EFFECTS OF CATECHOLAMINES ON THE MYOCARDIAL REDOX POTENTIAL

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Abstract —Using the redox potential of the myocardium (JEt) as a measure of the myocardial energy metabolism, the metabolic effects of three representative catecholamines, adrenaline (Adr), noradrenaline (Nor) and isoproterenol (Isp) were studied, in the canine heart-lung preparation supported by a donor. Adr and Nor produced an initial improvement of Jet, followed by a sustained debasement, while Isp produced only a debasement. Pretreatment of the preparation with phentolamine or dibenamine, resulted in an abolishment of the initial improvement, while the effect of Isp remained unchanged. After pretreatment of the preparation with practolol, the positive inotropic and chronotropic effect and the associated increase in the myocardial oxygen consumption were no longer seen with all the catecholamines tested. Under these conditions, Adr and Nor produced a sustained improvement of Jet, while Isp produced a transient debasement, which, in turn, was abolished by propranolol. When the heart was driven at a constant rate, the myocardial oxygen consumption did not increase with the catecholamines in most of the cases and a sustained improvement of Jet was observed with Adr and Nor; Isp produced a transient debasement. These findings indicate that catecholamines produce an improvement of the myocardial energy metabolism through activation of the adrenergic β-receptor.

Although the metabolic effects of catecholamines have been extensively studied both in vivo and in vitro (for references see Griffith (1) and Ellis (2), Williamson (3)), controversy still exists regarding the effects of these substances on the overall energy metabolism of the heart. Raab et al. (4) emphasized on the "oxygen-wasting" effects of catecholamines as an etiological factor of angina pectoris. However, some researchers have recently reported improvement of the myocardial energy metabolism produced by these compounds; Ribeilima et al. (5) found a consistent shift of the myocardial redox potential to more positive values after noradrenaline. Using a direct fluorometric technique for recording the intracellular oxidation-reduction state, Williamson and Jamieson (6) found transient changes of the pyridine nucleotides towards a more oxidized state after adrenaline.

In view of this discrepancy, we reexamined the effects of catecholamines on the myocardial energy metabolism using as a measure the redox potential of the lactate/pyruvate system of the blood perfusing the heart.

MATERIALS AND METHODS

Experiments were performed in the canine heart-lung preparation supported by a donor

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Mongrel dogs of either sex weighing between 7 and 12 kg were anesthetized with pentobarbital sodium 30 mg/kg administered intraperitoneally. Heart-lung preparations (HLP) were prepared according to the Krayer-Mendez modification of the original Starling method.

The level of the blood in the venous reservoir was kept constant at 10 cm above the right atrium throughout the course of the experiment, and the venous return was adjusted to 350–450 ml/min by a screw clamp placed around the rubber tubing connecting the venous reservoir with the venous cannula. To prevent the gradual deterioration of the preparation, the coronary sinus outflow of the preparation was forwarded to the femoral vein of another larger dog anesthetized with chloralose (45 mg/kg) and urethane (450 mg/kg) after premedication with a subcutaneous injection of 1.5 mg/kg of morphine hydrochloride, and was pumped back from the femoral artery as a fresh arterial blood to the venous reservoir of the HLP.

