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Adrenergic receptor density in brown adipose tissue of active and hibernating hamsters and ground squirrels

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Abstract

The ligand-binding characteristics (B_{max} and K_D) of α_1- and β_1/β_2-adrenoceptors were investigated in membranes prepared from brown adipose tissue (BAT) of warm-acclimated, cold-acclimated, hibernating and arousing ground squirrels (*Spermophillus undulatus*) and hamsters (*Mesocricetus auratus*) by specific binding of [^{3}H]prazosin and [^{3}H]CGP-12177, respectively. The physiological state did not change the affinity for the adrenoceptors in the BAT of ground squirrels and hamsters. There was a significant decrease in α_1-receptor density in arousing ground squirrels and a significant decrease in β_1/β_2 density in hibernating ground squirrels. The level of α_1-receptors was in all conditions higher than that of β_1/β_2 receptors. The results indicate a possible change in balance of adrenoceptor density in the processes of cold acclimation, hibernation and arousal. The balance between the various adrenoceptor subtypes may be important for the final effect of catecholamines in BAT in different physiological states.

Keywords: Adrenergic receptor; Brown adipose tissue (BAT); Cold acclimation; Ground squirrel; Hamster; Hibernation; [^{3}H]prazosin; [^{3}H]CGP-12177

1. Introduction

Hibernators exhibit marked depression of metabolism and periodically and spontaneously arouse from hibernation to restore euthermia using only endogenous thermogenesis (Lyman et al., 1982). Brown adipose tissue (BAT) (for review see Cannon and Nedergaard, 2004) plays a major role in producing the heat necessary for maintaining stable body temperature in cold-acclimated animals, and activation of this organ helps hibernators to arouse from deep torpor (Nedergaard and Cannon, 1984; Carneheim et al., 1989).

When an increased thermogenic capacity of BAT is needed, a recruitment process is initiated in the tissue. This process involves increased cell proliferation, mitochondriogenesis, increased UCP1 synthesis etc. In seasonal hibernators, such as the Yakutian ground squirrel (*Spermophillus undulatus*), the recruitment process can apparently occur in the absence of cold exposure and decreasing photoperiod, i.e. it is likely governed by a circannual system (Boyer et al., 1993). In the nonseasonal hibernator, the golden hamster (*Mesocricetus auratus*), brown adipose tissue is physiologically recruited by cold acclimation (Feist, 1970; Ricquier et al., 1979; Triandafillou et al., 1984; Nedergaard and Cannon, 1984,1992; Dicker et al., 1995).

Cold exposure in euthermic animals (and arousal from hibernation) induces a release of norepinephrine (NE) from the sympathetic nerve system innervating BAT. Interaction of NE with specific adrenoceptors (AR) on brown fat cell membranes triggers a complex cascade of intracellular signals leading to thermogenesis (Cannon and Nedergaard, 2004). In addition to controlling thermogenesis, different AR subtypes in BAT also govern proliferation, differentiation and apoptosis of brown adipocytes. These processes thus play an important role in cold adaptation in non-hibernators (Cannon and Nedergaard, 2004). In hibernators, the signals that lead to recruitment are not fully

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clarified. We therefore here wanted to examine the AR subtypes in BAT of hibernators in different recruitment states.

Three subtypes of β-AR (β_1, β_2, β_3) coexist in brown adipose tissue. All three subtypes are positively coupled to adenylyl cyclase by Gs proteins (Lafontan and Berlan, 1993). At least in experimental non-hibernators (mice and rats), it is these β-receptors that mediate most of the thermogenic response and much of the recruitment response, which includes extensive cell proliferation, cellular differentiation, mitochondriogenesis, as well as an increase in UCP1 amount (Rehnmark et al., 1990; Bronnikov et al., 1992; Zhao et al., 1994; Cannon et al., 1996). During brown adipocyte development, at the transition from proliferation to differentiation, a coordinated switch in the β-receptor subtype occurs from β_1 to β_3 (Bronnikov et al., 1999b).

The presence of α_1-receptors has been demonstrated in brown adipocytes (Mohell et al., 1983) and the different subtypes of α_1-AR (α_{1A}, α_{1B}, α_{1D}) have been observed in the tissue (Costain et al., 1996; Granneman et al., 1997; Kikuchi-Utsumi et al., 1997). The stimulation of α_1-AR leads via Gq proteins to Ca^{2+} mobilization from intracellular stores and to an increase in intracellular Ca^{2+} levels via an enhancement of phosphoinositide hydrolysis (Nedergaard et al., 1996). Activation of α_1-receptors leads to more effective expression of a number of genes important for hibernation and surviving in the cold: c-*fos* (Thonberg et al., 1994), UCP1 (Rehnmark et al., 1990), lipoprotein lipase (Kuusela et al., 1997) and thyroxine 5′ deodinase (Noronha et al., 1991). α_1-stimulation in brown fat cells from golden hamster may have a significant role in acute thermogenesis (Zhao et al., 1997). There is considerable evidence for α- and β-receptor cross-talk, which can play a significant role in the regulation of physiological events (Bronnikov et al., 1999a). It is also suggested that the balance between the various adrenoceptor subtypes may be important for the final effect of catecholamines in BAT.

