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Quantitative Evaluation of Gap Junctions in Rat Brown Adipose Tissue after Cold Acclimation

G. Schneider-Picard, J.-L. Carpentier*, and L. Girardier
Institute of Histology and Embryology* and Department of Physiology, University of Geneva Medical School, Geneva, Switzerland

Summary. The decrease in the metabolic capacity of rat brown adipose tissue during the late postnatal period can be reversed by cold acclimation of the animals. In order to find out whether a parallel decrease in capability for intercellular communication observed during this period is also reversed by cold acclimation, gap junction size and number per unit area of cell surface have been quantified in freeze-fracture replicas; cell diameters have been measured in semi-thin sections. It was found that the specific number of gap junctions remains unchanged during cold acclimation. However, the mean gap junction size increases by 75% and the ratio of gap junctional area per cell volume, an index for intercellular exchange capacity, is doubled. This result illustrates further the parallelism between metabolic capacity and cell communication in brown fat.

Key Words brown adipose tissue · gap junction · cold acclimation · freeze fracture

Introduction

Brown adipose tissue (brown fat) plays an important role in regulatory nonshivering heat production in newborns [7] and small mammals during cold stress [30]. In the rat, maximal respiratory capacity of brown fat is reached in the early postnatal period and then decreases at about one month after birth [2, 25], when other thermoregulatory mechanisms are fully developed [5].

The heat production in brown fat is mainly controlled by its direct sympathetic innervation [12], and since the adipocytes are electrically coupled [12, 20, 22, 26] it has been suggested that gap junctions might distribute the nerve stimulus throughout the tissue [26]. Indirect evidence for the syncitial character of the tissue came from the uniformity of membrane potential changes recorded with intracellular electrodes during tissue stimulation [11].

Quantitative evaluation of gap junctions during the postnatal period showed a close parallelism between the indexes of the capacity for heat production of the tissue (ultrastructural changes of the mitochondria [15, 32], specific activities of respiratory enzymes [3, 28, 32] and the capability for intercellular communication [23, 24], i.e., both increased after birth, showing a maximum between 2 and 17 days and then declined to adult level in animals not exposed to cold.

The high postnatal respiratory capacity can be restored in adult animals by cold exposure [3, 6, 13, 28, 30, 32]. The present study was undertaken to test whether the newly augmented respiratory capacity in brown fat of cold-acclimated rats is accompanied by a parallel variation in the capacity for intercellular communication.

Materials and Methods

Male Sprague-Dawley rats from the same litter were used. Thirty-four days after birth, four animals were transferred to a cold room at 10 °C and housed individually. Four animals stayed at 23 °C. After six weeks, the interscapular brown fat was removed, weighed, and prepared for freeze fracturing and phase contrast microscopy. The method of quantitative evaluation of gap junction frequency (i.e., the number of gap junctions per cell membrane area) and of mean gap junction size has been described in detail [24]. In brief, about 20 membrane faces of brown adipocytes were photographed from each animal at low magnification (6,000 ×) in a Philips EM 300. All gap junctions present in each membrane face were photographed at a higher magnification (10,000 ×). The areas of membrane faces and gap junctions were determined by planimetry. As already observed, gap junction configurations on brown adipocytes are quite variable (*see* Fig. 2*c* and *f* in ref. 24). In order to simplify the presentation of the present results, either large particle aggregates accompanied by small satellites or a "gap junctional plaque" containing several individual aggregates were considered as *one* gap junction unit. The area of these gap junction units is the sum of the individual areas. Most measurements were done on *P*-membrane faces; however, when *E*-faces were present on the micrographs, gap junctions were evaluated separately. This should serve as a control of the method. The cell size was evaluated on semi-thin sections of Epon embedded tissue and used for calculation of cell volume [33]. Means ± SEM are given. The sample sizes are indicated in parentheses in the results. Statistical analysis was done with the X^2 test.

Results

Table 1 shows that, in the cold-acclimated rat, the mass of interscapular brown fat is nearly doubled for similar body weight, whereas the cell size decreases by about 10%.

Figure 1 shows a typical gap junction between two adipocytes from a cold-acclimated rat. The micrograph also shows another characteristic ultrastructural feature of brown adipocytes, the numerous invaginations.

