INHIBITION OF CYCLIC AMP PHOSPHODIESTERASE OF THE RAT HEART AND BRAIN BY NEUROHORMONE C in vitro

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The effect of neurohormone C, a new coronary dilator isolated from bovine hypothalamus, on activity of the phosphodiesterase (PDE) of cyclic adenosine-3',5'-monophosphate (AMP) was investigated. Considerable inhibition of PDE was shown in the supernatant of rat brain and heart homogenates (2000g) under the influence of neurohormone C. The existence of correlation between the coronary dilator effect of the hormone and regulation of the cyclic AMP level under the influence of a change in PDE activity is postulated.

KEY WORDS: regulation of coronary circulation; hypothalamic neurohormone; phosphodiesterase of cyclic AMP.

The presence of new hypothalamic hormones acting as regulators of the cardiac circulation was demonstrated by the writers previously [2]. One such hormone, conventionally named neurohormone C, is a compound of low molecular weight isolated from the bovine hypothalamus and purified by electrophoresis and chromatography. Neurohormone C has a powerful and prolonged coronary dilator action in experiments on cats (in situ) after intravenous injection (0.1-1 μg/kg). It could be postulated on the basis of the study of the mechanisms of biochemical action of neurohormone C that it involves the participation, as is characteristic of most known hormones, of the cyclic AMP system, including adenylate cyclase (AC), the enzyme of biosynthesis, and phosphodiesterase (PDE), the enzyme of hydrolysis of cyclic AMP [14].

Cyclic AMP plays an important role in the regulation of cardiovascular activity. On the one hand, the regulatory role of cyclic AMP is manifested in the relaxation of smooth muscle induced by methylxanthines, papaverine, and many other pharmacological vasodilators. These relaxation processes are accompanied by powerful inhibition of PDE [10], which leads to an increase in the cyclic AMP concentration. A similar effect has also been observed in the coronary arteries [6], and the heart muscle and other tissues of mammals [11]. A marked vasodilator action is also shown by β-adrenomimetics [12], which are known to activate AC of various tissues considerably.

On the other hand, the regulatory function of cyclic AMP is exhibited during the positive inotropic and chronotropic effects of catecholamines [13-15], glucagon [8], and other agents stimulating AC, and also many substances inhibiting PDE [7]. Changes in PDE activity accompany changes in the tone of the coronary vessels [5].

In this investigation a possible connection between the coronary dilator action of neurohormone C and regulation of the cyclic AMP level by PDE was examined.

EXPERIMENTAL METHOD

Phosphodiesterase activity was determined from the quantity of cyclic AMP hydrolyzed during incubation with the enzyme. The character of hydrolysis was reflected in a decrease in the radioactivity of cyclic AMP-^{3}H. Experiments were carried out on rats weighing 200-300 g. The organs for testing (heart and brain) were

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TABLE 1. Changes in PDE Activity of Rat Brain and Heart Produced by the Action of Neurohormone C (M ± m, mean results of six determinations)

Experimental conditions	Organ	Content of tissue in sample, μg protein	Incubation time, min	Quantity of substrate hydrolyzed	
				%*	nmoles/mg protein
Control (bidistilled water)	Heart	4,6	40	70±8,1	77,6±8,9
Experiment (neurohormone C)				53±6,2	58,8±6,8
Control (bidistilled water)	Brain	1,7	20	62±7,2	186±21,5
Experiment (neurohormone C)				12±2,6	36±7,7

*Quantity of unhydrolyzed substrate taken as 100%.

