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Human Adipose Cells Have Voltage-dependent Potassium Currents

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Abstract. The whole-cell patch-clamp method was used to study the membrane electrical properties of human adipocyte cells obtained by differentiating from precursors of human abdominal and mammary tissues. All differentiated cells exhibited outward currents with sigmoidal activation kinetics. The outward currents showed activation thresholds between −20 to −30 mV and slow inactivation. The ionic channels underlying the macroscopic current were highly selective for K^+. Their selectivity was for typical K^+ channels with relative permeabilities of $K^+ > NH_4^+ > Cs^+ > Na^+$. No evidence of any other type of voltage-gated channel was found. The potassium currents (I_{KV}) were blocked reversibly by tetraethylammonium and barium. The IC_{50} value and Hill coefficient of tetraethylammonium inhibition of I_{KV} were 0.56 mM and 1.17 respectively. These results demonstrate that human adipose cells have voltage-dependent potassium currents.

Key words: Human adipose cells — Potassium channels

Introduction

Adipose tissue, once thought to function primarily as a passive depot for the storage of excess lipids, is now understood to play a critical role in the energy conversion process and homeostasis. Its functions enable adipocytes to influence the metabolic activity of tissues such as brain, liver and muscle. Several hormones and other factors are secreted from adipocytes. Some of these proteins are inflammatory cytokines, and some play a role in lipid metabolism, while others are involved in vascular homeostasis or the complement system (Trayhurn & Beattie, 2001). All this makes the metabolic function of white adipose tissue very complex and several aspects have not yet been adequately explained.

Many hormonal effects in eukaryotic cells are produced through changes in ionic permeabilities and subsequent changes in membrane potential. Although a great deal is known concerning white fat metabolism, there is little information available about its electrophysiology. The methodological problem for single-channel recording of these cells is that well adhered solitary cells are required. We developed an experimental model that allowed us to obtain mature white adipocytes from preadipocytes of epididymal tissue from rats (Ramírez-Ponce et al., 1996). In these cells we demonstrated the existence of voltage-dependent K^+ channels (K_v), using the whole-cell variant of the patch-clamp technique. These results were corroborated by other authors in isolated white adipocytes (Lee & Pappone, 1997; Ringer, Russ & Siemen, 2000). In a preliminary study carried out by means of intracellular recording, we showed that insulin and noradrenaline (NA) could modify the electrical properties of white adipocytes from rats (Ramírez-Ponce, Acosta & Bellido, 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. Insulin and NA also had opposite effects on the electrical properties of white adipose tissue from rats. NA reduced, whereas insulin increased the recovery time of membrane potential at the end of hyperpolarization current pulses. In later work we confirmed the effect of NA on electrical activity in white adipocytes from rats and obtained results that could implicate cAMP in the modulation of K^+ conductances in these cells (Ramírez-Ponce et al., 1998). Recently, we have presented evidence that insulin increases the density of K_v in white adipocytes, and that these channels may play a significant role in the development of such cells (Ramírez-Ponce, Mateos & Bellido, 2002).

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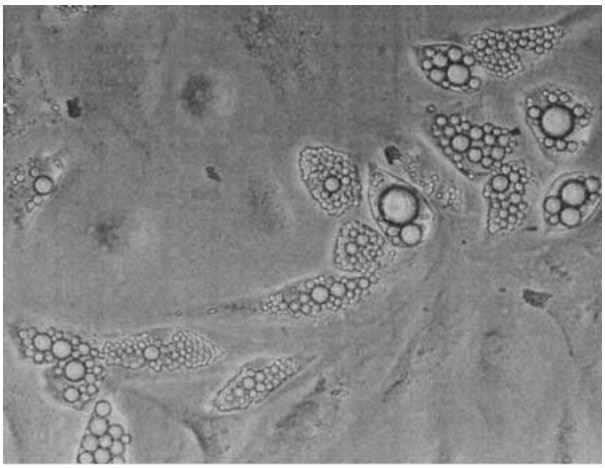

Fig. 1. Human adipose cells after 22 days growth in primary culture. Scale bar, 50 μm.

