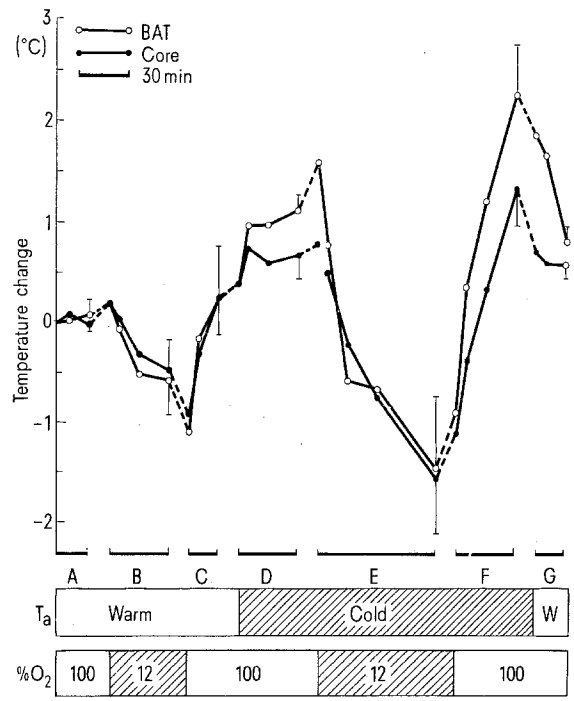
Hypoxic Inactivation of Cold-Induced Brown Fat Thermogenesis[1]

Brief exposure of anesthetized rats to hypoxia (12% oxygen) has been shown to effect an immediate reduction in the cold-induced thermoregulatory response of brown adipose tissue[2]. Moreover, this inhibition is readily reversible. The hypoxic inactivation of cold-induced brown fat thermogenesis may reflect a) tissue oxygen limitation of metabolic rate; and/or b) lack of maximal sympathetic stimulation of the tissue.

In an attempt to distinguish between these possibilities, the effect of low oxygen inhalation has been examined in unanesthetized, unrestrained rats under conditions wherein the brown fat was activated by cold and/or norepinephrine.



Temperature changes of brown adipose tissue (BAT) and core during successive exposure to varying chamber environmental test conditions as detailed in text. T_a refers to the temperature within the chamber (warm = 26 °C and cold = 12 °C). Each point represents the mean of 6 rats and selected SEM are also shown. Solid lines connect points which were recorded during the first 15 min of test A, C and G, the first 30 min of test B, D and F and the first 60 min of test E. Dashed lines connect points between tests and indicate BAT and core temperature drift that occurred during the subsequent 15 min following NE injections.

Copper-constantan thermocouples were implanted chronically in the interscapular brown fat pad (BAT) and adjacent to the carotid artery (core) in 12 cold-adapted, Long-Evans rats (350 ± 25 gm). The jugular vein was cannulated with polyethylene tubing (PE 50) for i.v. injection of norepinephrine (L-arterenol bitartrate, Sigma). 4 to 6 days following surgery, the thermogenic responses of these rats were examined. The unrestrained, unanesthetized animals were placed singly in lucite chambers (6 l volume). The environmental temperature of the chamber was controlled via a surrounding water bath and monitored with a thermocouple. The rats were exposed to either 100% oxygen or 12% oxygen in nitrogen (equivalent to an altitude of approximately 4,450 m) at a flow rate of 2 l/min. A 12 l/min flush was used when changing the gaseous environment within the chamber. Sodasorb granules were placed in the bottom of each chamber to prevent carbon dioxide accumulation. Core, chamber and brown fat temperatures were continuously monitored throughout the experiment.

In each experiment, the test conditions were varied in the following sequence: A, 100% O_2, warm (26 °C); B, 12% O_2, warm; C, 100% O_2, warm; D, 100% O_2, cold (12 °C); E, 12% O_2, cold; F, 100% O_2, cold; G, 100% O_2, warm. The times required to shift the chamber temperature and the gaseous environment were less than 10 and 2 min, respectively. Each experimental test environment was maintained until BAT and core temperatures stabilized (15–60 min). Immediately thereafter, the thermogenic responsiveness of the BAT was tested by injecting 10 μg NE. A NE-positive response was taken to be one in which the peak temperature of the BAT during the 5 min postinjection period was at least 0.3 °C above the mean temperature existing 5 min prior to injection. In 6 of the 12 surgically prepared rats, the temperature of the BAT rose in response to NE injection during conditions of 100% O_2 at 26 °C. The data from these animals are reported herein. Upon autopsy, it was found that the BAT thermocouples in the remaining 6 rats had advanced anterior to the tissue.

Brown fat and core temperatures remained quite constant (± 0.1 °C) during the 15 min exposure to 100% O_2 at 26 °C (Figure). However, 15–30 min after the O_2 concentration in the chamber had been reduced to 12%, the temperatures of both BAT and core had declined approx-

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Effect of varying chamber environmental test conditions on the thermogenic responsiveness of brown adipose tissue (BAT) to norepinephrine (10 μg) injection

Sequence of test conditions[a]	Warm			Cold			Warm
	100% O_2	12% O_2	100% O_2	100% O_2	12% O_2	100% O_2	100% O_2
	A	B	C	D	E	F	G
Rats tested	6	6	2	6	5	2	4
No. responding[b]	6	3	1	3	0	1	2
BAT Δ T (°C)[c]	0.91 ± 0.19	0.62 ± 0.14	0.80	0.37 ± 0.06	0.0	0.4	0.85 ± 0.07

[a] Test conditions A, B, C, D, E, F, G were maintained for 15, 30, 15, 30, 30, 30, 15 min, respectively prior, to 10 μg norepinephrine i.v. injections. [b] A positive response was considered to be a 0.3 °C rise in BAT temperature within 5 min following NE injection. [c] Values are means ± SEM of the positive responses.

imately 0.5 °C. This decrease was readily reversible upon re-exposure of the rat to 100% O_2.