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**Fig. 1.** General scheme of the canine heart-lung preparation supported by a donor (HLP c donor). The left upper half represents the usual heart-lung preparation. A Morawitz cannula was introduced via right atrium into the coronary sinus. The outflowing blood was sent to an extracorporeal circuit composed of a flowmeter and an oximeter placed in parallel and passed on to the femoral vein of another large dog, the donor. The fresh arterial blood from the femoral artery of the donor was pumped back to the venous reservoir of the heart-lung preparation. D: Donor dog A.C.: Arterial cannula A: Air cushion R: Pneumatic resistance W.B.: Water bath L: Lung V.R.(A): venous reservoir A V.R.(B): Venous reservoir B V.C.: Venous cannula S.V.C.: Superior vena cava J.V.C.: Inferior vena cava M.C.: Morawitz cannula P.B.: Pressure bottle S.A.(a): Side arm a S.A.(b): Side arm b G.C.: Graduated cylinder F.A.: Femoral artery F.V.: Femoral vein
For the determination of the blood lactate and pyruvate, blood samples were withdrawn at 2 places designated as (a) and (v) in Fig. 1. Two volumes of 6.5% perchloric acid (PCA) were added to the blood samples to precipitate the protein and the blood was centrifuged with a refrigerated centrifuge for 15 min at 3000 r.p.m. The pyruvic acid determinations were conducted in the following way: 0.5 ml of 1.5 mol Tris (hydroxymethyl) aminomethane base (Tris) (Boehringer Mannheim) solution was added to 0.2 ml of the protein-free extract and well mixed. After adding 0.5 ml of 0.64 mmol $\beta$-DPNH (Na$_2$ salt, Kyowa Hakko Kogyo) in 1.5 mol Tris, the optical density was read at 340 nm (OD$_{340}$) using a double-beam spectro-photometer (Hitachi Model 124). Then, 0.05 ml of lactic dehydrogenase (0.4 mg/ml suspension in (NH$_4$)$_2$SO$_4$, Sigma Chemicals) was added and the resulting changes in the OD$_{340}$ were followed until a steady minimum value was obtained. From the difference in the OD$_{340}$ (JOD$_{340}$), the pyruvate content was calculated using the following equation: pyruvate (mmol/ml of whole blood) = 0.72 × JOD$_{340}$. Determination of lactic acid was conducted in the following way: 1.0 ml of 7 mmol $\beta$-DPN (free acid, Kyowa Hakko Kogyo) in 1 mol glycine buffer (pH 9.2) with hydrazine (0.4 mol) and EDTA (5 mmol), 2.0 ml of redistilled H$_2$O and 0.05 ml of lactic dehydrogenase (same as above) were poured into test tubes and mixed well. Then, 0.2 ml of protein-free extract was added to 2.8 ml of this mixture and incubation was carried out for 1 hr at 37°C. After incubation, the OD was read at 340 nm. The lactate content of the blood was calculated as follows: Lactate content (mmol/ml of whole blood) = OD$_{340}$ × 7.25.

On the basis of these determinations, the redox potential of the coronary blood was calculated using the following equation: $E_h$ (mV) = 204 - 30.7 log (Lactate content/Pyruvate content) (8). Veno-arterial difference of the redox potential of the coronary blood ($\Delta E_h$) was taken to represent the myocardial redox potential (8).

Drugs used were: l-adrenaline hydrochloride (Sankyo), dl-noradrenaline hydrochloride (Sankyo), l-isoproterenol hydrochloride (Nikken Kagaku), phentolamine mesylate (CIBA-Geigy), dibenamine hydrochloride (Tokyo Kasei), dl-practolol (ICI Japan) and dl-propranolol hydrochloride (ICI Japan).

Catecholamines were infused continuously into the rubber tubing leading to the venous cannula of the preparation with the aid of an infusion pump (Harvard Apparatus Model 940). Blockers were injected into the venous reservoir.

RESULTS

Effects of catecholamines on $\Delta E_h$

In order to compare the metabolic action of the functionally-equipotent doses of three representative catecholamines, adrenaline, noradrenaline and isoproterenol, doses of these substances were chosen so as to produce coronary flow increase (Fig. 3) as well as the positive inotropic and chronotropic effects of a similar magnitude. Regarding chronotropic effects, the increase in heart rate was 64.4 ± 7.0 (S.E., n = 12) for isoproterenol, 62.2 ± 7.8 (n = 10) for adrenaline and 58.1 ± 6.4 (n = 10) for noradrenaline. These doses were 3–10 µg/min for adrenaline and noradrenaline and 0.3–1 µg/min for isoproterenol.
Effects of the above doses of catecholamines on the cardiohemodynamic parameters of the HLP donor and the effects of 10 \(\mu g/\text{min}\) of noradrenaline are illustrated in Fig. 2.

Effects of the catecholamines on the myocardial redox potential (\(\Delta E_h\)) are depicted together with the effects on the coronary flow and oxygen saturation of the coronary venous blood in Fig. 3.

Adrenaline and noradrenaline produced an initial improvement of \(\Delta E_h\) followed by a debasement, while isoproterenol produced a debasement. On an average, the initial improvement was more marked with noradrenaline than with adrenaline.

