CYCLIC PHOSPHODIESTERASE ACTIVITY AND THE ACTION OF PAPAVERINE

L. Triner, Y. Vulliemoz, I. Schwartz, and G. G. Nahas*

Department of Anesthesiology
College of Physicians & Surgeons, Columbia University
New York, N. Y. 10032

Received May 21, 1970

Summary

Papaverine, a smooth muscle relaxant, inhibits phosphodiesterase activity in homogenates of rabbit aorta, rat uterus and rat diaphragm. This inhibitory effect of papaverine is about 15-30 times greater than that of theophylline. In rat diaphragm, papaverine increased glycogenolysis to the same extent as epinephrine and had a synergistic effect with epinephrine on glycogen breakdown. These results suggest that the relaxing effect of papaverine might be due to its action on phosphodiesterase and are in agreement with the postulation that cyclic 3', 5'-AMP participates in the control of smooth muscle tone and contractility.

In the course of the study of the adenyl cyclase-phosphodiesterase system in smooth muscle, we have observed a decreased phosphodiesterase activity in the presence of papaverine. Since papaverine is a smooth muscle relaxant, such an effect would conform with the postulated role of cyclic 3', 5'-AMP in the regulation of smooth muscle tone and contractility (1, 2) and could explain the mechanism of action of papaverine. This report presents evidence of the inhibitory action of papaverine on phosphodiesterase.

Methods and Materials

Sherman rats of both sexes (as specified in the text), weighing 200-250 g, and male rabbits, 2500-3000 g, were used. Phosphodiesterase activity was assayed in homogenates of uterine, vascular, and striated muscle. Cyclic 3', 5'-AMP, marked by H^3 cyclic 3', 5'-AMP (New England Nuclear), was used as substrate. The reaction was

*L. Triner is a Senior Investigator of the New York Heart Association.

started by the addition of the enzyme (homogenate), carried on at 37° C. for 8 min and stopped at 0 and 8 min by boiling for 5 min in a water bath; cyclic 3', 5'-AMP was then separated on Dowex 50W-X4 Columns and by double precipitation with $Ba(OH)_2$ and $ZnSO_4$ (3) and counted in an Intertechnique liquid scintillation spectrometer. After correction for the recovery, the activity of the enzyme was calculated and expressed in μμMol of cyclic 3', 5'-AMP used/mg protein/min.

Protein was determined by Lowry's method (4) and glycogen by the method of Saifer (5).

