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Full Length Research Paper

Thermogenic response of guinea pig adipocytes to noradrenaline and β3-AR agonists

Ghorbani Masoud[1]* and Mehdi Shafiee Ardestani[2]

[1]Department of Research and Development, Pasteur Institute of Iran (Research and Production complex), 25th km Tehran Karaj Highway, Tehran, Iran.
[2]Department of Hepatitis and Aids, Pasteur Institute of Iran, Tehran, Iran.

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Brown adipocytes isolated from warm acclimated guinea pig do not respond to noradrenaline (NA) in spite of their marked thermogenic response *in vivo*. In contrast, in cold-acclimated guinea pigs, isolated brown adipocytes show substantial increase in capacity for a thermogenic response to NA. Chronic stimulation with a β3-AR agonist increased insulin-sensitivity of brown adipose tissue (BAT) in the guinea pig. To investigate the responsiveness of glucose transport to insulin, to NA, and to β3-AR agonists, we used BAT of guinea pigs as an animal known to be insulin-resistant. Results of this study showed that no β3-adrenergic agonist used, such as CL 316,243, BRL 37344, was able to stimulate oxygen uptake in BAT cells from cold-acclimated or new born guinea pigs. However, noradrenaline (NA), adrenaline (A) and isoproterenol (ISO) had a marked thermogenic effect on these cells. In contrast, in a comparative study in warm-acclimated rat BAT cells, CL 316,243 was even more potent than NA in stimulating oxygen uptake. We concluded that guinea pigs lack β3-ARs in their BAT. These results were interesting and noteworthy, suggesting that guinea pigs can be a natural model for β3-ARs knockout.

Key words: Brown adipose tissue (BAT), White adipose tissue (WAT), β3-ARs, guinea pig.

INTRODUCTION

Brown adipose tissue (BAT) cells from cold-adapted guinea pigs show a greater response to NA compared to cells from animals acclimated to room temperature (Hamilton and Doods, 2008). In most mammalian adipose tissues, the induction of lipolysis by catecholamines is mediated by β-adrenergic receptor (β-AR) subtypes such as β1- and β3-ARs (Rial and Nicholls, 1984). The guinea pig, however, shows low expression with lack of function of β3-ARs (Atgié et al., 1996). During cold-acclimation, there is a marked increase in cell number, mitochondrial content of each adipocyte, and the response of each mitochondrion to NA stimulation of adipose tissue. The increase of thermogenesis per mitochondrion is not because of an increase of respiratory chain, since cytochrome c oxidase remains unchanged. Instead, it is due to a marked increase in the uncoupling protein per mitochondrion (Himms-Hagen, 1995).

In these experiments, we prepared and incubated brown adipocytes from guinea pigs during the stages of cold adaptation and warm adaptation, and from new born guinea pigs.

The objective of this study was to assess whether noradrenaline (NA) and β3-AR agonists such as BRL and CL 316,243 could increase oxygen uptake in brown adipocytes of warm-acclimated guinea pigs, cold-acclimated guinea pigs and newborn guinea pigs.

MATERIALS AND METHODS

Female Dunkin-Hartley guinea pigs were obtained at 3 weeks of age. They were housed individually at 28°C, with free access to guinea pig chow and water. After one week, some guinea pigs were acclimated to 4°C for up to 4 weeks while other remained at 28°C during the same period. Pregnant guinea pigs were also obtained at

*Corresponding author. E-mail: mghorbani@irimc.org. Tel: +98-2616102999.

