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Increased thermoregulatory responsiveness in cold adapted but not in hyperthyroid hypermetabolic rats

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Cold-adapted (CA) rats, unlike non-adapted (NA) ones, give exaggerated metabolic response to acute cold exposure, with paradoxical "overshoot" core temperature (T_c) rise in the cold, and they also give enhanced hyperthermia to central injection of prostaglandin E_1 (PGE_1). The adaptation-dependent differences might be explained either by the high thermogenic capacity of peripheral tissues in CA rats or by differences in the central processing of regulatory signals. If high tissue metabolism sufficiently explains the extreme responses of CA animals, other hypermetabolic states (with high resting metabolic rate, RMR), e.g. hyperthyroidism, should also be accompanied by enhanced reactions. In the present study thermoregulatory responses to acute cold exposure or to PGE_1 were compared in hypermetabolic CA, similarly hypermetabolic thyroxine-treated (T_4) and control non-hypermetabolic NA rats (mean RMR = 8.12, 8.47 and 6.03 W kg^{-1}, respectively). Cold exposure was followed by paradoxical core temperature (T_c) rise of 0.5 to 0.7 °C only in CA rats, but by T_c fall (0.8 to 2.1 °C) in NA and T_4 animals. Identical central stimuli (PGE_1) induced larger elevations of T_c and metabolic rate in CA rats than in similarly hypermetabolic T_4 or in non-hypermetabolic NA animals (mean T_c rise of 1.9 °C in CA vs. 0.9 °C in T_4 and 1.0 °C in NA rats). Vasodilatation thresholds were also similar in NA and T_4, but lowered in CA animals. A hypermetabolic status, *per se*, does not seem to explain the enhanced thermoregulatory responsiveness of CA animals, adaptation-induced central regulatory changes may be more important for the "overshoot" phenomenon.

Keywords: thermoregulation, thermal responsiveness, cold-adaptation, cold exposure, hyperthyroidism, prostaglandin E, body temperature, metabolic rate

Cold-adapted (CA) rats transferred from thermoneutrality to cold have been demonstrated (10) to exhibit a characteristic "metabolic overshoot" reaction with consequent paradoxical rise in core temperature. In non-adapted (NA) rats cold

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exposure of comparable severity usually induces temperature fall. Apart from such acute cold exposures, central injections of prostaglandin E (PGE) also elicit exaggerated metabolic response and hyperthermia in CA rats as compared with their NA counterparts (7, 12). Since CA animals are known to have enhanced thyroid function and the thermogenic capacity of their peripheral tissues is also high (1–4), these enhanced reactions might possibly be explained by the high tissue thermogenesis, partly due to the enhanced thyroid function. Alternatively, not only the peripheral metabolic factors, but also the central regulation may be different in the CA status: in fact, CA rats exhibited enhanced central responses also to orexigenic stimuli (12).

On the one hand, if high tissue thermogenesis (without any change in central regulation) is indeed crucial in inducing the characteristic overshoot metabolic/thermal responses of CA rats, similar enhancement of cold-induced or PGE-induced responses should be expected to occur also in other hypermetabolic situations. Hyperthyroidism might be an example of such hypermetabolic states (8). On the other hand, changes in central regulatory factors might also be assumed to influence the metabolic functions of peripheral tissues. Accordingly, adaptation-dependent variances of central processing of thermoregulatory information in CA animals might possibly lead to extreme drive for tissue heat production in the course of acute cold exposure, as well as in response to central febrigenic stimuli, inducible e.g. by PGE (5, 11). However, in this case the drive is probably not modified by a hypermetabolic status *per se*, e.g. hyperthyroidism should not cause augmented responses to cold exposure or to PGE. In order to understand the mechanism of the process of adaptation to cold environment, it would be important to see whether or not central regulatory functions are involved or modified in this process. Such data might be expected to shed some light on the central and peripheral mechanisms of other adaptation processes as well.

In the present study the thermoregulatory responsiveness to cold or to PGE-treatment was compared in thyroxine-treated (T_4) hypermetabolic, CA hypermetabolic and NA non-hypermetabolic rats. Results of pilot studies (6) have been described earlier.

