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INHIBITION BY PAPAVERINE OF cGMP and cAMP PHOSPHODIESTERASES FROM THE RAT HEART

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Previous studies (1) have shown that papaverine reversibly inhibits the enzymatic hydrolysis of adenosine 3',5'-monophosphate (cAMP) by phosphodiesterase (PDE). The following increase in cAMP level in tissues seems responsible for the pharmacological properties of the drug, especially in the cardiovascular system (2-4). More recently attention has been drawn (5) to the role of guanosine 3',5'-monophosphate (cGMP), which often presents physiological properties opposite to those of cAMP. Since it has been reported (6-8) that cAMP-PDE also hydrolyses cGMP, we have now investigated the inhibitory effect of papaverine on the enzymatic hydrolysis of cGMP and cAMP by soluble PDE from rat heart.

Minced ventricles from 40 rats were homogenized at 4°C in 4 volumes of Tris-HCl buffer (0.04 moles/l, pH 8.0) containing sucrose (0.33 moles/l) and EDTA (1×10^{-6} moles/l), by 6 passes in a motor driven glass teflon Potter-Elvehjem homogenizer. After removal of the 3 000 g pellet, the preparation was centrifuged at 100 000 g for 1 h. The 50 % saturated $(NH_4)_2\ SO_4$ precipitate obtained from the 100 000 g supernatant was dissolved in a minimal volume of

0.4 moles/l Tris-HCl buffer (pH 8.0) containing Mg SO_4 (5 mmoles/l) and 2-mercaptoethanol (3.75 mmoles/l). It was then dialyzed for 18 h with 2 changes, against the same buffer.

PDE activities were measured at 37°C and pH 8.0, according to the method of Thompson and Appleman modified by Klotz and Stock (9). The enzyme concentration was chosen in order to obtain between 10 and 30 % of the substrate hydrolysed during the incubation time.

The cGMP and cAMP-PDE activities were assayed over two different ranges of substrate concentration, low (6.25×10^{-7} to 2.5×10^{-6} moles/l) and high (6.7×10^{-6} to 1×10^{-4} moles/l). This procedure was followed because the cAMP-PDE from most tissues, including the rat heart (10), show non linear kinetics when a wide range of cAMP concentration is tested. It allowed the determination of 2 apparent Michaelis constants (Km) and Maximum velocities (Vmax). The values were derived from regression analysis using the computer programme published by Cleland (11).

Figure 1 shows that papaverine inhibited PDE activities on both cAMP and cGMP, whether the substrate concentration ranges were high or low. The inhibition of cAMP hydrolysis seemed to be competitive while the inhibition of cGMP hydrolysis seemed to be either non competitive or mixed, depending on the substrate concentration range.

The data presented in Table I show that apparent Km, Vmax and Ki values were found in the case of each substrate, depending on its concentration range. However the differences between low and high apparent Km and Vmax values were larger for cAMP than for cGMP. On the other hand the apparent Km values were lower for cAMP-PDE than for cGMP-PDE, especially at low substrate concentration. The variations observed between the apparent Ki values according to the substrate and its concentration were relatively small and did not

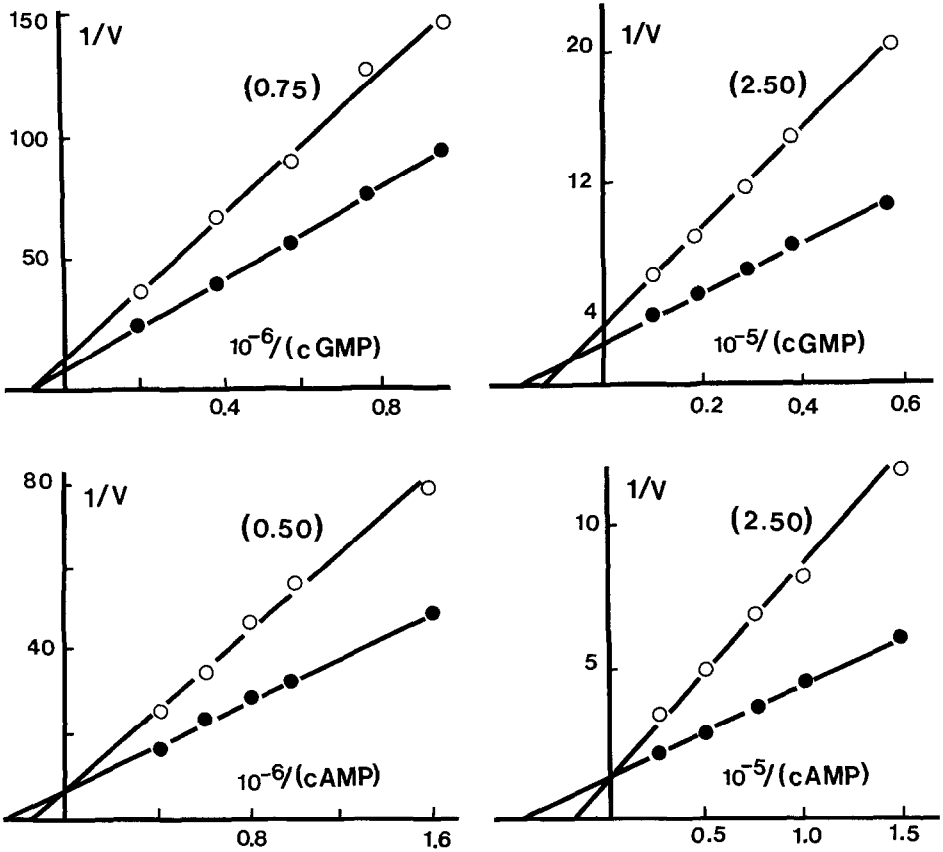

Figure 1. Lineweaver-Burk plots of the inhibition by papaverine of cGMP (top) and cAMP (bottom) hydrolysis at low (left) and high (right) substrate concentrations. ● substrate alone, ○ papaverine (concentration between brackets × 10^{-5} moles/l).

Table I. Kinetic properties and inhibition by papaverine of PDE from the rat heart.

Substrate		Apparent Km	Apparent Vmax	Apparent Ki
Concentration	Kind	× 10^{-5}moles/l	×10^{-9}moles/min/mg	× 10^{-5}moles/l
low	cAMP	0.4	1.5	0.6
	cGMP	2	1.7	1
high	cAMP	2	7.6	1
	cGMP	6	3.8	2

exceed the limits of accuracy of the method.

The Ki value is theoretically independant of the concentration and Km value of the substrate (12). Nevertheless we have precedingly found two apparent Ki of papaverine corresponding to two apparent Km of cAMP-PDE in different preparations from various tissues (13). We presently report that the apparent type of inhibition of PDE by papaverine varies according to the substrate. These findings are consistent with the existence in the tissues of various forms of PDE (possibly different enzymes) interacting differently with papaverine. Further experiments on more purified material are necessary in order to analyse the mechanism of action of papaverine.

So far it may be concluded that papaverine inhibits cAMP and cGMP hydrolysis by PDE from the rat heart with apparent Ki(s) which are close to each other in the case of both substrates.

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