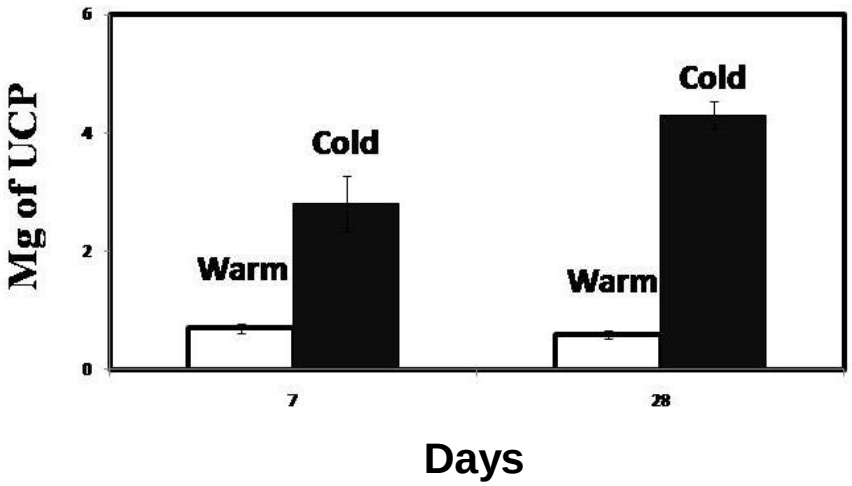

Figure 1. Total UCP of interscapular BAT of warm-acclimated guinea pigs and cold-acclimated (7 to 28 days) guinea pigs. At 7 days, it was 224 ± 35.6 for cold vs. 24.5 ± 4.3 for warm ($P < 0.001$); At 28 days, it was 150.0 ± 19.9 for cold vs. 15.4 ± 1.2 for warm ($P < 0.001$). Total mg protein changed slightly at 7 days, 84.5 ± 10.46 for cold vs. 42.0 ± 2.11 for warm ($P < 0.001$).

advanced stage of gestation. They were housed at room temperature with free access to food and water. The new born guinea pigs were used at the ages of 1, 2 or 3 days after delivery.

For measurement of metabolic rate, conscious guinea pigs were placed in a water-jacketed respiration chamber at 28°C with circulating air at the same temperature attached to an oxygen analyzer (Bechman Indistrial Oxygen Analyzer model 755) to monitor the oxygen uptake.

In all cases, BAT was isolated and transferred into Krebs-Ringer buffer (0.25 M sucrose, 0.2 mM dipotassium EDTA, and 1.0 mM HEPES, in distilled water, pH 7.2) and brown adipocytes were isolated by collagenase digestion of interscapular BAT and washed by flotation using a bicarbonate-buffered culture medium (Gibco DMEM 380-2320 AG) supplemented with 5.5 mM glucose, 1 mM ascorbic acid and 40 mg/ml fatty acid-free bovine serum albumin and equilibrated with 95% oxygen, 5% carbon dioxide, pH 7.4. Oxygen uptake of cells was measured polarographically (YSI Oxygen monitor) in the same medium.

Uncoupling protein (UCP) was measured in BAT homogenates by solid phase radioimmunoassay using antibody to purified hamster UCP and [^{125}I] protein A for detection.

The β-AR agonists we used were L-noradrenaline-D-bitartrate (Arterenol) (Sigma A 9512), L-isoproterenol-D-bitartrate (Sigma I2760), L-epinephrine-D-bitartrate (Sigma E4375), CL 316,243 (American Cyanamid Co), and BRL 37,344 (SmithKline Beecham).

Statistical analysis

Statistical analysis used Instat software to do one-way ANOVA, followed by Student-Newman-Keuls post hoc test. Significant differences are based on $P < 0.05$.

