β-Adrenergic Agonists Stimulate the Oxidative Pentose Phosphate Pathway in the Rat Heart

Heinz-Gerd Zimmer, Hans Ibel, and Ulrich Suchner

The oxidative pentose phosphate pathway is poorly developed in the rat heart compared with other organs, since the activity of glucose-6-phosphate dehydrogenase (G-6-PDH), the first and rate-limiting enzyme of the oxidative pentose phosphate pathway, is low. As a consequence, the available pool of 5-phosphoribosyl-1-pyrophosphate and the rate of adenine nucleotide biosynthesis are limited. Isoproterenol, 24 hours after subcutaneous administration at 0.1, 1, and 25 mg/kg, stimulated the activity of G-6-PDH in whole hearts dose-dependently from 4.3±0.16 (control) to 6.6±0.35, 10.3±0.82, and 11.5±0.56 units/g protein, respectively. The activity of 6-phosphogluconate dehydrogenase, another of the enzymes in the oxidative pentose phosphate pathway, remained unchanged. G-6-PDH activity started to increase 12 hours after isoproterenol application, when the glycogenolytic and functional response was over, and reached a peak value between 24 and 48 hours. This stimulating effect was also demonstrated in cardiac myocytes that were isolated 28 hours after isoproterenol application. β-Receptor blockade with atenolol reduced the isoproterenol-induced increase in cardiac G-6-PDH activity by 90%. Cycloheximide, which inhibits translation, and actinomycin D, which interferes with transcription, attenuated it by 83% and 78%, respectively. These results indicate that cardiac β-adrenergic receptors and enzyme protein synthesis are involved in this effect. Other β-sympathomimetic agents such as dopamine, dobutamine, fenoterol, salbutamol, and terbutaline also stimulated myocardial G-6-PDH activity in a time- and dose-related manner. The calcium antagonist D 600 (gallopamil) reduced the isoproterenol-elicited stimulation by 65%, and verapamil blunted the fenoterol-induced increase by 50%. This suggests that Ca2+ ions also contribute to the stimulation of the cardiac oxidative pentose phosphate pathway. (Circulation Research 1990;67:1525–1534)

The oxidative pentose phosphate pathway (PPP) is important, since it is the link between carbohydrate and fatty acid as well as purine and pyrimidine nucleotide metabolism (Figure 1). Glucose-6-phosphate (G-6-P), originating from glycolysis or from glucose taken up by the myocardial cell, is metabolized predominantly via the oxidative pentose phosphate pathway. A certain portion, however, enters the oxidative branch of the PPP of which glucose-6-phosphate dehydrogenase (G-6-PDH) is the first and rate-limiting enzyme. This pathway has two major functions to fulfill: 1) It provides reducing equivalents in the form of NADPH, which can be used for the synthesis of free fatty acids and for the conversion of oxidized glutathione to reduced glutathione. This is important for the detoxification of reactive oxygen species via glutathione peroxidase. 2) In this pathway, ribose-5-phosphate is generated, which can be transformed to 5-phosphoribosyl-1-pyrophosphate (PRPP), and this is an essential precursor substance for the synthesis of both pyrimidine and purine nucleotides. There are connections between the oxidative PPP and glycolysis at two levels via the transaldolase and transketolase reactions.

It has been inferred that the capacity of the oxidative PPP is low in the rat myocardium; this inference is drawn from the fact that adenine nucleotide biosynthesis is small because of the limited availability of PRPP. It has been shown for a variety of species, including humans, that the activity of myocardial G-6-PDH is low compared with that of 6-phosphogluconate dehydrogenase (6-PGDH). The critical step in the oxidative PPP, the G-6-PDH reaction, can be bypassed with ribose, which elevates via ribose-5-phosphate the PRPP pool and stimu-
lates adenine nucleotide biosynthesis. It has been demonstrated previously that an experimentally induced decline in the cardiac ATP pool can be attenuated or even prevented by application of ribose.

