Noradrenaline modulates the electrical activity of white adipocytes by a cAMP-dependent mechanism

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Abstract

We studied the effect of the external addition of noradrenaline (NA) on the electrical activity of white adipocytes from rat epididymal tissue. NA modified the active voltage response recorded by the current clamp technique, and decreased the macroscopic membrane potassium currents measured by a whole-cell configuration patch-clamp technique in cells obtained by culturing differentiating preadipocytes. To analyse if cAMP is implicated in the effect of this hormone on potassium conductances, the changes in electrical activity caused by

an increase in the intracellular concentration of cAMP were studied using intracellular recording. The addition of forskolin, or *N,N*-dibutyl cAMP plus 3-isobutyl-1-methylxanthine to the bath reduced active changes in membrane voltage responses to hyperpolarizing and depolarizing pulses. A similar effect was observed when vasoactive intestinal peptide was added to the superfusion chamber. These results suggest that NA could modulate K^+ conductances in white adipocytes, mediated by cAMP.

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Introduction

White adipose tissue is the major energy store in mammals. Its primary function is to store lipids and to release free fatty acids in response to various neural and hormonal stimuli. Breakdown of stored triglycerides to free fatty acids (lipolysis) is caused primarily by stimulation with catecholamines. In rodent white adipose tissue, three β -adrenoreceptors subtypes (β_1 , β_2 and β_3) have been found to coexist (Langin *et al.* 1991). In brown adipose tissue, responsible for regulatory nonshivering thermogenesis in most mammals of small body size, the same types of receptors have been identified (Lafontan & Berlan 1993). It is well established that the binding of catecholamines to these receptors induces lipolysis through their coupling with plasma membrane adenylyl cyclase. In brown adipocytes, the electrical membrane response to stimulation with noradrenaline (NA) is biphasic, and several types of channels with different physiological functions have been described (Ruß *et al.* 1993, for review see Koivisto *et al.* 1994); however, little information is available on the electrical properties of white adipocytes and their role in the metabolism of these cells.

Passive electrical properties of the adipocyte plasma membrane (membrane potential, input resistance and time constant) have been studied using intracellular recording, and it has been found that active changes can be evoked by electrical stimuli (Ramírez-Ponce *et al.* 1990). The active changes consisted of a rectification of the voltage response evoked by positive pulses and a slow

return to baseline at the offset of hyperpolarizing pulses. Both changes were dependent on extracellular K^+ , and were reversibly diminished when Co^{2+} replaced extracellular Ca^{2+} , and/or when 4-aminopyridine was added directly to the superfusion chamber. The same effect was obtained by external application of Ba^{2+} (Ramírez-Ponce *et al.* 1991). Such results suggested that white adipocytes could exhibit different voltage-dependent K^+ currents that represent the basis of their electrical response. Recently, the existence of voltage-dependent K^+ channels has been demonstrated using the whole-cell variant of the patch-clamp technique in mature adipocytes obtained by culturing differentiating preadipocytes from rat epididymal tissue (Ramírez-Ponce *et al.* 1996), and in isolated white adipocytes (Lee & Pappone 1997).

Insulin and NA are hormones known to act on the metabolic activity of white adipose tissue. A preliminary study by intracellular recording has shown that both can modify the electrical properties of white adipocytes (Ramírez-Ponce *et al.* 1991). Insulin produced an average hyperpolarization of 13.5 mV in the resting potential, and NA elicited a tendency to depolarize, up to 8.5 mV, in some cases. Insulin and NA also had opposite effects on the active electrical properties of white adipose tissue: NA reduced, whereas insulin increased the amplitude and the duration of the slow return to baseline. These results suggested that insulin and NA could modulate K^+ conductances in white adipocytes.

In this study, we confirmed the effect of NA on the active electrical response to negative pulses and its effect

on positive pulses. Our results, using the whole-cell variant of the patch-clamp technique in cells obtained by culturing differentiating preadipocytes, corroborated those findings.

cAMP is the most important intracellular mediator in the lipolytic response of the white adipose tissue to NA, and its concentration is diminished in the antilipolytic action of insulin on these cells (Abumrad *et al.* 1988). As cAMP can modulate ionic currents in other tissues (Bruggemann *et al.* 1993, Chung & Kaczmarek 1995, Deadwyler *et al.* 1995), we investigated its possible participation in the electrical activity of white adipocytes by using substances that increase the intracellular concentration of cAMP ($[cAMP]_i$), and have studied their effects on the active electrical properties of this tissue.