Until now there have been no electrophysiological data referring to human white adipocytes, and only indirect studies have suggested the existence of ionic channels in these cells (Shi et al., 1999; Shi et al., 2000; Vaughn et al., 2000; Xue et al., 2001). In metabolic studies, it is common to find distinct properties and regulatory mechanisms between different cell models derived from diverse species and development stages (Shi et al., 2000; Boone et al., 2000). For this reason, it would be interesting to know if human adipocytes have ionic channels. If adipocytes from both species possess channels with the same properties, interest in the electrophysiology of white adipose tissue and its possible physiological importance will grow. In this work, we demonstrate the existence of K_v in human white adipocytes and characterize their selectivity, kinetics, and pharmacological properties.

Materials and Methods

Subjects

Subcutaneous and visceral tissues of human fat were obtained from normal-weight subjects (BMI < 27 kg/m^2) undergoing abdominal surgery or surgical mammary reduction. Patients with malignant or chronic-inflammation diseases were excluded. All procedures were in accordance with current local guidelines and the Declaration of Helsinki.

Cell Culture

We devised a method for isolating preadipocytes from human adipose tissue, later differentiating them into adipocytes, based on methods previously described (Entenmann & Hauner, 1996; Ramírez-Ponce et al., 1996; Bornstein et al., 2000, Bastelica et al., 2002). The adipose tissue samples were immediately transferred to the laboratory in a solution |A| containing in mM: 130 NaCl, 5 KCl, 1 $CaCl_2$, 10 HEPES and 5 glucose. The pH of the solution was adjusted to 7.4. Visible fibrous material and blood vessels were carefully dissected, and the remaining adipose tissue was cut into small pieces. The minced material was incubated for 40 minutes at 37°C in 5 ml of solution |A| supplemented with 3% (w/v) bovine serum albumin (Sigma, Munich, Germany; fraction V) and 1.71 mg/ml collagenase type II (Sigma). The pH of the enzyme solution was adjusted to 7.4. Following digestion, the tissue was filtered through a 250 μm nylon gauze. After a centrifugation of $400 \times g$ for 10 minutes at 20°C, two fractions were obtained: a pellet in which stromal vascular cells were included, and a floating layer mainly comprising mature adipocytes. Floating cells were washed and centrifuged once again. Sedimented cells were resuspended in erythrocyte lysing buffer containing in mM: 155 NH_4Cl, 5.7 K_2HPO_4 and 0.1 EDTA, pH 7.3, incubated for no more than 10 min at 37°C, and centrifuged once again. The resulting pellet of stromal vascular cells was resuspended together with the floating cells in a proliferation medium, and plated on polylysine-treated glass cover slips. The composition of proliferation medium consisted of DMEM/Ham's F12 medium (1:1, v:v) supplemented with 10% neonatal calf serum, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 33 μM biotin, 17 μM pantothenate and 4% ITS liquid media supplement (insulin-transferrin-selenite 1×, v:v, Sigma). The cells were kept in a CO_2 incubator at 37°C. After three days the medium was changed to a defined serum-free medium to induce the differentiation into adipocytes. The adipogenic medium consisted of proliferation medium without neonatal calf serum supplemented by 10 μg/ml transferrin, 0.25 mM 3-isobutyl-1-methylxanthine, 100 nM hydrocortisone, 1.3 nM triiodo-thironine and 500 μU/ml insulin. This medium was changed every two days. Under these conditions, visible lipid accumulation starts within 6 to 8 days. Cells were studied between 20 to 35 days of incubation, when they contained larger lipid inclusions (adipocytes), and had changed their morphology to a spherical shape (Fig. 1).

