Cold Adaptation Modulates Ca^{2+} Signaling in Brown Preadipocytes

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One-week cold exposure of mice led to a 2-fold increase in the density of α_1-adrenoceptors in brown adipose tissue. The density of α_1-adrenoceptors returned to normal after adaptation to cold for 2 weeks. The reduced Ca^{2+} signaling in stem cells of brown fat activated via β-adrenoceptors and cAMP was transformed into the Ca^{2+}-system induced by α_1-adrenoceptors and similar to that in mature brown adipocytes.

Key Words: *brown preadipocytes; adrenoceptors; Ca^{2+} signaling; cold adaptation*

Cold exposure is a natural physiological factor inducing hyperplasia and hypertrophy of the brown adipose tissue (BAT). Adrenoceptors play a major role in the regulation of this process. Long-term cold exposure leads to pronounced hyperplasia of BAT and increased the percent of preadipocytes [8]. The hypothalamus regulates cold-induced activation of BAT via stimulation of norepinephrine secretion from sympathetic nerve endings [13]. Tissue norepinephrine not only modulates the immediate heat response in BAT via α- and β-adrenoceptors [14], but also stimulates tissue hyperplasia (*i. e.*, proliferation and differentiation of stem cells into mature brown adipocytes) [8]. Norepinephrine activates the cAMP signaling system in preadipocytes and mature brown adipocytes via β-adrenoceptors. The cAMP signaling system has the same function in these cells. However, our previous studies showed that norepinephrine activates different Ca^{2+} signaling systems in stem and differentiated cells [6]. The triggering mechanism for elevation of cytoplasmic Ca^{2+} concentration exists in mature brown adipocytes, but is absent in stem cells [6]. The slow low-amplitude Ca^{2+} signal in preadipocytes is realized via β-adrenoceptors. The adenylate cyclase pathway plays a major role in the regulation of Ca^{2+} concentration in immature cells [1,6].

The study of the mechanisms for adrenergic regulation in brown adipocytes showed that the rate of [^{3}H]-thymidine incorporation into mouse BAT DNA increases on day 3 of cold exposure and reaches maximum by the 8th day (7-fold increase) [11]. On day 13 of adaptation the rate of [^{3}H]-thymidine incorporation returned to normal, while tissue DNA content 3-fold surpassed the baseline level.

Cold adaptation of animals induces pronounced hyperplasia of BAT, but none of the physiological and cellular effects are realized via α_1-adrenoceptors [5,7, 10,12]. Here we studied the initial stages of transformation of the Ca^{2+} signaling system during proliferation and partial differentiation of brown preadipocytes.

MATERIALS AND METHODS

Male NMRI mice aging 3-5 weeks were kept in a vivarium at 24-28°C (control) or in a cold room at 4°C (cold stress). The animals were kept in cold for 3, 8, 14, and 21 days.

BAT cells were isolated by the collagenase method and purified by centrifugation. Changes in intracellular Ca^{2+} concentration were studied using Fura-2 fluorescent probe [1,2].

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Plasma membranes were isolated as described elsewhere [3,4]. α_1-Adrenoceptors were assayed by studying specific binding of [^{3}H]-prazosin to BAT membranes in 8 mice. BAT membranes (0.3 mg protein/ml) were incubated in duplicates with [^{3}H]-prazosin in 9 concentrations (0.02-5.30 nmol, 72 Ci/mmol, Amersham Pharmacia Biotech). Incubation was performed in 0.32 ml buffer at 30°C for 1 h. BAT membranes were filtered through a Whatman GF/C glass fiber filter on a Skatron cell harvester 7019 device (Skatron). The filters were washed with 6 ml incubation buffer and dried. Radioactivity was measured in 5 ml Emulsifier Scintillator Plus mixture (Packard Instrument Co.) on a Beckman scintillation counter. Nonspecific binding (B_{NSP}) was studied in the presence of 1000-fold excess phentolamine. Specific binding (B_{SP}) was estimated as the difference between total binding (B_T) and B_{NSP}.

