STUDIES ON THE MECHANISM FOR THE ISOPROTERENOL-INDUCED STIMULATION OF CARDIAC GLUCOSE-6-PHOSPHATE DEHYDROGENASE

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1. Introduction

According to results of previous investigations the catecholamines isoproterenol and dopamine induce in the rat myocardium an enhancement of glucose-6-phosphate dehydrogenase activity (EC 1.1.1.49) and an elevation of the available pool of 5-phosphoribosyl-1-pyrophosphate (PRPP), one of the endproducts of the hexose monophosphate shunt [1]. These findings indicated that catecholamines stimulate the myocardial hexose monophosphate shunt apart from their well-known effect on glycogenolysis [2–4]. Since only glucose-6-phosphate dehydrogenase, the first and rate-limiting enzyme of the oxidative branch of this shunt [5] showed a marked response to catecholamines, it was of interest to examine the mechanism that may be responsible for this stimulation.

Essentially two experimental approaches were utilized to study this question:

1. The time course of the isoproterenol-induced alterations in glucose-6-phosphate dehydrogenase activity was examined and compared to that of the changes in adenosine 3′,5′-monophosphate (cAMP) levels and in protein synthesis. Both parameters have been shown to be enhanced by catecholamines [2–4,6].
2. The effect of cycloheximide on the isoproterenol-induced enhancement of cardiac glucose-6-phosphate dehydrogenase activity was studied.

The results reported in this paper suggest that an increased new synthesis of enzyme protein is involved in bringing about the enhancement of glucose-6-phosphate dehydrogenase activity in the rat heart under the influence of isoproterenol.

2. Materials and methods

All experiments were done on female Sprague-Dawley rats (200–220 g body wt) maintained on a standard diet of Altromin® with free access to water. Isoproterenol was obtained from Fluka GmbH, Neu-Ulm, the β-receptor blocking agent atenolol was a gift from ICI-Pharma, Heidelberg. Cycloheximide was purchased from Sigma Chemie, München, [1-^{14}C]-glycine (spec. act. 58 mCi/mmol) from Amersham Buchler, Braunschweig. Isoproterenol was injected subcutaneously in a dose of 25 mg/kg either alone or simultaneously with atenolol (2 mg/kg). Cycloheximide was administered intraperitoneally 6 h prior to the application of isoproterenol as well as 6 and 12 h thereafter (0.6 mg/kg, respectively).

In experiments in which cardiac cAMP levels were to be determined, the animals were anesthetized with diethyl ether at different times after administration of isoproterenol, and the hearts were rapidly removed after thoracotomy and immersed in Freon® at −156°C. When the activity of glucose-6-phosphate dehydrogenase was to be measured, the rats anesthetized with diethyl ether were thoracotomized, a cannula was inserted into the ascending aorta, placed just above the aortic valve and tightly fixed. The hearts were rapidly excised, and the coronary arteries were purfused with ice-cold KCl solution (0.15 M containing 8 ml 0.02 M $KHCO_3$/l) to stop beating and

to remove blood. In experiments designed to evaluate protein synthesis, the animals were injected intravenously with [1-^{14}C]glycine (0.25 mCi/kg). After an exposure time of 60 min the hearts were rapidly excised and immersed in liquid nitrogen.

Myocardial cAMP was determined by the methods in [7]. The procedures for homogenisation of the hearts, centrifugation, dialysis of the supernatants and measurement of glucose-6-phosphate dehydrogenase activity were essentially those reported [8,9]. Protein concentration in the dialysate was determined using the modified biuret reaction [10]. The specific activity of glucose-6-phosphate dehydrogenase was expressed as units/g protein. Relative rates of cardiac protein synthesis were calculated by relating the radioactivity of total myocardial proteins to the mean specific activity of the intracellular glycine by the methods in [11]. The data on protein synthesis were expressed as percentage of the control values.

3. Results

Figure 1 shows the effects of isoproterenol on myocardial cAMP, on cardiac protein synthesis and on glucose-6-phosphate dehydrogenase activity. cAMP was maximally enhanced already within 1 h of isoproterenol administration and dropped to baseline levels after 12 h (fig.1A). Protein synthesis was increased after 5 h reaching maximal stimulation after 12 h (fig.1B). The activity of glucose-6-phosphate dehydrogenase showed the first response to isoproterenol after 12 h and attained the maximal increase after 48 h (fig.1C).