Patch-clamp Experiments

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), as described previously (Ramírez-Ponce et al., 1996). Data acquisition was performed by an ITC-16 computer interface (Instrutech Corp.) and (Pulse + Pulsefit) software (Heka electronik, Germany). Linear leak and capacitance currents were cancelled on-line using the P/4 procedure (Armstrong & Bezanilla, 1974). The composition of the external control solution was (in mM): 135 NaCl, 2.7 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 10 HEPES and 5 glucose; pH was adjusted to 7.35 ± 0.02 in all cases. To test the permeability to monovalent cations, cells were bathed in solutions containing 40, 80, 120, or 133 mM KCl, 133 mM NH_4Cl or 133 mM CsCl. In these solutions NaCl was replaced equimolarly by KCl, NH_4Cl or CsCl. In experiments where channel blockers were used, the external solution contained (in mM): 80 NaCl, 2.7 KCl 1 $CaCl_2$, 70 Trizma, 5 glucose and, depending on the experiment, 0.25, 5 or 50 TEA or 25 BaCl. The standard solution used to fill the electropipettes contained (mM) 80 potassium glutamate, 20 KF, 35 KCl, 2 $MgCl_2$, 10 HEPES and 5 EGTA; pH was adjusted to 7.25–7.30.

Results and Discussion

Cell Culture

Cell preparation has been a major methodological impediment to electrophysiological studies in white adipocytes. We previously devised a method for iso-

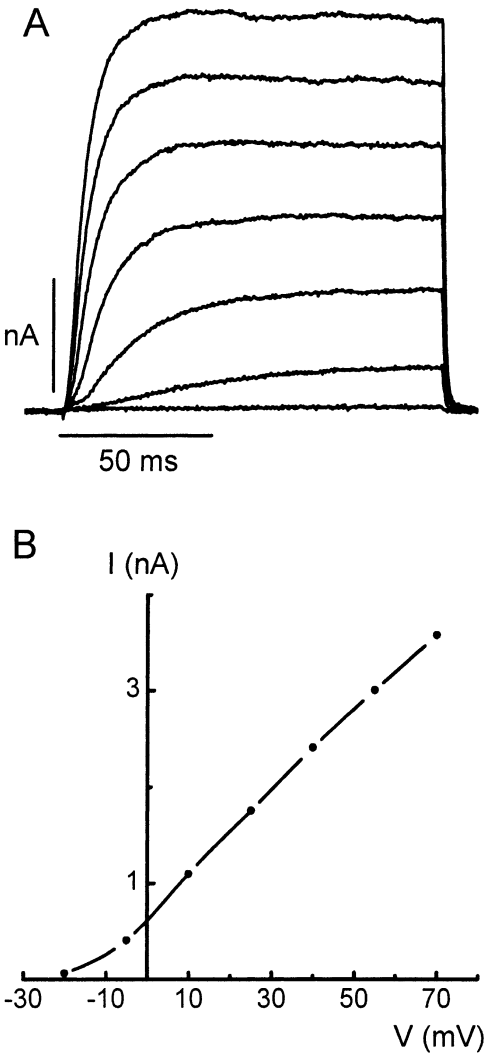

Fig. 2. Voltage-gated outward currents in a human adipocyte. (*A*) Superimposed current traces recorded every 8 s during 125 ms steps in 15 mV increments from −20 to +70 mV, applied from a holding potential of −70 mV. (*B*) Current-voltage relation obtained by measuring the peak amplitude of the traces shown in panel (*A*). External and internal control solutions used were as described in Materials and Methods.

lating preadipocytes from rat adipose tissue, later differentiating them into adipocytes (Ramírez-Ponce et al., 1996), based on the fact that isolated mature adipocytes added to the culture medium demonstrated differentiation-promoting activity (Carraro et al., 1990; Considine et al., 1996). On the assumption that human mature adipocytes could exhibit similar properties, we have implemented the method of obtaining adipocytes from human stromal vascular cells previously published by other authors (Entenmann & Hauner, 1996; Bornstein et al., 2000; Bastelica et al., 2002). In this way, we obtained well-adhered solitary mature human adipose cells that facilitated the use of patch-clamp techniques (Fig. 1).