**Fig. 2.** Effects of 10 \(\mu g/\text{min}\) of noradrenaline on the heart and coronary circulation. Tracings are from top to bottom: Systemic output (SOP), heart rate (HR), right atrial pressure (RAP), coronary flow (CF) and oxygen saturation of the coronary sinus blood (\(\text{VO}_2\)). Dog, 11 kg male, Heart weight 110 g.

**Fig. 3.** Effects of 0.3–1 \(\mu g/\text{min}\) of isoproterenol (\(n=12\)), 3–10 \(\mu g/\text{min}\) of adrenaline (\(n=10\)) and 3–10 \(\mu g/\text{min}\) of noradrenaline (\(n=10\)) on the myocardial redox potential (\(\Delta E_h\)), oxygen saturation of the coronary sinus blood (\(\text{O}_2\text{ Sat.}\)) and coronary flow (Cor. Flow). Vertical bars represent S.E. of means. *: significant at \(p<0.05\); **: significant at \(p<0.01\).
Due to the considerable increase in \( O_2 \) consumption, oxygen saturation of the coronary venous blood decreased after all three catecholamines, despite an increase in the coronary blood flow. The decrease produced by isoproterenol was minimal (statistically not significant) as compared with decreases produced by adrenaline and noradrenaline (Beginning with 5 min after infusion, the decreases were statistically significant).

**Effects of \( \alpha \)-blocker**

After pretreatment of the preparation with an adrenergic \( \alpha \)-blocker, phentolamine or dibenamine, which produced no significant effect on the cardiac actions of adrenaline and noradrenaline, but tended to augment the coronary flow increase produced by adrenaline and noradrenaline, an improvement of \( \Delta E_h \) was no longer seen after administration of adrenaline and noradrenaline; a debasement of \( \Delta E_h \) only was observed under this condition and was associated with less decrease in the oxygen saturation of the coronary venous blood. The effect of isoproterenol on \( \Delta E_h \) remained unchanged. Fig. 4 depicts the metabolic effects produced by the three catecholamines in the presence of 5 mg of phentolamine, together with the effects of this substance on the coronary blood flow and the coronary venous \( O_2 \) saturation.

**Effects of \( \beta \)-blocker**

Administration of 10–30 mg of practolol, an adrenergic \( \beta_1 \)-blocker, resulted in abolishment of increase in myocardial oxygen consumption and increase in the coronary blood flow produced by all three catecholamines. The positive inotropic and chronotropic effects were also abolished. Under these conditions adrenaline and noradrenaline produced only an improvement of \( \Delta E_h \), while isoproterenol induced a transient debasement (Fig. 5).

Further treatment of the preparation with 1–3 mg of propranolol resulted in a complete

![Fig. 4. Effect of 0.3–1 \( \mu \)g/min of isoproterenol (\( n=5 \)), 3–10 \( \mu \)g/min of adrenaline (\( n=7 \)) and 3–10 \( \mu \)g/min of noradrenaline (\( n=7 \)) on the myocardial redox potential, oxygen saturation of the coronary sinus blood and coronary flow after pretreatment of the preparation with 5 mg of phentolamine. Vertical bars represent S.E. of means. *: significant at \( p<0.05 \); **: significant at \( p<0.01 \)](image-url)
Fig. 5. Effect of 0.3–1 μg/min of isoproterenol (n = 7), 3–10 μg/min of adrenaline (n = 5) and 3–10 μg/min of noradrenaline (n = 5) on the myocardial redox potential, oxygen saturation of the coronary sinus blood and coronary flow after pretreatment of the preparation with 10–30 mg of practolol. Vertical bars represent S.E. of means. *: significant at p < 0.05; **: significant at p < 0.01

Fig. 6. Effects of 0.3–1 μg/min of isoproterenol (n = 6), 3–10 μg/min of adrenaline (n = 5) and 3–10 μg/min of noradrenaline (n = 6) on the myocardial redox potential, oxygen saturation of the coronary sinus blood and coronary flow after pretreatment of the preparation with 1–3 mg of propranolol. Vertical bars represent S.E. of means. *: significant at p < 0.05; **: significant at p < 0.01

abolishment of the debasement of JEH observed after isoproterenol, while the effects of noradrenaline or adrenaline remained essentially unchanged (Fig. 6).