As to the regulation of this pathway, it is known from studies on the liver that G-6-PDH is regulated by the NADP+/NADPH ratio, which exerts a "fine control." G-6-PDH is always inhibited by NADPH, and this inhibition can be overcome by oxidized glutathione. It has been demonstrated that the flow through the oxidative PPP in the isolated perfused rat heart can be enhanced acutely and rapidly by an oxidizing agent that increases the oxidized glutathione level and the NADP+/NADPH ratio.

It was the primary aim of this study to assess quantitatively the capacity of the oxidative PPP in the rat heart compared with other organs. To do this, the activity of G-6-PDH, the available pool of PRPP, and the rate of adenine nucleotide biosynthesis were determined (Figure 1). The second aim was to examine whether β-adrenergic agonists could influence the oxidative PPP. These agents have already been shown to activate glycogenolysis, to stimulate cardiac adenine nucleotide biosynthesis, and to have pronounced positive chronotropic and inotropic effects.

**Materials and Methods**

The experiments were done on female Sprague-Dawley rats (200–250 g body wt) obtained from Mus Rattus, Brunthal, F.R.G. They were fed a control rat chow diet (Altromin C 100, Altromin GmbH, Lage, F.R.G.) with free access to tap water. The substances were administered either as a subcutaneous injection or as a continuous intravenous infusion via a catheter (Vygon, Aachen, F.R.G.) that was positioned in the right jugular vein. The catheter was connected to a 20-ml syringe placed in an infusion pump (Infors AG, Basel, Switzerland). The infusion rate was 4 ml/kg/hr.

Isoproterenol was purchased from Boehringer Ingelheim, Ingelheim, F.R.G., or Sigma Chemie GmbH, Deisenhofen, F.R.G. It was dissolved in 0.9% NaCl containing acetic acid (0.2 g/l) and was subcutaneously injected in different doses. Dopamine was kindly provided by Giulini Pharma, Hanover, F.R.G. It was administered as a subcutaneous injection and as a continuous intravenous infusion. Dobutamine was a gift from Lilly GmbH, Giessen, F.R.G. Fenoterol was obtained from Boehringer Ingelheim; terbutaline was kindly provided by Draco Company, Lund, Sweden; and salbutamol was obtained from Kettelhack-Riker Pharma, Borken, F.R.G. These substances were administered as continuous intravenous infusions containing acetic acid. The syringes containing the substances were protected against light. Compound D 600 (administered as a subcutaneous injection) and verapamil (administered as a continuous intravenous infusion) were donated by Knoll AG, Ludwigshafen, F.R.G. [1-14C]Glycine (specific activity, 54.2 mCi/mmol), [8-14C]adenine (specific activity, 54.3 mCi/mmol), and [U-14C]adenine (specific activity, 282 mCi/mmol) were purchased from the Radiochemical Centre, Amersham, U.K., and were injected into the tail vein.

**Measurement of Metabolic Parameters**

The activities of G-6-PDH (EC 1.1.1.49) and 6-PGDH (EC 1.1.1.44) were measured according to the methods of Glock and McLean. After various periods of in vivo exposure to the different substances, the rats were anesthetized with ether, and a cannula was placed in the ascending aorta after thoracotomy and tightly fixed there. The hearts were quickly excised, and the coronary arteries were perfused via the cannula with an ice-cold KCl solution (0.15 M containing 8 ml of 0.02 M KHCO3/l) to remove blood and to stop the beating of the heart. After homogenization of the hearts in the perfusion medium, pH adjustment (7.0), and centrifugation (ultracentrifuge model L5-65, Beckman Instruments, Inc., Fullerton, Calif.) at 20,000 rpm for 30 minutes, the supernatants were dialyzed overnight. The enzyme activities were then measured spectrophotometrically. Protein concentration in the dialysate was determined by using the modified biuret reaction.
The mean specific activity of both enzymes was expressed as units per gram protein.

