TEMPERATURE REGULATION IN RATS EXPOSED TO COLD IN THE EARLY POSTNATAL PERIOD

V. G. Selyatitskaya, M. P. Moshkin, N. A. Romashov, T. V. Arshinova, and R. I. Salganik

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Many hormones and substrates induce expression of genes programming synthesis of corresponding adaptive enzymes in animal cells [6, 14]. Administration of genetic inducers, namely hormones or substrates, into animals in the early postnatal period has been shown to cause changes in the expression of genes induced by them, and these persist in adult animals for a long period of time [3, 4, 6]. For instance, injection of cortisol into newborn rats during the first 2 weeks of life causes a stable increase in activity of hormone-induced tyrosine aminotransferase in the liver of the adult animals [4]. Injection of large doses of galactose into newborn animals causes lasting changes in the activity of galactose-induced enzymes. Similar changes are found after neonatal induction of enzymes metabolizing amino acids and xenobiotics [3, 6]. This phenomenon has been called enzyme imprinting. Temperature and other external influences cause an increase in the rate of secretion of certain hormones which, inducing the synthesis of adaptive enzymes, maintain homeostasis of the internal medium of the body. For instance, when animals are exposed to cold, their synthesis and secretion of thyroid hormones, gluococorticoids, and catecholamines are intensified [10, 11]. Regular cooling of newborn rats during the first 7 days of life ("cold imprinting") leads to increased induction of tyrosine aminotransferase in the liver of the adult animals under the influence of cold, and the blood level of corticosterone in these animals also is consistently high [5]. It was decided to study whether these stable hormonal and metabolic shifts arising in adult animals as a result of "cold imprinting" are adaptive in character.

In the investigation described below several parameters of physical and chemical temperature regulation were studied in rats exposed to cold in the early postnatal period.

EXPERIMENTAL METHOD

Experiments were carried out in the winter on male Wistar rats. Immediately after birth the rats were divided into two experimental groups. Animals of one group (29 rats) were exposed daily for 7 days to cold at 2-4°C for 15 min as described previously [5]. Animals of the other group (20 rats) served as the control. At the age of 2 months all the rats were weighed and kept in single cages. Eight animals from each group were then placed in a temperature chamber and kept for 7 days at 4-5°C to investigate their capacity for cold adaptation. Regular conditions of illumination were maintained in the animal house: 12 h of light and 12 h of darkness. The body weight, and the length and diameter of the tail of all the experimental animals were measured at the age of 4 months, and after sacrifice the weight of the brown fat tissue (BFT) in the interscapular region was measured. In six rats from each of the four groups the temperature-regulating response to intraperitoneal injection of noradrenalin (NA) in a dose of 800 μg/kg body weight was studied. For the noradrenalin test the animals were kept at a temperature of 26°C. Thermophysical parameters were measured at intervals of 10 min 30 min before injection of NA and during 1 h after the injection. Heat emission from the body surface, with the exception of heat loss by evaporation, was measured by direct calorimetry [1]. The cylindrical differential calorimeter consisted of a working chamber and a comparison chamber, with eight thermocouples on the surface of each. To abolish heat disturbances both chambers were placed in a thermostat with water jacket. The calorimeter was calibrated with a space heater. During the measurements the animal's tail was outside the working chamber. Simultane-

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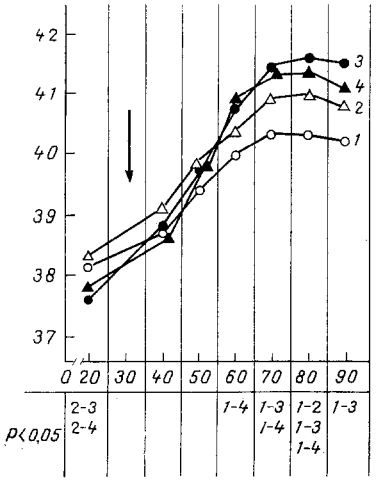