The histograms in Fig. 2 show the distributions of gap junction sizes in the cold-acclimated animals and their litter mates at 23 °C. In brown fat from the control animals, the smallest gap junctions ($<3\times10^{-2}$ μm^2) represent about 45% of all gap junctions measured, and this proportion is decreased to about 20% in the cold-acclimated rats. The size distributions on *P*- and *E*-faces are similar for each group. The mean gap junction size, calculated from these distributions according to [24], is nearly doubled in brown fat of cold-acclimated animals, whereas the number of gap junctions per 100 μm^2 membrane area is unchanged (Table 2).

In order to evaluate probable functional intercellular communication, i.e., the capacity for exchange of small molecules between cells, not only the size of the gap junctions but also the cell size has to be taken into account. A quantitative estimation of intercellular communication has been proposed by Sheridan [27]. This author has shown that the ratio of gap junction area to cell volume determines the speed at which the concentration of a substance inside a cell may be changed, an increase in this ratio reflecting an increase in the "capability" for communication [27]. This treatment was developed for a two-cell system; but, it can be shown that such an estimation can also be used in the more general, three dimensional cell system [23]. As shown in the appendix, if one assumes that a density n (of small molecules) satifies the diffusion equation, then the change of density can be approximated by $\partial n/\partial t = D\Delta n$, with $D = csd$ as diffusion coefficient, where s = gap junctional area per unit membrane area, d = cell diameter, c = constant which may vary slowly with time. However, in order to transmit a message throughout a cell assembly, it may be that it is not the distance that is of importance, but the number of cells over that distance. In that case, one can define a "messenger diffusion coefficient," $D' = cs/d$, which is equivalent to Sheridan's capability for communication in the two-cell system [27].

In order to compare the index D' from tissue from cold-acclimated animals to those from the postnatal period, the cell diameters of the adipo-

Table 1. Body weight, brown fat weight, and cell size

	Cold acclimated (10 °C)	Control (23 °C)
Body weight (g)	324 ±10 (4)	332 ±4 (4)
Interscapular brown fat (mg)	502 ±36[a] (4)	262 ±22 (4)
Cell diameter (μm)	30.0±0.2[a] (476)	34.1±0.2 (421)

The numbers in parentheses indicate sample size.

[a] $P<0.001$ cold acclimated *vs.* control.

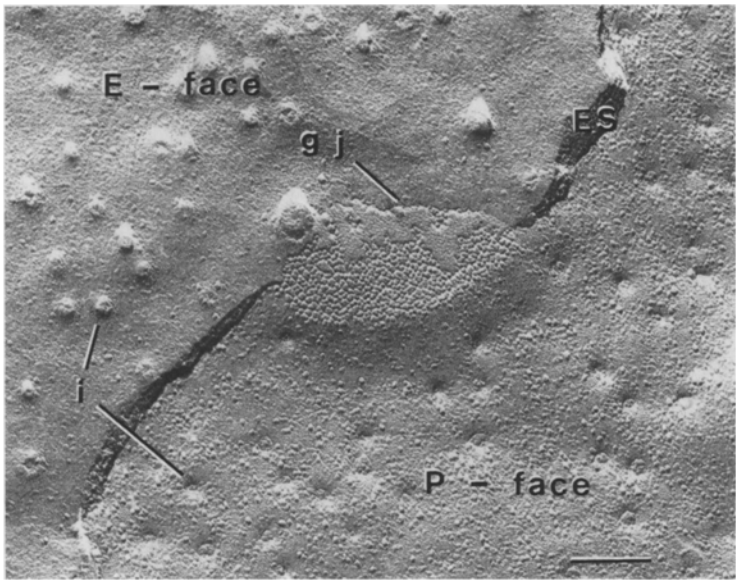

Fig. 1. Plasma membranes (*P-face* and *E-face*) of two neighboring brown adipocytes from a cold-acclimated animal. A gap junction (*gj*) in a *P-E* fracture-face transition is seen in a region of reduced extracellular space (*ES*). *i* indicates plasma membrane invaginations. Calibration bar corresponds to 0.2 μm

cytes have been determined (Table 1). Table 2 shows that in the cold-acclimated rat the ratio gap junction area per cell volume is doubled as compared to control animals and is of the same order of magnitude as in brown fat from 2 and 17 day old animals [23, 24].

