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Involvement of α_2-adrenergic activities in thermogenic responses to feeding, feeding level, and ambient temperature

Einbeziehung von α_2-adrenergen Aktivitäten in die thermogenen Reaktionen auf die Fütterung, das Fütterungniveau und die Umgebungstemperatur)

Summary To investigate the α_2-adrenergic effect on the metabolic rate, young bulls were exposed to environmental variants (feeding levels of 1.0 and 1.6 times the ME_m and ambient temperatures of 18°C and 4°C) and treated preprandially with a α_2-adrenergic agonist (clonidine) in each case. The heat production (HP) was continuously measured by indirect calorimetry using climatized respiratory chambers. Post-clonidine, the preprandial HP fell in all variants but the strongest decrease occurred at 4°C, 1.6 times the ME_m. The postprandial HP rose 1.3-fold the HP of animals received the carrier (saline) at 4°C, 1.6 times the ME_m. Animals exposed to 18°C, 1.6-fold the ME_m did not significantly increase the postprandial HP after clonidine administration, suggesting different sympathetic outflow corresponded to differing resting metabolic rate, occurring in the environmental variants. Circulating fuels (glucose, non-esterified fatty acids) responded to α_2-adrenergic reduction of the sympathetic outflow but did not parallel the HP changes. Studies on monocytes revealed a linear correlation ($r^2 > 0.9$) between resting metabolic rate and expression of sulfonylurea receptors, the constitutive component of ATP-sensitive K^+ channels (K_{ATP}) suggesting a function of K_{ATP} in coupling the systemic HP with cellular metabolism.

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Zusammenfassung Um den α_2-adrenergen Einfluß auf die Wärmeproduktion (WP) zu untersuchen, wurden Jungbullen Umgebungstemperaturen von 4°C und 18° sowie Ernährungsintensitäten des 1,0 und 1,6-fachen des energetischen Erhaltungsbedarfs (ME_m) ausgesetzt und präprandial der α_2-adrenerge Agonist Clonidin verabreicht. Die präprandiale WP fiel nach Clonidingabe stark ab, am stärksten bei Tieren, die 4°C (1,6-fache des ME_m) ausgesetzt waren. Die postprandiale WP stieg 5 h nach der Clonidininfusion bei diesen Tieren um das 1,3-fache der WP bei Verabreichung von physiologischer Salzlösung, während Tiere bei 18°C und 1,6-fachem an ME_m nicht signifikant reagierten. Die WP-Stufen in den Umweltvarianten korrelierten nur schwach mit den Spiegeln an Glukose, freien Fettsäuren, Schilddrüsenhormonen und Kortisol, jedoch direkt und eng mit der Expression von Sulfonylharnstoffrezeptoren bei Monozyten. Die Ergebnisse zeigen, daß die WP maßgeblich von der α_2-adrenergen Aktivität abhängt und deren regulatorische Funktion auch auf die Expression von Sulfonylharnstoffrezeptoren gerichtet zu sein scheint.

Key words Thermogenesis – α_2-adrenergic activity – ATP-sensitive K^+ channels

Schlüsselwörter Thermogenese – α_2-adrenerge Aktivität – ATP-sensitive K^+ Kanäle

Introduction

The resting and adaptive metabolic rate appears to be under centralnervous control, mediated mainly by sympathetic activity, ventilatory system and cardiac output (5). However, the perfusion of tissue with blood via capillary activities, regulated by some tissues themselves, can be limiting for the metabolic rate (5, 11). ATP-sensitive K^+ channels (K_{ATP}) seem to play a deciding role for the HP regulation at cellular level as they set the membrane potential in many cells. They release ATP which may be converted to adenosine bound by adenosine receptors with tonic function, and their activity is under control of cell's metabolic rate as they are closed by increasing intracellular level of ATP (1, 2). Adrenoceptors may influence the K_{ATP} activity via G-Proteins (1) so that the activity of this channel type is linked to the adrenergic component of the sympathetic neurotransmission. However, the coordination of the whole body HP with cell's metabolism is not well-understood. Therefore, the aim of this study was to elucidate the implication of the α_2-adrenergic thermoregulatory component of the sympathetic system in the whole body HP and in the expression of K_{ATP} in monocytes as indicator cells.