Levels of cyclic AMP (cAMP) were determined according to the method of Gilman. The content of G-6-P was measured using the method of Lang and Michal. The available pool of PRPP was assessed from the radioactivity of adenine nucleotides after 5, 10, and 15 minutes of in vivo exposure of the rats to intravenously injected [8-14C]adenine or [U-14C]adenine. Since PRPP is consumed in the reaction by which adenine is converted to AMP catalyzed by adenine phosphoribosyltransferase (EC 2.4.2.7) and since AMP is in rapid equilibrium with ADP and ATP via the myokinase reaction (EC 2.7.4.3), the total radioactivity of the adenine nucleotides ATP + ADP + AMP is an indirect measure of the available pool of PRPP. After the indicated periods of vivo exposure, the hearts were excised in ether anesthesia and frozen in liquid nitrogen. After tissue extraction, adenine derived from adenine nucleotides by hydrolysis was separated, and the radioactivity was counted in a tri-carb liquid scintillation spectrometer (model 3380, Packard Instruments, Downers Grove, Ill.)

The de novo synthesis (biosynthesis) of adenine nucleotides was measured on the basis of the incorporation of [1-14C]glycine (250 μCi/kg body wt injected into the tail vein) into adenine nucleotides. After an in vivo exposure time of 60 minutes, the hearts were excised in ether anesthesia and frozen in liquid nitrogen; the radioactivity was measured after extraction, separation, and purification of the adenine nucleotides, according to methods described earlier. Rates of de novo synthesis were calculated by relating the total radioactivity of adenine nucleotides due to the incorporation of [1-14C]glycine to the mean specific activity of the tissue glycine precursor pool, which was determined with an amino acid analyzer (model BC 200, BioCal München, F.R.G.). Rates of protein synthesis were determined in the same hearts in which adenine nucleotide biosynthesis was measured by relating the radioactivity of total cardiac proteins to the mean specific activity of the amino acid precursor pool.

Measurement of Left Heart Function

When the function of the left heart was measured, the rats were anesthetized with 80 mg/kg i.p. thiobutabarbital sodium (Inactin, Byk Gulden, Konstanz, F.R.G.). The depth of anesthesia was tested by eliciting reflexes in the legs by forceps. After tracheotomy, a catheter was placed into the trachea to maintain airway patency, to allow suction of secretions, if necessary, and to institute artificial respiration in case of an emergency.

The ultraminiature catheter pressure transducer (model PR-249, Millar Instruments, Inc., Houston) was used for left heart catheterization. This catheter was inserted into the right carotid artery and advanced upstream from the aorta into the left ventricle. It was attached to a Millar control unit (model TC-100), which was connected to an HSE ultraminiature catheter pressure transducer (model PR-249, Millar Instruments, Inc., Houston) was used for left heart catheterization. This catheter was inserted into the right carotid artery and advanced upstream from the aorta into the left ventricle. It was attached to a Millar control unit (model TC-100), which was connected to an HSE ultraminiature catheter pressure transducer (model PR-249, Millar Instruments, Inc., Houston) was used for left heart catheterization. This catheter was inserted into the right carotid artery and advanced upstream from the aorta into the left ventricle. It was attached to a Millar control unit (model TC-100), which was connected to an HSE ultraminiature catheter pressure transducer (model PR-249, Millar Instruments, Inc., Houston) was used for left heart catheterization. This catheter was inserted into the right carotid artery and advanced upstream from the aorta into the left ventricle. It was attached to a Millar control unit (model TC-100), which was connected to an HSE ultraminiature catheter pressure transducer (model PR-249, Millar Instruments, Inc., Houston) was used for left heart catheterization. This catheter was inserted into the right carotid artery and advanced upstream from the aorta into the left ventricle. It was attached to a Millar control unit (model TC-100), which was connected to an HSE ultraminiature catheter pressure transducer (model PR-249, Millar Instruments, Inc., Houston) was used for left heart catheterization. This catheter was inserted into the right carotid artery and advanced upstream from the aorta into the left ventricle. It was attached to a Millar control unit (model TC-100), which was connected to an HSE ultraminiature catheter pressure transducer (model PR-249, Millar Instruments, Inc., Houston) was used for left heart catheterization. This catheter was inserted into the right carotid artery and advanced upstream from the aorta into the left ventricle. It was attached to a Millar control unit (model TC-100), which was connected to an HSE ultraminiature catheter pressure transducer (model PR-249, Millar Instruments, Inc., Houston) was used for left heart catheterization. This catheter was inserted into the right carotid artery and advanced upstream from the aorta into the left ventricle. It was attached to a Millar control unit.
measured (Figure 2). There was a parallel behavior of these three parameters in the organs examined. They were all highest in kidney, followed by liver, heart, and skeletal muscle. From these results, it appears that the capacity of the oxidative PPP is lowest in heart and skeletal muscle. To substantiate this, ribose was administered. It did not alter the PRPP availability and adenine nucleotide biosynthesis in kidney and liver. In heart and skeletal muscle, however, there was a marked increase (Figure 2, middle and bottom panels).