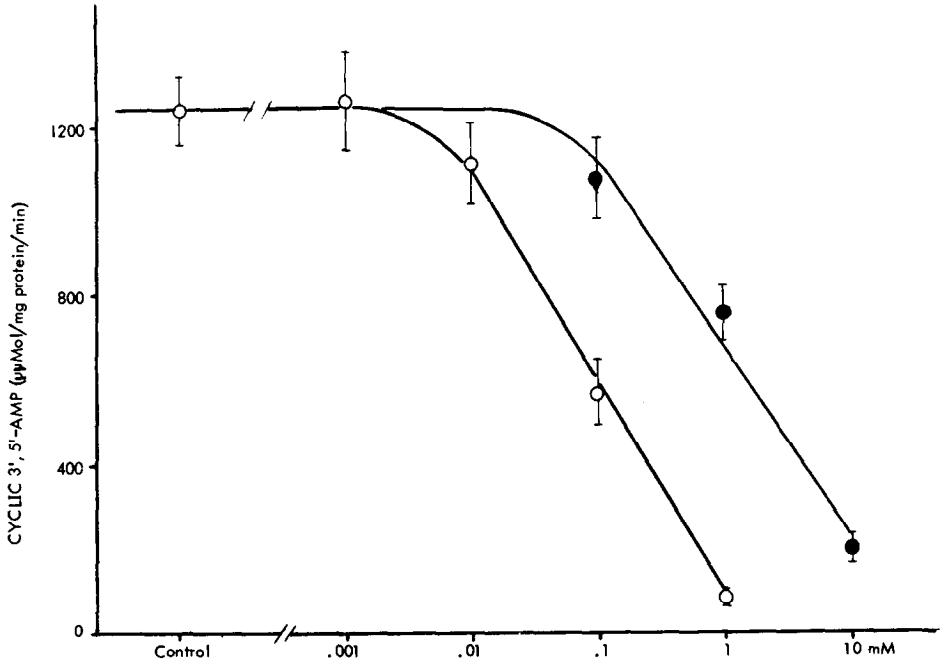

Fig. 1: Effect of papaverine o—o—o and theophylline ●—●—● on phosphodiesterase activity in homogenates of rabbit aortic smooth muscle. Each point represents the mean of 6 experiments ± S. E.

Results and Discussion

The control activity of phosphodiesterase in rabbit aorta, assayed in the presence of 33 μMolar concentration of cyclic 3', 5'-AMP was 1.24 ± 0.08 mμMol/mg protein/min. Papaverine in 10, 100 and 1000 μMolar concentrations decreased phosphodiesterase activity by 11, 54 and by more than 90% respectively (Fig. 1). When compared to the effect of theophylline under the same conditions, papaverine appears to be about 15 times stronger an inhibitor of phodphodiesterase; estimated concentration of papaverine and theophylline for 50% inhibition are 80 μMol and 1.4 mMol

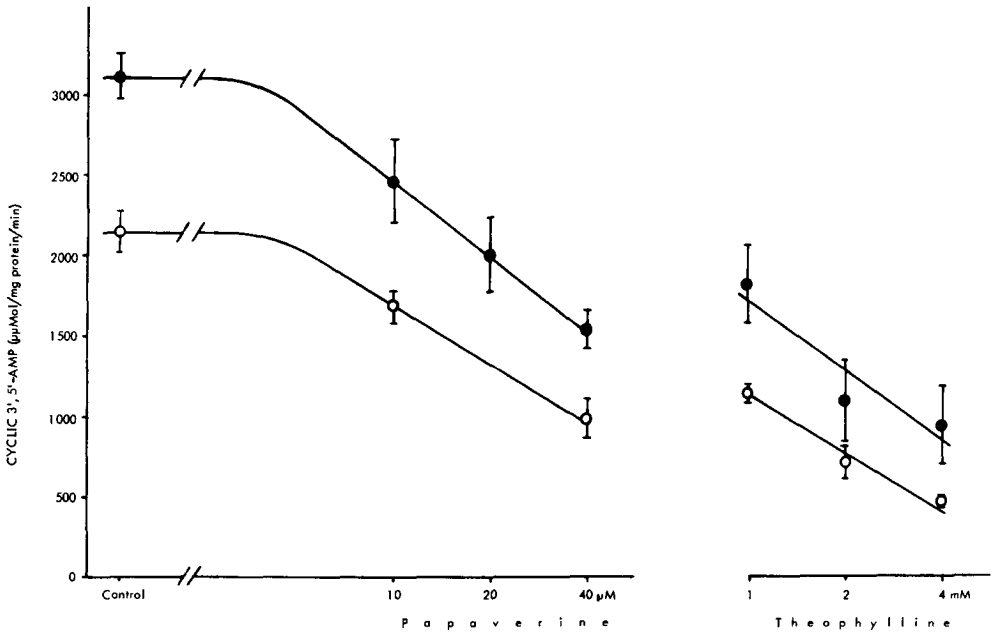

Fig. 2: Effect of papaverine and theophylline on phosphodiesterase activity in homogenates of rat uterine muscle in the presence of 165 μMolar ●——●——● and 82.5 μMolar o——o——o concentration of substrate. Each point represents the mean of 6 experiments ± S.E.

respectively. Similar effects of papaverine and theophylline and a similar ratio of concentrations for 50% inhibition were observed with uterine smooth muscle phosphodiesterase (Fig. 2).

