

Effect of electrical stimulation on lipolysis of human white adipocytes

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Abstract: The goal of the present study was to investigate the effect of 30 min of electrical stimulation on the activation of lipolysis in human white adipocytes. Two stimulation protocols (S1, S2) were conducted in vitro on isolated human white adipocytes. Subcutaneous adipose tissue was obtained from female subjects undergoing abdominal adipose tissue reduction. Adipose tissue of 10 female subjects (mean age, 38.7 ± 9.1 years) and 6 female subjects (mean age, 37.2 ± 11.3 years) was obtained for S1 and S2, respectively. All subjects fasted overnight before tissue removal. The control conditions were a basal and a β -adrenergic stimulation (isoproterenol (ISO), 10^{-6} mol·L $^{-1}$) of lipolysis. For S1, the 3 electrostimulation conditions consisted of a monopolar square-wave pulse current for 30 min at intensities of 4, 8, and 20 mA, respectively. In S2, the 2 electrostimulation conditions consisted of a bipolar square-wave alternating current for 30 min at intensities of 4 and 6 mA, respectively. Fat cell lipolysis was measured by quantifying the release of glycerol from adipocytes for 3 trials in each experimental condition. For S1, 4 mA significantly increased lipolysis 1.5 times over basal values ($p \leq 0.01$), 8 mA and 20 mA did not increase lipolysis significantly, and no significant difference ($p > 0.05$) was found between ISO and 4 mA. For S2, 4 mA ($p \leq 0.05$) and 6 mA ($p \leq 0.01$) significantly increased lipolysis by 1.8 and 2.3 times above basal, respectively. Our results demonstrate that both monopolar (4 mA) and bipolar (4 and 6 mA) electrical stimulations significantly activated in vitro lipolysis. Our findings suggest the existence of a new lipolytic pathway that may involve K_v channels shown to exist in human white adipocytes.

Key words: adipose tissue, electrical stimulation, white adipocytes, fat cell lipolysis.

Résumé : Cette étude se propose d'évaluer l'effet d'une stimulation électrique d'une durée de 30 min sur l'activation de la lipolyse des adipocytes blancs chez l'humain. On élabore deux protocoles de stimulation (S1, S2) in vitro d'adipocytes blancs humains isolés. On prélève du tissu adipeux sous-cutané chez des femmes admises en clinique pour une adipectomie abdominale. Pour le protocole S1, on prend le tissu adipeux de 10 femmes (âge moyen, $38,7 \pm 9,1$ ans) et pour le protocole S2, on prend le tissu adipeux de 6 femmes (âge moyen, $37,2 \pm 11,3$ ans). Tous les sujets sont soumis au jeûne durant toute la nuit précédant le prélèvement du tissu. Les conditions témoins consistent en une situation de base et en une stimulation bêta adrénergique (isoprotérénol (ISO), 10^{-6} mol·L $^{-1}$) de la lipolyse. En S1, les 3 conditions d'électrostimulation consistent en des courants d'impulsions rectangulaires monopolaires d'une durée de 30 min aux intensités suivantes 4, 8 et 20 mA, respectivement. En S2, les 2 conditions d'électrostimulation consistent en des courants d'impulsions rectangulaires bipolaires d'une durée de 30 min aux intensités suivantes 4 et 6 mA, respectivement. On évalue la lipolyse des cellules adipeuses par la quantité de glycérol libéré des adipocytes mesurée en triplicata dans chaque condition expérimentale. En S1, à une intensité de 4 mA, la lipolyse augmente significativement de 1,5 fois ($p \leq 0,01$) par rapport aux valeurs de base; à une intensité de 8 mA et de 20 mA, on n'observe pas d'augmentation significative de la lipolyse et on ne note aucune différence significative ($p > 0,05$) entre les conditions ISO et 4 mA. En S2, aux intensités de 4 mA et de 6 mA, on observe respectivement une augmentation significative de 1,8 fois ($p \leq 0,05$) et de 2,3 fois ($p \leq 0,01$) par rapport aux valeurs de base. Selon nos observations, les 2 régimes d'électrostimulation, monopolaire (4 mA) et bipolaire (4 et 6 mA), activent significativement la lipolyse in vitro. Nos observations suggèrent l'existence d'une nouvelle voie lipolytique pouvant impliquer les canaux K_v dont on connaît l'existence dans les adipocytes blancs chez l'humain.

