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Electrical Activity in White Adipose Tissue of Rat

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Intracellular recording of white adipocytes was performed in an *in vitro* preparation. Resting potential, input resistance and membrane time constant averaged: -34 ± 9 mV, 295 ± 161 MΩ, and 58 ± 19 ms respectively (mean ± SD, n = 32). Intracellular injection of positive and negative square current pulses elicited membrane voltage responses, characterized by a rectification of the voltage change evoked by positive pulses, and a slow return to baseline at the offset of hyperpolarizing pulses. The amplitude and duration of the slow return to resting potential was dependent on membrane potential, pulse duration, and extracellular K^+ concentration. This response was depressed when external Ca^{2+} was replaced by Co^{2+}, and by external application of 4-aminopyridine. These results indicate that white adipocytes can generate membrane voltage responses which may mostly be a consequence of the activity of ionic channels. The properties of the slow return to baseline suggest that it may be due to a transient K^+ current.

Key words: White adipocyte, Electrophysiological properties, Potassium conductance.

Some hormones acting on white adipose tissue metabolic activity are known to affect both its transmembrane ionic gradients (10, 17) and its membrane potential (3, 5). Experimental modifications of extracellular ionic concentrations, which could be expected to produce changes in the resting potential, induce metabolic changes in adipose tissue similar to those evoked by hormones (4, 14). Moreover, these experimental changes also modify the tissue response to hormonal stimulation (6, 8, 13, 18, 20). Membrane potential may therefore play a significant role in the basal metabolic activity of white adipocytes and in its response to hormones, but only a few data on the electrical characteristics of fat cells are available (2, 12). Recently, the existence of Ca^{2+} dependent K^+ channels has been suggested in adipocyte plasma membranes by measuring the fluxes of $^{86}Rb^+$ (17). The aims of this work have been directly to study the passive and active membrane electrical pro-

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perties of adipocytes by intracellular recording with glass microelectrodes.

Materials and Methods

Distal segments of epididymal adipose tissue were excised from fed male Wistar rats weighing 120-180 g. A 0.5 cm^2 piece of tissue was placed in a recording chamber and superfused with a solution of the following composition (in mM): 124 NaCl, 5 KCl, 1.3 $MgSO_4$, 1.2 $NaPO_4H_2$, 2.4 $CaCl_2$, 25 $NaCO_3H$, and 10 glucose.

This solution was bubbled with 95 % O_2 and 5 % CO_2 to increase its oxygen contents and to maintain a pH 7.4. Temperature in the chamber was kept at 35-37 °C. Since tissue tended to float up, it was necessary to fix it to the bottom of the chamber with a plastic grate placed over it. The intracellular recording amplifier had a constant current pump for current injection through the microelectrode and electronic compensation of stray capacitance and voltage drop across the microelectrode. Good impalements were characterized by a sudden change of potential (> 20 mV) that remained stable for at least 15 min. The electrical characteristics of the microelectrodes were systematically checked after withdrawal from the cells.

Results

Resting potential of fat cells averaged −34 ± 9 mV. The mean values of input resistance and time constant were 295 ± 161 MΩ, 58 ± 19 ms (mean ± SD, n = 32). The last two parameters were measured from the voltage drop induced by hyperpolarizing current pulses of 0.1 to 0.3 nA.

The application of current pulses induced membrane voltage changes (fig. 1A). The responses obtained by depolarizing pulses had a smaller amplitude than those with hyperpolarizing ones, which can be clearly seen in the current-voltage curve of figure 1B. This indicates the existence of outward rectification in adipocytes. At the offset of hyperpolarizing pulses, membrane voltage did not follow

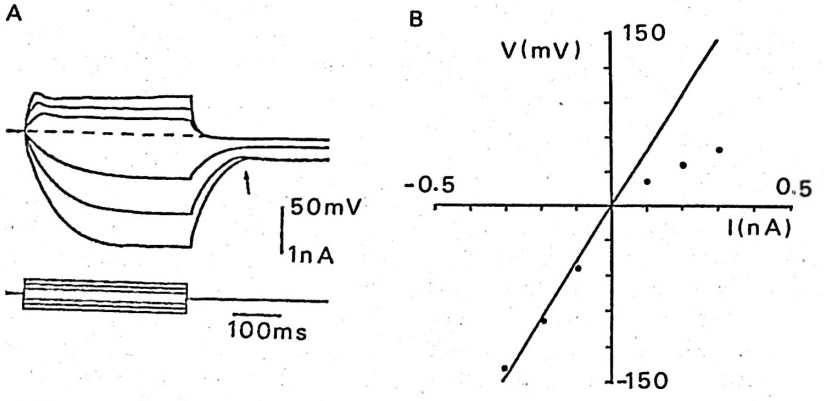

Fig. 1. *Electrical responsed evoked in an adipocyte by injection of positive and negative square current pulses.*
Note in A the slow return to baseline indicated by the arrow. B. Current-voltage plot made with values measured with the recording of points of the fig. A. At negative voltage the data points are fitted by a straight line with a slope of 466 MΩ which is the input resistance of the cell. Resting potential of −40 mV.

