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EFFECT OF ISOPROTERENOL ADMINISTRATION ON RAT HEART GLUTATHIONE STATUS

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The intraperitoneal administration of 3, 10 and 80 mg/Kg isoproterenol produced in the cardiac muscle a dose dependent increase of GSH content and a slight elevation of GSSG content. In addition, the treatment with the catecholamine at the doses of 3 and 10 mg/Kg produced a slight decrease of the mixed glutathione disulfides level, whilst at the dose of 80 mg/Kg, this effect was more pronounced. These changes were not accompanied by modifications of the activities of the enzymes glutathione peroxidase, glutathione reductase and glutathione S-transferase.

Key words: isoproterenol; glutathione; mixed disulfides; glutathione peroxidase; glutathione reductase; glutathione S-transferase; heart

Abbreviations: XSSG, protein-bound glutathione mixed disulfides; NEM, N-ethylmaleimide.

INTRODUCTION

Glutathione is the most abundant thiol in cells and its content in the cardiac muscle is approximately 1–2 mM as reduced glutathione (GSH) and 20–40 μM as oxidized glutathione (GSSG)[1].

In the heart these levels are maintained constant under a range of perfused conditions able to produce marked changes in the pattern of energy producing metabolism[2] or of glucose availability[1]. More recently variations in the heart GSH and GSSG contents following exposition of the cardiac muscle to anoxic or ischemic perfusions have been reported[3,4]. Moreover, the fact that the cardiac glutathione reductase activity is low, corresponding to 10% of the hepatic activity[5], it is possible that the intracellular GSSG level can increase, especially if the heart is submitted to hydroperoxide stress[5]. This characteristic probably allows the formation of mixed disulfide of glutathione (X-SSG). In fact, in the heart muscle the concentration of X-SSG is larger that the

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combined GSH + GSSG level, a condition opposite to that present in the liver tissue[6]. The level of rat heart X-SSG seems to change only slightly under the effect of starvation[6], but relatively little is known about the identity of such mixed disulfides. In the present study we have investigated the effect of isoproterenol administration on glutathione metabolism examining in addition the status of the cellular X-SSG.

MATERIALS AND METHODS

Male rats (Sprague–Dowley) weighing 200 g were injected intraperitoneally with isoproterenol dissolved in 0.9% NaCl. After 4 hours the hearts were quickly removed from the animals anesthetized with diethylether and perfused retrogradely through the aorta with cold saline solution in order to remove the blood. Portions of the heart were homogenized with Ultra-Turrax homogenizer in 1 M $HClO_4$-2 mM EDTA for GSH — GSSG measurements; for GSSG measurements, the acid solution contained 50 mM N-ethylmaleimide (NEM) in order to trap the GSH. The acid extracts were neutralized with 2 M K_2CO_3–0.3 M N-morpholino propane sulfonic acid and assayed immediately for GSH + GSSG content according to the method of Tietze[7]. The assay mixture for glutathione determination contained: 50 mM potassium phosphate buffer, pH 7.4, 1 mM EDTA; 0.1 mM 5′5-dithiobis (2-nitrobenzoic acid); 0.15 mM NADPH; 6 units/ml glutathione reductase and an appropriate volume of neutralized sample. After 1 min, the increase in absorbance at 412 nm was measured for 3 min using a double beam Perkin–Elmer spectrometer model 559.

The content of GSH + GSSG was calculated on the basis of a GSH calibration curve. For GSSG determination, the neutralized samples were previously extracted with diethylether in order to remove the excess of NEM. GSH content was calculated by subtracting the content of GSSG from the GSH — GSSG sum. The content of protein-bound glutathione mixed disulfides (X-SSG) was determined according to Harrish *et al.*[8]. The tissue homogenized in 3 vol of 0.1 M Tris/HCl, pH 8.0 was treated with 150 mg $NaBH_4$, 2 ml 8 M guanidine and two drops of n-octanol. After incubation at 40°C for 30 min, the excess of $NaBH_4$ was removed with 1.0 ml of 60% $HClO_4$. The supernatant collected after centrifugation at 20,000 g for 45 min was neutralized with 2M K_2CO_3–0.3 M N-morpholino propane sulfonic acid and estimated for total glutathione content (TG) as above described. Each single estimation was calibrated by the addition of a known amount of GSH (internal standard) to the cuvette. XSSG is calculated by the formula XSSG = TG − (GSH + GSSG).

For the measurements of enzymatic activities, portions of the heart tissues were homogenized in 10 vol ice-cold potassium phosphate buffer, pH 7.4, 30 mM KCl by using an Ultra-Turrax homogenizer. The suspensions were then sonicated three times at 40 watt for 20 sec (15 sec interval) and the supernatants obtained by centrifugation at 20,000 g for 15 min were utilized for measuring enzymatic activities. Glutathione peroxidase activity was measured by using cumene hydroperoxide as substrate[9] and glutathione reductase activity following the oxidation of NADPH as described by Mize and Langdon[10]. Glutathione S-transferase activity was assayed by monitoring the formation of S-(2-4-dinitrophenyl)-glutathione at 340 nm according to Habig *et al.*[11].

