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Thermogenic effect of two β-adrenoceptor blocking drugs, propranolol and carvedilol, on skeletal muscle in rats. A microcalorimetric study ☆

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Abstract

This study investigated the influence of the prototype $\beta_1\beta_2$-adrenoceptor antagonist propranolol and the novel carvedilol ($\beta_1\beta_2$- and α_1-antagonist) on the thermogenesis of skeletal muscle *ex vivo*. During 1 week propranolol was given orally to 14 rats, 5 mg kg^{-1} once daily, and carvedilol was given to 23 rats, 3 mg kg^{-1} once daily; 19 rats received saline. Gastrocnemius muscle was obtained after decapitation. Carbogen-saturated Krebs–Ringer bicarbonate buffer containing glucose–insulin was pumped via heat exchangers through the microcalorimetric ampoule during the measurement procedure, starting within 50 min after the biopsies. The results showed that the mean resting heat production at 37°C was lower ($p < 0.02$, ANOVA) after propranolol, 0.45 mW g^{-1} wet muscle, than after carvedilol, 0.63 mW g^{-1}, and also lower than in the control group ($p < 0.05$), 0.62 mW g^{-1}. Muscle ATP content was not changed and showed no relationship to the heat production. The muscle utilized ≈10% of its total energy for the Na–K pump as assessed by ouabain inhibition. In conclusion, the lower heat production from muscle in the free-fed rat after propranolol agrees with some human studies using whole-body oxygen consumption measurements. Carvedilol had no influence, probably because of its combined β- and α_1-blocking activity. The results indicate that the sympathetic nervous system is implicated in the regulation of resting skeletal muscle thermogenesis. Microcalorimetry of isolated muscle may help to understand the metabolic action of different adrenoceptor antagonists.

Keywords: Adrenergic receptor; ATP; Carvedilol; Propranolol; Skeletal muscle; Thermogenesis

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1. Introduction

β-Adrenoceptor blocking agents constitute an important group of drugs commonly used in clinical practice, particularly in hypertension, angina pectoris and hyperthyroidism. They have pronounced influence on lipolysis and glycogenolysis. Sometimes their use is limited by the occurrence of side effects, such as muscle fatigue in the lower extremities, general tiredness and impaired physical performance. Probably many factors contribute to these symptoms. Using microcalorimetry, we demonstrated that after eight days of oral medication the non-selective $\beta_1\beta_2$-adrenoceptor blocker propranolol caused a decrease of thermogenesis in human skeletal muscle samples, whereas no change was noted with a non-selective drug with partial β_2-agonist activity (pindolol) or with a β_1-selective drug (atenolol) [1]. These results were achieved after performance of light physical work and did not represent the true basal state.

In an effort to extend our previous findings, we have now examined the effect of β-blockade on the resting muscle thermogenesis with the purpose of differentiating between two different $\beta_1\beta_2$-blockers, the prototype propranolol and the novel carvedilol. Carvedilol has an interesting profile and shows in addition to its β-blocking effect a marked vasodilating action, primarily from α_1-adrenoceptor blockade [2]. It appears further to have few metabolic side effects [2] and probably no influence on physical performance [3]. The muscle samples were taken in free-fed rats and examined by a direct calorimetric technique [4]. We also evaluated ATP levels and the energetics of the Na–K pump in muscle after ouabain suppression. The drug effects were compared with those of saline treatment.

2. Experimental

Propranolol, 5 mg kg^{-1} once daily (Inderal, Zeneca Ltd., Macclesfield, UK), was given orally to 14 Wistar rats and carvedilol, 3 mg kg^{-1} once daily (Boehringer Mannheim GmbH, Mannheim, Germany), was given to 23 rats; 19 control rats were given 1 ml of 0.9% NaCl. Decapitation was performed at 10.00 a.m. after seven days' treatment, 2 h after drug intake. Carvedilol has a longer half life than propranolol, and 12 of the rats were given the last dose of carvedilol 18 h before sampling in order to compare the peak effect afte 2 h with the effect at the end of the dosing interval.

The rats were kept in cages 40 cm × 30 cm × 15 cm; they had free access to water and standard food. Mean body weight was 290 mg (S.D. 39 mg). Muscle specimens were excised from the middle portion of the gastrocnemius muscle. One specimen was immediately frozen in liquid nitrogen for measurement of ATP using a luminometric method (Luminometer 1251, LKB-Wallac, Turku, Finland) [5]. Another sample was handled in Krebs–Ringer bicarbonate buffer (values in mol dm^{-3}): 0.12 NaCl, 0.005 KCl, 0.0015 $CaCl_2$, 0.015 $NaHCO_3$, 0.001 $MgCl_2$, 0.001 NaH_2PO_4, pH $\approx$ 7.4, supplemented with 0.0056 mol dm^{-3} of glucose and insulin (Actrapid, Novo, Denmark) in a final concentration of 0.1 unit cm^{-3} buffer [6]. The

sample was freed from visible connective tissue and fat, carefully blotted on filter paper and thereafter quickly weighed just before the calorimetric measurement; mean muscle weight was 32 mg (S.D. 7 mg). According to data on solubility and diffusiveness of oxygen at 37°C [7], it was calculated that this muscle size should allow adequate oxygenation. The study was approved by the Lund University Ethical Committee for Animal Research.

