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Increased myocardial oxygen consumption and resting heat production, as measured by microcalorimetry, after propranolol and carvedilol treatment: Is there a partial agonistic effect in the rat?

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Abstract

This study investigated the influence of β-blockade on the resting heat production of myocardial tissue by microcalorimetry. During one week, propranolol ($\beta_1\beta_2$-adrenoceptor antagonist) was orally given to 14 rats – 5 mg kg^{-1} once daily, and carvedilol ($\beta_1\beta_2$- and α_1-antagonist) to eight rats – 3 mg kg^{-1} once daily; 36 rats were controls. Thin slices of cardiac tissue, $\approx$10 mg, were removed from the apex. Carbogen-saturated Krebs–Ringer bicarbonate buffer with glucose as substrate was pumped through the microcalorimetric ampoule during the measurement at 37°C.

Unexpectedly, the mean resting heat production was higher after both propranolol, 1.25 mW g^{-1} wet tissue ($p < 0.01$, ANOVA) and carvedilol, 1.19 mW g^{-1} ($p < 0.05$) treatments, than in the control group, 1.01 mW g^{-1}. The same applied to oxygen consumption. The calculated anaerobic fractions were 16, 8 and 24% in the respective groups, but differences were not significant. Also, when added in vitro, propranolol caused an enhanced myocardial resting heat production by an average of 23%. As resting myocardial metabolism contributes to the overall cardiac energetics to a relatively minor extent, the net result of treatment will probably be of only marginal physiological importance.

The experimental outcome is indicative of a stimulation of resting myocardial metabolic activity after propranolol and carvedilol, rather than a predicted decrease. We hypothesize that the absence of anything to depress in the non-beating heart tissue, reveals a small degree of partial β-agonist activity, possibly via the newly discovered β_3-adrenoceptor. © 1997 Elsevier Science B.V.

Keywords: β-Adrenergic blockade; Carvedilol; Heat production; Myocardium; Oxygen consumption; Propranolol

1. Introduction

Myocardial oxygen consumption is determined by many factors of which the contractile state, the wall tension and the beating rate are the most important [1]. Also contributing to cardiac energetics is the resting or basal metabolic rate [2], which may account for 1/5 to 1/3 of the total myocardial metabolism in an animal at rest [3,4]. During β-adrenergic blockade a direct cardiodepressant action may be involved. A fall in resting metabolism would then be anticipated. There is, however, no documentation concerning such effects. Chronic β-blockade influences lipolysis resulting in a diminished availability of free fatty acids; it has also a cardiac dilatory effect that augments the left ventricular wall tension and oxygen

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requirements. One unrecognized factor, which might indirectly have an oxygen-sparing effect on the heart, is the finding of a substantial fall (by 27%) in resting heat production of skeletal muscle after chronic β-adrenergic blockade [5].

The purpose of this study was to try to quantify, by direct microcalorimetry, the myocardial resting heat production of tissue slices and to examine the influence of β-adrenergic blockade. The hypothesis proposed that continuous treatment would suppress the resting heat production of myocardium, such as in skeletal muscle [5]. Oxygen consumption was also measured. Two different $\beta_1\beta_2$-blockers were studied, the prototype propranolol and the novel carvedilol. Carvedilol has an interesting profile, possessing α_1-adrenoceptor blocking property with a marked vasodilating action [6]; it has revealed a greater cardioprotective action than propranolol in experimental models of myocardial infarction [7]. Propranolol was shown not only to increase the density of β-adrenoceptors in the rat heart [8,9], but also the number of α_1-adrenoceptors [10,11]. The physiological relevance of this remains to be determined. Possibly, the α_1-adrenoceptors may serve as a reserve mechanism to maintain myocardial responsiveness to catecholamines under conditions where β_1-adrenoceptor function is compromised, unless tolerance develops [6,12].

2. Experimental

Drugs were administered by gastric feeding once daily during seven days; decapitation and sampling was performed on the eighth day at 10.00 a.m., 2 h after drug intake. Experiments were made in the following series: first, 16 controls, then 14 rats treated with propranolol (Inderal®, Zeneca, Macclesfield, U.K.), 5 mg kg^{-1}; thereafter, 12 controls, followed by eight rats treated with carvedilol, 3 mg kg^{-1} (Boehringer Mannheim GmbH, Mannheim, Germany), and finally another eight controls. Doses were slightly higher in comparison with those commonly used in clinical practice. Treatment was not blinded to the observer. The rats (Wistar) were kept in cages (40 × 30 × 15 cm) and had free access to water and standard food; the mean body weight was 290 g (S.D. 39). Experiments were performed in accordance with the Ethical Committee for Animal Research at the Lund University.