Fig. 1

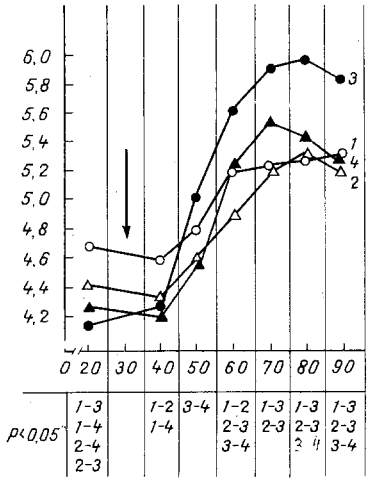

Fig. 2

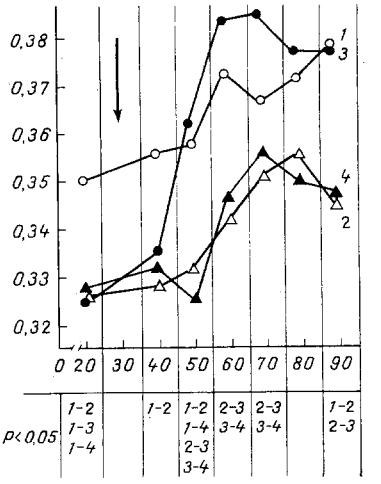

Fig. 3

Fig. 1. Changes in rectal temperature in response to injection of NA. Abscissa, time after placing animal in calorimeter (in min); ordinate, body "core" temperature (in °C). Arrow indicates time of injection of NA; P) significance of differences between groups. 1) Control animals; 2) animals exposed to "cold imprinting"; 3) control animals after 7 weeks of cold adaptation at 4-5°C; 4) animals exposed to "cold imprinting" after 7 weeks of cold adaptation.

Fig. 2. Changes in heat loss after injection of NA. Abscissa, time (in min); ordinate, heat loss (in mW/cm^2). Remainder of legend as to Fig. 1.

Fig. 3. Changes in coefficient of heat loss following injection of NA. Abscissa, time (in min); ordinate, coefficient of heat loss (in $mW/deg\ C \cdot cm^2$). Remainder of legend as in Fig. 1.

ously with heat emission, the body "core" temperature also was determined rectally with a copper–Constantan thermocouple (the detector was introduced to a depth of 5-6 cm. Heat emission was calculated by the equation:

$$\text{Heat emission } (mW/cm^2) = \frac{Q}{9.13 \cdot P^{2/3} - S},$$

where Q is the reading of the calorimeter (in mW); P the body weight (in g); $9.13 \cdot P^{2/3}$ is the area of the body surface of the rat (in cm^2); S the surface area of the tail, measured as the surface area of a cone (in cm^2). The coefficient of heat loss also was calculated by the equation:

$$\text{Coefficient of heat loss } (mW/cm^2 \cdot deg\ C) = \frac{\text{heat loss}}{T_{rect} - T_{amb}},$$

where T_{rect} is the "core" body temperature (in °C) and T_{amb} is the temperature in the thermostat (in °C). The significance of the difference between the experimental groups was calculated by Student's t test.

EXPERIMENTAL RESULTS

In mouse-like rodents the surface area of the tail, which performs the function of an organ of physical temperature regulation, varies depending on the physiological conditions: In regions with a cold climate the length and, correspondingly, the surface area of the tail of these animals are less than in regions with a warm climate [7].

In adult rats exposed to cold in the early postnatal period, the relative surface area of the tail has been found to be significantly less than in the control: 6.5 ± 0.3 compared with $7.3 \pm 0.2\%$ ($P < 0.05$).

In animals of all groups in response to injection of NA a significant increase ($P < 0.001$) was observed in the body temperature as a result of the calorigenic reaction. It will be clear from Fig. 1 that at the peak of the calorigenic reaction (40-50 min after injection of NA) the smallest rise of temperature was observed in the control animals; adaptation of the control rats to cold for 7 weeks caused a sharp rise in their ability to respond to injection of NA by a rise of body temperature (Fig. 1). Animals exposed to "cold imprinting" responded by a rapid rise of body temperature to injection of NA, independently of cold adaptation.

TABLE 1. Relative Weight of BFT in Animals of Different Experimental Groups (in g/100 g body weight)

Exposure in early postnatal period	Conditions of rearing at a mature age		P
	22°C	7 weeks of cold adaptation	
Control	0.185±0.013 (11)	0.340±0.021 (8)	<0.001
Cold imprinting	0.204±0.009 (8)	0.258±0.011 (6)	<0.001
P		<0.01	

Legend. Number of animals given in parentheses.

In response to injection of NA heat loss from the body surface also increased in rats of all groups ($P < 0.01$), but the greatest increase was observed in control animals adapted to cold at a mature age (Fig. 2). The maximal increase in heat loss and body temperature in response to injection of NA, which was observed in control rats undergoing cold adaptation, is evidence of a marked increase in the calorigenic effect of NA during adaptation to cold at a mature age, and it is in good agreement with the extensive data on this question [2, 7, 8].

In rats exposed to "cold imprinting" subsequent adaptation to cold did not change the level of heat loss (Fig. 2). In animals of both groups, undergoing "cold imprinting," the coefficient of heat loss, incidentally, was much lower than in the rats of the corresponding control groups (Fig. 3).

