EFFECT OF NITROGLYCERIN ON PHOSPHORYLASE ACTIVITY OF THE MYOCARDIUM

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The effect of nitroglycerin on myocardial phosphorylase activity in rats was studied in vitro and in vivo. Nitroglycerin ($2 \cdot 10^{-3}$ M) does not affect phosphorylase activity in vitro. If injected intravenously in a dose of 1 mg/kg nitroglycerin increases the relative percentage of phosphorylase *a* after 1 min but reduces the total phosphorylase activity. The increase in the activity of phosphorylase *a* produced by nitroglycerin indicates excitation of the β-adrenergic structures of the myocardium. Comparison of these findings with the observed ability of nitroglycerin to liberate catecholamines reveals correlation between the increase in the relative percentage of phosphorylase *a* and noradrenalin liberation. These results confirm the view that the mechanism of action of nitroglycerin includes an adrenergic component.

Previous investigations have shown that the cardiovascular effects of nitroglycerin are brought about with the participation of central and peripheral adrenergic processes. Nitroglycerin has been shown to interfere with the metabolism of the biogenic monoamines. In particular, nitroglycerin can liberate chatecholamines in the brain and heart tissues [1]. Catecholamines stimulate the adrenergic structures of the myocardium, modifying their metabolism by increasing glycogenolysis. Excitation of the myocardial β-adrenergic receptors is usually accompanied by an increase in phosphorylase activity [5-7].

The present investigation was accordingly carried out to study the effect of nitroglycerin on the phosphorylase activity of the myocardium.

EXPERIMENTAL METHOD

Rats weighing 250-300 g were anesthetized with pentobarbital (40 mg/kg, intraperitoneally) in order to eliminate stress reactions. Nitroglycerin was injected into the caudal vein in a dose of 1 mg/kg. The heart was removed to determine the phosphorylase activity 1, 5, 10, and 20 min after the injection of nitroglycerin. In a special series of experiments nitroglycerin in a concentration of $2 \cdot 10^{-3}$ M was added to the heart muscle extract in a test tube. Phosphorylase activity was determined by Cori's method [4]. Inorganic phosphate was determined by Sumner's method [10].

To obtain the tissue extract the heart was frozen with liquid nitrogen, ground into a powder, and extracted with 4 volumes of triphosphate buffer, pH 6.8, for 10 min.

EXPERIMENTAL RESULTS AND DISCUSSION

In the experiments in vitro in which nitroglycerin was added to heart muscle extract, no change was found in the phosphorylase activity, indicating that nitroglycerin has no direct action on phosphorylase. After intravenous injection of nitroglycerin into the rats a redistribution of the *a* and b forms of phosphorylase was observed, with a relative increase in the *a* form (Table 1).

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TABLE 1. Effect of Nitroglycerin on Phosphorylase Activity of Myocardial Tissue

	Number of exprmts.	Phosphorylase *a*	Phosphorylase b	Phosphorylase *a* + b	Percent of phosphorylase *a*[1]	Percent of phosphorylase b
		in μg-atoms/g tissue				
Control	10	34 (43–25)	23 (31–15)	56 (68–44)	62 (70–54)	38 (48–28)
Nitroglycerin in concentration of $2 \cdot 10^{-3}$ M (in vitro)	5	32 (37–27)	20 (28–12)	51 (57–45)	63 (67–59)	37 (41–33)
Nitroglycerin in dose of 1 mg/kg, intravenously after 1 min	8	26 (30–22)	12 (16–8)	38 (47–29)	71 (80–62)	29 (38–20)
» 5 »	5	27 (32–22)	23 (39–7)	50 (67–33)	54 (62–46)	46 (54–38)
» 10 »	5	30 (37–23)	17 (26–8)	47 (57–37)	65 (75–55)	35 (50–20)
» 20 »	5	29 (36–22)	14 (20–8)	43 (51–35)	68 (75–60)	32 (45–19)

Note: Confidence limits for phosphorylase *a* at P = 0.05% are mean values of all data for relative percentage of phosphorylase *a* in each experiment.

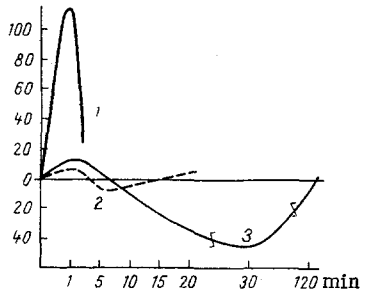

Fig. 1. Effect of nitroglycerin on catecholamine content and phosphorylase activity in the rat myocardium. Abscissa, time after injection of nitroglycerin; ordinate, values of parameters (in % of control): 1) noradrenalin in perfusion fluid; 2) phosphorylase *a*; 3) noradrenalin in myocardium.

The increase in the relative percentage of phosphorylase *a* was presumably connected with the adrenergic action of nitroglycerin, i.e., with its induction of the liberation of catecholamines which, acting on the β-adrenergic structures of the myocardium, activate adenyl cyclase. This, in turn, leads to activation of phosphorylase. This hypothesis was confirmed by the parallel course of the changes in the relative percentage of phosphorylase *a* and in the concentration of catecholamines in the myocardium observed after administration of nitroglycerin. Data for the changes in the total catecholamine content in the myocardium, the free noradrenalin determined in the perfusion fluid flowing from the isolated heart, and the change in phosphorylase activity in the myocardium of the rats under the influence of nitroglycerin are shown in Fig. 1. It will be clear from Fig. 1 that during the first minute after the injection of nitroglycerin catecholamines were liberated from the myocardium. The increase in the concentration of free noradrenalin in the perfusion fluid flowing from the isolated rat heart reached a maximum by the end of the first minute and returned to its initial level 3 min after the injection [2]. A gradual decrease in the total catecholamine content was observed in the myocardial tissue. The relative percentage of phosphorylase *a* also increased during the first minute after the injection of nitroglycerin. Correlation was thus observed between the time of liberation of catecholamines and the increase in the relative proportion of active phosphorylase *a* in the myocardium under the influence of nitroglycerin.

These findings agree with the results of investigations indicating that nitroglycerin gives sympathomimetic effects manifested by an increase in the frequency and strength of the cardiac contractions [1, 8, 9].

However, despite the increase in the relative percentage of phosphorylase *a*, the absolute phosphorylase activity fell and remained below its initial level 20 min after the injection of nitroglycerin, when the normal relative percentage of phosphorylase *a* had been restored. This fact indicates that the mechanism of action of nitroglycerin on myocardial metabolism is not limited to its adrenergic effect.

The possibility cannot be ruled out that the decrease in phosphorylase activity is linked with the reorganization of myocardial metabolism in the direction of stimulation of oxidation [11]. With an increase in the oxygen consumption and in the intensity of oxidative conversions the activity of glycolytic enzymes, including phosphorylase, may be reduced [3]. The action of nitroglycerin on the phosphatase of phosphory-

lase is another possibility. Fluctuations in the activity of phosphorylase b and phosphorylase *a* 5-10 min after the injection of nitroglycerin can be attributed to the activity of phosphatase.

These experiments thus confirm the presence of a peripheral adrenergic component in the mechanism of action of nitroglycerin.

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