Similar effects of hypoxia were observed when the chamber temperature had been lowered to 12 °C. Under conditions of 100% O_2 and 30 min of cooling the chamber, there occurred an average rise in temperature of the BAT of 0.7 ± 0.15 °C and of the core, 0.3 ± 0.24 °C. These cold-induced thermogenic responses were abolished when the atmosphere was changed from 100% to 12% O_2. In such an environment, the temperature of both the brown fat and core fell precipitously (after 1 h, BAT temperature was down 3.0 °C and core, 2.3 °C). Furthermore, after 15 min of exposure to hypoxia, the temperature of the BAT no longer exceeded that of the core. Return to a 100% O_2 atmosphere (still at 12 °C) resulted in a rapid increase in both the BAT and core temperatures and after 5 min, the temperature of the BAT again exceeded that of the core in all rats. At the end of the experiment, 4 of the 6 rats were returned to warm conditions (while breathing 100% O_2) and within 15 min the temperature of the brown fat had decreased to approximately that of the core.

These rapid and reversible hypoxic-induced changes in the temperatures of the brown fat and core are similar to those previously reported for rats under anesthesia[2]. Additionally, in the present study, exposure to hypoxia depressed or abolished the response of brown fat to injected NE (Table). That is, although a positive response to NE was observed in all 6 animals breathing 100% O_2 at 26 °C, only 3 of these rats exhibited such a response when breathing 12% O_2 at 26 °C, and none when exposed to 12% O_2 at 12 °C.

These data thus indicate that the hypoxic-induced depression of brown fat thermogenesis does not reflect lack of sympathetic activation at the level of the tissue. Rather the absence of a thermogenic effect of injected NE on the brown fat of cold exposed rats breathing low O_2 supports the view that the metabolism of the tissue is affected directly by the hypoxic environment. That this effect may be mediated via oxygen-limitation of cellular respiration is a conclusion similar to that reached by HEIM and HULL[3] for their observations in the newborn rabbit. Accordingly, the hypoxic inactivation of cold-induced brown fat thermogenesis is most likely a direct reflection of the concomitantly lowered arterial pO_2, although other indirect effects of low oxygen breathing (e.g. altered pCO_2) are not excluded by the data presented. Decreased regional blood flow to BAT would further limit oxygen delivery to the tissue. Such an effect for 8% O_2 inhalation has been observed in the unanesthetized euthermic woodchuck[4] but not in the anesthetized rat[5].

Résumé. Chez des rats adaptés au froid, non anesthésiés, placés brièvement dans une atmosphère de 12% d'oxygène, on observe un arrêt immédiat de la thermogénèse de la graisse brune. Cet effet semble s'expliquer par le manque d'oxygène au niveau cellulaire plutôt que par l'absence d'activation par le système sympathique. L'absence d'une réaction de thermogénèse en réponse à une injection de noradrénaline chez des rats soumis au froid et dans des conditions hypoxiques plaide en faveur de cette explication.

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The Presence of Octopamine in the Brain of *Helix aspersa* and its Action on Specific Snail Neurones

Octopamine, *p*-hydroxy-*β*-hydroxyphenylethylamine, was first identified in the salivary glands of *Octopus vulgaris*[1]. The distribution of octopamine in the octopus nervous system has recently been determined[2]. Octopamine has been found to be a normal constituent of rat sympathetic nervous system[3] and brain[4] and to be present in a wide range of species[4]. Octopamine would appear to be largely confined to nervous tissue since following denervation the octopamine content of an organ falls drastically[4]. Both dopamine and noradrenaline have been shown to be present in the *Helix* nervous system[5,6], but the present of octopamine has not been reported.

Materials and methods. All experiments in this study were made on the garden snail, *Helix aspersa*. A sensitive enzymatic assay for octopamine, developed by MOLINOFF, LANDSBERG and AXELROD[7], was used in the present study. The levels of noradrenaline were assayed using the method of SAELENS, SCHOEN and KOVACSICS[8]. The isolated snail brain was prepared for electrophysiological recording by a method previously described[9]. Intracellular recordings were made from identifiable neurones using glass microelectrodes filled with molar potassium acetate. Bio-electric potentials were amplified and displayed on a Tektronix 502A oscilloscope. Permanent traces were made on an AEI pen oscillograph. Drugs were dissolved in snail Ringer and added close to the preparation in a volume of 0.2 ml. The isolated snail brain was bathed in 10 ml Ringer. The Ringer was changed following each application of drug. The composition of the snail Ringer has previously been described[9].

Results and discussion. The levels of noradrenaline and octopamine were determined for brain and heart. The levels of noradrenaline in the brain were 0.82 ± 0.18 μg/g protein and for heart were 2.04 ± 0.35 μg/g protein. The results are the mean of 12 determinations. The levels of octopamine in the brain were 0.75 ± 0.15 μg/g protein and for heart were 1.32 ± 0.45 μg/g protein. The results are the mean of 6 determinations. The standard deviation is indicated in each case.

Octopamine, with a threshold of 50 pmol, was found to mimic the action of dopamine and noradrenaline on

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