RESULTS

UCP content of interscapular BAT was markedly increased in cold acclimated guinea pigs (Figure 1). Cold acclimation induced a significant increase in UCP content in both times ($P < 0.001$). The increase is based on the mitochondrial concentration of UCP in µg per mg mitochondrial protein. Since UCP is a specific marker for BAT activity, the increase of UCP content confirms that cold-acclimation has a stimulatory effect on BAT and activates the tissue to increase the UCP concentration in mitochondria to generate heat to fight against cold exposure. In another experiment, we demonstrated the substantial thermogenic response of brown adipocytes isolated from BAT of cold-acclimated but not of warm-acclimated guinea pigs to NA. In warm-acclimated guinea pigs, there was no response to NA by brown adipocytes (Table 1), whereas there was a substantial increase in oxygen consumption and thermogenesis in brown adipocytes isolated from cold-adapted guinea pigs (Table 1 and Figure 2). The experiment was continued with adrenaline (A) and isoproterenol (ISO) (Figure 2). None of these agonists had any effect on isolated brown adipocytes from warm-acclimated guinea pigs. Because of variation in the rate of oxygen consumption from one preparation to another, data are expressed as % of maximum for each agonist in each experiment. Isolated brown adipocytes were then incubated with two selective β3-agonists, BRL 37,344 and CL 316,243 in the same situation and preparations that responded to NA. Surprisingly, there was no response to these β3-AR selective compounds (Figure 3).

Thinking that there might have been a loss of β3-ARs during the isolation of brown fat cells, in another experiment, intact conscious cold-acclimated guinea pigs received a subcutaneous injection containing CL 316,243 and their oxygen uptake measured. Results showed that injection of 10 mg CL 316,243 per kg body weight had no effect on oxygen uptake (Figure 4). In contrast, injection

Table 1. Relative potencies (EC_{50}) of different β-adrenergic agonists in stimulating the respiration of brown adipocytes isolated from BAT of guinea pigs.

Agonists	Warm-acclimated guinea pig	Cold acclimated guinea pig
	Dose response (EC_{50})	Dose response (EC_{50})
Isoproterenol	No response	5.0×10^{-7}*
Adrenaline	No response	2.0×10^{-6}*
Noradrenaline	No response	1.5×10^{-6}*
CL 316,243 – β3-agonist	No response	No response
BRL 26810 - β3-agonist	No response	No response

Iso > A = NA are considered as β1-adrenergic agonists.* Concentration of agonist in M.

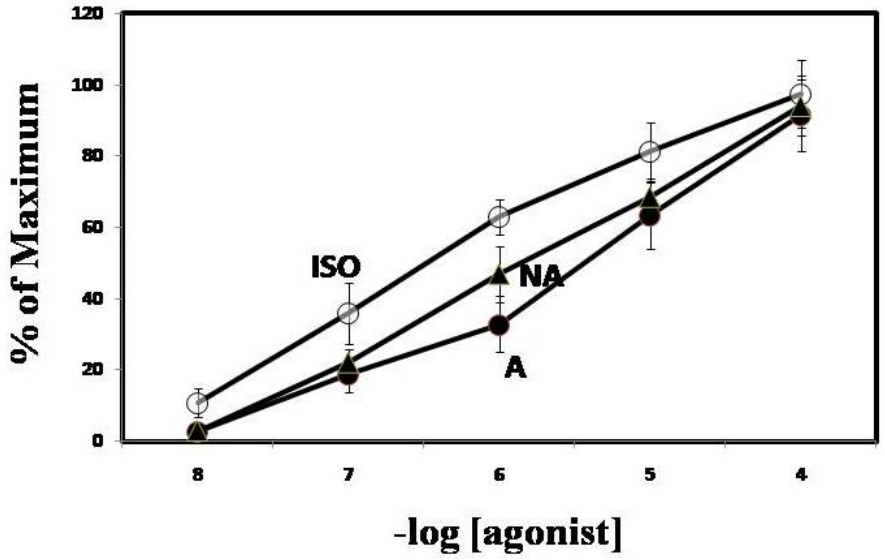

Figure 2. Thermogenic effect of adrenergic agonists on brown adipocytes isolated from cold-acclimated guinea pigs. Symbols: Isoproterenol (ISO) ○, adrenaline (A) ●, noradrenaline (NA) ▲. Actual maximum rates (in nmol per 10^6 cells, n = 6 cells preparations) were 247 ± 46.8 for ISO, 212 ± 34.0 for A, and 191 ± 43.7 for NA. Calculated EC_{50} was 5.0×10^{-7} M for A, and 1.5×10^{-6} for NA.

of NA or cold exposure increased oxygen uptake in guinea pigs by 87 and 108%, respectively (Figure 4).