Discussion

Gap junctions, identified in freeze-fracture replicas as aggregates of intramembranous particles in *P*-faces and corresponding pits on *E*-faces are thought to be the structures responsible for electrical coupling and/or for exchange of low molecular weight molecules [4, 16]. In nonexcitable tissues it seems that it is the latter function that can more likely be attributed to the junction [8, 10, 14, 18]. Indeed, it has been shown that small gap junctions are sufficient to maintain electrical coupling [21] and that an increase in the size of gap junctions improves metabolic coupling [9, 34].

Brown fat from the adult rat exposed to a cold environment undergoes a number of morphological and biochemical changes [6, 32]. The decreased cell size in tissue of cold acclimated animals is explained by decrease of lipid content [17]. Furthermore, it has been shown that there is an increase in respiratory capacity during cold acclimation, as expressed by proliferation of mitochondria [15, 31, 32] and an increase in the activity of respiratory chain enzymes [3, 28, 32] similar to that during early postnatal life. Interestingly, we find a similar

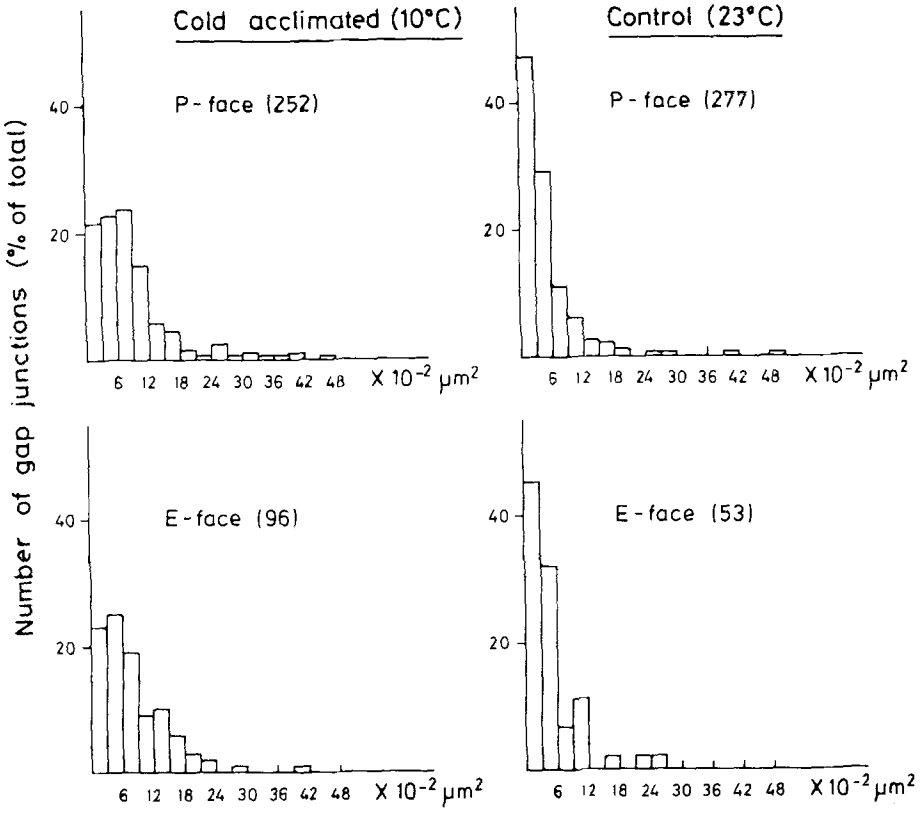

Fig. 2. Distribution of gap-junction areas in plasma membrane *P*- and *E*-faces of brown fat from cold acclimated (10 °C) and control (23 °C) animals. The numbers in parentheses represent the number of gap junctions measured

Table 2. Gap junction quantification

	Cold acclimated (10 °C)		Control (23 °C)	
	P-face	*E*-face	*P*-face	*E*-face
Membrane area sampled (μm^2)	2,636.5	823.3	2,717.0	401.4
Number of gap junctions per 100 μm^2	11.7 ±0.7	12.6 ±1.2	11.6 ±0.7	13.7 ±1.8
Mean gap junction area ($\times 10^{-3}$ μm^2)	82.1 ±5.2[a]	80.0 ±8.2	47.0 ±2.9	48.0 ±6.7
Cell membrane area occupied by gap junctions (%)	0.96±0.09[a]	1.01±0.15	0.55±0.05	0.66±0.13
Gap junction area per cell volume ($\times 10^{-3}$ μm^{-1})	1.88±0.17[a]	1.97±0.29	0.96±0.08	1.16±0.23

[a] $P<0.0001$ cold acclimated *vs.* control.

pattern of size distribution of gap junctions and the same capability for communication in the tissue of the cold-acclimated animals as in the early postnatal period, when tissue capacity for heat production is maximal [23, 24].