Changes in the calorigenic effect of NA observed during adaptation to cold correlated with changes in weight of BFT (Table 1). In control rats 7 weeks of adaptation to cold induced not only an increase in the calorigenic action of NA, but also an almost twofold increase in the relative weight of BFT. In animals exposed to "cold imprinting" prolonged exposure to a low temperature at a mature age led to a considerably smaller increase in weight of BFT and to a considerably smaller thermogenetic reaction to NA. The relationship of cause and effect between these two parameters has recently been well attested [12].

The results are evidence that exposure to cold in the early postnatal period, irrespective of subsequent conditions of rearing, induces stable changes in the mechanisms of physical temperature regulation aimed at increasing the heat insulation of the animals. This is reflected in a decrease in the relative surface area of the tail and a decrease in the coefficient of heat loss for the remainder of the body surface. Maintenance of body temperature by this method is energetically advantageous [2].

Adaptation of rats to cold (5°C) is known to reduce the increase in body weight of the animals [13]. It is an interesting fact that the increase in body weight in animals exposed to "cold imprinting" when kept for a period of 7 weeks at a temperature of 4-5°C was much greater than in control rats kept under similar conditions (64.0 ± 7.3 and 36.0 ± 6.8 g, $P < 0.01$). It is probably the reduction of energy expenditure on the maintenance of body temperature that determines the much more rapid increase in body weight of animals previously exposed to "cold imprinting" compared with control rats when exposed to a long period of cooling.

There is thus reason to suppose that "cold imprinting" leads to stable adaptation of animals to a low ambient temperature.

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PLATELET ADHESION AND AGGREGATION ON SURFACES COATED WITH HUMAN COLLAGENS OF TYPES I, III, IV, AND V

F. Misselwitz, V. L. Leitin, S. P. Domogatskii, O. V. Merzlikina, I. D. Novikov, and V. S. Repin

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Interaction between platelets and the collagen components of the connective-tissue matrix of the injured vessel wall is the key factor in initiation of hemostasis and thrombosis. Four genetic types of collagen, namely I, III, IV, and V (CI, CIII, CIV, and CV) are found in arteries [5, 10]. These collagens differ in the composition of the chains, location in the vessel wall, and capacity for fibrillogenesis [2, 5, 10]. CV is located on the endothelial surface facing the lumen of the vessel, CIV and CV, which do not form fibrillary structures in vivo, in the basement membrane. CI and CIII form fibrils in the intima, media, and adventitia [2, 5, 10]. The ability of different collagens to induce platelet aggregation in suspension depends on the form in which the collagens are present – monomeric (triple-chain) and fibrillary, for only fibrillary collagens induce aggregation [3, 4, 7, 11, 14].

In this investigation scanning electron microscopy (SEM) and radioisotopic methods were used to study platelet adhesion on surfaces coated with monomeric human collagens CI, CIII, CIV, and CV, polymeric-fibrillary CI and CIII, or polymeric-amorphous CIV and CV. The collagen substrates differ considerably in their general level of adhesion and in their ability to induce changes in shape of adherent platelets and the formation of large stratified (thrombus-like) aggregates. These differences are entirely due to the genetic type of collagen and are independent of whether the immobilized collagen is in the polymeric or monomeric form. Brief details about these cultures were published in the form of abstracts [9].

EXPERIMENTAL METHOD

CI, CIII, CIV, and CV were isolated from a pepsinized homogenate of human placenta by differential salting out with 0.7-4.5 M NaCl at neutral and acid pH values, followed by chromatography on DEAE-cellulose [6, 13]. Wells (16.4 mm in diameter) in "Multiwell" No. 3008 cultural plates (Falcon Plastics, USA) were coated with monomeric CI, CIII, CIV, and CV, polymeric fibrillary CI and CIII, polymeric amorphous CIV and CV, gelatin-denatured CI or CIII, and ovalbumin. The collagens were dissolved in 100 mM acetic acid (pH 2.8) in a concentration of 1-4 mg/ml. Gelatin was prepared by heating solutions of collagens in acetic acid for 1 h at 56°C. To prepare the monomeric coating an aliquot of an acid solution of collagen or gelatin was diluted with 200 mM carbonate buffer (pH 9.6) to a final concentration of 10 μg/ml, poured into the wells in a volume of 0.5 ml, and incubated for 12-18 h at 4°C. Wells coated with monomeric collagen and gelatin were treated additionally with ovalbumin to bind with areas of the polystyrene that were not coated with collagen [12]. The ovalbumin was dissolved in 30 mM phosphate buffer containing 150 mM NaCl (pH 7.4) in a concentration of about 1 mg/ml, poured into the wells in a volume of 0.5 ml, and incubated for 30 min at 22°C [12]. To obtain a polymeric coating 0.3-

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