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Effects of Noradrenaline and Insulin on Electrical Activity in White Adipose Tissue of Rat

M. P. Ramírez-Ponce*, J. Acosta and J. Bellido

Departamento de Fisiología
Fisiología Médica y Biofísica
Facultad de Medicina
Universidad de Sevilla
41009 Sevilla (Spain)

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An active change in membrane voltage responses to hyperpolarizing pulses has been identified by intracellular recording on an *in vitro* preparation of white adipose tissue. This change was characterized by a slow return to baseline at the offset of the pulses. Amplitude and duration of the slow return to baseline were dependent on extracellular K^+ concentration, and were diminished by external application of Ba^{2+}. Such properties suggest that this electrical response can be mainly due to activation of transient K^+ conductances. The effects that noradrenaline and insulin have over the slow return to baseline have been also studied. While external addition of noradrenaline decreased amplitude and duration of this electrical response, insulin produced the opposite effect. These results suggest that noradrenaline and insulin could modulate K^+ conductances in white adipocytes.

Key words: Adipose tissue, Potassium conductance, Noradrenaline, Insulin

Many hormonal effects in eukaryotic cells are produced through changes in ionic permeabilities and subsequent changes in membrane potential. Electrophysiological techniques developed in past years have made possible to study the electrical properties of a number of cells, and their participation in cellular responses to hormonal stimuli (8, 13, 15).

The passive electrical properties of the adipocyte plasma membrane (Vm, input resistant and time constant) have been studied by intracellular recording, and it has been found that also active changes can be evoked by electrical stimuli. These active changes consist of a rectification on the voltage change evoked by positive pulses, and a slow return to baseline (SRB) at the offset of hyperpolarizing pulses (14).

Some hormones acting on the metabolic activity of white adipose tissue are known to affect both its transmembrane ionic gradient (7, 12) and its membrane poten-

* To whom all correspondance should be addressed

tial (3, 5). The electrical properties of adipocytes may therefore play a significant role in basal metabolic activity and in the response of these cells to hormones. The present work shows that the SRB is mainly generated by K^+ conductances which are blocked by Ba^{2+}, and that its characteristics are modified by noradrenaline and insulin.

Materials and Methods

The technique used for intracellular recording of white adipose tissue has been described previously (14), and therefore only a brief account of the experimental arrangement is given. 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 where it was continuously superfused with a solution of the following composition (in mM): 124 NaCl, 5 KCl, 1.3 $MgSO_4$, 1.2 NaH_2PO_4, 2.4 $CaCl_2$, 25 $NaHCO_3$, and 10 glucose. This solution was bubbled with 95 % O_2 and 5 % CO_2 to increase its oxygen content and to maintain a 7.4 pH. Temperature in the chamber was kept at 35-37 °C. In the experiments where the effects of Ba^{2+}, noradrenaline and insulin were tested, these substances were added directly to the chamber from stock solutions. Noradrenaline was diluted immediately before use.

Amplitude and duration of SRB were measured by a digital storage oscilloscope (Gould 1604). Areas were calculated using a waveform processor adapted to the same oscilloscope.

Bovine insulin and noradrenaline were purchased from Sigma, the rest of the chemicals were from Merck.

Results

Direct stimulation of adipocytes by negative square current pulses elicits an active electrical response characterized by a slow return to baseline (SRB) at the offset of the pulse (fig. 1A). The trace illustrates that the termination of the pulse had two clear components. The first fast component was due to the discharge of the membrane capacitance and reached a level 10-20 mV below the resting potential of the cell. At this point, there was a marked inflection in the voltage signal (arrow), followed by a second smooth slow component, that lasted for up to 15 to 20 s. A previous study (14) showed that the SRB seen in adipocytes is possibly due to activation of transient K^+ currents. In low external K^+, the SRB was enhanced (compare fig. 1, A and B). The external addition of Ba^{2+}, which is known to block many K^+ selective channels (4), depressed the SRB (compare C and D).

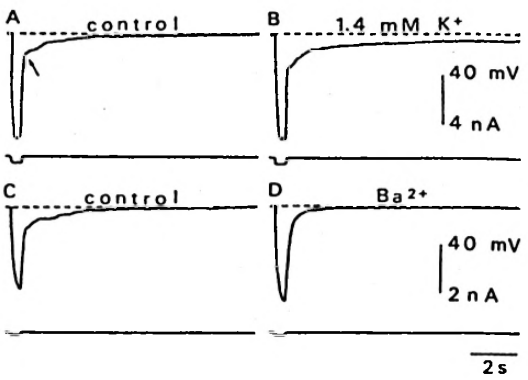

Fig. 1. *Electrical responses evoked in adipocytes by injection of negative square current pulses. Participation of K^+ conductances.*

Dependence of the slow return to baseline on the external potassium concentration (A and B) and effect on this response of 2.5 mM Ba^{2+} (C and D). Note that external Ba^{2+} reduces the amplitude and the duration of the slow return to baseline. Resting potentials in B and D were maintained at the same values as their control recordings, by DC membrane potential. Records A and B belong to a cell with a resting potential of −32 mV. Records C and D are from a different cell with a resting potential of −40 mV.

