tension adjusted such that the atria were beating at the peak of the length-tension curve. The preparation was allowed to stabilize for a period of 0.5–1 h at which time the atrial rate was counted. The chronotropic response to catecholamines was tested by adding epinephrine (E) or norepinephrine (NE) to the bath to bring the bath content of E to the following concentrations: 10^{-7} M, 10^{-6} M, 10^{-5} M or of NE to these concentrations: 10^{-7} M, 10^{-6} M. The atrial rate was then counted after stabilization. Statistical analyses were done according to methods described by Schefler[10].

Results. The spontaneous rate of atria isolated from sedentary controls was 235 ± 7.8 beats/min (mean $\pm$ SEM). The rate from exercised animals was 194 ± 5.7 beats/min. The difference between sedentary and exercised was significant ($p < 0.001$).

The figure shows these basal rates as well as the responses of atria to the various concentrations of E and NE. Because atria from exercised animals beat at a significantly lower rate than atria from controls, we analyzed the data by means of a factorial analysis of variance. By this test, the response to E (with the exercise effect factored out) was significant ($p < 0.005$) at all concentrations except 10^{-7} M. The response to NE was significant at all concentrations. However, interaction effects between exercise and catecholamines were not significant at any concentration indicating that chronic exercise does not alter the chronotropic response to catecholamines. Nevertheless, trained atria always beat at a significantly ($p < 0.01$) lower rate than did untrained atria at all concentrations of E and NE. The rate of trained atria only exceeded the basal rate of control atria by a significant amount ($p < 0.05$) when in the presence of E in a concentration of 10^{-5} M.

Discussion. Our results corroborate those of Bolter et al.[11] which show that isolated atria from exercise-trained rats beat at a slower rate than atria from sedentary controls. The mechanism for exercise bradycardia therefore exists at least in part at the level of the atrium itself. This bradycardia could certainly be related to the increased amount of acetylcholine found in the exercised heart tissue. At least a part of the bradycardia effect might be explained in terms of altered sensitivity of the pacemaker to the autonomic neurotransmitters. However, our results indicate that atria from exercised animals are equally as sensitive to catecholamines as atria from sedentary rats. Whatever anti-adrenergic mechanism exists in these exercised hearts, it is not exerted by a reduction in chronotropic responsiveness to catecholamines. It is noteworthy, however, that the rate of trained atria was always less than untrained atria and that only with E in a concentration of 10^{-5} M did exercised atria beat at a faster rate than the basal rate of non-exercised controls. These results are consistant with an anti-adrenergic role of exercise as expressed by Raab et al.[9].

1 This study was supported in part by a grant from the Genesee Valley Heart Association.
2 C.M. Tipton and B. Taylor, Am. J. Physiol. *208*, 480 (1965).
3 Y.C. Lin and S.M. Horvath, J. appl. Physiol. *33*, 796 (1972).
4 C. de Schryver and J. Mertens-Strythagen, Experientia *31*, 316 (1975).
5 D.C. Smith and A. El-Hage, Experientia *34*, 1027 (1978).
6 C. de Schryver and J. Mertens-Strythagen, Am. J. Physiol. *217*, 1589 (1969).
7 R. Gordon, S. Spector, A. Sjoerdsma and S. Udenfriend, J. Pharmac. exp. Therap. *153*, 440 (1966).
8 A.S. Leon, W.D. Horst, N. Spirt, E.B. Wiggan and A.H. Womelsdorf, Chest *67*, 341 (1975).
9 W. Raab, E. DePaula, P. Silva, H. Marchet, E. Kimura and J.K. Starcheska, Am. J. Cardiol. *5*, 300 (1960).
10 W.C. Scheffler, Statistics for the Biological Sciences. Addison-Wesley Pub. Co., Reading 1969.
11 C.P. Bolter, R.L. Hughson and J.B. Critz, Proc. Soc. exp. Biol. Med. *144*, 364 (1973).

Complete cold substitution of noradrenaline-induced thermogenesis in the Djungarian hamster, *Phodopus sungorus*[1]

H. Böckler, St. Steinlechner and G. Heldmaier

Zoological Institute, J. W. Goethe-University, Siesmayerstr. 70, D-6000 Frankfurt/Main (Federal Republic of Germany), 1 July 1981

Summary. The thermogenic response to injections of noradrenaline at thermoneutrality was substituted by thermogenesis at low ambient temperatures. This demonstrates that noradrenaline-induced heat production is equivalent to physiologically induced nonshivering thermogenesis during cold exposure.