It was of interest to determine whether the oxidative PPP in the heart could be enhanced in intact rats so that more PRPP would be provided for nucleotide metabolism. In a first attempt, the effect of isoproterenol on the activity of cardiac G-6-PDH and 6-PGDH was tested. Isoproterenol stimulated the activity of G-6-PDH in a dose-dependent man-

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<th>G-6-PDH (units/g protein)</th>
<th>6-PGDH (units/g protein)</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>28</td>
<td>4.3±0.16</td>
<td>11.2±0.25</td>
</tr>
<tr>
<td>ISO</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 mg/kg</td>
<td>7</td>
<td>6.6±0.35*</td>
<td>11.3±0.33</td>
</tr>
<tr>
<td>1.0 mg/kg</td>
<td>11</td>
<td>10.3±0.82*</td>
<td>12.3±0.52</td>
</tr>
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<td>25.0 mg/kg</td>
<td>12</td>
<td>11.5±0.56*</td>
<td>12.2±0.64</td>
</tr>
</tbody>
</table>

Values are mean±SEM; n=number of experiments; G-6-PDH, glucose-6-phosphate dehydrogenase; 6-PGDH, 6-phosphogluconate dehydrogenase; ISO, isoproterenol.

*p<0.0005 vs. control.

ner but had no influence at all on the activity of 6-PGDH (Table 1).

The changes in metabolic and functional parameters that occurred over a time period of 72 hours subsequent to the highest dose of isoproterenol are shown in Figure 3. There was an immediate increase in the cAMP content as well as in the content of G-6-P. Parallel to these metabolic alterations, heart rate and LV dP/dt max were also elevated. After 12 and 24 hours, these metabolic and functional parameters had returned to the control level. However, adenine nucleotide biosynthesis, which was also enhanced very early, remained elevated, although at a somewhat lower level. With a time lag there was an increase in cardiac protein synthesis, and when this was clearly enhanced, the activity of myocardial G-6-PDH started to be stimulated. The peak was reached quite late, after 24 and 48 hours.

After 28 hours of in vivo exposure to isoproterenol (25 mg/kg, subcutaneously applied), G-6-PDH activity was measured in freshly isolated cardiac myocytes obtained from the left ventricle and compared with that of NaCl-injected rats. In myocytes from control rats, it was 0.3±0.05 units/g protein (n=7); in those from isoproterenol-treated rats, it was significantly increased (p<0.0005) to 3.2±0.55 units/g protein (n=6).

From the time course (Figure 3), it is suggestive that activation of G-6-PDH in the heart is dependent on protein synthesis and thus may reflect an increased new synthesis of enzyme protein. Therefore, the effect of inhibitors of protein synthesis on G-6-PDH was examined. Cycloheximide and actinomycin D, which had no effect on cardiac G-6-PDH activity by themselves, attenuated the isoproterenol-induced increase by 83% and 78%, respectively (Table 2).

β-Receptor blockade with atenolol reduced the isoproterenol-elicted enhancement of G-6-PDH activity by 90%. The calcium antagonist compound D 600 (gallopamol) blunted it by 65% (Figure 4). When dopamine was administered either as subcutaneous injection or as continuous intravenous infusion, there was a time- and dose-dependent stimulation of cardiac G-6-PDH activity, which was almost completely abolished with atenolol (Table 3). Dobutamine also
stimulated G-6-PDH activity, though not as markedly as did dopamine (Table 3).