Material and methods

Young bulls 280±10 kg in weight at the beginning of the experiments were exposed to different ambient temperatures (4°C, 18°C) and fed a diet (8), consisting of 20 % barley and 80 % hot air dried grass (crude fiber: 24 %, crude protein: 15 %, ME : 10.6 MJ/kg DM). The HP was measured by indirect calorimetry (climatized respiratory chambers). The animals were jugularis vein-catheterized by polyurethane catheters (Braun-Melsungen). The health state was monitored via blood immune protein levels (CRP, α_2M, C3c, IgG), rectal temperatures, and the heart rate, which was recorded by a Sport-tester (Polar, Finland). The experiments started with an infusion of saline, the carrier of clonidine. On the next day, the α_2-adrenergic agonist clonidine (3, 4) was administered (20 nmol kg^{-1}, 8 min). The animals were fed 1 h and 5 h post-infusion. Glucose and non-esterified fatty acids (NEFA) were enzymatically determined, thyroid hormones (T_3, T_4) and cortisol by EIA (9). Mononuclear leukocytes were prepared and monocytes were flow cytometrically analyzed (10) by incubation with dihydrorhodamine (DHR) (0.75 µM) and with bis-(1,3-dibutylbarbiturate) trimethine oxonol (DiBaC, 200 nM). Sulfonylurea receptors were detected by 40 nM fluorescent glibenclamide (glibenclamide-Bodipy, MoBiTec) via flow cytometry (9). Binding was saturable up to 40 nM and was completely displaced by 200-fold excess of unlabeled glibenclamide.

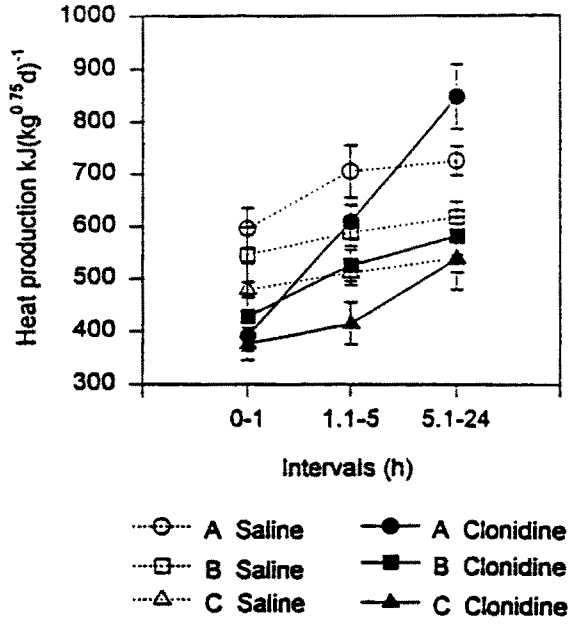

Fig. 1 Clonidine or saline were administered young bulls exposed to A (n=8) 4°C and fed 1.6-fold the ME_m; B (n=11) 18°C, 1.6-fold the ME_m; C (n=6) 18°C 1.0-fold the ME_m with ME_m = 450 kJ $(kg^{0.75}d)^{-1}$. Means and their standard deviations (small bars) of the heat production (HP) are shown during the preprandial interval (0-1 h), the interval after the first meal (1.1-5 h), and the second meal (5.1-24 h). Gas exchange was measured in 10 min intervals. For comparison, the HP was calculated per day in each case.

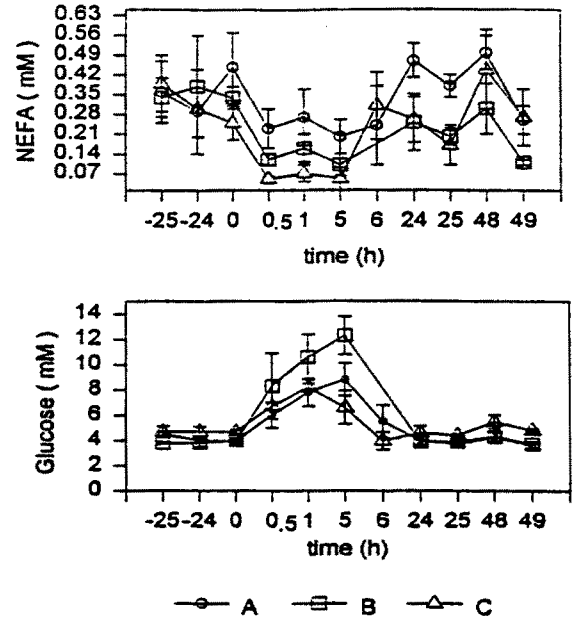

Fig. 2 Temporal course of the fuel levels post-saline (25, 24 h) and post-clonidine at the next day (0 indicates the start of the infusion) and the following days are shown. NEFA stand for non-esterified fatty acids. A, B, C symbolize the experimental variants defined in Fig. 1. Means (symbols) and standard deviations (small bars) are shown.

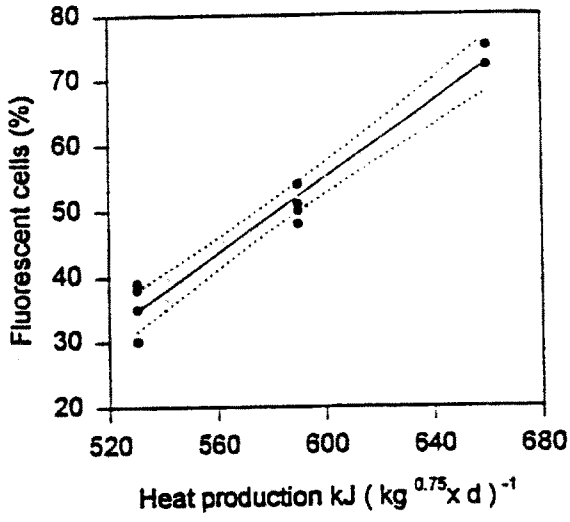

Fig. 3 Symbols represent means (pooled cells from 3 or 4 animals) and repeated experiments. The left hand symbols stand for 18°C, 1.0-fold the ME_m, the medial symbols for 18°C, 1.6-fold the ME_m, the right-hand symbols for 4°C, 1.6-fold the ME_m. Dotted lines demonstrate the confidence interval ($P < 0.05$) of the regression (coefficient of regression b=1.29 and of determination r^2=0.958). The binding of the sulfonylurea receptor ligand glibenclamide-Bodipy was flow cytometrically detected.