In small mammals, nonshivering thermogenesis (NST) is the dominating pathway for thermoregulatory heat production, and shivering thermogenesis is only used when heat production by NST is insufficient[2,3]. The most important site of NST appears to be brown adipose tissue (BAT)[4], where heat is liberated by oxidation of fatty acids following a stimulation of BAT cells by noradrenaline (NA) released from the sympathetic nerve endings of BAT[5,6]. This mechanism of NST induction is deduced from the fact that NST may also be induced artificially by injections of NA or it may be inhibited by injections of β-adrenergic inhibitors like propranolol[7,8]. The artificial stimulation of NST is most commonly used for quantitative determination of NST. Provided that an optimum dosage of NA is used, NST may be stimulated to its maximum in a curarized or deeply anesthetized mammal even in a warm environment. However this artificial stimulation always leaves the question whether the observed calorigenic response to NA is equivalent to the physiologically available NST in an unrestrained mammal during cold exposure.

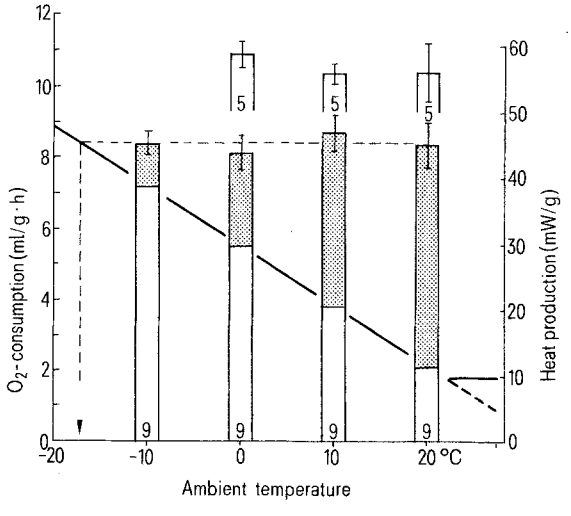
If NA-induced NST corresponds to the cold-induced NST, than the calorigenic effects of exogenous NA and endogenous NA should be able to substitute for each other, i.e. if NA is injected during cold exposure the same NST maxima should be obtained as at thermoneutrality (for theoretical considerations see also Mejsnar and Jansky[9]). Differing from previous authors we want to use the term 'cold substitution' instead of 'temperature substitution' for a more precise description of the phenomenon. A complete cold substitution of the calorigenic response to exogenous

NA would indicate that a) NA-induced calorigenesis is equivalent to physiologically available NST, b) NA is the transmitter for cold-induced NST, and c) only NST and no shivering thermogenesis has been activated at the level of the T_a presently investigated. Previously published data on cold substitution are far from uniform and there seem to exist interspecific differences as to whether a complete substitution or an additive relation is found. The aim of the present study was to determine the cold substitution in the Djungarian hamster, an animal whose NST capacity is extremely large, and therefore can be tested over a wide range of T_a[10].

A total number of 51 Djungarian hamsters was used in this experiment. The animals were kept under natural photoperiodic conditions at constant T_a (23 °C = 'inside') or outdoors at natural ambient temperatures and light according to the season (= 'outside'). They were kept in individual cages with woodshavings as bedding material and were provided with food and water ad libitum. For the NA-tests the hamsters were placed on PVC-grids in small plastic boxes (2.8 l) inside a climate chamber. The metabolic rates of the unanestetized and unrestrained hamsters were determined using an open flow system with a paramagnetic O_2-analyser and an infrared CO_2-analyser (for further details see Heldmaier and Steinlechner[11]). The NA-tests were performed at T_a's of 20, 10, 0, and −10 °C in hamsters from 'inside' (December 1978), while 'outside' animals were tested only at 20, 10, and 0 °C (February 1979). Preliminary experiments had shown that at T_a's higher than 20 °C the hamsters became hyperthermic or even died after injection of NA. Recent investigations indicate that injections of catecholamines may inhibit their release from sympathetic nerve terminals[12]. Therefore, experiments on cold substitution require an optimal dosage of NA, which compensates for its possible inhibitory effect on endogenous release of NA. Dose-response tests with hamsters from both 'inside' and 'outside' showed maximal stimulation of NST between 0.4 and 0.8 mg NA/kg when injected s.c.[13].