Materials and Methods

Intracellular recording experiments

The technique used for intracellular recordings in white adipose tissue has been described previously (Ramírez-Ponce *et al.* 1990). Briefly, distal segments of epididymal adipose tissue were excised from fed male Wistar rats weighing 120–180 g. A 0.5-cm² piece of tissue was placed in a recording chamber, where it was continuously superfused with a solution containing 124 mM NaCl, 5 mM KCl, 1.3 mM MgSO₄, 1.2 mM NaH₂PO₄, 2.4 mM CaCl₂, 25 mM NaHCO₃, and 10 mM glucose. This solution was bubbled with 95% O₂ and 5% CO₂ to increase its oxygen content and to maintain pH 7.4 the temperature in the chamber was kept at 35–37 °C. NA, forskolin, *N,N*-dibutyryl cAMP (db-cAMP) plus 3-isobutyl-1-methylxanthine (IBMX) or vasoactive intestinal peptide (VIP) were added directly to the chamber from stock solutions. All chemical products were purchased from Merck (Darmstadt, Germany) and Sigma Chemical (St Louis, MO, USA).

The current and voltage signals were stored on videotape. For the final reproduction of the data, the signals from the videotape were digitized by an ITC-16 computer interface (Instrutech Corp., NY, USA) and software (Pulse+Pulsefit) developed by Heka elektronik GmbH (Lambrecht, Germany).

Patch-clamp experiments

Preadipocytes from rat epididymal fat pads were isolated by incubation for 40 min at 37 °C in the presence of collagenase as described previously (Ramírez-Ponce *et al.* 1996). Dissociated cells were centrifuged and, after three washes, the floating cells were suspended in culture medium consisting of DMEM supplemented with 10% neonatal calf serum, 2 mM glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin, and plated on polylysine-treated glass cover slips. The cells were studied between the 5th and 12th days of incubation.

Figure 1 Effect of external addition of 4 µM NA on electrical responses elicited in white adipocytes by injection +0.5 nA (A–C) or –0.5 nA (D–F) current pulses. (A, D) Controls. (B, E) After NA treatment ($n=6$ experiments). (C, F) After washing with control solution. All records are from a cell with a resting potential (RP) of –25 mV. (G, H) Ionic currents from a white adipose cell. (G) Whole-cell current during a 125-ms voltage step from –70 to +20 mV, applied from a holding potential of –70 mV in control solution ($n=7$ experiments). Superimposed on this trace are the current produced after 40 µM NA was added to the bath solution and the current recorded after washing with control solution. (H) Current–voltage relation obtained by the measurement of the peak amplitude of the current signals recorded every 8 s during 125-ms voltage steps in 10-mV increments from –20 to +70 mV, applied from a holding potential of –70 mV. ■, Control solution; ●, after addition of 40 µM NA; ▲, after washing with control solution.

Ionic currents were recorded using the whole-cell configuration of the patch-clamp technique (Hamill *et al.* 1981), with an EPC-7 patch-clamp amplifier (List Electronics, Darmstadt, Germany). Data acquisition was performed by an ITC-16 computer interface (Instrutech Corp.) and (Pulse+Pulsefit) software (Heka elektronik GmbH). Linear leak and residual uncompensated currents through the membrane capacitance were cancelled on-line using the P/4 procedure (Armstrong & Bezanilla 1974).

The composition of the control external solution was 130 mM NaCl, 2.7 mM KCl, 2.5 mM CaCl₂, 1 mM MgCl₂, 10 mM Hepes and 5 mM glucose; pH was adjusted to 7.35. In experiments in which NA was added, this was previously diluted in 5 mM ascorbic acid to avoid

Figure 2 Effect of forskolin (fors) ($50\ \mu\text{M}$) on electrical responses evoked in white adipocytes by injection of current pulses ($n=5$ experiments). (A–C) Responses to a stimulus of $+0.4\ \text{nA}$ in a cell with an RP of $-30\ \text{mV}$. (D–F) Responses to a stimulus of $-0.4\ \text{nA}$ in a cell with an RP of $-44\ \text{mV}$.

oxidation. The standard solution used to fill the electro-pipettes contained 80 mM potassium glutamate, 20 mM KF, 2 mM MgCl_2 , 10 mM Hepes and 5 mM EGTA, pH 7.25 in all cases.