We have earlier preliminarily reported possible effects of hibernation on α_1-receptor density in the BAT of a nonseasonal hibernator, the golden hamster (Nedergaard et al., 1996). To examine possible differences in AR regulation between seasonal and nonseasonal hibernators, we have in the present study investigated the density and affinity of α_1 and β_1/β_2 adrenoceptors in BAT of a seasonal hibernator (the ground squirrel (*S. undulatus*)) and a nonseasonal hibernator (the golden hamster (*M. auratus*)). These animals were investigated in conditions differing in adrenergic stimulation intensity: under low endogenous stimulation by NE in summer-active (warm-acclimated) and in hibernating animals, and under high endogenous stimulation by NE in cold-acclimated animals and in animals arousing from hibernation.

2. Material and methods

2.1. Source of BAT

Ground squirrels of both sexes were trapped near Yakutsk (Siberia, Russia) in late summer and housed in individual cages at 20–22 °C under natural photoperiod. Food, water and nesting material were available *ad libitum*. In November, the animals were transferred to a dark room with a temperature of 2–4 °C. BAT was obtained (as described in 2.2) from cold-acclimated or hibernating animals in December–January (on days 3 to 5 of a hibernation bout). In 9 hibernating ground squirrels, arousal was artificially evoked by transferring the animals to 10 °C and manipulating them by applying leads for electrocardiography and body temperature measurement (Popov et al., 1999). From 5 of these, BAT was obtained when the rectal temperature was about 8–9 °C and the heart rate was 90–100 beats per minute (Fig. 1) (referred to as “arousing”). At this time, BAT temperature was measured to be about 15 °C. The BAT from summer-active animals was obtained the following July.

Hamsters of both sexes from the Wenner-Gren Institute’s colony were housed one per cage either at 20 °C (warm-acclimated) or at 4 °C (cold-acclimated or hibernating), for at least 6 wk, with a 6:18-h light–dark cycle. All animals had free access to food (a diet mainly based on sunflower seeds) and water.

2.2. Membrane preparation from BAT

Semi-purified membrane fractions were obtained principally following the procedure of Costain et al. (1996). Animals were decapitated and the interscapular, subscapular, cervical and axillary BAT was rapidly removed, cleaned of adhering tissues, weighed and homogenized in a Potter–Elvehjem homogenizer with a Teflon pestle in 0.25 M sucrose, 10 mM Tris–HCl, 1 mM EDTA, 0.1 mM PMSF (pH 7.4). The 10% (w/v) homogenate was filtered through one layer of silk cloth and the filtrate was centrifuged at 9000 ×*g* for 10 min at +4 °C. The fat layer at the top of the centrifuge tube was removed, and the supernatant fraction was then centrifuged at

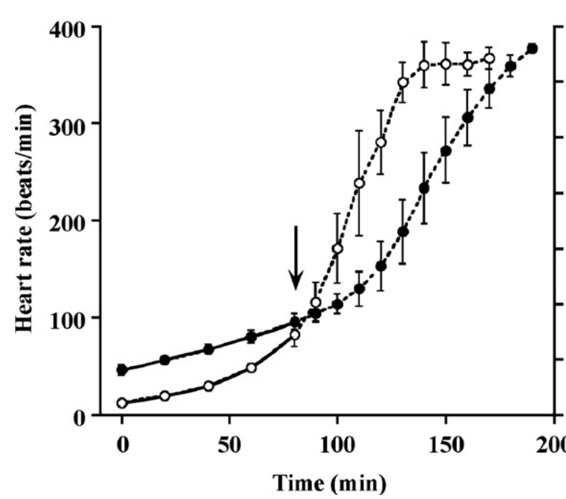

Fig. 1. Artificial arousal from natural hibernation in ground squirrels. At time 0, ground squirrels were transferred from the cold room to +10 °C, and heart rate and rectal temperature were followed during the thus induced arousal. Filled circles indicate the time-course of rectal temperature, obtained by electrical monitoring; open circles indicate the time-course of heart rate, obtained by electrocardiography. Each point on the solid curves (-) is the mean ± SE of 9 animals and on the dotted curves (- - -) is the mean ± SE of 4 animals. The remaining 5 animals were killed at the time point indicated by the arrow.