The heart was quickly removed and ca. 0.2 mm thin slices of tissues were cut from the apex, using a razor blade. The specimen was handled in Krebs–Ringer bicarbonate buffer (values in mmol l^{-1}): 120 NaCl, 5 KCl, 1.5 $CaCl_2$, 15 $NaHCO_3$, 1 $MgCl_2$, 1 NaH_2PO_4, supplemented with 5.6 glucose and insulin (Actrapid® Novo, Denmark) in a final concentration of 0.1 unit ml^{-1} buffer [13]; insulin was not used during the perfusion in the calorimeter. The slices were carefully blotted on filter paper and quickly weighed just before the measurements. Mean specimen weight was for the calorimetry 9.6 mg (S.D. 1.9) and for the oxygen consumption measurements 5 mg. The same procedure was used for all three groups. Rates of heat production and oxygen consumption are expressed on a wet-weight basis. Specimens were also taken from the gastrocnemius muscle for microcalorimetry, the result of which has been published elsewhere [14].

2.1. Calorimetry

The microcalorimeter used in the present work was of the thermopile heat-conduction type (Thermal Activity Monitor 2277, Thermometric AB, Järfälla, Sweden), arranged as twin instrument with one of the units containing the reactive system and the other containing the reference ampoule with water [15]; the differential signal was registered. Measurements were performed at 37°C. A 3 ml flow-through vessel was used for liquid perfusion. Good baseline stability, established with the vessel charged with water, was obtained, the change being ±0.3 μW over 10 h. The voltage output was amplified 1000 times by an operational amplifier. Calibration was carried out electrically. The calorimetric vessel was thermostated in three consecutive heat exchange positions before reaching the thermopile zone after ≈30 min. The perfusion medium (above) was introduced into the ampoule, 5 ml h^{-1}, by the use of a peristaltic pump (LKB Perpex, Sweden). Carbogen (O_2 : CO_2 = 95 : 5) was bubbled through the medium outside the calorimeter. The tissue specimens were held in place in the middle of the measurement ampoule by a thin stainless steel needle. Measurement started 68 min (S.D. 10) after tissue sampling and lasted 2.5–3 h. The pH was measured intermittently by a

radiometer type G297/G7 capillary electrode (Copenhagen, Denmark) during the registration period and was 7.3 in the effluent, which means only a very slight decrease. A constant rate of heat production was usually achieved for the myocardial slices after ca. 1 h. The heat production rate, P, as calculated from the power–time curves, (dQ/dt), refers to the second hour after start of the calorimetric measurements and is expressed in units of mW g^{-1} wet tissue. The coefficient of variation for the method was calculated in duplicate experiments, using two almost identical microcalorimeters [16] with 1 ml ampoules, perfusion rate 5 ml h^{-1}.

In simultaneous experiments outside the calorimeter (without perfusion), using the same medium with no insulin, the rate of myocardial oxygen consumption was measured polarographically by a Clark electrode unit (Hansatech, Surrey, U.K.) in a 1 ml stirred vessel, for ≈1 h at maximum oxygen saturation and 37°C. The recorded values are expressed in nmol g^{-1} s^{-1} wet tissue. The oxygen-dependent heat energy produced, $P(O_2)$ was calculated in mW g^{-1} from the product of the oxygen consumption rate and the enthalpy change, $\Delta H = -2931$ kJ mol^{-1} glucose, ΔH being the caloric equivalent of oxygen for the combustion of glucose to CO_2 and water [17].

In a series of calorimetric experiments ($n = 8$) with rat heart slices (8–10 mg), propranolol hydrochloride (ICI-Diagnostika, Falkenberg, Sweden) was added in vitro to the KRB buffer in a final concentration of 10^{-5} mol l^{-1}, using glucose as substrate (without insulin). A microcalorimeter with a 1 ml perfusion vessel was used [16], the perfusion rate being 5 ml h^{-1}. Similarly, noradrenaline was added in six experiments in a final concentration of 10^{-4} mol l^{-1}. In both series, the exposure time was 2 h.

2.2. Statistics

Means and standard deviations (S.D.) or 95% confidence interval are given. Intergroup differences were tested by analysis of variance (ANOVA). The significance was further defined by calculating the 95% confidence interval for the difference. The 36 controls were regarded as one group. Correlation coefficients were calculated by a linear regression analysis. Wilcoxon's test was used for the drug experiments in vitro.