Results

Effects of noradrenaline

The application of current pulses induced membrane voltage changes in white adipose tissue. The typical control responses to depolarizing and hyperpolarizing pulses are illustrated in Fig. 1A and D; with positive pulses, the records showed smaller amplitudes than those with negative pulses. At the offset of hyperpolarizing pulses, the profile of membrane voltage did not follow an exponential curve of increasing over the entire membrane discharge, as would be expected for a passive RC circuit; instead, the cells remained hyperpolarized for a greater period of time, exhibiting a slow return to baseline (arrow in Fig. 1D). The parameters that we used to characterize the slow return to baseline were its amplitude (voltage difference between the resting potential and the potential at onset of the slow return to baseline), and its duration (time elapsed between the onset of the slow return to baseline and the point at which the resting potential was attained).

Figure 1B and E shows the effects of external addition of $4\ \mu\text{M}$ NA on the voltage response in current-clamp experiments. With depolarizing pulses, the voltage rectification disappeared and the membrane behaved like a passive resistor–capacitor (RC) circuit, with an increase in voltage amplitude; this implies an increase in the membrane resistance to ionic currents. With hyperpolarizing pulses, the effect was seen at the end of the pulse,

decreasing the amplitude (by 53%) and duration (by 44%) of the slow return to baseline, and again showing nearly passive electrical behaviour. When NA was washed from the bath, the effects were partially reversed in 95% of cases when negative pulses were applied (Fig. 1F), but recovery could not be obtained with positive pulses.

Figure 1G and H illustrates the results of the voltage-clamp experiments on macroscopic membrane currents of white adipocytes obtained by culturing differentiating preadipocytes from rat epididymal tissue. In all differentiated cells, outward currents exhibiting sigmoidal activation kinetics were observed. In previous work (Ramírez-Ponce *et al.* 1996), we demonstrated that these ionic currents in white adipocytes were the consequence of the existence of voltage-dependent K^+ channels in these cells. Figure 1G shows that external application of NA ($40\ \mu\text{M}$) blocked the current by 30% at 20 mV. The peak current recovered to 106% of control when NA was washed from the bath. In these experiments, a greater concentration of NA was required than for intracellular recordings, presumably because factors essential to the NA response are more easily dialysed during whole-cell recording. The relationship between peak current and voltage is shown in Fig. 1H. The threshold for current activation in this cell was around $-20\ \text{mV}$, and did not change after the addition of NA. Other cells showed an activation threshold of around $-40\ \text{mV}$ and were not affected by NA.

Effects of cAMP

We wanted to investigate whether changes in $[\text{cAMP}]_i$ would produce any effect on the electrical activity of white adipose tissue. Among the substances that increase intracellular cAMP, we chose to use forskolin because it activates adenyl cyclase (Madison & Nicoll 1986). For

Figure 3 Effect of IBMX (10 μ M) and db-cAMP (500 μ M) on electrical responses evoked in white adipocytes by injection of negative or positive square-wave current pulses ($n=12$ experiments). (A–D) Responses to a stimulus of -0.3 nA in a cell with an RP of -24.5 mV. (E) Response to a stimulus of $+0.5$ nA in a cell with an RP of -26 mV.

these experiments, forskolin was dissolved in a solution of dimethyl sulphoxide (DMSO) 1 : 2000; we had previously checked that the solvent at this concentration did not produce changes in the electrical parameters to be studied. External forskolin (50 μ M) reduced the voltage rectification with depolarizing pulses, which was seen as an increase in the voltage of the membrane, similar to that produced by an RC circuit (Fig. 2B), and diminished the amplitude of the slow return to baseline by 70% and its duration by 67% (Fig. 2E). When forskolin was washed from the bath, the effects were partially reversed in 89% of cases (Fig. 2C and F).

The effect of forskolin on the electrical responses of adipocytes could be due to an increase in $[cAMP]_i$ and/or to the direct action of the drug on K^+ channels, as has been observed in other cells (Harris-Warrick 1989, Garber *et al.* 1990). To examine the isolated effect of modification of $[cAMP]_i$ on the electrical responses in white adipose tissue, we increased the concentration of cAMP by means of its liposoluble derivative, db-cAMP. The external addition of

Figure 4 Effect of VIP (250 nM) on electrical responses evoked in an adipocyte by injection of (A) positive ($+0.2$ nA) or (B) negative (-0.2 nA) square-wave current pulses ($n=4$ experiments). The cell had an RP of -39 mV.

db-cAMP in various concentrations (from 1.25 μ M to 1 mM) did not evoke significant changes in the electrical response of white adipocytes to positive and negative pulses (data not shown).