RESULTS

β-Adrenoceptors and α_1-adrenoceptors on brown preadipocytes are involved in the Ca^{2+} response. The signal induced by β-specific isoproterenol has a greater amplitude compared to that realized via α_1-adrenoceptors in response to α_1-specific cirazoline (Fig. 1, *a*).

Cold exposure for 3 days had no effect on the Ca^{2+} response. However, the Ca^{2+} response of brown preadipocytes underwent significant changes after 8-day cold exposure (Fig. 1, *b*). We observed an increase in the rate of Ca^{2+} influx stimulated by antagonists. The cell reaction to norepinephrine estimated by changes in intracellular $D[Ca^{2+}]_i$ concentration increased more than by 2 times after 20 min. The response to isoproterenol and cirazoline increased by 2 and 3 times, respectively. These data show that the Ca^{2+} response initiated via β-adrenoceptors and α_1-adrenoceptors did

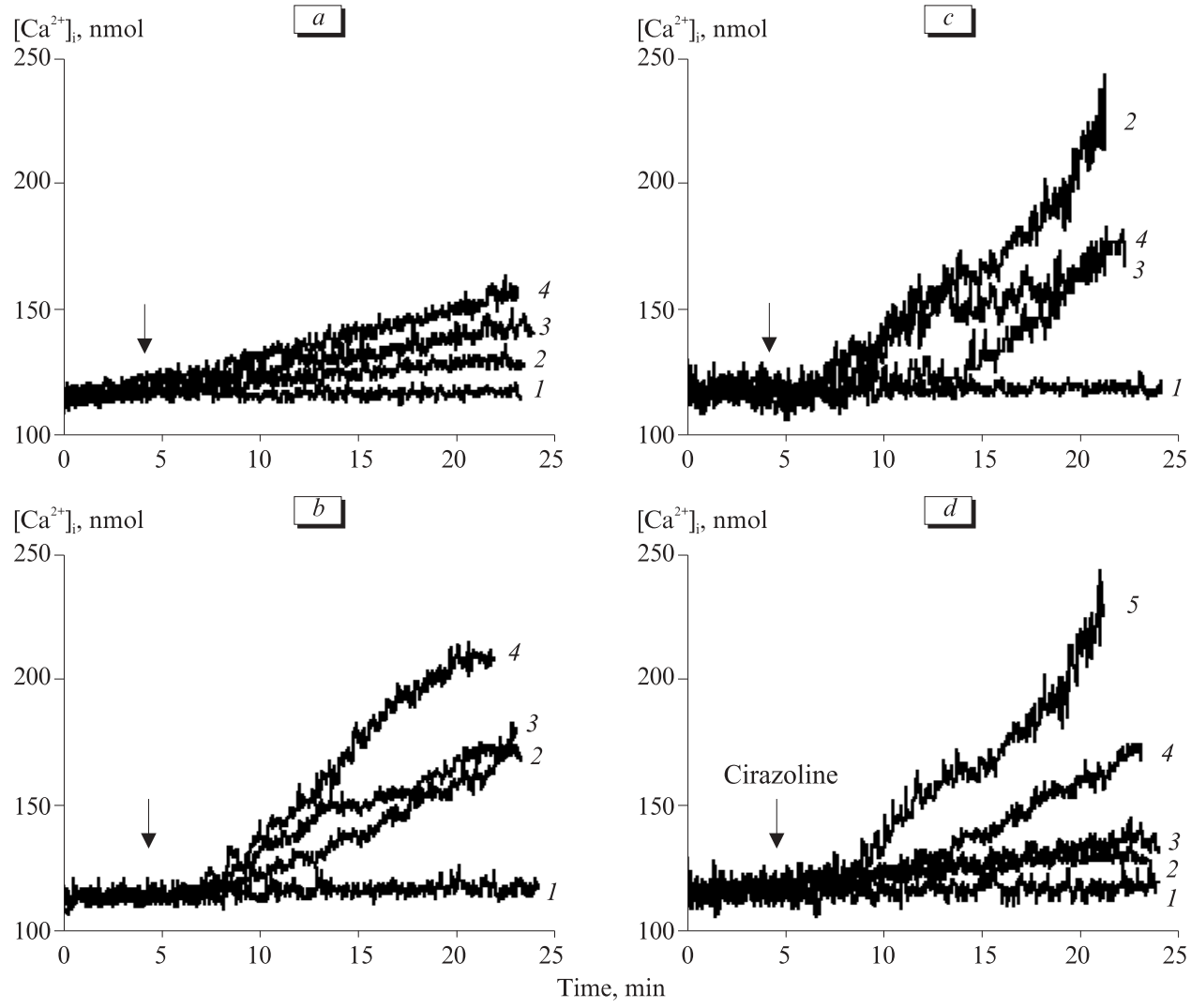

Fig. 1. Increase in Ca^{2+} concentration in brown fat preadipocytes induced by 10 μM adrenergic agonists: without agonists (*1*), cirazoline (*2*), isoproterenol (*3*), and norepinephrine (*4*). Control, warm conditions (*a*); cold adaptation for 8 (*b*) and 14 days (*c*); cell response to α_1-selective cirazoline (*d*). Control (*2*) and adaptation for 3, 4-8, and 5-14 days (*3*, *d*). Arrow: treatment with agonists.

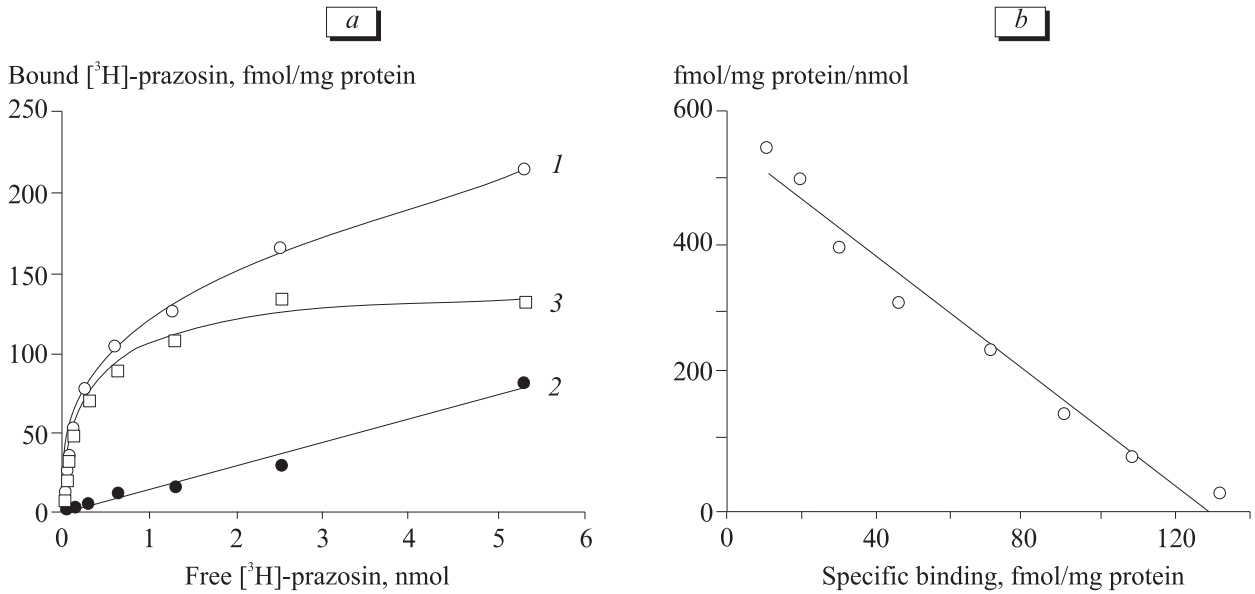

Fig. 2. Binding of [^{3}H]-prazosin to brown preadipocyte membranes in cold-adapted mice. Total (*1*), nonspecific (*2*), and specific binding (*3*) as a function of [^{3}H]-prazosin in increasing concentrations (*a*). Specific binding isotherm in Scatchard coordinates (*b*). Ordinate: bound prazosin/free prazosin (*b*).