2.1. Calorimetry

The microcalorimeter used in the present work was of the thermopile heat-conduction type, arranged as a twin instrument with one of the units containing the reactive system and the other containing the reference ampoule with water [8]. Measurements were performed at 37°C. A 0.7 cm^3 flow-through vessel for liquid perfusion was used. Good baseline stability, established with the vessel charged with water, was obtained, the change being $< 2\ \mu W$ during 12 h. The voltage output was amplified with a Keithley 150B Microvoltmeter (Keithley Instruments Inc., Cleveland, OH, USA), 10 μV range, calibration was carried out electrically. The calorimetric vessel was thermostated in two consecutive heat exchange positions before reaching the thermopile zone after ≈ 15–20 min. The perfusion medium (above) was introduced very close to the resting muscle samples by the use of a peristaltic pump (LKB Perpex, Sweden) in order to keep the pH constant; perfusion rate was 5 $cm^3\ h^{-1}$. Carbogen ($O_2 : CO_2 = 95:5$) was bubbled through the medium outside the calorimeter. The tissue specimens were held in place inside the measurement ampoule by a 5 mm stainless steel needle. Perfusion started within 50 min after the biopsies and lasted ≈ 3 h. During the registration period, the pH in the effluent was measured intermittently by a Radiometer type G297/G7 capillary electrode (Copenhagen, Denmark). 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 muscle [4]. The coefficient of variation for the method is 4.2% [6].

2.2. Energy expenditure of the Na–K pump

Ouabain octahydrate (10^{-4} mol dm^{-3}) was added to the perfusion medium after incubation of the muscle samples for 2 h in the calorimetric vessel. The ouabain-induced depression of heat production (ΔP) was calculated as a percentage of the total resting heat production rate [9].

2.3. Statistics

Means and standard deviations (S.D.) or 95% confidence interval are given. Intergroup differences were tested by analyses of variance (ANOVA). The significance was further defined by calculating the 95% confidence interval for the difference. The correlation coefficients were calculated by a linear regression analysis.

Table 1
Effects of 7 days of oral treatment with propranolol (Pr.) and carvedilol on resting heat production (P = power) of muscle (gastrocnemius), measured *ex vivo* by perfusion microcalorimetry at 37°C, and muscle ATP in free-fed rats

Number of cases	NaCl $n = 19$	Propranolol $n = 14$	Carvedilol $n = 23$	Significance of difference Pr. − NaCl
Muscle heat production rate				ANOVA
P/(mW g^{-1} wet wt.)	0.62 (0.52–0.73)	0.45 (0.36–0.55)[a]	0.63 (0.55–0.72)	<0.05
ATP/(μmol g^{-1} dry wt.)	20.3 (4.0)	18.8 (4.1)	18.9 (4.2)	>0.3, NS

Values are means with S.D. or 95% confidence intervals in parentheses. NS = not significant.
[a] Significance vs. carvedilol: $p < 0.02$.

3. Results

3.1. Heat production (Table 1)

The mean resting heat production rate of skeletal muscle was lower after propranolol treatment in comparison with controls and carvedilol treatment by an average of 27% ($p < 0.05$) and 28% ($p < 0.02$), respectively. The 95% confidence interval for the difference between propranolol and control values was 0.02–0.31 mW g^{-1} muscle, and between propranolol and carvedilol values 0.05–0.31 mW g^{-1} muscle. No relationship ($r = -0.08$) was found between heat production and the muscle sample weight (range 18.6–46.7 mg; $n = 19$) in the control group. In the carvedilol treated group there was no difference in heat production between biopsies taken 2 h and 18 h after drug intake.

Incubation with ouabain decreased thermogenesis consistently in muscle. The mean ΔP for muscle was in the control group −9.9% (95% confidence interval 6.2–13.6%) and in the carvedilol group −11.5% (10.0–12.9%), which is not significantly different.

3.2. ATP (Table 1)

Mean ATP values were slightly lower in the two actively treated groups compared with the controls but values did not differ significantly. ATP content in muscle did not correlate with the heat production rate ($r = 0.1$; $n = 34$).