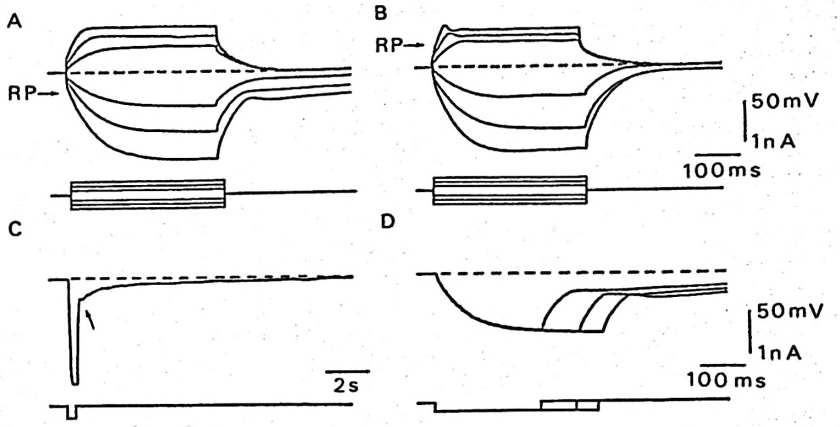

Fig. 2. *Electrical properties of voltage changes elicited by injection of square current pulses.* Electrical responses evoked in adipocytes by injection of positive and negative square current pulses applied on top of DC membrane depolarizing (A) and hyperpolarizing (B). C. Complete time course of the slow return to baseline. D. Dependence of the slow return to baseline on the duration of the hyperpolarizing pulses. From A to C, records belong to a cell with a resting potential of −40 mV. Record D is from different cells with resting potential of −44 mV.

an exponential trajectory, as could be expected for a passive RC circuit, but the cells remained hyperpolarized for a variable time period and they exhibited a slow return to baseline (SRB, arrow in fig. 1A). As it is also shown in this figure, the SRB became slower as membrane potential during the preceding pulse was more negative.

The effect of DC current injection on the current/voltage relations are shown in figure 2A and B. Outward rectification evoked by depolarizing pulses was smaller when the cell was depolarized (fig. 2A) than when it was held at a hyperpolarized potential level (fig. 2B). On the contrary, the SRB was smaller with negative (fig. 2B) than with positive DC current (fig. 2A). The time course of the SRB is shown in figure 2C at a slow time base. The trace illustrates that at the break of the negative pulse (arrow) the membrane potential remained hyperpolarized and that a full recovery was not attained after about 15 to 20 s. This response was enhanced as the duration of the preceding negative pulse increased (fig. 2D).

The properties of the SRB resemble voltage responses previously recorded in other preparations which, as it has been suggested, are due to activation of transient K^+ currents (1, 7, 9, 14). Thus, it was checked in the adipose tissue preparation the effect of changes in external K^+. Figure 3 shows that in low external K^+ the SRB was enhanced (compare traces A and B). The same figure also illustrates the effects of several blockers of transient K^+ conductances. Both replacement of Ca^{2+} by Co^{2+} (compare traces C and D) and external addition of 4-aminopyridine (4-AP) (compare traces E and F) depressed the SRB. The effects of Co^{2+} and 4-AP were additive as shown at a slower time base in figure 4.

Discussion

The *in vitro* preparation used in this report is a novel approach to the study of the electrophysiological characteristics of adipocytes. The resting potentials recorded in these experiments fit well with other