removed, washed, weighed, and homogenized in a glass homogenizer with a Teflon pestle in 10 volumes of 160 mM Tris-HCl buffer, pH 7.5, containing 5 mM $MgCl_2$. The homogenate was then centrifuged (2000g, 10 min) and the supernatant was used as the source of PDE. The enzyme was incubated with the substrate at 37°C for various times (0-40 min). Because of the high PDE level in the brain the supernatant of this tissue was diluted 1 : 10 with buffer, whereas that from heart tissue was diluted 1 : 2. Since the radioactivity of the hydrolysis product 5'-AMP-^{3}H also reflects PDE activity, to prevent further decomposition of the 5'-AMP-^{3}H by the action of 5'-nucleotidase, incubation was carried out in the presence of 2 mM unlabeled 5'-AMP. Neurohormone C (or bidistilled water in the control test) was first mixed with the supernatant of the homogenates before addition to the incubation samples. The method of Pöch [11], in a micromodification [1] involving fractionation of the hydrolysis products by ascending thin-layer chromatography on Silufol UV-254 plates (Czechoslovakia), was used. The final volume of the incubation mixture was 10 μl. The composition of the incubation mixture was as follows (in moles): Tris-HCl buffer (pH 7.5) 0.1, $MgCl_2$ $3 \cdot 10^{-3}$, cyclic AMP $0.5 \cdot 10^{-4}$, cyclic AMP-^{3}H 10^{-6} (0.2 μCi), 5'-AMP $2 \cdot 10^{-3}$, neurohormone C 2 μl (about 5 ng) or bidistilled water 2 μl, heart homogenate 110 μg of tissue, or brain homogenate 30 μg of tissue. The reaction was started by addition of the enzyme (5 μl) to the reaction mixture. The reaction was stopped by immersion in boiling water for 3 min. After cooling to 20°C the samples were centrifuged (3000g, 15 min). The supernatant (3 μl) was chromatographed in a system of: n-butanol–acetone–NH_4OH (8 : 2 : 1). The radioactivity of the hydrolysis products was counted on SL-30 liquid scintillation spectrometers (France) in toluene scintillator. The total count of the chromatograms was usually $2.5 \cdot 10^4$–$3 \cdot 10^4$ cpm. When the enzyme activity was calculated, the results of chromatography of the labeled preparation of cyclic AMP-^{3}H and also of samples conventionally incubated for 0 min were taken into consideration. The protein content was determined by Lowry's method. The following were used: cyclic AMP from Buchs SG (Switzerland), cyclic AMP-^{3}H with a specific activity of 22.1 Ci/mmole from New England Nuclear (USA), 5'-AMP from Reanal (Hungary), and theophylline from Sigma (USA).

EXPERIMENTAL RESULTS

The experimental results are given in Table 1. They show that under the influence of neurohormone C heart PDE was inhibited by 24% whereas brain PDE was inhibited by 81% relative to the control.

When the action of neurohormone C was compared with that of theophylline (10 mM), a known PDE inhibitor, it was found that under the experimental conditions used neurohormone C had an equally strong inhibitory action on the brain preparation. In the experiments on the heart the action of neurohormone C was weaker than that of theophylline. However, it must be remembered that the optimal inhibitory concentration of theophylline is much higher than that of the concentrations of neurohormone C studied.

During incubation in the presence of $2 \cdot 10^{-3}$ M 5'-AMP clear correlation was observed between the decrease in label in the chromatographic spot of cyclic AMP-^{3}H and a corresponding increase in the label of 5'-AMP-^{3}H formed during incubation. The adenosine level did not exceed 4-5%. It was also found that 5'-AMP, in the concentrations specified, has no such effect on PDE activity. These results are in good agreement with those of Pöch [9], who showed that 5'-AMP, in concentrations below $2.7 \cdot 10^{-3}$ M, does not affect the inhibition of PDE by theophylline, papaverine, and other preparations.

In the present experiment the initial velocity of hydrolysis of cyclic AMP by the action of cardiac PDE in the control and experimental series was 2.96 ± 0.41 and 1.84 ± 0.29 nmole/mg protein/min, respectively; hydrolysis of cyclic AMP under the influence of brain PDE took place with an initial velocity of 11.4 ± 1.5 and 2.7 ± 0.63 nmole/mg protein/min, respectively.

The presence of such a powerful regulator of PDE activity as neurohormone C in the hypothalamus is most interesting. The AC and PDE of the brain exhibit higher activity than the AC and PDE of all other vertebrate tissues; moreover, even though PDE activity is many times greater than AC activity [3], the brain tissue can accumulate cyclic AMP very rapidly in concentrations more than 10 times higher than the basal level [4]. One of the probable mechanisms of this effect may be inhibition of PDE not only by endogenous ATP, pyrophosphate, and citrate, but also through hormonal regulation. The results suggest that besides other hormones, neurohormone C may also play a role in the hormonal regulation of PDE activity. Preliminary investigations have shown that the cyclic AMP content in rat brain homogenates after incubation with exogenous ATP in the presence of neurohormone is appreciably higher than in the control. Inhibition of PDE activity by papaverine is known [12] to precede the onset of relaxation of smooth muscle. Papaverine, which has no coronary action in the guinea pig heart, is known not to change PDE activity [10]. However, it is a noteworthy fact that some known PDE inhibitors do not affect the coronary circulation.

In the study of the mechanisms of the coronary dilator action of neurohormone C it is important to discover whether relaxation of the muscles of the coronary vessels arising under the influence of the neurohormone is connected with inhibition of PDE.

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