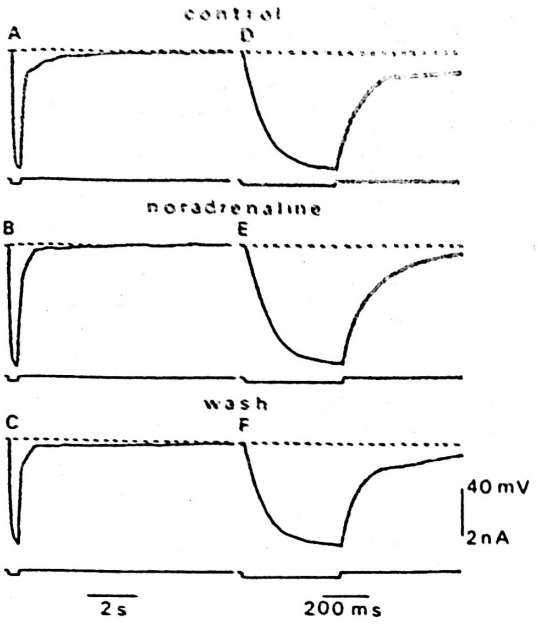

Fig. 2. *Effect of external addition of 4 μM noradrenaline on the slow return to baseline.*
Records B and B were obtained after 8 min of noradrenaline treatment, and C and F were obtained after 30 min washing with a control solution. Resting potential in B and E was maintained as in the control situation, by DC membrane potential. All records belong to a cell with a resting potential of −25 mV.

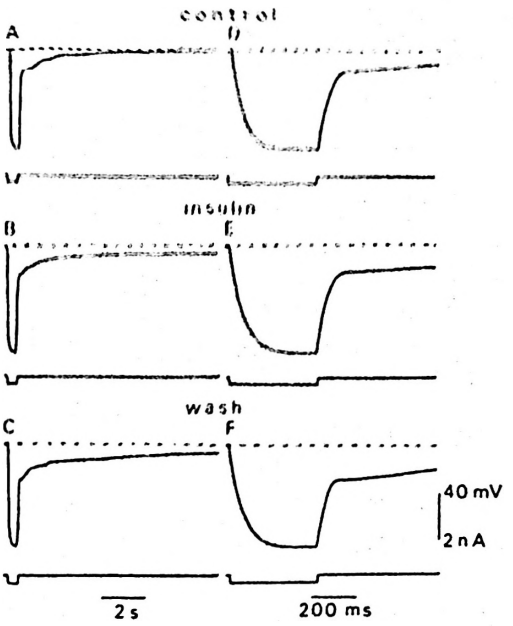

Fig. 3. *Effect of external addition of 8.7 nM insulin on the slow return to baseline.*
Records B and E were obtained after 10 min of insulin treatment, and C and F were obtained after 40 min washing with a control solution. Resting potential in B-F was maintained as in the control recording, by DC membrane potential. Records belong to a cell with a resting potential of −30 mV.

When noradrenaline and insulin were added externally, this electrical response was modified. Noradrenaline depressed the amplitude and the duration of SRB, 8 min later since addition (fig. 2, compare traces A and B, and at a faster time base traces D and E). This effect was observed in all of the five cells registered. The action of noradrenaline was reversible after 30 min washing with a control solution (C and F) in four cells among the five ones. On the contrary, 8 min after addition, insulin increased the amplitude and the duration of SRB (compare traces A and B, D and E in fig. 3). This effect was observed in all of the six cells registered; although it was more evident in four of them, those of a longer time in the register, at least 30 min. As shown in fig. 3C and 3F the effect of insulin did not recover even 30 min after switching to the control solution; it seems that a longer time would be necessary to obtain the recovery, but good impalements could never be maintained more than 45 min after this treatment. Noradrenaline and insulin had also opposite effects on the resting membrane potential of the cells. Noradrenaline produced a tendency to depolarization, which in some cases reached up to 8.5 mV. Insulin elicited an average hyperpolarization of 13.5 mV; this value fits well with those

Table I. *Effects of noradrenaline and insulin on SRB*

The numbers in parenthesis correspond to the cells selected. The values are expressed in percentage of change (mean ± SD) related to the control.

	Amplitude[a] (mV)	Duration[b] (s)	Area[c] (mV.s)
Noradrenaline (4)			
control	1.00	1.00	1.00
4 μM	0.53±0.12	0.33±0.10	0.27±0.10
Insulin (6)			
control	1.00	1.00	1.00
8.7 nM	1.39±0.22	1.96±0.61	1.87±0.74

[a] It was measured between resting potential and the onset of the SRB.
[b] It was determined between the onset of the SRB up to the point in which the resting potential was reached.
[c] It was calculated the area enclosed by the curve between the onset of the SRB and the point in which the resting potential was reached.

obtained by other authors using an equal concentration (2). The catecholamine and the hormone effects on the properties of the SRB are summarized in table I, where the cells with a recovery after a noradrenaline treatment were chosen. The values given in the table are related to the percentage of change after treatment against control.