To examine whether the available pool of PRPP may also be affected by these substances, the incorporation of [14C]adenine into myocardial adenine nucleotides was determined under the influence of isoproterenol, dopamine, and dobutamine. Isoproterenol increased the incorporation of labeled adenine both after 5 and 24 hours, subsequent to subcutaneous injection. Also, dopamine and dobutamine stimulated this process after 48 hours of continuous intravenous infusion (Figure 5).

Since dopamine and dobutamine are used clinically, it was of interest whether other sympathomimetic agents that are given to patients may affect the cardiac PPP. Fenoterol, salbutamol, and terbutaline stimulated G-6-PDH activity, though to different

![Table 2](image)

**Table 2. Activity of Myocardial Glucose-6-phosphate Dehydrogenase After a Single Subcutaneous Injection of Isoproterenol and the Effect of Cycloheximide and Actinomycin D in Control and Isoproterenol-Treated Rats**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>G-6-PDH (units/g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28</td>
<td>4.3±0.16</td>
</tr>
<tr>
<td>ISO</td>
<td>12</td>
<td>11.5±0.56*</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>3</td>
<td>4.4±0.17</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>4</td>
<td>4.9±0.29</td>
</tr>
<tr>
<td>ISO+cycloheximide</td>
<td>5</td>
<td>5.5±0.21†</td>
</tr>
<tr>
<td>ISO+actinomycin D</td>
<td>6</td>
<td>5.9±0.40†</td>
</tr>
</tbody>
</table>

Values are mean±SEM; n=number of experiments. G-6-PDH, glucose-6-phosphate dehydrogenase; ISO, isoproterenol. ISO was administered in a dose of 25 mg/kg. Cycloheximide (0.6 mg/kg) and actinomycin D (0.3 mg/kg) were injected intraperitoneally for the first 5 hours before ISO administration. The same doses of cycloheximide and actinomycin D were given 6 and 12 hours after ISO injection. All measurements were made 24 hours after ISO administration.

*p<0.0005 and t<0.025 vs. control.

![Figure 3](image)

**Figure 3.** Graphs showing effects of isoproterenol on the levels of cardiac cyclic AMP (cAMP, ——) and glucose-6-phosphate (G-6-P, ——) (top panel), on heart rate (——) and left ventricular (LV) dP/dt max (—–—) (second panel from top), on myocardial adenine nucleotide synthesis (middle panel), on protein synthesis (second panel from bottom), and on the activity of cardiac glucose-6-phosphate dehydrogenase (G-6-PDH) (bottom panel) in rats. Values are mean±SEM; the number of animals at each point in time is given in parentheses.

![Figure 4](image)

**Figure 4.** Bar graph showing activity of myocardial glucose-6-phosphate dehydrogenase 24 hours after subcutaneous injection of isoproterenol (ISO) alone and in combination with the β-receptor blocker atenolol or the calcium antagonist compound D 600 (gallopamil). Atenolol (1 mg/kg s.c.) and D 600 (10 and 5 mg/kg s.c.) were given twice, 12 hours apart. Values are mean±SEM; number of experiments is indicated in parentheses.
degrees (Figure 6). None of these substances had an influence on cardiac 6-PGDH activity (data not shown).

Since fenoterol had the most marked effects, further experiments were done with this drug. Fenoterol stimulated cardiac G-6-PDH activity in a dose-dependent manner (Figure 7). To examine whether this increase can be influenced by blocking the calcium channel, the calcium antagonist verapamil was used. Verapamil antagonized the hemodynamic response to fenoterol effectively. Heart rate and LV systolic pressure were even lower than control values when verapamil was administered in combination with fenoterol (Figure 8). Yet, G-6-PDH activity was still increased by 30% in the presence of verapamil, while the pressure-rate product was even lower than control values (Figure 9).