Since brown adipocytes of warm-acclimated guinea pigs had no thermogenic response to NA, due to very low level of UCP, it was not feasible to assess their response to β3-AR agonists. It has previously been shown that the stimulation of adenylate cyclase activity in crude membranes isolated from BAT of both warm- and cold-acclimated guinea pigs had no response to various β-adrenergic agonists including CL 316,243, whereas it was stimulated by ISO, A, NA and salbutamol in membranes of both warm- and cold-acclimated guinea pigs (Duffaut et al., 2006; Carpéné et al., 1994). Because β3-ARs might have been present in young guinea pigs and lost with aging, as occurs in other precocial mammals such as bovine (Rafael et al., 1986), we also studied the effects of NA and CL 316,243 on brown adipocytes isolated from BAT of newborn guinea pigs. β3-AR agonists also failed to stimulate oxygen uptake in BAT cells from cold acclimated or new born guinea pigs, while NA had a marked effect on increasing oxygen uptake as well as stimulation of adenylate cyclase activity in membrane of these cells. Therefore, it is suggested that the stimulation of thermogenesis by ISO, A, and NA in both warm- and cold-acclimated guinea pigs is mediated by β1-ARs and not by β3-receptors, and these animals do not express β3-AR.

DISCUSSION

Present results show chronic exposure to cold increases mitochondrial uncoupling protein synthesis in BAT of guinea pigs and induces thermogenesis in isolated BAT

