Effect of isoprenaline on the phosphodiesterase activity of the rat heart

The intracellular level of cyclic AMP is regulated by the synthesizing enzyme adenylate cyclase and the hydrolysing enzyme phosphodiesterase (Drummond & Perrot-Yee, 1961). Marked stimulation of adenylate cyclase activity with an increased level of cyclic AMP in response to various catecholamines is well documented (Robison, Butcher & Sutherland, 1971). Lee, Kuo & Greengard (1971) have shown that isoprenaline increases the myocardial cyclic AMP level in the rat and in calf heart slices. It is possible that the elevated cyclic AMP levels produced by catecholamines in the heart may not be dependent on increased adenylate cyclase activity alone but may also be affected by alterations in phosphodiesterase activity. We have investigated the effect of isoprenaline on the phosphodiesterase activity of the rat heart.

Phosphodiesterase is mostly located in the cytoplasm (Thompson & Appleman, 1971). In most tissues studied there are at least two separate forms each possessing different Km values (Beavo, Hardman & Sutherland, 1970; Loten & Sneyd, 1970; Lagarde & Colobert, 1972). Initial experiments were performed to determine the Km values of phosphodiesterase activity of the rat heart using a wide range of concentrations of 3′ 5′-cyclic AMP.

Albino rats, 120–150 g, of either sex were killed by a sharp blow on the head and the heart rapidly excised. All subsequent procedures were at 0–4°. Ventricular muscle was passed through a tissue press and homogenized in a glass homogenizer with twice its volume of glass distilled water. The homogenate was centrifuged at 100 000 *g* at 4° for 1 h and the supernatant fraction was collected. The protein content of the homogenate and the supernatant fraction was estimated by the method of Lowry, Rosebrough & others (1951).

Phosphodiesterase activity was determined by the method of Huang & Kemp (1971). Pilot experiments to determine the incubation time and optimum protein concentration of the enzyme source led us to adopt an incubation time for the enzyme of 5 min. Enzyme kinetics for determining Km values were studied using 3′5′-cyclic AMP over a range of 0·05–40·0 $\times$ 10^{-6}M in the incubation medium, the supernatant fraction was used as the enzyme source. In two studies each of 6 experiments, two distinct Km values were observed: 35·0 $\pm$ 3·95 $\times$ 10^{-6}M, and 1·0 $\pm$ 0·1 $\times$ 10^{-6}M with the respective V_{max} being 12·9 $\pm$ 2·2 and 1·13 $\pm$ 0·28. Subsequent experiments were made utilizing substrate concentration ranges at both these Km values. Phosphodiesterase activity was also estimated in the homogenate. The apparent activities at high and low Km were 7·77 $\pm$ 1·92 and 0·82 $\pm$ 0·19 n mol of cyclic AMP converted per mg of protein per 5 min, respectively. No significant change in the enzyme activity could be observed following overnight dialysis in 20mM tris buffer at 4° of the 100 000 *g* supernatant fraction.

The effect of isoprenaline over a wide dose range was studied with the nondialysed 100 000 *g* supernatant fraction. In the presence of high substrate concentration, isoprenaline doses varying between 10^{-10} to 10^{-4}M did not affect the enzyme activity (Table 1). On the other hand, in the presence of lower concentrations of the substrate, isoprenaline produced a distinct effect and in concentrations of 10^{-10} to 10^{-6}M of isoprenaline significantly decreased the phosphodiesterase activity. At higher concentrations (10^{-5} to 10^{-4}M) the suppression of phosphodiesterase activity was evident but was not statistically significant (Table 1).

The existence of phosphodiesterase in more than one form has been observed earlier. Beavo & others (1970) observed that in the crude particulate fraction of heart, phosphodiesterase had two different Km values for 3′, 5′-cyclic AMP of 0·8

Table 1. *Effect of isoprenaline on phosphodiesterase activity.* n mol of cAMP converted per mg of protein per 5 min.

	Control	Isoprenaline (M) 10^{-10}	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}
High Km substrate (n = 6)	10·23 ± 0·54	10·39 ± 0·80	11·33 ± 2·44	10·78 ± 2·15	10·28 ± 1·41	10·81 ± 1·80	10·98 ± 2·30	9·62 ± 2·53
Low Km substrate (n = 6)	1·52 ± 0·25	1·10 ± 0·23 *	1·31 ± 0·18	1·19 ± 0·20 *	0·79 ± 0·10 *	1·17 ± 0·27 *	1·21 ± 0·14	1·25 ± 0·17

* Paired analysis with the control values: $P < 0{\cdot}05$.

and 25·0 μM. The differential responsiveness of two types of phosphodiesterase as observed in the present study is not unprecedented. Loten & Sneyd (1970) observed that insulin affects the two types of phosphodiesterase of adipose tissue in a qualitatively different manner. Uzunov, Shein & Weiss (1973), working with the soluble supernatant fraction of cloned astrocytoma cells of the rat, observed two peaks of adenosine 3′, 5′-monophosphate phosphodiesterase and noradrenaline affected one and not the other.

Isoprenaline has been shown to be taken up by various tissues including the heart (Callingham & Burgen, 1966; Foster, 1969). The present investigation shows that isoprenaline depresses one of the two types of phosphodiesterase activity that is present in the 100 000 *g* supernatant fractions of the rat heart. Thus, isoprenaline-induced increases in cyclic AMP level in heart tissue may be a reflection of the combined result of increased adenylate cyclase activity as well as a decreased phosphodiesterase activity. The question why isoprenaline in the rat heart in the present study and noradrenaline in cloned astrocytoma of rat (Uzunov & others, 1973) affect only one form of phosphodiesterase is yet to be solved.

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