### Table 3. Influence of Dopamine and Dobutamine on the Activity of Cardiac Glucose-6-phosphate Dehydrogenase in Rats

<table>
<thead>
<tr>
<th>Time period (hr)</th>
<th>n</th>
<th>G-6-PDH (units/g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14</td>
<td>4.5±0.17</td>
</tr>
<tr>
<td>Dopamine (500 mg/kg s.c.)</td>
<td>24</td>
<td>5.8±0.33*</td>
</tr>
<tr>
<td>Dopamine (500 mg/kg s.c.)</td>
<td>48</td>
<td>10.3±0.96†</td>
</tr>
<tr>
<td>Dopamine (500 mg/kg s.c.)+atenolol</td>
<td>48</td>
<td>5.0±0.45</td>
</tr>
<tr>
<td>Dopamine (1 mg/kg/hr i.v.)</td>
<td>48</td>
<td>5.7±0.25*</td>
</tr>
<tr>
<td>Dopamine (10 mg/kg/hr i.v.)</td>
<td>48</td>
<td>8.3±0.54†</td>
</tr>
<tr>
<td>Dobutamine (10 mg/kg/hr i.v.)</td>
<td>48</td>
<td>6.0±0.23*</td>
</tr>
</tbody>
</table>

Values are mean±SEM; n=number of experiments. G-6-PDH, glucose-6-phosphate dehydrogenase. Dopamine was administered as a single subcutaneous injection or as continuous intravenous infusion. Dobutamine was applied only as continuous intravenous infusion. Atenolol was injected subcutaneously in a dose of 10 mg/kg three times. Measurements of G-6-PDH activity were made after the indicated periods of time.

*tp<0.025 and †p<0.0005 vs. control.

### Discussion

Our comparative studies have shown that the capacity of the oxidative PPP was lower in heart and skeletal muscle than in kidney and liver. The assessment of this pathway was based on three independent parameters, G-6-PDH activity, PRPP availability, and adenine nucleotide biosynthesis, which were measured in different rats. The magnitude of these parameters corresponded very closely in each rat organ examined (Figure 2). The G-6-PDH activity was lower in the myocardium than in the kidney and liver.14 This supports earlier findings obtained in dog heart muscle homogenates,22 in isolated cardiac myocytes from rats,23 and in the rat during development.24 In the latter study, the activities of both G-6-PDH and 6-PGDH were always lower in the heart than in the liver.

The first and rate-limiting enzyme, G-6-PDH, seems to be responsible for the limited capacity of the oxida-
tive PPP in the heart, so that the available pool of PRPP and the rate of adenine nucleotide biosynthesis are very small.\(^4\) This limitation can be overcome by ribose, which bypasses the critical step, the G-6-PDH reaction. As a consequence, the PRPP pool and the rate of adenine nucleotide biosynthesis were elevated by ribose (Figure 2).\(^6,25\) Ribose has been shown to restore, partially or entirely, the adenine nucleotide pool in various pathophysiological situations, such as stimulation with isoproterenol,\(^6\) postischemic recovery,\(^7\) and myocardial infarction.\(^8\)

In skeletal muscle, G-6-PDH activity was even lower than in the heart,\(^26\) and adenine nucleotide biosynthesis was not measurable under control in vivo conditions. When ribose was administered, it was of the same order of magnitude as in the heart in the control situation (Figure 2). The conditions for measuring adenine nucleotide biosynthesis may be different in the isolated rat extensor digitorum longus muscle\(^27\) and in the perfused rat hindquarter preparation.\(^28\) In these preparations, adenine nucleotide biosynthesis was readily measurable.