The activity of phosphodiesterase in uterine smooth muscle was 2.15 ± 0.13 and 3.12 ± 0.15 mμ Mol/mg protein/min when assayed in the presence of 82.5 and 165 μMolar concentrations of cyclic 3', 5'-AMP respectively. At both substrate levels the estimated concentrations of papaverine and theophylline required to inhibit by 50% is 40 μMol and 1.2 mMol respectively.

The inhibitory effect of papaverine on phosphodiesterase was tested in other systems which are known to be regulated by cyclic 3', 5'-AMP. An example is given in Fig. 3. In rat diaphragm papaverine, in a concentration which inhibits phosphodiesterase (100 μMol papaverine inhibited phosphodiesterase by 50%: from a control

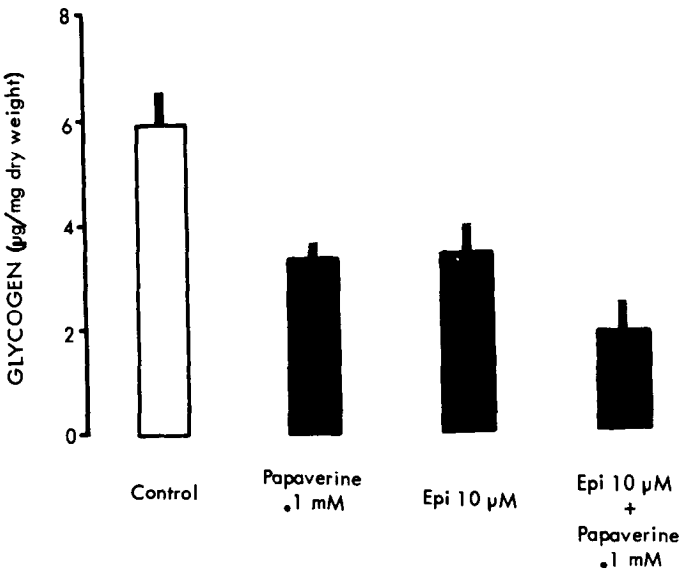

Fig. 3: Changes in glycogen content in rat (male) diaphragm muscle after incubation with papaverine (.1 mMol), epinephrine (10 μMol), and both drugs combined. The columns represent means of 6 experiments ± S.E.

value of 439 to 227 μμMol/mg protein/min, $P < 0.001$), decreased glycogen content to the same extent as epinephrine, 10 μMol, a concentration known to stimulate adenyl cyclase (from a control value of 5.96 μg/mg dry weight to 3.31 and 3.37 μg/mg dry weight respectively). Both drugs, when combined, exerted a synergistic effect, as one would expect when stimulation of adenyl cyclase is coupled with inhibition of phosphodiesterase.

The results demonstrate that papaverine has an inhibitory effect on phosphodiesterase in vascular and uterine smooth muscle and that papaverine is a more potent inhibitor of this enzyme than theophylline. This finding is in agreement with the results reported by Kukowetz et al (6) for myocardial muscle and coronary vessel and also by others (7) for brain phosphodiesterase. In addition, papaverine in concentrations which inhibit phosphodiesterase in striated muscle brought about the expected biochemical consequences, i.e., increased glycogenolysis and a synergistic effect with epinephrine on glycogen breakdown. In view of a previous report which led to the postulation that cyclic 3', 5'-AMP participates in the control of smooth muscle tone and contractility (1, 2), and in view of the present results showing the inhibitory effect of papaverine on phosphodiesterase in vascular and uterine muscle, we suggest that the relaxing effect of papaverine (referred to as general, nonspecific) might be exerted through its action on phosphodiesterase. Such an effect is accompanied by an accumulation of cyclic 3', 5'-AMP in the cell, which in turn would lead to a decreased tone and contractility of smooth muscle as we reported earlier (1, 2, 8, 9, 10). Experiments to determine whether there is a causal relationship between the effect of papaverine on phosphodiesterase and its relaxing effect on smooth muscle are now being carried out.

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