As can be seen in the figure, there is a complete cold substitution in the hamsters from both inside and outside, i.e. the increase in O_2-consumption induced by injection of NA at 20 °C is reduced at lower T_a-levels by the amount of the corresponding resting metabolic rate elevated by cold exposure. In hamsters from 'inside' there is a mean maximum O_2-consumption after NA-injection from 8.1 to 8.7 ml/g · h^{-1}; in hamsters from 'outside' these values are between 10.4 and 10.9 ml/g · h^{-1}. At −10 °C there was no significant increase in muscular activity before injection of NA, as confirmed by EMG recordings. Similar results in mice were found by Mejsnar and Jansky[14] if the animals were injected with an optimal dosage of NA, as well as in the golden hamster[15], in voles[16], guinea-pigs[2] and in rats[17]. Jansky et al.[18], testing various species, report contradictory results. They could prove complete cold substitution only in the guinea-pig, while in hedgehogs and mice additive relations were found. At least in the mouse this result might be due to a suboptimal dosage of NA[19] or hypothermia of the animals, whereas the additive effects observed in the hedgehog were confirmed by Wünnenberg et al.[20] and Werner and Wünnenberg[21]. These authors also regard corticosteroids, in addition to NA, as mediators of NST in the nonhibernating hedgehog.

With all prerequisites met, such as optimal NA-dosage, wide temperature range, normothermy of the animals after NA-injection, exclusion of muscular activity, testing without narcotics and restraint, we could demonstrate a complete cold substitution of the calorigenic response to NA in the Djungarian hamster. Therefore, we conclude, that a) the NA-test is a suitable measure for quantitative determination of physiologically available NST, showing that b) NST in vivo is mediated by NA, and c) that NA-dependent NST is the only pathway for thermoregulatory heat production in the Djungarian hamster exposed to moderate cold.



Maximal O_2-consumption of Djungarian hamsters after injection of 0.8 mg/kg NA at different ambient temperatures (dotted bars = inside; open bars on top = outside). Open part of lower bars indicate resting metabolic rate at each temperature tested. Solid line shows regression line of metabolic rate versus T_a (y = 5.45-0.166 x) and BMR (1.99 ml/g · h^{-1}). The regression line for metabolic rate versus T_a was the same for hamsters from 'inside' and 'outside'. The broken line represents mean maximal NA-induced NST (8.43 ml/g · h^{-1}). With this NST capacity the Djungarian hamster (inside) can maintain homeothermy down to a T_a of −18 °C as indicated by the arrow. Values are means ± 1 SEM with N for each group given within the bars.

1 Supported by the Deutsche Forschungsgemeinschaft (He 990).
2 E. Zeisberger and K. Brück, Pflügers Arch. *296*, 263 (1967).
3 G. Heldmaier, in: Proc. Int. Symp. Environ. Physiol., p. 79. Ed. R.E. Smith. Fed. Am. Soc. exp. Biol., Baltimore 1972.
4 D.O. Foster and M.L. Frydman, Can. J. Physiol. Pharmac. *56*, 110 (1978).
5 A.C.L. Hsieh and L.D. Carlson, Am. J. Physiol. *190*, 247 (1957).
6 J. Seydoux and L. Girardier, Experientia *33*, 1128 (1977).
7 L. Jansky, Biol. Rev. *48*, 85 (1973).
8 T. Heim and B. Hull, J. Physiol. *187*, 271 (1966).
9 J. Mejsnar and L. Jansky, in: Nonshivering thermogenesis, p. 27. Proc., Prague 1970.
10 S. Steinlechner and G. Heldmaier, Verh. dt. Zool. Ges. *1980*, 308 (1980).
11 G. Heldmaier and S. Steinlechner, J. comp. Physiol., in press (1981).
12 J.A. Bevan, Fedn Proc. *37*, 187 (1978).
13 H. Böckler, unpublished results.
14 J. Mejsnar and L. Jansky, Physiologia bohemoslov. *20*, 157 (1971).
15 S. Vybiral and L. Jansky, Physiologia bohemoslov. *23*, 235 (1974).
16 D.D. Feist and M. Rosenmann, Can. J. Physiol. Pharmac. *54*, 146 (1976).
17 J. Mejsnar, S. Gregorova and L. Jansky, Physiologia bohemoslov. *21*, 153 (1972).
18 L. Jansky, R. Bartunkova, J. Kockova, J. Mejsnar and E. Zeisberger, Fedn Proc. *28*, 1053 (1969).
19 G. Heldmaier, Z. vergl. Physiol. *73*, 222 (1971).
20 W. Wünnenberg, G. Merker and K. Brück, Pflügers Arch. *352*, 11 (1974).
21 R. Werner and W. Wünnenberg, Pflügers Arch. *385*, 25 (1980).