Mots-clés : tissu adipeux, stimulation électrique, adipocytes blancs, lipolyse des cellules adipeuses.

[Traduit par la Rédaction]

Introduction

Adipose tissue lies at the center of a complex network that influences energy homeostasis. To achieve this goal, it acts

as an energy reserve and is mainly controlled by the activity of lipoprotein lipase (insulin regulated) for the hydrolysis of circulating triacylglycerols and CD36 for the incorporation of fatty acids into fat cells. On the other hand, the release of

Received 27 July 2010. Accepted 21 January 2011. Published at www.nrcresearchpress.com/apnm on 13 April 2011.

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fatty acids from adipose tissue, namely fat cell lipolysis, is largely controlled by catecholamines (noradrenaline (NA) and adrenaline) when energy is needed. The release of fatty acids depends on the ability of catecholamines to activate adrenergic receptors and their enzymatic cascade. This cascade is increased through β -adrenoceptors coupled to Gs proteins, and is decreased through α_2 -adrenoceptors coupled to Gi proteins and through insulin receptors in their activation of intracellular type III phosphodiesterases (Arner 2005). All 3 receptors are present at the membrane surface of human fat cells. Among these, β -adrenoceptor dysfunction is often reported in obesity (Clément et al. 1995; Lacasa et al. 1984; Large et al. 1997; Lönnqvist et al. 1992) and could lead to an attenuated increase of fatty acid release when an energy surge is experienced, such as during exercise. While the absolute rate of fatty acid release may be as high (even higher) in obese compared with nonobese subjects, the increase in lipolytic rate induced by β -adrenergic stimulation is often lower in obese subjects (Blaak et al. 1994).

Although much is known about white adipose tissue metabolism, little information is available and few studies have been conducted about its electrophysiology. Studies have reported that most cellular functions are accompanied by ionic fluxes across the membrane (Bleicher et al. 1966a, 1966b; Clausen 1970; Clausen and Kohn 1970; Ziegler et al. 1980). Transmembrane ion fluxes initiate changes in the physicochemical balances, promoting or suppressing molecular interactions (Hille 1992).

The hormonal regulation of fat cell lipolysis is also known to have an effect on ionic fluxes (Touabi and Jeanrenaud 1970), on ionic gradients across the membrane (Hales and Perry 1970; Pershadsingh and McDonald 1979), and on membrane potentials (Beigelman 1965; Beigelman and Hollander 1965; Cheng et al. 1981). Membrane potential that is modified through hormonal changes may play a significant role in the basal metabolic activity of the white adipose tissue. Furthermore, agents used to inhibit ion flow through the membrane can mimic some hormonal effect on adipocytes (Ho and Jeanrenaud 1967; Ho et al. 1967; Mosinger and Kujalova 1966). However, changing the concentration of the different cations (Mg^{2+} , Ca^{2+} , K^{+} and Na^{+}) in the incubation medium showed no direct effect on the lipolytic rate in isolated hamster brown fat cells (Nedergaard 1981).

However, Ramírez-Ponce et al. (1990) were the first to study the electrophysiological characteristics of white adipocytes. Using the "whole cell variant of the patch clamp" technique, they showed that rat white adipocytes contain voltage-dependent potassium channels (K_v) of the delayed rectifier type (Ramírez-Ponce et al. 1996). This finding has since been corroborated by other authors in isolated white adipocytes (Lee and Pappone 1997b; Ringer et al. 2000).

Ramírez-Ponce et al. (1991) also demonstrated that insulin and NA could modify the passive and the active electrophysiological properties of the rat white adipocyte. NA was shown to successfully depolarize the membrane at levels up to 8.5 mV over its resting potential, whereas insulin produced a mean hyperpolarisation of 13.5 mV. Insulin and NA had opposite effects on the recovery time of membrane potential at the end of hyperpolarisation intracellular current pulses. NA reduced this parameter, whereas insulin increased it (Ramírez-Ponce et al. 1998).

Furthermore, Ramírez-Ponce et al. (2003) was the first to demonstrate that human white adipocytes, obtained from subcutaneous and visceral adipose tissues from normal-weight subjects (body mass index (BMI) $< 27 \text{ kg}\cdot\text{m}^{-2}$), also contain K_v channels. The properties of these channels appear to be the same as the K_v channels found in rat adipocytes, except for a higher density in human cells. To our knowledge, no studies have reported the effects of K_v channels and the membrane potential on the activity of fat cell lipolysis.