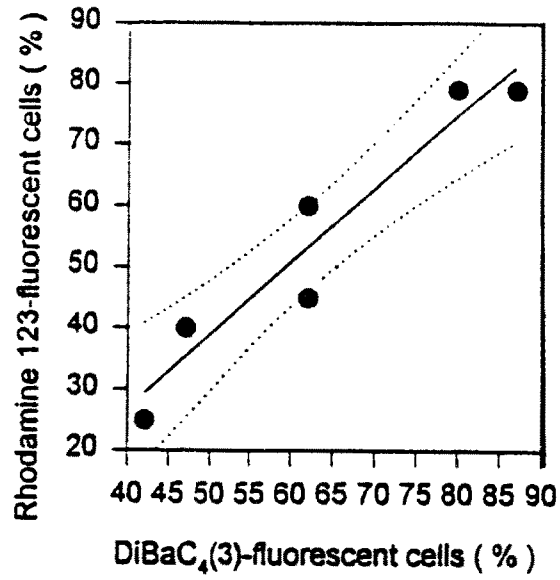

Fig. 4 Symbols stand for means (pooled cells from 3 or 4 animals) of repeated experiments. Mononuclear blood cells were incubated with DHR oxidized within the cell to fluorescent rhodamine 123 that is sequestered by active mitochondria in dependence on the electrical transmembrane potential difference of mitochondrial membranes (6). The cells were also incubated with the fluorescent anionic oxonol dye $DiBaC_4(3)$ repelled from plasma membranes, dependent on the transmembrane potential difference (12). Monocytes were fluorimetrically analyzed by flow cytometry. The cells were from animals exposed to environmental conditions, differing in feeding level and ambient temperatures. Right-hand symbols stand for 18°C, 1.0-fold the ME_m; medial symbols for 18°C, 1.6-fold the ME_m; left-hand symbols for 4°C, 1.6-fold the ME_m. Dotted lines represent the confidence interval ($P < 0.05$) of the regression (coefficient of regression b=1.19 and of determination r^2=0.921).

Results and discussion

The differences (and their standard deviations) of the resting metabolic rate between animals exposed to the environmental variants A, B, C defined by Fig. 1 were [$kJ(kg^{0.75}d)^{-1}$] A-B 50±21, A-C 117±26, B-C 67±25, and the mean differences expressed by standard deviations of the difference were 2.38, 4.5, 2.68 indicating a statistical significance at $P < 0.01$ level. Animals exposed to A responded to a α_2-adrenergic reduction of the sympathetic outflow stronger with both the thermogenic decrease during the first hour post-clonidine (Fig. 1) and the meal-evoked HP which was 1.3-fold the HP post-saline (Fig. 1, 5.1-24 h), suggesting a higher sensitivity of the sympathetic thermoregulatory component in animals exposed to A. We are unaware of any studies which reported such clearly defined implication of the sympathetic nervous system in the pre- and postprandial HP, dependent on feeding level and ambient temperature. The resting level of the major fuels were not differing among A, B, C (Fig. 2). During the clonidine-caused HP decrease, a strong elevation of the glucose level occurred, confirming previous results, indicating α_2-adrenergic involvement in the regulation of the glucose level (4). In contrast, the NEFA levels fell. The recovery of the glucose level was not dependent on environmental conditions in contrast to the NEFA response (Fig. 2). However, postprandial NEFA decrease (note the values 24 and 25 h or 48 and 49 h post-clonidine) was evident in all environmental variants (Fig. 2). This is consistent with reports using monogastric animals for studies on the α_2-adrenergic effects on glucose and NEFA levels (7). The T_3, T_4, and cortisol levels reacted to clonidine but the response did not markedly covary with the HP (data not shown). At cellular level, the resting metabolic rate of A, B, C was related to the expression of monocyte sulfonylurea receptors (Fig. 3). The receptors are components of ATP sensitive K^+ channels (K_{ATP}) which couple electrical membrane events with the mitochondrial activity (1, 2). Thus, we related monocyte's transmembrane potential difference to the electric potential of mitochondrial membranes in intact cells from animals exposed to A, B, C. A significant linear relationship was found (Fig. 4). In this way, an indirect evidence was obtained that the K_{ATP} expression may play a role for the thermoregulation associated with the sympathetic activity in different environmental conditions.

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