

## The action of cinnarizine and of phentolamine on the noradrenaline-dependent calcium influx in vascular smooth muscle

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It has been proposed that cinnarizine, a non-competitive antagonist of noradrenaline, was acting by inhibiting the opening of  $\text{Ca}^{++}$ -channels evoked by membrane depolarization (Godfraind, Kaba & Rojas, 1973). The present experiments were designed in order to determine whether the cinnarizine induced depression of the tonic contraction evoked by noradrenaline in rat aorta was quantitatively related to a reduction of noradrenaline-dependent calcium influx.

The lanthanum method has been introduced in order to estimate the cytoplasmic calcium content in smooth muscle (Breemen *et al.*, 1973), however, recent observations on intestinal smooth muscle have shown that the La-resistant Ca is only one of several intracellular fractions (Burton & Godfraind, 1974). Therefore, the action of lanthanum on Ca content and efflux has also been studied.

Strips 4 cm long were prepared by spiral section of rat aorta, they were bathed at 37°C in physiological solution (mM): NaCl 122,  $\text{NaHCO}_3$  15, KCl 5.9,  $\text{CaCl}_2$  1.25 and glucose 11, pH 7.2, aerated by 95%  $\text{O}_2$  + 5%  $\text{CO}_2$ . They were transferred to the same solution containing  $^{45}\text{Ca}$  and thereafter washed in a lanthanum solution (mM): NaCl 122, KCl 5.9,  $\text{MgCl}_2$  1.25, glucose 11,  $\text{LaCl}_3$  50, Tris-maleate 15, pH 6.8. The residual radioactivity of muscle was measured by liquid scintillation counting.

$^{45}\text{Ca}$ -efflux was not completely blocked in lanthanum solution. When the sodium pump was inhibited by ouabain  $10^{-3}$  M, there was a net gain of  $^{45}\text{Ca}$ ; this gain was recovered in the La-resistant Ca fraction when the washout period did not exceed 5 minutes. This indicates that the slowing

of Ca efflux in lanthanum solution was sufficient to estimate the Ca turnover of intracellular Ca stores.

The turnover rate was estimated in resting aortae by measuring the difference in La-resistant  $^{45}\text{Ca}$  content after 5 and 7 min in radioactive solution: it was equal to  $5.52 \mu\text{mol } ^{45}\text{Ca kg}^{-1} \text{ min}^{-1}$ . When noradrenaline ( $10^{-5}$  M) was added to the bathing solution between the 5th and the 7th min, it increased up to  $35.27 \mu\text{mol } ^{45}\text{Ca kg}^{-1} \text{ min}^{-1}$ . This increase was dose-dependent, the  $\text{ED}_{50}$  was similar to that found for contractions ( $2 \times 10^{-8}$  M). In the presence of phentolamine the dose-effect curves of the noradrenaline-dependent Ca turnover were displaced to the right in a competitive manner: the  $\text{pA}_2$ , estimated according to Arunlakshana & Schild (1959) was 7.8 (it was 7.9 for the contraction). In the presence of cinnarizine, there was a dose dependent depression of the maximum noradrenaline-dependent increase of Ca turnover. A 50% reduction was observed for cinnarizine  $10^{-6}$  M which depressed the maximum tonic contraction by about 50%.

The present results show that the alpha adrenergic stimulation of aorta evoked an increased rate of Ca exchange across plasma membrane. They also indicate that the action of cinnarizine might be attributed to the blockade of calcium channels.

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## References

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