Protein concentration was estimated by the method of Bradford[12] using bovine serum albumine as standard.

TABLE I
Effect of isoproterenol administration on rat heart glutathione and mixed disulfides contents

	GSH[a]	GSSG[a]	X-SSG[b]
Control	2.55 ± 0.4	0.13 ± 0.07	0.36 ± 0.03
3 mg/Kg	4.28 ± 0.6°	0.25 ± 0.05	0.16 ± 0.02
10 mg/Kg	5.77 ± 1.0°°	0.27 ± 0.09	0.15 ± 0.06
80 mg/Kg	6.20 ± 1.0°°	0.25 ± 0.06	0.13 ± 0.03°

[a] nmol/mg prot;
[b] nmol/mg tissue.
Each result is expressed as mean of four experiments ± SE; °$p<0.05$; °°$p<0.01$ significantly different from control.

RESULTS

Table I shows the effect of isoproterenol administered to rats at doses of 3, 10, and 80 mg/Kg, respectively on the cardiac content of GSH, GSSG and X-SSG. Isoproterenol caused a significant dose dependent elevation of the heart GSH level; the concentration of GSSG also tends to increase due to the effect of isoproterenol, reaching a similar cellular level for all the doses of isoproterenol employed. For these effects, the ratio GSH/GSSG remained close to control value after administration of 3 and 10 mg/Kg isoproterenol, whilst at the dose of 80 mg/Kg the catecholamine produced a sensible increase of the GSH/GSSG ratio. The injections of isoproterenol caused a decrease of the cardiac X-SSG content with a more marked significant effect at the dose of 80 mg/Kg.

Table II shows the effect of isoproterenol injections on the activities of enzymes related to the metabolism of glutathione. No changes were detectable for glutathione peroxidase, glutathione reductase and glutathione S-transferase activities. Only glutathione reductase activity was slightly reduced following injections of 80 mg/Kg isoproterenol.

DISCUSSION

The present study indicates that the administration of isoproterenol significantly increases the concentrations of GSH in a dose dependent manner. Isoproterenol also

TABLE II
Effect of isoproterenol administration on rat heart glutathione related enzyme activities

	Glutathione[a] Peroxidase	Glutathione[a] Reductase	Glutathione[b] S-transferase
Control	170.5 ± 6.2	20.2 ± 0.6	17.5 ± 0.6
3 mg/Kg	173.8 ± 10.4	19.3 ± 0.9	17.1 ± 0.4
10 mg/Kg	168.2 ± 12.0	18.8 ± 1.0	18.4 ± 0.5
80 mg/Kg	168.7 ± 10.1	18.1 ± 1.2	17.7 ± 1.0

[a] nmol NADPH oxidized/min·mg prot;
[b] nmol coniugated formed/min·mg prot.
Each result is expressed as mean of four experiments ± SE.

slightly increases GSSG level, whilst produces a diminuition of the X-SSG content. Such a perturbation of the heart glutathione status cannot be provoked by an alteration of the enzymatic activities such as the glutathione peroxidase, reductase and S-transferase, since these enzymes are not affected by the administration of the catecholamine. More likely isoproterenol increases the cardiac GSH level by enhancing the availability of NADPH for the glutathione reductase activity. In fact, the β-agonist is able to stimulate the heart glucose 6-phosphate dehydrogenase activity and the hexose monophosphate shunt[13]. On the other hand the cardiac turnover of glutathione is very low and little dependent on the endogenous synthesis[14]. As a consequence of this fact, it is also possible that isoproterenol enhances the GSH level by augmenting the cardiac uptake of circulating glutathione. The diminuition of the X-SSG level induced by the administration of isoproterenol, especially at higher doses, is difficult to explain, but it is probable that the catecholamine favours the remotion of GSH from the mixed disulfide deposit by enhancing the level of NADPH necessary for the activities of the coupled enzymes thiol transferase and glutathione reductase. It is also possible that isoproterenol varying the concentration of cellular cyclic AMP may control the cardiac disulfide-sulfhydryl ratio[15]. Thus isoproterenol appears to be an important effector able to shift the thiol cellular status towards a more reducing condition. At this level the metabolic effectors which are able to modify the GSH/GSSG ratio or the X-SSG level are considered important signals able to modulate enzyme activities or protein functions[16]. In fact, the formation of mixed disulfide between glutathione and enzymes catalyzed by the enzyme thiol transferase may regulate the metabolic activity[17] particularly of the carbohydrate metabolism[18]. In conclusion, isoproterenol by changing the cardiac status of glutathione can affect the metabolism of the heart muscle and in this respect it could be important to underline that glutathione has been implicated in the control of muscle contractility[19] and in the stimulation of the inotropic activity elicited in the cardiac muscle by catecholamines[20].

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