not differ in animals exposed to cold for 8 days. The cell response to β-adrenergic and α_1-adrenergic agonists was additive. However, the Ca^{2+} response initiated by norepinephrine was realized mainly via α_1-adrenoceptors. It should be emphasized that in control animals the Ca^{2+} response was generated by β-adrenoceptors. Cold adaptation for 14 days more significantly increased Ca^{2+} response realized via α_1-adrenoceptors (Fig. 1, *c*). The Ca^{2+} response surpassed the reaction initiated not only by β-adrenergic agonists, but also by norepinephrine. Therefore, stimulation of the cellular Ca^{2+} response after cold adaptation was related to an increase in the relative role of α_1-adrenoceptors (Fig. 1, *d*). These changes reflect reduced Ca^{2+} signaling in brown preadipocytes.

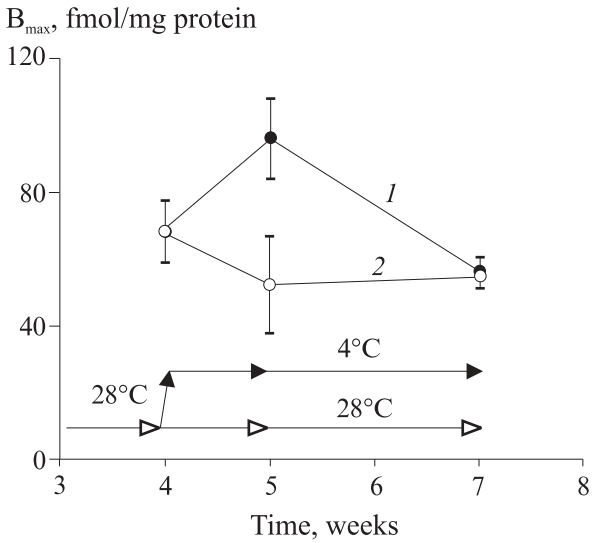

Fig. 3. Density of α_1-adrenoceptors in brown adipocytes of cold-adapted mice (*1*) and animals kept in warm conditions (*2*). Arrow: change of the temperature regimen.

Experiments with equilibrium binding of α_1-specific [^{3}H]-prazosin to mouse BAT membranes revealed a considerable number of α_1-adrenoceptors (B_{max}= ~60 fmol/mg protein). The density of tissue α_1-adrenoceptors increased by 2 times after 1-week cold adaptation (Fig. 2). However, further adaptation decreased the density of BAT α_1-adrenoceptors to a control level (Fig. 3).

It is believed that mitogenesis is induced by growth factors whose receptors possess tyrosine kinase activity. It should be emphasized that G protein-coupled receptors (*e. g.*, adrenoceptors) also modulate the regulation of cell proliferation [9]. Previous studies showed that norepinephrine and cAMP stimulate proliferation of cultured brown preadipocytes [3,5]. The Ca^{2+} signaling system in brown fat cells is reduced and provided by α_1-adrenoceptors and β-adrenoceptors. cAMP plays a role in the Ca^{2+} response. Stimulation of α_1-adrenoceptors on mature brown adipocytes leads to activation of phospholipase C, formation of inositol-1,4,5-triphosphate, and intracellular Ca^{2+} mobilization. Ca^{2+} ions play a role of tertiary messengers in preadipocytes and stimulate cell proliferation (similarly to cAMP). The model of cold adaptation is convenient for estimation of the sequence of cellular events leading to tissue growth. The study of the early stage of brown adipocyte differentiation on the model of cold adaptation allows evaluation of the type of messengers involved in the regulation of this process. The initiation of Ca^{2+} signaling after 1-week adaptation to

cold is mainly associated with an increase in the number of α_1-adrenoceptors on the plasma membrane of brown fat preadipocytes. Proliferation is completed after 2 weeks, and the density of α_1-adrenoceptors returns to normal. The increase in the Ca^{2+} signal during this period is related to transition of adipocytes from one Ca^{2+} system into another more potent system. The observed changes serve as a marker of cell maturity and reflect their differentiation.

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