4. Discussion

We found clear evidence of decreased resting metabolic heat production of rat gastrocnemius muscle, as assessed by microcalorimetry following 7 days' oral propranolol intake, whereas the new vasodilating $\beta_1 \beta_2$- and α_1-antagonist carvedilol

had no influence. Caution must of course be taken in extrapolating in vitro results to the intact organism. Regarding the experimental conditions in vitro, we recently analysed the potassium content in human teased muscle biopsy samples and found a non-significant decrease of only ≈3% after 2–3 h incubation at 37°C in Krebs–Ringer phosphate buffer in the presence of glucose–insulin (unpublished data; see [10–12]). The measured heat production of human muscle samples has further been found to reflect reliably the in vivo conditions [4], as the thermogenic rates determined with the present microcalorimetric method are in close agreement with estimations of oxygen consumption in the resting human leg [13] and forearm [14].

Whatever the precise mode of action of the reduced metabolism, an attractive hypothesis can be derived from findings that propranolol ("acutely" administered) can decrease cyclic AMP and glycogen phosphorylase *a* in human muscle [15], and can apparently reduce the availability of glycogen as a source of energy both at rest [16,17] and after short intensive exercise [15,18] when glycogen is the preferred substrate. Degradation of glycogen to lactate is mediated by β_2-receptors, leading to energy (ATP) production and liberation of heat. In rats, total body glycogen content has been shown to be reduced by more than 25% after administration of propranolol for 9 weeks [19].

Other theories should be considered as well. Muscle thermogenesis may be inhibited in an indirect way, via blockade of adrenergic β_1-receptors in adipose tissue [20], resulting in a diminished availability of free fatty acids and the reduced rates of oxidation in muscle.

Using oxygen consumption measurements, propranolol seemed to have no effect on basal metabolic rate in numerous human trials [21–26]. In fact, only a few studies report a reduction, which probably was dependent on the preceding diet [27–29]. We found previously by microcalorimetry in fasting humans a non-significant decrease by an average 17% in basal thermogenesis of the vastus lateralis muscle after 7 days' treatment with propranolol [10]. Also, when added in vitro in increasing concentrations, propranolol had no influence on heat production of human muscle [10].

The sympathetic nervous system has been regarded as playing only a minor role in the regulation of basal metabolic rate (i.e. "essential" and "endothermic" heat). This must be distinguished from the clear reduction in "facultative thermogenesis" that has often been demonstrated under β-blockade. Propranolol significantly inhibited the stimulated whole-body respiratory exchange (i.e. "diet-induced thermogenesis") in numerous trials [23–26,30–32], although again there are contradictory results, both in humans [21,22,33–37] and animals [38]. The magnitude of the present decline in muscle heat production of free-fed rats was 27%; the significant result, in contrast to our previous finding in fasting humans [10], is probably attributable to a more pronounced inhibitory response to propranolol after food intake by the rats, *which means from a level above basal fasting.*

Documentation is sparse concerning the role of α-adrenergic receptors in the regulation of energy balance. Seaton et al. [34] reported that oxygen consumption increased after $\alpha_1\alpha_2$-adrenoceptor blockade with phentolamine, whereas no change

was noted when phentolamine was administered together with propranolol. Regarding carvedilol, we can only speculate whether the lack of hypometabolic effect might stem from the fact that this $\beta_1\beta_2$-antagonist has a potent α_1-adrenoceptor blocking activity [2]. Otherwise, a high dose per kg was administered in comparison with the doses commonly used in clinical practice.

The rat gastrocnemius muscle utilized under resting conditions $\approx 10\%$ of its overall energy for the Na-K pump, with no difference after carvedilol treatment. We have previously reported that the mean ΔP for human vastus lateralis muscle after propranolol medication (6%) was similar to that for a placebo (10; see also [9,39]). Finally, only slight changes in mean ATP content in muscle were observed compared to control rats. However, the net result of ATP determinations is of course dependent on both the utilization (muscular contractile activity) and the production rate of ATP.

5. Conclusions

The present findings in the free-fed rat indicate that the sympathetic nervous system is implicated in the regulation of resting skeletal muscle thermogenesis. The demonstrated lower resting heat production rate (metabolic activity) from isolated muscle samples after one week of oral treatment with propranolol are in line with some previous human studies using whole-body oxygen consumption measurements. Carvedilol, in contrast, had no influence, probably because of its combined β- and α_1-blocking activity. The practical implications of these findings are unknown, especially as many physiological situations with high adrenergic drive are so different from the present state. Direct microcalorimetry may help in understanding the metabolic action of different adrenoceptor blockers on striated muscle [1,10,40] and other cells [41].

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