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On the mechanism of papaverine action on the control of vascular smooth muscle contractile activity by extracellular calcium

It has been suggested recently that the relaxing effects of papaverine on smooth muscle might be exerted through cyclic phosphodiesterase (PDE) inhibition and consecutive accumulation of cyclic-3′,5′-AMP (Triner, Vulliemoz & others, 1970). Since relaxation probably follows the decrease of intracellular free Ca^{2+} ions, it still remains to be explained how cyclic AMP influences calcium movements (Stoclet, Peguet & Waeldele, 1971). Kukovetz & Pöch (1970) suggested that cyclic AMP probably increases Ca^{2+} uptake by the membranes and might enhance active Na-exclusion in vascular smooth muscle.

We now report the influence of papaverine and exogenous cyclic AMP (*N*-2′-*O*-dibutyryl adenosine-3′,5′-monophosphate) on noradrenaline-induced isometric responses of isolated aortic strips from the rat in different extracellular calcium concentration salines.

The thoracic aortae of 10 to 12 weeks old male rats (EOPS OFA) were removed from the left carotid to the diaphragm and prepared (Godfraind & Kaba, 1969). Isometric responses elicited by noradrenaline ($7{\cdot}5 \times 1^{-7}$M) were recorded under an initial tension of 1 g. Papaverine or cyclic AMP were added to the bath 10 min before noradrenaline. The reference response of each aortic strip was obtained in "normal" Krebs-bicarbonate medium (Ca content 2·5 mM). Except when $CaCl_2$ was added during the record of noradrenaline effect, any change in calcium concentration of the saline was followed by a period of equilibration to obtain a constant response to noradrenaline. With calcium concentrations lower than normal, the aortae were previously depleted by incubations for 1 h in calcium-free Krebs bicarbonate containing ethylene glycol bis amino-2-ethylether-*NN*′-tetra-acetic acid (EGTA, 1 mM).

The response elicited by noradrenaline included a phasic component which was not directly influenced by extracellular calcium concentration, and a tonic component which was sustained for 20 min or more and varied with extracellular calcium concentration. It increased with calcium concentration rising from 0 to 2·5 mM and decreased slightly when calcium concentration further rose.

Papaverine inhibition of noradrenaline-induced vasoconstriction was characterized by the decrease with time of the tonic component. This effect appeared at a concentration of papaverine (5×10^{-6}M) which decreased only moderately both phasic and tonic components during the first minutes of noradrenaline action. Both these components were more completely and readily depressed by higher concentrations of papaverine, but for 5×10^{-6}M the tension stayed at a level which was approximately constant for some time. Both this plateau duration and the slope of the later relaxation varied with extracellular calcium concentration. In 1·25 mM Ca^{2+} Krebs bicarbonate, the tonic component was more rapidly abolished than in normal or 5 mM Ca^{2+} saline. A sudden increase of calcium concentration up to 10 mM just after the plateau strongly increased the rate of relaxation caused by papaverine.

Exogenous cyclic AMP mimicked papaverine's inhibitory effects, but induced rhythmic activity during relaxation and reduced to a lesser extent the maximal tension during the tonic component of the response. The rate of relaxation was strongly increased and the rhythmic activity abolished by raising extracellular calcium up to 10 mM.

Although the direct proof (actual determination of cyclic AMP) is still lacking, these results bring further evidence that papaverine exerts its inhibitory effects on vascular smooth muscle through the increase of cyclic AMP concentration. The qualitative differences between papaverine and dibutyryl-cyclic AMP action might correspond to differences of cellular repartition of cyclic AMP from endogenous or exogenous origin.

Both papaverine and cyclic AMP enhanced the inhibitory effect of calcium on contractile activity. Since this inhibitory effect is probably the consequence of membrane "stabilization", a tentative hypothesis is that cyclic AMP decreased or abolished the coupling between excitation and contraction by increasing the calcium uptake by the membrane of vascular smooth muscle. This membrane action of cyclic AMP might play a role in the control of contractile activity by extracellular calcium and occur simultaneously with the modification of actomyosin responses reported by Uchida & Mommaerts (1963).

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