The goal of this study was then to investigate the effect of electrical stimulation on the activation of lipolysis in human white adipocytes. The hypothesis was that a specific level and type of electrostimulation would increase lipolysis in isolated in vitro human white adipocytes.

Materials and methods

Subjects

Two stimulation protocols (S1, S2) were conducted in vitro on isolated cells. Different subjects were recruited for the 2 studies that were conducted sequentially, S1 before S2. We recruited female subjects who were undergoing subcutaneous adipose tissue reduction, either through liposuction or surgery. Protocol S1 comprised 10 female subjects aged between 25 and 54 years old (mean age, 38.7 ± 9.1 years), and protocol S2 comprised 6 female subjects aged between 42 and 52 years (mean age, 37.2 ± 11.3 years). For both groups (S1 and S2), subcutaneous adipose tissue taken from the abdominal region only was used. Furthermore, in both S1 and S2, all subjects fasted overnight before tissue removal. The subcutaneous adipose tissue samples were obtained from private liposuction clinics that could not or would not provide patient information other than the sex and the region from where the tissue sample was extracted. We recognize that the lack of clinical data on the subjects leaves open a range of possibilities that could confound interpretation of the results, including BMI, drug use, and health status, for example. This represents a limit of this project, especially in light of the different results between S1 and S2 for the isoproterenol (ISO) condition presented below. However, it does not minimize the effect found with the electrostimulation conditions. The study was approved by the university's committee on human research (Université du Québec à Montréal, Montreal, Que., Canada).

Experimental conditions

In both S1 and S2, the lipolytic effect of electrostimulation was compared with 2 control conditions: basal (cells without any external stimulus) and a β -adrenergic stimulation of lipolysis by ISO at a concentration of $10^{-6} \text{ mol}\cdot\text{L}^{-1}$ showed to maximally stimulate fat cell lipolysis in our experimental conditions. In the electrostimulation conditions, isolated fat cells were exposed to 5 conditions. First, in S1, monopolar square-wave pulse currents at intensities of 4, 8, and 20 mA were used. Second, in S2, bipolar square-wave pulse alternating currents at intensities of 4 and 6 mA were applied. Two independent protocols were deemed necessary to compare the efficacy of a monopolar current relative to a bipolar alternating current at a given intensity, i.e., 4 mA. S2 also allowed to test for another intensity (i.e., 6 mA) closer to the one found to be efficient in S1 (i.e., 4 mA).

In both S1 and S2, the protocols were applied to different cell suspensions that were extracted from a given subject (within-subject design). Furthermore, still both in S1 and S2, square-wave pulse currents were delivered from a custom built current generator (voltage range 0–30 V, current range 0–50 mA). The frequency of stimulation was set at 1 Hz (i.e., using a 500-ms on- and 500-ms off-duty cycle) and maintained for a period of 30 min while the cell suspension was shaken (40 repetitions per minute) in a water bath that was maintained at a temperature of 37 °C. The current intensity and pulse shape were monitored using an ampere meter (FLUK 179) and an oscilloscope (Tektronix TDS2022), respectively.

Experimental measurements

Fat cell lipolysis was measured as previously reported (Savard et al. 1987). Briefly, lipolysis was measured in triplicates by quantifying the release of glycerol from adipocytes in each experimental condition. Glycerol was enzymatically transformed and the production of NADH was measured by spectrophotometry at 340 nm (Pharmacia LKB-Novaspec). Glycerol content was thereafter expressed in $\mu\text{mol} \cdot (10^6 \text{ cell})^{-1} \cdot (30 \text{ min})^{-1}$. Cellular viability and cell number were verified after each condition using trypan blue (0.4% in normal saline) and counted in Neubauer's hemacytometer. Cells exposition to ISO stimulation also allowed the verification of their viability. The electrodes and solution impedance were also measured.

Procedures

Adipocytes were collagenase-isolated according to the technique described by Rodbell (1964). Briefly, tissue fragments were incubated in the presence of collagenase ($5 \text{ mg} \cdot \text{g}^{-1}$ adipose tissue) for 30 min at 37 °C under 100 strokes $\cdot\text{min}^{-1}$ shaking in KREBS buffer. KREBS buffer also contained glucose ($0.5 \text{ g} \cdot \text{L}^{-1}$) and fatty acid free BSA fraction V ($40 \text{ g} \cdot \text{L}^{-1}$). After washing 4 times, isolated adipocytes were suspended in fresh KREBS buffer. The medium was adjusted to obtain 500–1000 adipocytes per 50 μL of cell suspension.