Voltage-gated Ionic Currents

In all human white adipose cells voltage-clamped in this study ($n = 57$), the primary membrane current was an outward ionic current as shown in Fig. 2. The currents activated in response to depolarizing voltage steps showed a sigmoidal activation time course and after reaching a maximum were slowly inactivated. The degree of inactivation varied from cell to cell. With the cell membrane potential held at −70 mV, the threshold for current activation in this cell was −20 mV, although other cells showed activation thresholds of −30 mV. The average current density at +70 mV was 2.2 ± 0.3 (pA/μm^2 ± SE, $n = 17$), calculated as described previously (Ramírez-Ponce et al., 2002). This value is significantly higher (at the 5 × 10^{-4} level, $P = 2.3 \times 10^{-4}$) than that obtained in rat adipocytes grown in a medium supplemented with insulin, 1.1 ± 0.1 pA/μm^2 (Ramírez-Ponce et al., 2002). On the assumption that the channels in both species have the same properties, these differences indicate that human adipocytes could have higher density channels than rat adipocytes.

In order to examine if these channels had the same properties as rat adipose cells, we tested their selectivity for potassium ions, replacing external Na^+ with increasing concentrations of K^+. Figure 3*A* shows the outward currents generated by the substitution of external Na^+ with 133 mM K^+ and the internal solution decreased to 90 mM K^+. Reversal potentials for the currents as a function of external/internal K^+ concentration are plotted in Fig. 3*B*. The reversal potentials were measured by the value of the membrane voltage for zero ionic current obtained in *I-V* curves (*data not shown*) and were generated with the values of the peak amplitude of the currents for different external/internal concentrations of potassium, as described in the legend. The data were fitted by linear regression with a slope that agreed with the predicted value for a K^+-selective channel at 25°C (58.07 mV/10-fold change in external/internal K^+), indicating that these channels are highly selective for K^+.

The monovalent cation selectivity of channels in human white adipocytes was tested by measuring the tail currents when all the external Na^+ of the bath solution was replaced by K^+, NH_4^+ or Cs^+. The currents recorded are shown in Fig. 4*A–D*, and the curves obtained from the peak amplitude of their tail currents are shown in Fig. 4*E*. The reversal potentials were calculated from these curves. Permeability ratios for each test cation relative to K^+ (P_X/P_K) were calculated from the average change in reversal potential measured from tail currents, using a modified Goldman (1943) and Hodgkin & Katz (1949) equation for equal external monovalent test ion concentration: $P_X/P_K = e^{\Delta E_R F/RT}$ where ΔE_R is the shift in reversal potential and F, R, and T are Faraday constant, gas constant, and absolute temperature, respectively. Fig. 4*C* shows that NH_4^+

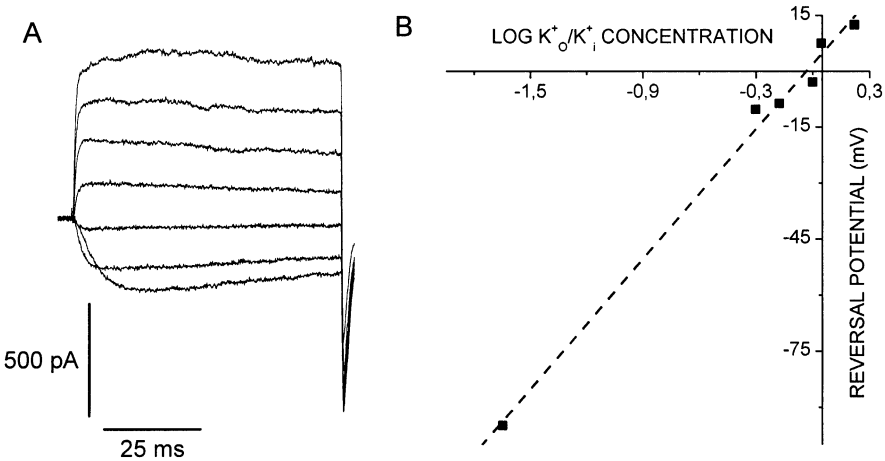

Fig. 3. Selectivity for K^+ ions. (*A*) Appearance of inward K^+ currents when external Na^+ was replaced by 133 mM K^+ and the concentration of internal potassium was decreased to 90 mM. The pulse protocol was the same as Fig. 2. (*B*) Plot of the reversal potential versus $\log(K_o^+/K_i^+)$ using different external/internal concentrations (in mM): 2.7/130, 40/130, 133/130, 2.7/90, 40/90 and 133/90.