Discussion

Recently, active electrical responses have been found in white adipocytes (outward rectification and SRB). The properties of SRB suggested that they could express the activity of transient K^+ currents. The replacement of Ca^{2+} by Co^{2+} and external addition of 4-aminopirydine (4-AP) had an inhibitory effect (14). In this report, fig. 1 shows that the amplitude and the duration of SRB were dependent on external K^+ concentration and this response was strongly depressed by bath application of Ba^{2+}. Therefore, the generation of the SRB in adipocytes could be proposed as a result of the activation of K^+ conductances: a C^{2+}-dependent transient K^+ current and a second component similar to 4-AP sensitive A-current.

Moreover, the characteristics of SRB were modified by bath application of noradrenaline and insulin. According to table I, noradrenaline depressed the amplitude and the duration of SRB and insulin increased them. These results indicate that noradrenaline and insulin may modulate the activating or inactivating process of the K^+ channels in white adipose cells.

Insulin has been said to increase cytosolic calcium (6, 10). An elevated internal Ca^{2+} concentration could open Ca^{2+} dependent K^+ channels. The existence of Ca^{2+} dependent K^+ channels has been suggested in adipocyte plasma membranes by measuring the fluxes of $^{86}Rb^+$ (11). In addition insulin appears to produce a decrease in levels of cAMP in the antagonizing effect of this hormone over fatty acid transport stimulation by catecholamine in adipocytes (1). Many hormones and neurotransmitters attenuate cAMP accumulation in intact cells by inhibition of adenylate cyclase via activation of GTP-binding proteins (G_i) (9). There is a substantial evidence that G_i proteins are involved in some insulin actions (16) which may explain the insulin effect over the SRB by activation of K^+ channels regulated by G_i proteins. The opposite effect or noradrenaline over SRB could be a consequence of the interaction of this substance with its receptor and subsequent activation of G proteins linked to stimulation of adenylyl cyclase.

Although further experimental works are necessary to establish a relationship between SRB and hormonal action on white adipocytes, the present results could serve as a starting point.

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Resumen

Se identifica, mediante registro intracelular en una preparación *in vitro* de tejido adiposo blanco, un cambio activo en la respuesta del potencial de membrana a pulsos hiperpolarizantes, caracterizado por una vuelta lenta al potencial de membrana tras finalizar el pulso. La amplitud y duración de esta vuelta lenta es dependiente de la concentración extracelular de K^+ y disminuye por la acción externa de Ba^{2+}. Tales propiedades sugieren que esta respuesta eléctrica puede ser debida fundamentalmente a la activación de conductancias transitorias al K^+. Se estudian también los efectos que la noradrenalina y la insulina tienen sobre la vuelta lenta al potencial de membrana, observando que la adición externa de noradrenalina deprime su amplitud y duración y la insulina produce el efecto opuesto. Estos resultados sugieren que la noradrenalina y la insulina podrían modular la conductancia al K^+ en adipocitos blancos.

Palabras claves: Tejido adiposo, Conductancia al potasio, Noradrenalina, Insulina.

References

1. Abumrad, N. A., Harmon, C. M., Barnela, U. S. and Whitesell, R. R.: *J. Biol. Chem.*, 263, 14678-14683, 1988.
2. Beigelman, P. M. and Hollander, P. B.: *Proc. Exptl. Biol. Med.*, 110, 590-595, 1962.
3. Beigelman, P. M. and Hollander, P. B.: *Acta Endocrinol.*, 50, 648-656, 1965.
4. Castle, N. A., Haylett, D. G. and Jenkinson, D. H.: *TINS*, 12, 59-65, 1989.
5. Cheng, K., Groarke, J., Osotimehin, G., Haspel, H. C. and Sonenberg, M.: *J. Biol. Chem.*, 256, 649-655, 1981.
6. Clausen, T.: *Horm. Metab. Res. Suppl.*, 2, 66-70, 1970.
7. Hales, C. N. and Perry, M. C.: *Horm. Metab. Res. Suppl.*, 2, 63-65, 1970.
8. Light, D. B., Corbin, J. D. and Stanton, B. A.: *Nature*, 344, 336-339, 1990.
9. Limbird, L. E.: *FASEB J.*, 2, 2686-2695, 1988.
10. McDonald, J. M., Bruns, D. E. and Jarett, L.: *Proc. Natl. Acad. Sci. USA*, 73, 1542-1546, 1976.
11. Pershadsingh, H. A., Gale, R. D., Delfert, D. M. and McDonald, J. M.: *Biochem. Biophys. Res. Commun.*, 135, 934-941, 1986.
12. Pershadsingh, H. A. and McDonald, J. M.: *Nature*, 281, 495-497, 1979.
13. Petersen, O. H. and Maruyama, Y.: *Nature*, 307, 693-696, 1984.
14. Ramírez-Ponce, M. P., Acosta, J. and Bellido, J. A.: *Rev. esp. Fisiol.*, 46, 133-138, 1990.
15. Ritchie, A. K.: *J. Physiol.*, 385, 611-625, 1987.
16. Rothenberg, P. and Kahn, C. R.: *J. Biol. Chem.*, 263, 15546-15552, 1988.