Statistical analysis

Descriptive statistics (means and the standard deviations) were calculated for all trials and conditions. All conditions were compared using *t* tests (because of unequal *n*) for repeated measures (within-subject design) adjusted using the Bonferroni correction (Abdi 2007). Differences with a *p* value reaching 0.05 or 0.01 are reported and were deemed statistically significant.

Results

Cell count in S1, investigating monopolar currents, revealed that the 4-mA stimulation had no effect on the number of viable cells. However, the 6-mA condition showed a small decrease of viable cells. In S2, the 4- and 6-mA bipolar stimulation conditions had no effect on cell viability (results not shown). The data support the fact that monopolar and bipolar stimulations of isolated fat cells have little or no effect on their viability *in vitro*.

For S1, results show (Fig. 1) that the 4-mA monopolar current stimulated adipocyte lipolysis at a mean level of 1.5

Fig. 1. Histogram showing the mean (and SE) glycerol release in the 5 experimental conditions: no stimulation (BASAL), isoproterenol stimulation (ISO), and 4-, 8-, and 20-mA monopolar electrical stimulation conditions. Symbols indicate significant difference from basal: *, $p \leq 0.05$, **, $p \leq 0.01$.

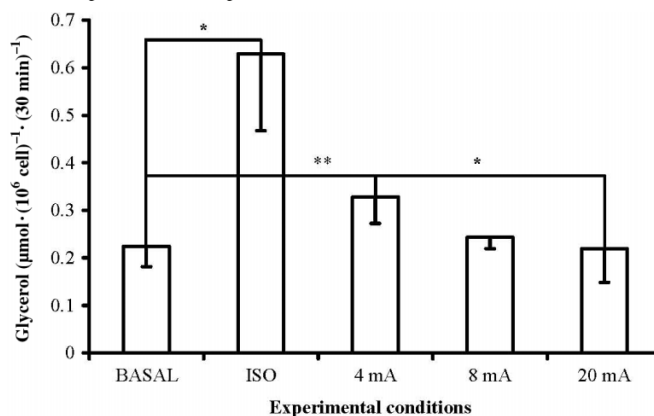
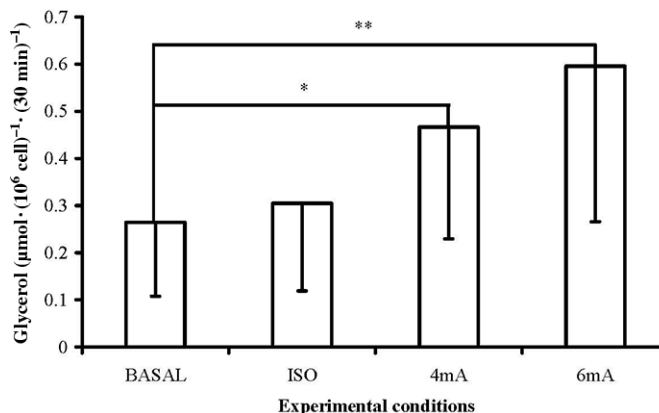


Fig. 2. Histogram showing the mean (and SE) glycerol release in the 4 experimental conditions: no stimulation (BASAL), isoproterenol stimulation (ISO), and 4- and 6-mA bipolar electrical stimulation conditions. Symbols indicate significant difference from basal: *, $p \leq 0.05$, **, $p \leq 0.01$.



times that of basal ($p = 0.004$) and that of the 20-mA current ($p = 0.012$) conditions. The 8- and 20-mA conditions did not significantly increase lipolysis compared with the basal control condition, revealing *p* values of 0.890 and 0.142, respectively. On the other hand, ISO ($10^{-6} \text{ mol} \cdot \text{L}^{-1}$) produced levels of adipocyte lipolysis greater (2.8-fold) than that of basal ($p = 0.020$). However, the 1.9-fold difference between the ISO ($10^{-6} \text{ mol} \cdot \text{L}^{-1}$) and 4 mA conditions did not reach the statistically significant difference level ($p = 0.067$).