Brown adipocytes have been shown to be electrically coupled [20, 22, 26], which indicates that the structures identified in freeze-fracture replicas as gap junctions may be permeable to small molecules. Although the morphological data do not demonstrate the permeability of the gap junctions studied, the parallelism between the variations of the indices of capacity for intercellular communication and heat production in the postnatal period [23, 24], together with the similar magnitude of this index in the early postnatal period and after cold acclimation, suggest that a modulation of gap junctions accompanying changes in metabolic capacity occurs in brown fat.

There appears, however, to be an upper limit of respiratory capacity as indicated by the similarity of these indices after cold acclimation and during early postnatal life in the rat. Once the maximal metabolic capacity of the tissue is reached, an increase of total activity can be achieved only by the increase of tissue mass due to proliferation [2, 6, 13]. Our result of an increase of mass of interscapular brown fat with concomittant decrease in cell size is in accordance with tissue hyperplasia during cold acclimation.

It has been shown that the sympathetic nervous system plays a permissive role in the trophic response of brown fat to cold [1, 17]. In view of the observation of an increase in gap junction size in cell culture in response to cAMP [9], one could speculate that the sympathetic nervous input on the tissue might influence gap junction modulation via cAMP, which is the mediator of the acute metabolic response in brown fat [19] and whose basal level is increased in brown fat from cold-acclimated animals [29].

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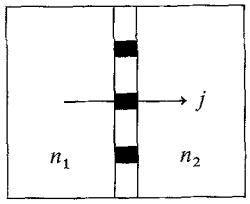
Appendix

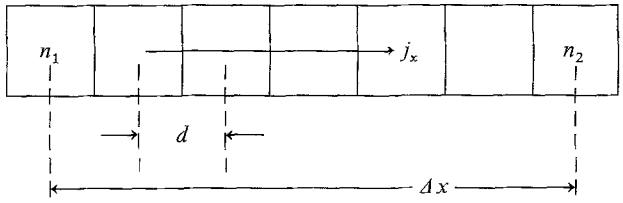
First, two cells, connected by gap junctions, are considered. The conductivity for molecules crossing the intercellular junctions should be proportional to the gap junction area. Assuming that the cells contain molecules with constant densities n_1 and n_2, and that the gradients within the cells are negligibly small, then the flux density of molecules is given by

$$j = -cs(n_2 - n_1)$$



where c = constant, s = gap junction area/unit membrane area.

Let us now consider a "mathematical cell" containing many physical cells:



$k_x = \Delta x/d$ is the number of cells on the length Δx; d is the average cell diameter. Obviously, the conductivity of the mathematical cell is k-times smaller then that between two cells, and one obtains for the current density in the x-direction:

$$j_x = cs/k_x(n_2 - n_1) = csd(n_2 - n_1)/\Delta x.$$

For sake of simplicity, we take as an approximation its limiting value, namely

$$j_x = -csd\,\partial n/\partial x \quad \text{Fick's I. law.}$$

For the current densities in the y and the z directions the same results are obtained.

With the help of the continuity relation

$$\partial n/\partial t = -\nabla \vec{j}$$

one finally obtains Fick's II. law:

$$\partial n/\partial t = csd\Delta n = D\Delta n$$

with $D = csd$ as diffusion coefficient, which is a measure for the speed with which the molecules might diffuse through the tissue. However, in order to transmit a message throughout the cell assembly, not the distance x might be important, but the number of cells over that distance, namely $k_x = x/d$. In that case the diffusion equation should be represented in the following form:

$$\partial n/\partial t = cs/d\,(\partial^2 n/\partial k_x^2 + \partial^2 n/\partial k_y^2 + \partial^2 n/\partial k_z^2)$$

and a "messenger diffusion coefficient" (D') can be defined as

$$D' = cs/d.$$