That G-6-PDH is the rate-limiting enzyme of the oxidative PPP in the heart is also supported by our results obtained with isoproterenol. This B-adrenergic agonist induced a dose-dependent increase in G-6-PDH activity but did not affect 6-PGDH activity (Table 1). Parallel to the increase in G-6-PDH activity, the PRPP pool was elevated (Figure 5), and adenine nucleotide biosynthesis was enhanced (Figure 3). Our time course studies, however, revealed that the temporal relations were more complex. From the data in Figure 3, it appears that there were two distinct phases subsequent to B-receptor stimulation: The first phase, lasting about 5 hours, was characterized by the enhancement of glycogenolysis as evidenced by the elevation of cardiac cAMP and G-6-P levels, by the positive chronotropic and inotropic response, by the elevation of the PRPP pool (Figure 5), and by the immediate increase in adenine nucleotide biosynthesis. There was no change in G-6-PDH activity (Figure 3). During the second phase, after 12 hours subsequent to isoproterenol, glycogenolysis and heart function had become normalized, but adenine nucleotide biosynthesis was still increased, though at a lower level. The PRPP pool was also elevated (Figure 5), and protein synthesis as well as G-6-PDH activity started to increase steeply (Figure 3). Thus, the increase in the available PRPP pool and in adenine nucleotide biosynthesis coincided with the stimulation of glycogenolysis during the first phase and with the enhancement of G-6-PDH activity during the second phase.

Since G-6-PDH activity was unchanged during the first phase, a mechanism other than an enhanced flow through the oxidative PPP may be responsible for the increase in the PRPP pool and in adenine nucleotide biosynthesis. One possibility is that not only glycogenolysis but also glycolysis is stimulated by isoproterenol. This could then lead to an increased availability of PRPP through the nonoxidative PPP via the transaldolase and transketolase reactions. The activities of these enzymes were shown to be much higher than those of G-6-PDH and 6-PGDH in muscle.\(^26\) Furthermore, the large amounts of NAD\(^+\) present in the heart (29) may favor glycolysis, whereas the small quantity of NADP\(^+\) may limit the oxidative PPP. In this context it is interesting to mention that triiodothyronine did not affect cardiac G-6-PDH activity,\(^29\) yet the cAMP level as well as adenine nucleotide and protein synthesis were in-

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PRPP Pool Increase (%)</th>
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<tbody>
<tr>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>Fenoterol 1 mg/kg</td>
<td>0.5</td>
</tr>
<tr>
<td>Fenoterol 10 mg/kg</td>
<td>10</td>
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Figure 6. Bar graph showing effect of fenoterol, salbutamol, and terbutaline (1 mg/kg/hr each), administered as a continuous intravenous infusion for 48 hours, on the activity of cardiac glucose-6-phosphate dehydrogenase in rats. Values are mean±SEM; number of animals is indicated in parentheses.

Figure 7. Bar graph showing dose-related increase in the activity of cardiac glucose-6-phosphate dehydrogenase in rats after 48 hours of continuous intravenous infusion of fenoterol. Values are mean±SEM; number of animals is indicated in parentheses.
increased, suggesting stimulation of glycolysis and of the nonoxidative branch of the PPP.

Our time-course study (Figure 3) also provided some hints as to the mechanism for the increase in G-6-PDH activity. G-6-PDH activity did not increase before protein synthesis was enhanced. From this temporal relationship it can be suggested that activation of G-6-PDH in the heart depends on protein synthesis and thus may reflect an increased new synthesis of enzyme protein. Both interference of transcription with actinomycin D and inhibition of translation by cycloheximide attenuated the isoproterenol-induced G-6-PDH stimulation by about 80% (Table 2). Thus, it appears that synthesis of new enzyme protein, that is, enzyme induction, is involved in the observed stimulation of G-6-PDH activity. But what is the mechanism for the enhancement of protein synthesis? In this regard, the β-receptor-mediated cAMP elevation seems to play an important role. When the increase in cAMP was prevented by β-blockers, the isoproterenol-induced enhancement in adenine nucleotide and protein synthesis and in G-6-PDH (Figure 4) was almost entirely prevented. Also, the dopamine-elicited stimulation was abolished by atenolol (Table 3). Therefore, it may be that β-adrenergic agonists, via elevation of intracellular levels of cAMP, induce the transcription of the respective genes by activating a cAMP-dependent protein kinase and by phosphorylation of a protein factor that links kinase activation with transcription of cAMP-responsive genes.

