 36–37°C and contractions were recorded isometrically. Methoxamine HCl (2 μg/ml), 5-hydroxytryptamine creatinine SO_4 (50 μg/ml) and $BaCl_2$ (1 mM) each caused rhythmic contractile activity (frequency = 1.3–4.8/min, amplitude = 0.02–2.38 g tension). In Krebs-Henseleit from which $CaCl_2$ had been omitted, none of these drugs produced a response. As the $[Ca^{2+}]o$ was raised gradually, contractions were observed when the $[Ca^{2+}]o$ exceeded a threshold of 0.3 mM in different experiments. Both the amplitude and frequency of these contractions then increased dramatically in the range 0.5–1 mM over this threshold.

High concentrations of verapamil HCl were required to inhibit rhythmic contractions produced by methoxamine (Figure 1(a)), 5-HT or $BaCl_2$. The amplitude was reduced by 5–30 μg/ml, while the frequency was inhibited only with 50–100 μg/ml verapamil. In most experiments, there was a slight increase in frequency at concentrations of verapamil in the range 5–10 μg/ml.

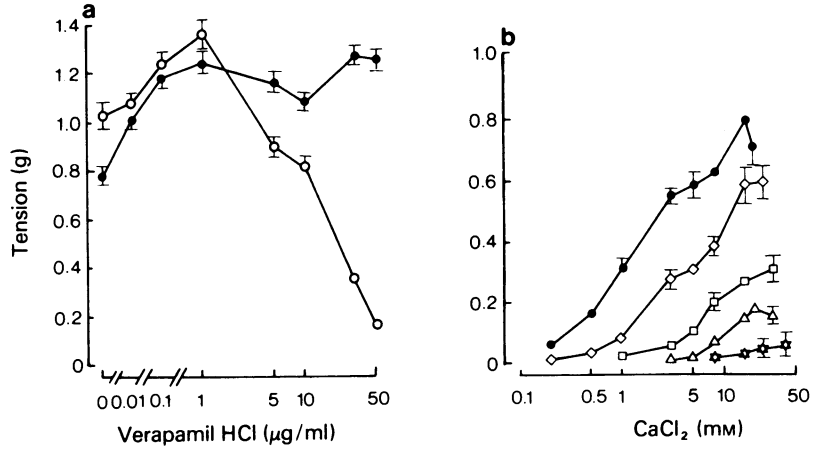

Figure 1 (a) Effect of cumulatively increasing concentrations of verapamil on rhythmic contractions induced by methoxamine HCl (2 μg/ml) in isolated rat vasa deferentia. One vas served as the control (●) while the contralateral vas received verapamil (○). Each point is the mean ± s.e. mean of 4 experiments. (b) Effect of verapamil on the dose-response curve for $CaCl_2$ on depolarized rat vasa deferentia. (●) control, (◇) 0.1 μg/ml verapamil, (□) 1 μg/ml verapamil. (△) 10 μg/ml verapamil, (✡) 50 μg/ml verapamil. Each point is the mean ± s.e. mean of 3 experiments.

Contractions were obtained when $CaCl_2$ was added cumulatively to rat vasa deferentia suspended in a depolarising solution (Na^+ = 16.2, K^+ = 127.6, Hepes = 10.0, Glucose = 11.1, Cl^- = 130.0, Mg^{2+} = 1.2 and SO_4^{2-} = 1.2 mM). These contractions were inhibited by verapamil in much lower concentrations than those required to inhibit rhythmic contractions. The maximum of the Ca^{2+} dose-response curve was depressed by 18 ± 2%, n = 3, by verapamil 0.1 μg/ml and by 60 ± 4%, n = 3, by verapamil 1 μg/ml (Figure 1(b)).

These results show that in the rat vas deferens rhythmic contractions do not require entry of Ca^{2+} through varapamil-sensitive channels, the calcium probably being supplied from an intracellular store. Because low concentrations of verapamil block $CaCl_2$ contractions in depolarized tissues this suggests a selective inhibition of transmembrane Ca^{2+} flux, whereas the block of rhythmic contractions by higher concentrations is probably a non-specific action, such as a local anaesthetic effect (Haeusler, 1972). Nevertheless, the dependence of the rhythmic contractions on $[Ca^{2+}]o$ suggests a superficial Ca^{2+} store is essential and the possibility remains that Ca^{2+} may enter through a separate channel that is not blocked by verapamil (Golenhofen & Hermstein, 1975).

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Withdrawal of centrally acting antihypertensives in conscious dogs

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The occurrence of rebound hypertension and tachycardia on the abrupt withdrawal of clonidine therapy in man is now well established. An animal model for this syndrome has however been difficult to obtain and the results have been contradictory. Dix & Johnston (1977) and Oates, Stoker, Monaghan & Stokes (1978) demonstrated the phenomenon in rats while Cavero, Fenard, Finch, Lefevre & Roach (1977) could not. Using the Alderley Park strain of Wistar rats and SHR of the Okamoto strain we have been unable to demonstrate rebound even when following the protocols described in the papers of Dix & Johnston (1977) or Oates *et al.* (1978).

In beagle dogs a rebound effect on heart rate (HR) has been consistently observed following clonidine withdrawal after 10 days of dosing (100–200 μg/kg 2× or 3× daily). Blood pressure (BP) and HR were measured on days 1, 4 and 9 of treatment and following the withdrawal at 4 h intervals for 28 h and then daily for the next 2–3 days. Nine dogs have been studied, in four of which only HR was measured. BP was recorded from indwelling catheters and during treatment diastolic BP fell by 23 ± 3.4% (mean ± s.e. mean) ($P < 0.005$) from a control value of 87.4 ± 4.5 mmHg. The control HR was 75.4 ± 3.3 bts/min and it varied condiserably during therapy. When measured 3 h after dosing HR was depressed consistently. This reduction varied between 48.5 ± 7.1% ($P < 0.01$) on day 1 and 32.2 ± 4.6% ($P < 0.001$) on day 9. The HR was depressed 8 h after the initial doses but by day 9 HR was 37.6 ± 8.8% ($P < 0.005$) above controls at this time.

On withdrawal of clonidine the BP returned to control with no significant overshoot while HR showed a marked rebound. Eight h after the last dose HR was elevated by 38.0 ± 12.1% ($P < 0.025$). HR reached a maximum 16 h after the last dose, the elevation being 59.9 ± 18.8% ($P < 0.005$), before returning to control levels over the next 2–3 days.

Similar experiments have been performed with ICI 106270 (600 μg/kg 3× daily) a new centrally acting antihypertensive (Clough, Hatton, Pettinger, Samuels & Shaw, 1978). Ten dogs have been used in 5 of which only HR was measured. During treatment diastolic BP fell by 18 ± 3.9% ($P < 0.025$) from a control value of 113.7 mmHg. The control HR was 88.9 ± 4.1 beats/min and during therapy it did not increase significantly above control levels at any time. As with