Materials and Methods

Adult (223–287 g) female Wistar rats were kept, for at least three weeks, at a room temperature of 24–26 °C (NA group, n=9) or in a cold chamber of 3–5 °C (CA group, n=8), with a 12/12-h light-dark cycle (lights switched on at 6:00). The rats had food and water supply *ad libitum*. Further NA animals received daily subcutaneous injections of 100 μg D-L-thyroxine (Reanal) (T_4 group, n=9). All animals were habituated to handling and accustomed to a wire-mesh restraining-cage in which they could not turn around, but their other movements were not blocked. About 10 days before the first thermoregulatory test a metal guide cannula was implanted into the right lateral cerebral ventricle (i.c.v.) of each rat under ketamine + xylazine anesthesia (78+13 mg kg^{-1} intraperitoneally) (12). On the morning of the tests the unanesthesized rats were semi-

restrained in the usual cage and, together with the cage, they were placed into an open-circuit metabolic chamber at their thermoneutral temperatures (i.e. 25, 30 and 20–25 °C, for CA, NA and T_4 groups, respectively, according to our previous practice) (8,10). The standard temperature was secured by a thermostatically controlled water-bath. A Kipp-Noyons diaferometer was used for continuous measurement of CO_2 production (indicating metabolic rate, MR). Along with MR, the temperatures of the colon, the tail skin and the metabolic chamber (T_c, T_s and T_a, respectively) were also continuously measured by copper-constantan thermocouples. Vasodilatation threshold was judged from a sudden rise of T_s, as compared with the standard T_a.

Acute cold test: after reaching a metabolic/thermal equilibrium in the chamber, the water-bath was cooled by added ice (NA rats to 15 or 5 °C, all others to 5 °C) for 60 min, then it was re-warmed to thermoneutrality for further 30 min. In other tests the rats were given i.c.v. injections of 100 ng PGE_1 (Sigma) or the solvent 0.9% NaCl in a volume of 5 μl through an extension of the cannula (in order to avoid acute disturbance of the animals); the injections were given at thermoneutrality, MR and temperatures were measured continuously for 1 hour.

All animals went through a similar protocol. A 3-week adaptation period to cold (CA, n=8) or room temperature (NA, n=9; and T_4, n=9) was followed by a test-period. The i.c.v. cannula was implanted to each rat during the second week of the adaptation period, while thyroxine was given to T_4 rats from the last week onwards without interruption until the end of the experiment. During the first week of the test period cold tests were performed. CA and T_4 rats had one exposure to 5 °C. The NA rats were exposed to cold twice: once to the same 5 °C, once to 15 °C (in random order); the latter one to see their response to a cold, which causes comparable strain (similar MR rise in % of RMR) as that observed in cold exposed CA rats. On the following week the animals had two i.c.v. injection tests (two days apart) in random order: once PGE_1, the other time solvent was given.

At the end of the tests the animals were given an overdose of urethane and the location of the implanted cannula was macroscopically checked. In all experimental procedures the permission and rules of the Local Ethical Committee for Protection of Animals in Research (BA02/2000-20/2001) were followed.

One-way ANOVA with Scheffe's *post hoc* tests were used for statistical analysis of the data. Differences were accepted statistically significant at the level of $p<0.05$. In the figures means ±S.E.M. are shown.

Results

At the corresponding thermoneutral environments, the resting MR (RMR) was significantly higher in CA than in NA rats, but slightly lower than in T_4 animals: 8.12±0.58, 6.03±0.39 and 8.47±0.44 W kg^{-1}, respectively. Upon being transferred to intense cold (5 °C for CA and T_4, 15 °C for NA animals) MR increased in all rats (Fig. 1). The extent of rise, in % of RMR, was comparable between the CA and NA

groups (slightly smaller in the NA group). This indicates that 15 °C meant almost similarly strong strain for NA rats, as 5 °C did for CA animals. For NA rats a 5 °C environment was not only intense, but extremely severe cold and, although it evoked further MR rise, it induced a further T_c fall. MR in T_4 rats increased only by 60–70% and reached the same absolute level as the MR of NA rats at 15 °C (absolute values are not shown in the figure). The resting T_c was lowest in CA, higher in NA rats, and still higher in rats of the T_4 group (37.9±0.1, 38.2±0.1 and 38.7±0.1 °C, respectively).