3. Results

The normal mean resting heat production value was 1.01 mW g^{-1} (S.D. 0.19) myocardial tissue. Duplicate experiments were performed in glucose/KRB buffer and a coefficient of variation in myocardial heat production of 13% was calculated between the individual samples from eight untreated rats. When β-OH butyrate was used as substrate in the KRB buffer in a final concentration of 3 mmol l^{-1}, a mean value of 1.06 mW g^{-1} was obtained in three experiments, which is not different from that measured with glucose as substrate. There was no relationship ($r = 0.04$) between heat production and myocardial sample weights (range 5–12 mg; $n = 58$).

Noradrenaline, added in vitro in six experiments, resulted in significantly ($p < 0.03$) higher heat production by 85% (or, by 0.73 mW g^{-1}, 95% confidence interval 63–106%).

3.1. β-Blockade experiments

The resting heat value was increased after propranolol and carvedilol medication in comparison with controls by an average of 24% ($p < 0.01$) and 18% ($p < 0.05$), respectively (see Table 1); the 95% confidence interval for the differences between propranolol and control values was 0.11–0.37 mW g^{-1} myocardial tissue, and between carvedilol and controls 0.03–0.33 mW g^{-1}.

The myocardial oxygen consumption was also found to be higher after propranolol treatment in comparison with controls by an average of 39% ($p < 0.05$), and still higher ($p < 0.02$) after carvedilol treatment. The calculated $P(O_2)$ values were lower than P. The contribution of anaerobic heat production was indirectly calculated by subtracting the heat produced by aerobic metabolism from the total heat: it was ≈24% in the control group, and 16 and 8% in the propranolol and carvedilol groups, respectively; differences are not significant.

Propranolol, when added in vitro to the KRB-buffer in eight perfusion experiments, caused a significant ($p < 0.05$) increase of myocardial heat by average 23% (95%, confidence interval 3–43%), from a mean value of 1.01 (S.D. 0.17) to 1.23 (S.D. 0.23) mW g^{-1}.

Table 1
Effects of seven-day oral treatment with propranolol and carvedilol on resting heat production (P = power) and oxygen consumption of slices of rat myocardium (apex); biopsies taken 2 h after drug intake. P was measured by perfusion microcalorimetry at 37°C in KRB-buffer with glucose as substrate, and oxygen consumption by a Clark electrode unit in parallel experiments. $P(O_2)$ = oxygen-dependent heat energy. (Values are means (S.D.))

Group	Control $n = 36$	Propranolol $n = 14$	Carvedilol $n = 8$
P (mW g^{-1} wet wt.) -direct calorimetry	1.01 (0.19)	1.25 (0.22)[c]	1.19 (0.20)[a]
Oxygen consumption (nmol g^{-1} s^{-1})	1.53 (0.79)	2.15 (0.90)[a]	2.23 (0.75)[b]
$P(O_2)$ (mW g^{-1})	0.77 (0.38)	1.05 (0.44)[a]	1.09 (0.37)[b]

[a] $p < 0.05$; [b] $p < 0.02$; [c] $p < 0.01$ vs. control (ANOVA).

4. Discussion

Further basic metabolic work on isolated myocardial tissue is needed before one can be sure to what extent the resting heat production is representative of the metabolism of the heart. Different calorimetric methods have previously been applied on heart preparations [18–20]. We have used the present methodology and reported significant differences in resting metabolic activity of heart tissue slices from diabetic vs. non-diabetic rats [21], and from hibernators vs. non-hibernators [22]. Further work on the conditions in vitro is in progress. Besides, the technique has been extensively used by our group on isolated skeletal muscle-fibre bundles to investigate the metabolic action of different adrenergic agents [5,23,24]. Values of heat production from slices of human skeletal muscle ex vivo [24] were, for instance, found to agree with in vivo measurements of muscle oxygen consumption. Regarding the integrity of tissues slices, we have found in experiments of human teased vastus lateralis muscle at 37°C that a non-significant decrease in potassium content of only ≈3% occurs after 2–3 h perfusion of Krebs–Ringer phosphate buffer in the presence of glucose–insulin (unpublished data).

In the present study, we found unexpectedly that the myocardial heat production ex vivo, following continuous treatment with propranolol, rose by 24% compared with controls, reflecting an increased resting metabolic rate. This was associated with an enhanced oxygen consumption.

The mechanisms behind the observed increase in cardiac resting energy state are not clear. New hypothesis have to be generated, especially as the in vitro exposure of the slices to a high propranolol concentration elicited an almost immediate increase in heat (by an average of 23%). This would indicate an accelerated utilization of glucose and precludes an up-regulation of cardiac β-receptors, which normally occurs during continuous medication [8,9], as responsible for the result.