In S2, results demonstrate (Fig. 2) that the 6-mA bipolar stimulation significantly ($p = 0.01$) increased lipolysis by 2.3-fold over the basal control condition. Adipocyte lipolysis also increased by 1.8-fold for the 4-mA stimulation condition compared with basal values ($p = 0.03$). On the other hand, ISO at $10^{-6} \text{ mol} \cdot \text{L}^{-1}$ did not succeed to stimulate significantly lipolysis, increasing it by only 15%.

Discussion

To our knowledge, this project represents the first attempt to induce fat cell lipolysis in human adipocytes through elec-

trical stimulation. The most important finding of our study is that both monopolar (4 mA) and bipolar (4 and 6 mA) square-wave pulse stimulations significantly activated in vitro lipolysis. The increase in lipolysis with the protocol using monopolar stimulation (S1) was shown not to be statistically different from the β -adrenergic agonist stimulation (ISO at 10^{-6} mol·L $^{-1}$). In S2, using bipolar stimulation, the level of lipolysis was significantly increased by an electrical stimulation but not by ISO (10^{-6} mol·L $^{-1}$). Although the present study did not directly investigate β -adrenergic receptors, the latter lipolytic resistance to the ISO stimulation could be the result of a dysfunction of β -adrenoceptors (Langin et al. 1996). Indeed, β -adrenoceptor dysfunctions are often reported in obesity (Clément et al. 1995; Lacasa et al. 1984; Large et al. 1997; Lönnqvist et al. 1992). Hence, this dysfunction could explain the ISO results in S2. Whether the number of receptors, their sensitivity, or the transmembrane signal is involved in the present results is not a question investigated in our study. However, since fat cell lipolysis was activated by an electrical stimulation, it is likely that the mechanism involved is at variance with the β -adrenergic stimulation of fat cell lipolysis.

Indeed, the major focus of the present study was the electrostimulation of fat cell lipolysis. Much is known about white fat cell metabolism, but little information is available regarding its electrophysiology. In the last few years, white adipocytes have been shown to have membrane K_v channels both in rats (Lee and Pappone 1997a, 1997b; Ramírez-Ponce et al. 1996; Ringer et al. 2000) and in humans (Ramírez-Ponce et al. 2003). Furthermore, NA and insulin are known to change the passive and the active electrical membrane properties in white adipocytes (Ramírez-Ponce et al. 1996). The modulation of K^+ conductance has been proposed to be a consequence of the raise of cAMP concentration in the adipocyte (Ramírez-Ponce et al. 1998). The primary human white adipocyte membrane current is an outward ionic current (Ramírez-Ponce et al. 2003), indicating the existence of K_v channels of the delayed-rectifier type.

Since the physiological role that K_v channels may play in white adipocyte is unknown, it is premature to conclude to its involvement in the present results. However, it has been shown that these K_v channels could be implicated in the development and growth of brown adipocytes (Lee and Pappone 1999; Wilson et al. 2000) and in the development of white adipocytes in rats (Ramírez-Ponce et al. 2002).

On the other hand, electrical stimulation is known to depolarize the cell membrane and change the membrane electrical properties by acting on ionic voltage dependent channels when such channels are present. Several studies demonstrated that various cellular functions are activated when cells are exposed to low voltage stimulation for a long period of time (Kim et al. 1998; Korenstein et al. 1984; Lehmann and Berg 1998). Consequently, knowing that human white adipocytes present K_v voltage-dependent channels, that NA depolarizes the cell membrane, that the depolarization of adipocyte membrane could be cAMP dependent, and that both NA and cAMP are well known for their capacity to increase adipocyte lipolysis, one can hypothesize that the electrostimulation used in the present study could have induced ionic fluxes that have stimulated the lipolytic cascade in a manner that still remains to be clarified.

Conclusion

To our knowledge, this study represents the first attempt to induce fat cell lipolysis in human white adipocytes using electrical stimulation. Our results demonstrate that both monopolar (4 mA) and bipolar (4 and 6 mA) stimulations significantly activated in vitro lipolysis. Based on our results, and inspired by the ground-breaking work of Ramírez-Ponce et al. (2003), we are developing a new line of research on the electrophysiology of the adipocyte and its function. Identifying the role and channel type of this new current-dependent, membrane-bound lipolytic activity will certainly represent one of the primary focuses of this new and stimulating research program.

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