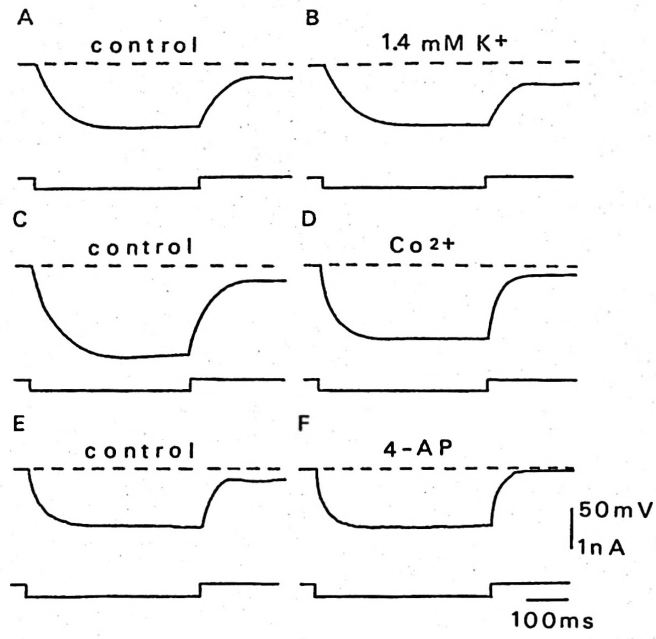

Fig. 3. *Dependence of the slow return to baseline on external K^+ and effect on this response of K^+ channel blockers.*
Note that low external K^+ increases the amplitude of the slow return to baseline (compare traces A and B). Both, external Co^{2+} (2.4 mM) and 4-AP (3 mM) reduce the amplitude of the slow return to baseline. Resting potential in B, D, and F were maintained at the same values as in control recording. Records A and B belong to a cell with resting potential of −32 mV. Record C-F are from different cells with resting potentials of −24 mV.

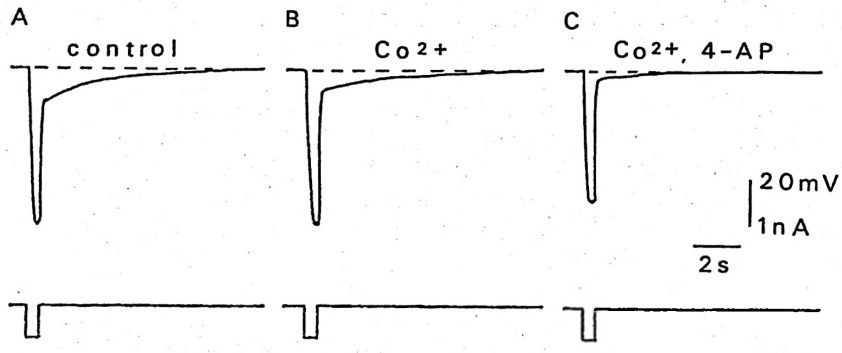

Fig. 4. *Reduction of the slow return to baseline by external Co^{2+} and 4-AP.*
Note in C that both agents have additive effect. Resting potential in B and C was maintained at the same value as in A (control recording, −24 mV).

previously reported (2, 3, 12). However, it has been found in addition electrical responses in adipocytes (outward rectification and SRB) which suggest the existence of voltage dependent ionic channels in these cells.

Voltage responses similar to SRB of adipocytes have been observed in several electrically excitable cells (1, 7, 9, 14, 19) and it has been suggested that they represent the activity of transient K^+ currents. The response found in the adipocytes was strongly depressed by bath application of 4-AP, which resemble that previously found in other preparations (7, 9, 14). However, replacement of external Ca^{2+} by Co^{2+} also had an inhibitory effect. Evidences for the existence of Ca^{2+}-dependent transient K^+ current have been recently reported in a number of preparations different than those of adipose tissue (1, 19).

Therefore, among the conductances that can generate the SRB in adipocytes it appears that K^+ conductances like A-type current and a Ca^{2+}-dependent transient current may participate.

The electrical properties of adipocytes could play a significant role in their metabolic functions. It is known that insulin hyperpolorizes fat cells, and it has been proposed that it also increases cytosolic calcium (6, 11). An elevated internal Ca^{2+} concentration may open Ca^{2+}-dependent K^+ channels which could be responsable for the hyperpolarizing effect of insulin. Thus a further characterization of the electrical properties of adipocytes and the relationship between membrane electrical events and cell metabolism will surely be of interest to understand the physiology of fat cells.

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Resumen

Se desarrolla una técnica de registro intracelular para preparaciones *in vitro* de tejido adiposo blanco. Los valores calculados para el potencial de membrana, resistencia de entrada y constante de tiempo oscilan entre −34 ± 9 mV, 295 ± 161 MΩ y 58 ± 19 ms, respectivamente (media ± DS, n = 32). La inyección intracelular de pulsos cuadrados de corriente positiva y negativa provocan respuestas en el potencial de membrana caracterizados por una rectificación del cambio de voltaje inducido por pulsos positivos, y una vuelta lenta al potencial de membrana tras la aplicación de pulsos negativos. La amplitud y duración de la vuelta lenta al potencial de membrana es dependiente del valor del potencial de membrana, duración del pulso y de la concentración extracelular de K^+. Esta respuesta decrece cuando se reemplaza el Ca^{2+} extracelular por Co^{2+}, y/o se añade 4-aminopiridina directamente en la cámara de perfusión. Estos resultados indican que los adipocitos blancos pueden generar respuestas de voltajes que son posiblemente consecuencia de la actividad de canales iónicos. Las propiedades de la vuelta lenta al potencial de membrana sugieren que puedan ser debidas a una corriente transitoria de K^+.

Palabras clave: Adipocito blanco, Propiedades electrofisiológicas, Conductancia de potasio.

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