Effects of pacing

As demonstrated in our previous paper (7), when the heart was driven at a constant rate of 137.5 ± 2.0/min on the average after crushing the sino-atrial pacemaker, increase in
FIG. 7. Effects of 0.3–1 μg/min of isoproterenol (n=8), 3–10 μg/min of adrenaline (n=8) and 3–10 μg/min of noradrenaline (n=7) in paced heart on the myocardial redox potential, oxygen saturation of the coronary sinus blood and coronary flow. Vertical bars represent S.E. of means. *: significant at p<0.05; **: significant at p<0.01

the myocardial oxygen consumption was not observed after administration of the catecholamines, in most of the experiments. There were no significant changes in the myocardial oxygen consumption or the myocardial redox potential after pacing, since the heart was driven at a rate which was only slightly higher than the respective spontaneous rate. Fig. 7 illustrates the effects of the three catecholamines on the myocardial redox potential (ΔΕh), under this condition. Noradrenaline and adrenaline produced an improvement of the ΔΕh, while isoproterenol produced a transient debasement.

DISCUSSION

The redox status of pyridine nucleotides (DPN and DPNH, to be described collectively as DPN in the following section) is a sensitive indicator of the functional state of cellular oxidation. However, direct measurements of the tissue content of these substances do not necessarily supply the required information; they fail to differentiate between the free and bound nucleotides (only free DPN participates in redox reactions) and they provide no information on the distribution of these substances between the various cell compartments, which is known to be uneven (9). Both difficulties may be overcome by measuring the ratio of the concentrations of the oxidized and reduced metabolites of suitable DPN-linked dehydrogenase systems that are in equilibrium or near-equilibrium with the pyridine nucleotides (10). The lactate and pyruvate system was chosen for the present experiment since Bücher and Russmann (11) demonstrated that this system gives most reliable values for the cytoplasmic DPN/DPNH ratio under a variety of conditions. In the present experiment, the concentrations of these substances in the blood entering and leaving the heart were determined, instead of determining the tissue content of these metabolites, since our aim was
to assess moment to moment changes in the redox state in actual ‘in vivo’ situations and it is not practical, or perhaps impossible to conduct sequential determinations of the tissue content at short time intervals. The use of the blood lactate/pyruvate ratio as a measure of the tissue lactate/pyruvate ratio would provide more exact values, if lactate and pyruvate freely and equally permeate cell membranes, so that changes in their intracellular levels will be reflected by similar changes in the blood. However, Henderson et al. (12) found apparent differences in the relative rates of efflux of these substances from the myocardium; the efflux rates of pyruvate were 10 times greater than those of lactate, despite the similarity of the molecules, and the intracellular concentration of lactate was higher than would have been predicted on the basis of passive diffusion and quite independent of the changes in the blood level (13). The L/P ratio was found to be three times higher in the tissue of isolated perfused rat heart than in the perfusate (14). In spite of these objections, it was demonstrated by Opie and Mansford (15) in the isolated perfused rat heart that the direction of changes of the perfusate lactate/pyruvate ratio (L/P ratio) generally moved in the same direction as the heart L/P ratio. Furthermore, they concluded that extracellular L/P ratio changes are a most useful index of the transition from aerobiosis to anaerobiosis, since the only condition causing a large rise in the extracellular L/P ratio was anoxia, if nutritional factors influencing these ratios are taken into account and, in particular, the diabetic state is excluded.