Figure 2 demonstrates that the enhancement of glucose-6-phosphate dehydrogenase activity seen after 24 h (fig.1C) was almost completely prevented when the β-receptor blocker atenolol was applied simultaneously with isoproterenol. Cycloheximide did also

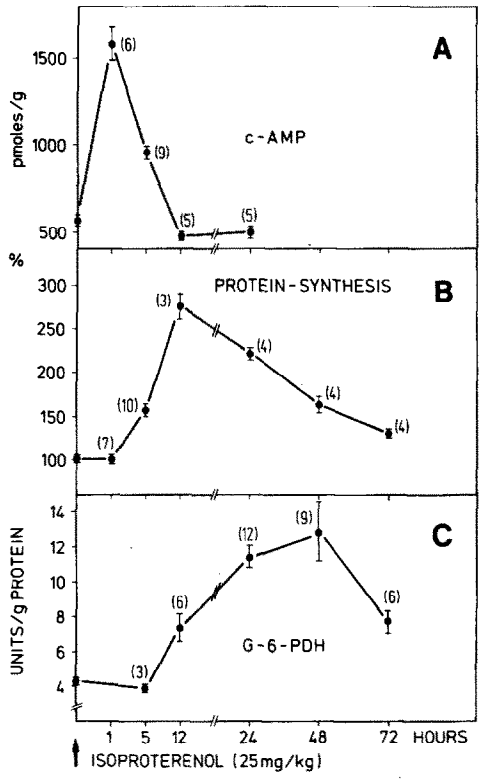

Fig.1. Effects of isoproterenol (25 mg/kg, administered subcutaneously) on cAMP levels, protein synthesis and glucose-6-phosphate dehydrogenase activity in rat hearts. Mean values ± SEM, number of experiments in parentheses.

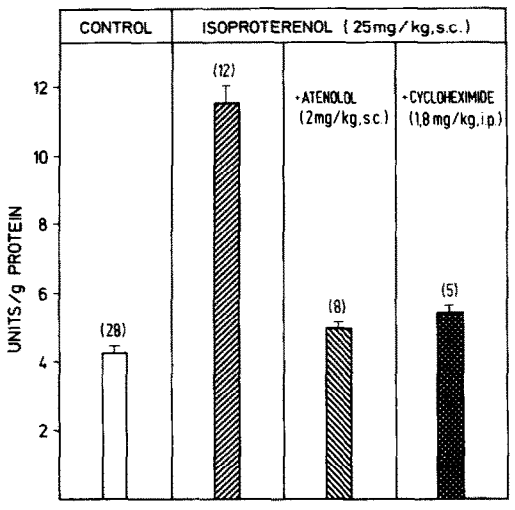

Fig.2. Myocardial glucose-6-phosphate dehydrogenase activity 24 h after subcutaneous administration of isoproterenol either alone or in combination with atenolol or cycloheximide. Atenolol was applied subcutaneously at the same time as isoproterenol. Cycloheximide was administered intraperitoneally 6 h prior to isoproterenol application (0.6 mg/kg) and 6 and 12 h thereafter (0.6 mg/kg, respectively). Mean values ± SEM, number of experiments in parentheses.

markedly attenuate the isoproterenol-induced stimulation of glucose-6-phosphate dehydrogenase activity. It did not affect the enzyme activity when given alone (data not shown).

4. Discussion

From the time course of changes given in fig.1 it is evident that glucose-6-phosphate dehydrogenase activity increased with a considerable time lag. It seemed therefore reasonable to assume that some metabolic processes, probably protein synthesis, must be enhanced in order to bring about this stimulation in our experimental condition. In fact, isoproterenol caused an enhancement of protein synthesis [6] that occurred prior to the increase in glucose-6-phosphate dehydrogenase activity. Moreover, cycloheximide which blocks protein synthesis at the translational level [12,13] and which prevented the isoproterenol-induced elevation of protein synthesis (data not shown), markedly attenuated glucose-6-phosphate dehydrogenase stimulation (fig.2). From these results it appears that this enhancement is mainly due to increased new synthesis of enzyme protein.

From the time-dependent alterations presented in fig.1 it is furthermore evident that the elevation of cAMP is clearly the first event that occurred immediately after stimulation of cardiac β-adrenergic receptors with isoproterenol. When this effect was antagonized by β-receptor blockade with atenolol (fig.2), the stimulation of cardiac glucose-6-phosphate dehydrogenase activity was prevented. Thus it may be concluded that the enhancement of glucose-6-phosphate dehydrogenase activity is mediated via cardiac β-receptors and that cAMP is involved in bringing about the enhancement of protein synthesis and eventually of glucose-6-phosphate dehydrogenase.

Although the mechanisms involved are not known, it is tempting to speculate that isoproterenol may affect RNA synthesis via stimulation of nuclear protein (histone and nonhistone) phosphorylation through the cAMP-dependent protein kinase system [14] thus resulting in increased synthesis of glucose-6-phosphate dehydrogenase. Such a sequence of events would eventually lead to stimulation of the hexose monophosphate shunt and thus contribute to keep the available pool of PRPP elevated. The sustained increase of the available PRPP pool can be considered to be responsible for the maintenance of the enhanced purine biosynthesis which has been shown to occur under the influence of isoproterenol [15].

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