This lack of effect of db-cAMP could have been due to the action of cytosolic esterases, which hydrolyse the db-cAMP (Valverde *et al.* 1992). To avoid this situation, we used IBMX (an inhibitor of phosphodiesterase) in addition to db-cAMP. Figure 3 shows the effect of db-cAMP (500 μ M) with IBMX (10 μ M). Active electrical phenomena evoked by depolarizing and hyperpolarizing pulses were reduced reversibly (Fig. 3C and E). The amplitude and the duration of the slow return to baseline diminished by 50% and 76% respectively. These effects were partially recovered in 75% of cases when db-cAMP plus IBMX were washed from the bath (Fig. 3D and E). IBMX alone did not produce any significant effect (Fig. 3B).

It is known that VIP generates a lipolytic action on white adipose tissue, and cAMP participates in this action as the principal intracellular mediator (Green *et al.* 1985), as is also the case with NA. Therefore we chose also to study the effect of VIP on the electrical activity of this tissue. The external addition of VIP (250 nM) to white adipocytes produced results similar to those obtained with forskolin and db-cAMP plus IBMX (Fig. 4A and B). The effect of depolarizing pulses was partially reversible in 50% of cases. The amplitude of the slow return to baseline was reduced by 22% and the effect persisted after VIP was washed from the bath. No significant effect on the duration of the slow return to baseline was found.

Discussion

In this study we have confirmed the effect of NA on electrical activity in white adipocytes and obtained results

that could implicate cAMP in the modulation of K^+ conductances in these cells. Intracellular recordings showed that NA modified all typical responses to depolarizing and hyperpolarizing pulses, and the patch-clamp technique demonstrated that NA decreased the outward currents.

In previous work, it has been demonstrated that K^+ conductances could participate in the generation of electrical activity in white adipocytes (Ramírez-Ponce *et al.* 1990, 1991). The existence of a Ca^{2+} -activated K^+ conductance and of a transient current, such as the A-type current, was suggested from the time-course of the membrane potential changes induced by injection of a current, and from the modifications of the electrical response produced by the external application of several blockers of voltage-dependent K^+ channels. Recently, the existence of K^+ channels in white adipocytes has been corroborated in adipocytes obtained from preadipocytes differentiated in culture (Ramírez-Ponce *et al.* 1996) and in isolated white adipocytes (Lee & Pappone 1997). Our results may therefore indicate that NA could block K^+ channels in white adipocytes.

When we increased the intracellular concentration of cAMP by the external addition of forskolin or db-cAMP plus IBMX, we obtained a decrease in the active electrical responses in white adipocytes recorded by intracellular recording. Both treatments reversibly depressed the outward rectification recorded by application of positive pulses and the amplitude and duration of the slow return to baseline produced by application of negative pulses.

Our results implicate cAMP in the modulation of K^+ conductances in white adipocytes. Examples exist of other tissues in which cAMP exerts a modulator effect over K^+ channels, such as the inhibition of the potassium A-current in hippocampal neurons (Deadwyler *et al.* 1995). In GH4 cells, it incapacitates voltage-dependent K^+ channels (Chung & Kaczmarek 1995) and in the mutant of *Drosophila* 'ether-a-go-go', it modulates a permeable channel of K^+ and Ca^{2+} (Bruggemann *et al.* 1993).

The effect of VIP on the electrical activity of white adipose tissue further supports this concept. VIP reduces the electrical activity of the adipocytes. It is known that VIP evokes lipolytic and anti-insulinic activity, thereby increasing the concentrations of cAMP in white adipocytes (Green *et al.* 1985). Moreover, in other tissues, VIP modulates Ca^{2+} -dependent K^+ channels through cAMP (Lechleiter *et al.* 1988).

The described effects of NA and insulin on the electrical activity of white adipocytes can thus be explained in a similar way. NA, in its lipolytic action, increases the concentrations of cAMP, which partially block K^+ conductances and hence cause the cells to depolarize. Insulin, in its antilipolytic action, reduces the concentrations of cAMP; this in turn activates K^+ conductance, which would explain the hyperpolarization that has been observed in white adipocytes after the addition of insulin

(Ramírez-Ponce *et al.* 1991, Beigelman & Hollander 1962).

Although further experimentation is necessary to establish that the electrical activity of white adipose tissue participates in the metabolism and hormonal response of the tissue, the present results offer new data that indicate a link between them.

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