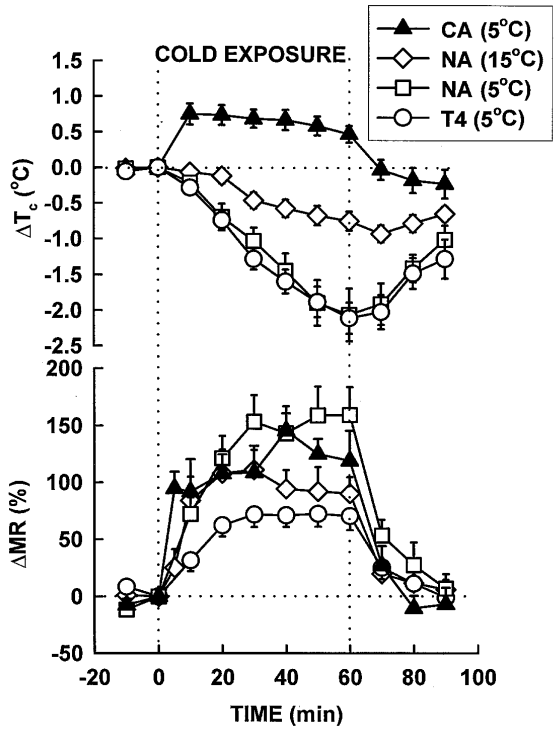

Fig. 1. Changes of core temperature (upper plane, ΔT_c, °C) and metabolic rate (lower plane, ΔMR, in % of the initial value) in T_4 (circles, n=9), control NA (squares, n=9) and CA (filled triangles, n=8) rats in the course of a 60-min exposure from thermoneutral to cold of 5 °C (or NA to 15 °C, diamonds), and during the 30-min re-transfer to thermoneutrality. Initial values in T_4, NA and CA animals for T_c are 38.7±0.1, 38.2±0.1, 37.9±0.1 °C, and for MR are 8.47±0.44, 6.03±0.39, 8.12±0.58 W kg^{-1}, respectively

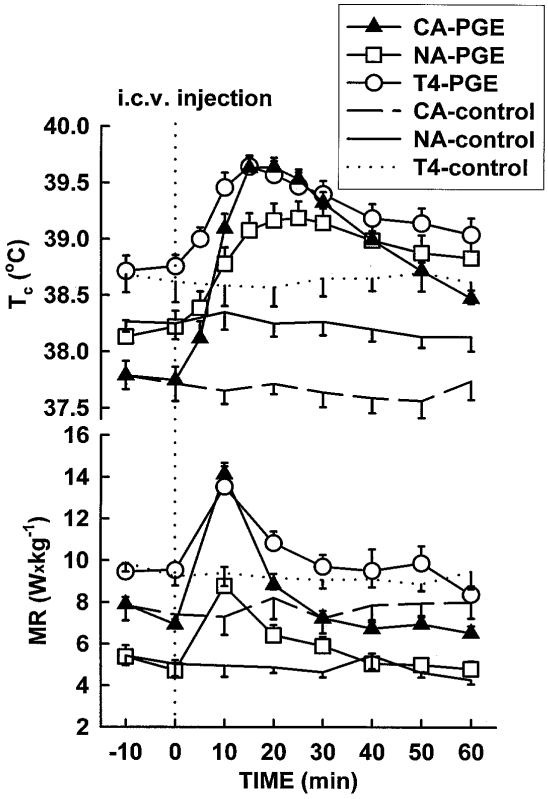

Fig. 2. Changes of T_c (upper plane, °C) and MR (lower plane, W kg^{-1}) in T_4 (circles, n=9), control NA (squares, n=9) and CA (filled triangles, n=8) rats following i.c.v. injection of 100 ng PGE_1 at the corresponding thermoneutral ambient temperatures. Dotted, continuous, and dashed lines represent T_c- and MR-changes following i.c.v. injections of the vehicle to the same T_4, NA and CA animals, respectively

In the course of the 1-hour intense cold exposure T_c rose by 0.5±0.1 °C in CA, but fell by 0.8±0.1 °C in NA and by 2.1±0.2 °C in T_4 animals; in the NA group exposure to 5 °C was followed by similarly pronounced hypothermia as in the T_4 group. The MR-rises in NA and T_4 animals were slower and could not prevent the development of hypothermia. The T_c rise in CA rats persisted throughout the duration of exposure, and it was significantly different from all other groups. Upon re-warming to thermoneutrality, a sudden fall in MR was characteristically accompanied by a T_c fall in CA but never in NA or T_4 rats.

An i.c.v. injection of 100 ng PGE_1 to CA rats induced sharp rises in both T_c and MR, significantly exceeding the corresponding rises in NA or T_4 animals: 1.9±0.1 vs. 1.0±0.2 or 0.9±0.1 °C and 7.10±0.55 vs. 4.06±1.05 or 4.00±1.11 W kg^{-1}, respectively (Fig. 2). Vehicle injections were without effect in all three groups.

Chamber temperature (T_a, which primarily determines T_s and mean skin temperature before skin vasodilatation) and T_c values at the onset of skin vasodilatation (prior to cold exposure or i.c.v. injection) are demonstrated in Fig. 3: T_c and T_s are main determinants of central heat loss threshold function. Similarly to NA rats, in T_4 animals tail skin vasodilatation started at a high T_c (even slightly higher since the thermoneutral T_a was relatively low), unlike in CA rats, in which lower vasodilatation T_c thresholds were observed (despite the also low T_a). Only cases with clear-cut threshold values are demonstrated (in some tests stabile vasoconstriction or -dilatation, or slow/gradual warm-up of the tail skin did not allow threshold determination).