All the three catecholamines used produced a shift to more negative values of myocardial redox potential and a decrease in the O₂ saturation of the coronary venous blood. This is in agreement with the finding of Takenaka (16) who reported that adrenaline produced a shift of the myocardial redox potential to more negative values. Although the debasement of \( \frac{\text{JEh}}{\text{O}_2} \) is inseparably associated with the augmentation of cardiac function and resultant increase in \( \text{O}_2 \) consumption, as is evident from the results obtained in paced-hearts and in the hearts pretreated with \( \beta \)-blockers, it is unlikely that a hypoxia of the myocardium could have been the cause of the observed debasement of \( \frac{\text{JEh}}{\text{O}_2} \), for, as we demonstrated in our previous paper (7), a definite hypoxia is not conceivable at least in the case of isoproterenol, since the augmented \( \text{O}_2 \) consumption produced by this compound is virtually compensated for by a corresponding increase in the coronary flow. Furthermore, it was shown by Lundsgaard-Hansen (17) that to produce significant changes in the L/P ratio of the blood leaving the heart, extreme degrees of arterial hypoxaemia with coronary sinus \( \text{pO}_2 \) values 10 mm Hg or below are required. This contradicts our findings with adrenaline and nor-adrenaline; the fall of the oxygen saturation of the venous blood was minimal despite of the large increase in myocardial oxygen consumption. The most plausible explanations would be that the augmented cardiac function resulted in a metabolic hyperactivity with resultant debasement of \( \frac{\text{JEh}}{\text{O}_2} \). Key event of the metabolic hyperactivity could be the augmented utilization of free fatty acid, since it is demonstrated in the isolated perfused rat heart (3), that increased fuel requirement imposed on the heart by positive chronotropic and inotropic effects of catecholamines was provided partly by an increase in the rate of FFA utilization. According to Garland (18) and Garland and Randle (19), an accelerated
utilization of FFA resulted in an inhibition of pyruvate dehydrogenase through accumulation of acetyl CoA and DPNH, with a decrease in the rate of pyruvate entry into the citrate cycle (20). Under these conditions L/P ratio of the perfusate of the isolated rat heart was found to be increased (21). This sequence of events may probably explain the sustained debasement of $\Delta$Eh observed after administration of the three catecholamines. The fact that the debasement was largely abolished by practolol was compatible with this interpretation, since the augmentations of lipolysis in the adipose tissue and calorigenic action in the rat are mediated by adrenergic $\beta_1$-receptors (22–24).

An initial transient debasement of the $\Delta$Eh, produced by isoproterenol in the paced hearts as well as in the presence of practolol, (not observed after treatment of the preparation with propranolol), may be attributed to a well-known augmentation of glycogenolysis, since adrenergic receptors in the muscle subserving glycogenolysis is generally considered to belong to $\beta_1$-type receptor (25), and according to Williamson (3), the duration of augmented glycogenolysis produced by catecholamines in the heart is rather short.

In addition to a debasement, an improvement of myocardial redox potential was observed after adrenaline and noradrenaline in the present experiments. This is in agreement with the previous reports by Ribellima et al. (5) and Williamson and Jamieson (6).

It seems unlikely that the increased coronary blood flow produced by catecholamines renders blood concentrations of one or both substances nonrepresentative of tissue concentration, thus making a shift of the calculated myocardial redox potential to a more positive value, for the same improvement of the myocardial energy metabolism was recorded using a direct fluorometric technique of the myocardial DPNH (26).

The improvement was more marked with noradrenaline than with adrenaline. This may be due to a lack of augmentation of glycogenolysis after noradrenaline; as is mentioned above, glycogenolysis in muscle is mediated through stimulation of $\beta_1$-receptor and noradrenaline has little stimulating action on the $\beta_1$-receptor.

The improvement of $\Delta$Eh persisted after practolol and in the paced hearts as well as after propranolol, and was found to be inhibited by adrenergic $\alpha$-blocking agents. It is possible that the improvement may have resulted from an augmented utilization of glucose produced by these compounds, since catecholamines reportedly produce an augmentation of myocardial utilization of glucose, which is not blocked by $\beta$-blocking agents (3, 27, 28). Although there is apparently no documentation showing that the increased myocardial utilization of glucose induced by catecholamines is mediated through $\alpha$-adrenergic receptor, there is evidence that the utilization of glucose in the peripheral tissue is regulated by $\alpha$-adrenergic receptor (29, 30). As regards the mechanism of augmentation of glucose utilization, the findings of Pastan et al. (31) demonstrated that the oxidation of reduced forms of DPN by the mitochondrial-microsomal preparation of the rat thyroid gland was accelerated under the catalytic influence of catecholamines, and that the increased oxidation of pyridine nucleotides resulted in an augmentation of glucose metabolism. A similar mechanism may possibly be operative in the improvement of $\Delta$Eh observed after adrenaline and noradrenaline.
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