It is interesting that the catecholamines epinephrine and isoproterenol also stimulated the oxidative PPP in the monkey palm eccrine sweat gland and that this could be suppressed by the β-receptor blocker propranolol.

Apart from activation of the adenylate cyclase–cAMP system, Ca2+ ions seem also to contribute to the stimulation of G-6-PDH activity. When the calcium antagonists D 600 (gallopamil) and verapamil were applied in combination with isoproterenol or fenoterol, the catecholamine-induced increase in G-6-PDH activity was attenuated by 65% (Figure 4) and 50% (Figure 9), respectively. Similarly, D 600 blunted both the isoproterenol-induced increase in cAMP and in adenine nucleotide biosynthesis by 50%. The remaining stimulation of G-6-PDH activity occurred independent of the function of the heart: The isoproterenol-mediated stimulation of G-6-PDH activity took place at a time when the positive chronotropic and inotropic effect was over (Figure 3). Moreover, the fenoterol-induced increase in heart function was abolished by verapamil to such an extent that heart rate and LV systolic pressure (Figure 8) and the pressure–rate product (Figure 9) were even lower than control, yet the G-6-PDH activity was still elevated. Thus, there was a dissociation between function and metabolism of the heart in these experimental conditions.

It is interesting that not only isoproterenol but also clinically applied sympathomimetic substances stimulate the oxidative PPP. Dopamine and dobutamine were applied for 24 or 48 hours, since the time-course studies with isoproterenol had revealed the peak G-6-PDH activity to be during this period of time (Figure 3).
These agents act directly at cardiac $\beta_1$-receptors. Accordingly, the effect of dopamine on myocardial G-6-PDH activity was prevented by the $\beta_1$-selective blocker atenolol (Table 3). Also, sympathomimetic agents that interact with $\beta_2$-receptors, such as fenoterol, salbutamol, and terbutaline, are used for the treatment of bronchoconstrictive disease-stimulated G-6-PDH activity after an in vivo exposure time of 48 hours (Figures 6 and 7). Considering the high doses that were applied in our studies, this effect may be due to the influence on cardiac $\beta_1$-receptors. Apart from their positive inotropic effect, all these sympathomimetic agents had metabolic effects that ultimately tend to restore the cardiac ATP pool (Figure 1). This can be considered as a beneficial effect in the treatment of heart failure.

A final aspect concerns the cellular compartment in which the oxidative PPP is localized within the myocardium. It has been shown in this study that G-6-PDH activity was present in freshly isolated cardiac myocytes and that it was increased about 10-fold after 28 hours of in vivo exposure to a high dose of isoproterenol. Since G-6-PDH activity was lower in isolated cardiac myocytes compared with whole heart, other cell elements in the myocardium must also have this enzyme. The coronary endothelial cells can be excluded as a potential site, since the biosynthesis of adenine nucleotides, which is dependent on the oxidative PPP, was inhibited by isoproterenol. A likely cellular compartment may therefore be the connective tissue. Since isoproterenol in the doses applied induces focal myocardial cell lesions, the observed increase in G-6-PDH could well have occurred in inflammatory or connective tissue cells. It is known that the oxidative PPP is increased markedly subsequent to experimental myocardial infarction. However, when the isoproterenol-induced focal myocardial injury was prevented by simultaneous administration of a calcium antagonist, G-6-PDH activity was still enhanced (Figure 4). Furthermore, the isoproterenol-induced percent increase was much higher in isolated cardiac myocytes than in the whole heart. That the oxidative PPP is active in isolated cardiac myocytes and that it can be altered experimentally has also been shown in a previous study.

In summary, all $\beta$-adrenergic agonists tested stimulated the oxidative PPP in the rat heart in vivo in a dose- and time-related manner. An increase in the oxidative PPP has also been observed during the development of cardiac hypertrophy due to aortic constriction. Interestingly, other positive inotropic agents such as ouabain (authors' unpublished observations) and triiodothyronine had no effect. Therefore, the stimulation of the oxidative PPP seems to be a new metabolic effect that is characteristic for sympathomimetic agents.

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References


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