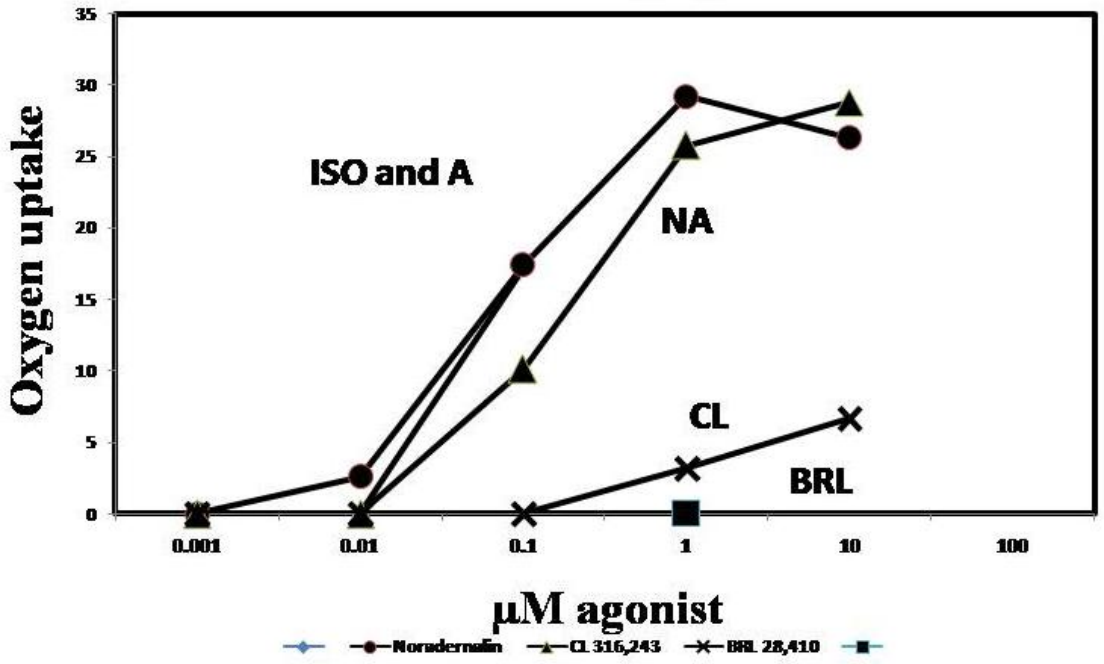

Figure 3. Effect of β-adrenergic agonists on respiration of isolated brown adipocytes from cold-acclimated guinea pigs. Symbols: Isoproterenol (ISO) ○, adrenaline (A) ●, noradrenaline (NA) ▲, CL 316,243 (CL) ×, BRL 28,410 (BRL) ■, Oxygen uptake is in nmol per 10^6 cells per minute.

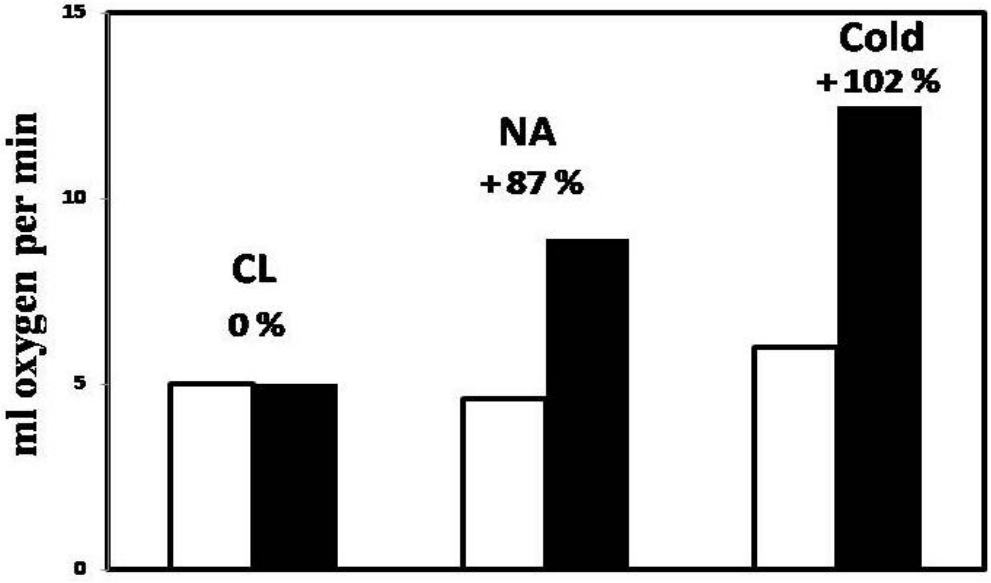

Figure 4. Effect of cold-acclimation and β-adrenergic agonists on energy expenditure of conscious cold-acclimated guinea pigs. Animals were either exposed to cold, infused with CL 316,243 (CL) or infused with noradrenaline (NA). Open bars show the oxygen uptake before injection or cold exposure and solid bars show the oxygen uptake after injection or cold exposure.

cells. Our data also confirmed the remarkable increase in thermogenic responsiveness to NA of brown fat cells isolated from BAT of cold-acclimated guinea pigs (Hamilton and Doods, 2008). Since, NA is able to stimulate lipolysis in brown fat cells of both warm- and cold-acclimated guinea pigs (Cunningham et al., 1986;