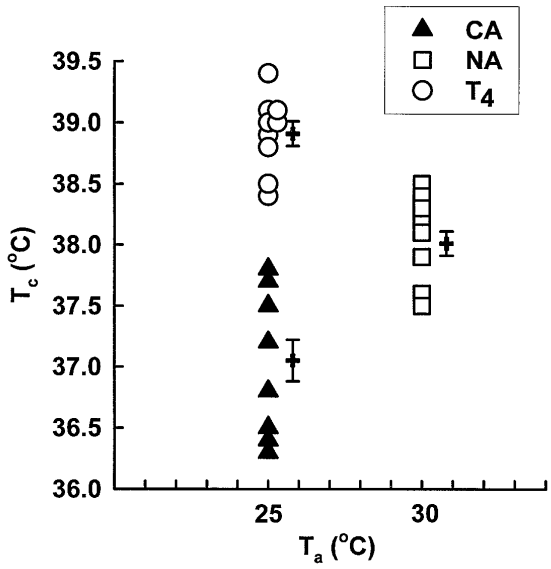

Fig. 3. Temperature of the chamber (T_a) and the colon (T_c) at the onset of tail skin vasodilatation in T_4 (circles), NA (squares) and CA (filled triangles) rats

Discussion

The response of CA rats to acute cold exposure appears to be paradoxical, in the sense that MR starts rising immediately, without any initial decrease in T_c, and it may increase so much that T_c actually becomes elevated, instead of falling like in NA ones (9, 10). In NA rats the T_c fall at 15 °C is not due to metabolic exhaustion: the extent of the MR rise (in %) is similar as that seen in cold exposed CA rats with paradoxical T_c rise, and NA rats can further elevate (at 5 °C) their MR (i.e. they should be able to prevent a T_c fall at 15 °C).

In the course of re-warming CA rats from intense cold to thermoneutrality, the high T_c is followed by a paradoxical T_c fall, as a characteristic inverse overshoot phenomenon. This is also absent in the T_4 and NA groups. In fact, artificially pre-heated CA rats with high resting T_c (exceeding the resting T_c of T_4 rats), did not show any overshoot to cold, but upon transfer to thermoneutrality from cold, they still exhibited a T_c drop, quite the same way as their CA counterparts without pre-heating (10). Thus, the higher resting T_c of T_4 rats probably cannot explain the lack of the overshoot phenomenon.

Enhancement of peripheral cold sensitivity was suggested (9) to participate in the overshoot phenomenon, but it did not appear to offer a full explanation, and did not exclude a possible role for high tissue thermogenic capacity or for increased central responsiveness.

The high level and high sensitivity of tissue thermogenesis in CA animals (1–4). might indeed help the manifestation of any MR rise, including this MR overcompensation. Still, the large thermogenic capacity, in itself, could not explain the overshoot metabolic response to cold, or the exaggerated PGE-hyperthermia, since neither phenomenon was observed when the high RMR was due to a hyperthyroid state. Thus, in the overshoot response of CA rats an important role must be assumed for regulatory factors.

Increased responsiveness of central thermoregulatory structures to various (thermal and non-thermal) stimuli has been concluded from experiments in which enhanced thermal responses to centrally applied prostaglandin E were found in CA rats as compared with NA animals (12). Greater feeding responses to central injections of neuropeptide Y were also observed. In these studies, identical amounts of the centrally applied substances elicited different responses, depending on the adaptation status.

The present data clearly show that CA rats are more sensitive to various stimuli: not only the comparable peripheral stimuli induce greater central drive for increasing MR, but also the comparable central ones. The increased central regulatory sensitivity is by no means confined to enhancement of heat production. The shift in vasodilatation threshold emphasizes that the perception or central transformation of peripheral warm signals may as well be altered by cold adaptation. Under appropriate conditions (warm or thermoneutral environment), CA rats can also lose heat more easily than their NA or T_4 counterparts. Of course, in a standard cold environment (during the adaptation period or during acute exposure to cold), the enhanced tendency for heat production prevails. Thus, in CA rats, in addition to the higher peripheral cold sensitivity and higher basic tissue thermogenic activity, the central regulatory changes are also in favour of the enhanced responses to various stimuli.

When CA rats are placed into a thermoneutral environment the peripheral cold stimuli are acutely missing and the regulated level of MR is suppressed to the possible minimum, although RMR remains still high due to changes at tissue level. Still, due to the central regulatory changes, heat loss is activated earlier, altogether resulting in a lower resting T_c than in NA rats. In T_4 rats due to primary hypermetabolism and continuous hyperthermia the warm receptors are stimulated chronically (but heat loss is

not activated proportionally), similarly as in warm-adapted animals. Taken together, the thermoregulatory functions of hypermetabolic T_4 rats resemble those of NA ones, and do not mimic those of CA animals.

In summary, the enhanced responsiveness of CA rats to cold or PGE is likely to be due to regulatory changes of central origin and the hypermetabolism that accompanies cold-adaptation appears to play only a minor role in the overshoot phenomenon.

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