Could the absence of any depressant in the non-beating heart tissue uncover a small degree of partial β-agonist activity? From animal work, it was reported that partial agonists, derived chemically from pindolol, cause part of their stimulant effects (at high concentrations) through heart β-receptor subtypes that are neither β_1- nor β_2-adrenoceptors [25]; these effects were not antagonized by propranolol. As a matter of fact, several $\beta_1\beta_2$-antagonists like propranolol are exhibiting weak and partial agonistic effects at the β_3-adrenergic receptor, expressed in Chinese hamster ovary cells [26]. The role of this receptor may differ between species but functional β_3-adrenoceptors also appear to be present in humans, at least in the regulation of fat metabolism and gastrointestinal function [27].

The resting heat of myocardial slices is low compared to the functioning organ and the increase because of propranolol and carvedilol will amount to only a few percent units of the total intact heart metabolism. It is therefore unlikely that the change in oxygen consumption is measurable in the working situation as depressant effects on function are involved after β-blockade: a lowered heart rate, reduced contractility and wall tension, and increased coronary arteriolar resistance.

Clinically, there are some divergent effects of compounds like propranolol on the intact human heart. In

ischaemic heart disease, propranolol decreased cardiac oxygen consumption by an average of 22% due to a decline in contractility and heart rate [28], while in another study, using single doses of propranolol (0.2 mg kg^{-1} i.v.), myocardial oxygen uptake was increased compared with control values for corresponding rate–pressure products [29]. If heart rate after intravenously administered propranolol [30], atenolol [31], or timolol [32] is kept constant by fixed rate pacing, the expected fall in overall myocardial oxygen consumption was abolished. For comparison, the β_1-blocker metoprolol decreased oxygen requirements in idiopathic dilated cardiomyopathy even during fixed rate atrial pacing [33]. In rabbits, propranolol had no influence on energy production of isometric and isotonic cardiac contractions as measured by thermopiles [34].

Has chronic dosing any special consequences on myocardial specimens compared to the effects on skeletal muscle? Regarding propranolol, given in one dose per day for a week, we found a suppression of skeletal muscle heat production in the rat [14], which was rather similar to that observed earlier in human experiments with different doses [5], indicating effective chronic blockade in the present experimental situation as well. Carvedilol, in contrast, had no suppressive effect on skeletal muscle heat production ex vivo [14].

The resting heat production rate P reflects heat from both aerobic and anaerobic metabolic processes. Under physiological conditions there is little use of anaerobic metabolism in the intact heart, probably less than 5% of total energy output [19]. When the isolated dog heart was studied during contractions, production of heat due to anaerobiosis appeared under certain conditions, namely in hypoxia and after arterial alkalization, irrespective of hypoxia [35]. Usually, fatty acids are the most important cardiac substrate. With no substrate available, the isolated but oxygenated and perfused heart can maintain contractility for over 40 min by oxidizing endogenous lipids [19,36]. From the results of the present two methods, run in parallel with glucose as the only substrate, we calculated a fairly high contribution of anaerobic metabolism for the energy supply, approximately 24% in the control group. It tended to be less in the propranolol and carvedilol groups, 16% and 8%, respectively. Whether this reflects changed overall capacity to cope with ischemia during β-blockade could only be speculated on. Even if these percentages are rough approximations, it is possible that during β-adrenergic blockade anaerobic metabolic pathways are less stimulated in the rat. It was shown in hyperthyroid patients that an enhanced anaerobic heat production in lymphocytes was suppressed with β-adrenergic blockade [37].

In aerobic metabolism the amount of energy yield per unit volume of O_2 varies only slightly with the type of substrate oxidized [38]. The heart has a high capacity for glycolysis and when the availability of fatty acids is reduced, the rate of aerobic glycolysis will increase substantially [39]. In the present study, no influence on the energy values was noted after a change of substrate from glucose to β-OH butyrate.

In conclusion, resting cardiac total metabolism, as measured by microcalorimetry on slices of tissue from the rat apex, was unexpectedly augmented after a seven-day oral treatment with two $\beta_1\beta_2$-antagonists, propranolol and carvedilol. Results were supported by parallel oxygen consumption measurements and also by direct exposure of the slices to high propranolol concentrations. This is indicative of a stimulation of myocardial metabolism. We hypothesize that the absence of any depressant, in a nonbeating heart tissue, uncovered a small degree of partial β-agonist activity, possibly via the β_3-adrenoceptor. As resting myocardial metabolism contributes to the overall cardiac energetics to a relatively minor extent, the net result will probably be of less physiological importance. It is, however, of interest to understand what factors might influence different compartments of energy expenditure in the heart, physiologically and in diseased states. We consider microcalorimetry as being a new approach to the problem of studying the thermogenic action of catecholamines on isolated cardiac tissue and the influence of anti-adrenergic drugs.

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