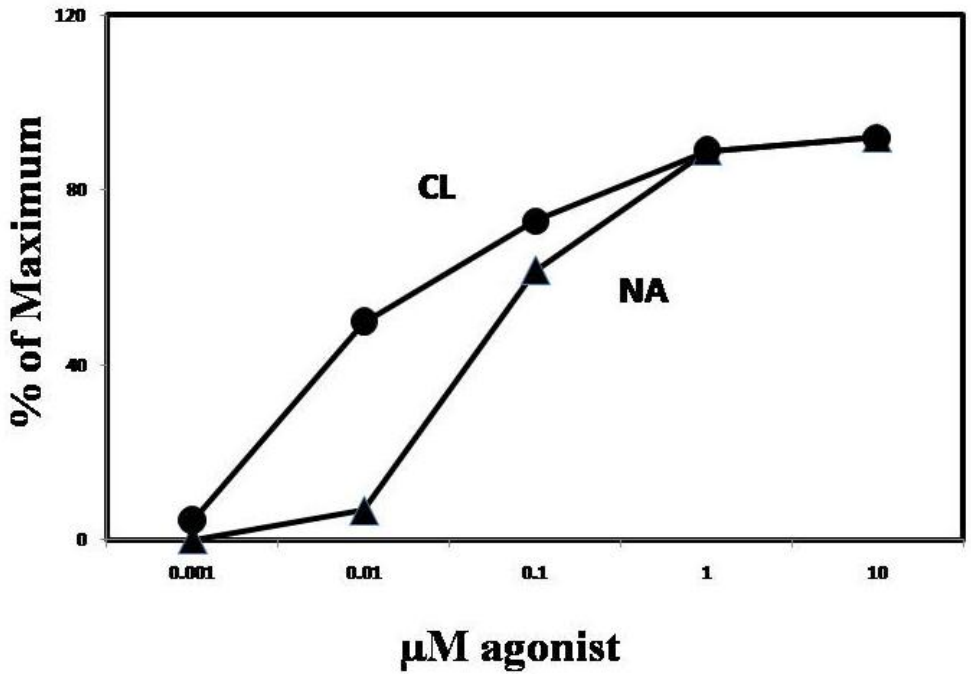

Figure 5. Effect of β-adrenergic agonists on oxygen uptake of brown adipocytes isolated from rats. Symbols: noradrenaline (NA) ▲, CL 316,243 (CL) ●.

Pond and Mattocks, 1991) and they exhibit no responses to all the β3-agonists tested, we conclude that the β-adrenergic responsiveness of guinea pig fat cells essentially involves β1- and β2-ARs and not β3-receptors (Carpéné et al., 1999).

It is also known that β3-adrenergic agonists are inactive on lipolysis in white adipose tissue (WAT) from guinea pigs (Lafontan et al., 1992), in contrast to their lipolytic effect on rat WAT cells. Differences in the adrenergic regulation of lipolysis have also been reported between rat and guinea pigs (Rafael et al., 1986).

However, there is evidence of existence of β3-AR mediating relaxation of various segments of the gastro-intestinal tract of guinea pigs (Bond and Vanhoutte, 1992).

Since, β3-ARs are present in intestinal tissue and absent in adipose tissues of guinea pigs, we conclude that the regulation of β3-ARs expression is different in guinea pigs and rodents, in which they are present in both adipose tissues and intestine. In contrast to guinea pig, in a comparative study in rat BAT cells reported previously (Himms-Hagen et al., 1994), the capability of CL 316,243 was even more potent than NA in stimulating of oxygen uptake (Figure 5). The guinea pig thus differs from rodents by the absence of β3-adrenergic effects in its adipose tissue and thus resembles the human and bovine species (Strosberg and Pietri-Rouxel, 1996; Himms-Hagen et al., 1996). More recent studies on cloning and sequencing of the guinea pig β3-AR gene have revealed a slightly higher amino acid sequence similarity with the human than with the rodent β3-ARs (Atgié et al., 1996). β3-ARs in guinea pig ileum have the pharmacological characteristics of human β3-ARs (Emorine et al., 1989). It is now known that the pharmacological characteristics of human β3-ARs differs from that in rodents (Strosberg and Pietri-Rouxel, 1996; Emorine et al., 1992) in which β3-adrenergic signalling acutely down regulates adipose triglyceride lipase in brown adipocytes (Deiuliis et al., 2010). It is noteworthy that the guinea pig is not a rodent, thus, it is not surprising that it does not possess rodent-type β3-ARs.

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