ions have a permeability ratio relative to K^+ of 0.118 (± 0.03, $n = 8$), Cs^+ (Fig. 4*D*) of 0.077 (± 0.01, $n = 8$), and Na^+ (Fig. 4*A*) was almost impermeant, with an upper limit for their permeability ratios of 0.002. This selectivity sequence of $K^+ > NH_4^+ > Cs^+ > Na^+$ is similar to that found in the channels of white adipose cells from rats (Ramírez-Ponce et al., 1996), which indicates the existence in human adipose cells of voltage-dependent K^+ channels of the delayed-rectifier type. The results reported here, together with those in white (Ramírez-Ponce et al., 1996; Lee & Pappone, 1997; Ringer et al., 2000) and brown adipocytes from rat (Lucero & Pappone, 1989), indicate that all adipocytes express these voltage-gated K^+ conductances.

The voltage-dependent ionic potassium currents (I_{KV}) were blocked reversibly by TEA and Ba^{2+} (blockers of voltage-dependent K^+ channels). Figure 5*A* shows that external TEA (50 mM) blocked the current by approximately 93% ($n = 8$). The effect of 25 mM Ba^{2+} was additive to that of TEA, reaching a blocking level of up to 97% ($n = 4$). The current recovered to 66% ($n = 4$) of the control level when the TEA and Ba^{2+} were washed from the bath. The dose-response relationship for blocking the current peak using TEA is shown in Fig. 5*B*. The IC_{50} value and Hill coefficient of inhibition of I_{KV} by this agent, determined as was described previously (Ramírez-Ponce et al., 2002), were 0.56 mM and 1.17, respectively. These values are similar to those obtained in rat adipocytes, such as in mature cells with single-channel recording in outside-out configuration (Ringer et al., 2000), and in adipocytes obtained by differentiating preadipocytes with whole-cell recording (Ramírez-Ponce et al., 2002). Under our recording conditions, we found no evidence of any other type of voltage-gated channel. In particular, there were no indications of an inward current that could reveal the presence of significant voltage-gated Na^+- or Ca^{2+}-conductances in these cells, although preceding studies have suggested the existence of calcium channels in human adipocytes that could have a significant role in the physiology of white adipose tissue (Shi et al., 2000; Xue et al., 2001). Nevertheless, our results are in agreement with those previously obtained using the same technique as in rat adipocytes (Ramírez-Ponce et al., 1996; Lee & Pappone, 1997), which failed to find calcium channels in these cells.

These data constitute the first study of the electrical properties of human adipose cells, and demonstrate the existence of voltage-dependent K^+ currents in these cells. Previous studies have suggested the existence of potassium channels in human adipocytes (Vaughn et al., 2000), but there have not been any experiments that had shown such electrical activity.

The possible physiological role of K_V in human adipocytes remains unknown. In rat, the data suggest that insulin increases the density of these channels in adipose cells, and that these channels may be necessary for normal growth in culture (Ramírez-Ponce et al., 2002). These results allow us to add adipose cells to the growing list of cell types in which potassium channels have been implicated in cellular development. In brown fat cells, functional voltage-gated K^+ channels may be necessary for their proliferation and differentiation, and purinergic modulation of I_{KV} may be important in altering adipocyte growth and development (Wilson & Pappone, 1999; Wilson et al., 2000). Wilson et al. (2000) found that in brown adipocytes ATP and β-adrenergic stimulation enhanced voltage-gated K-current inactivation, suggesting that P2-receptor and β-adrenergic-receptor stimulation may converge in altering the activity of voltage-gated K currents, and this could have a modulating effect on adipose tissue growth. Thus, these authors proposed that determining the function and mechanism of P2 purinergic or β-adrenergic modulation of I_{KV} gating in brown adipocytes may be important for understanding

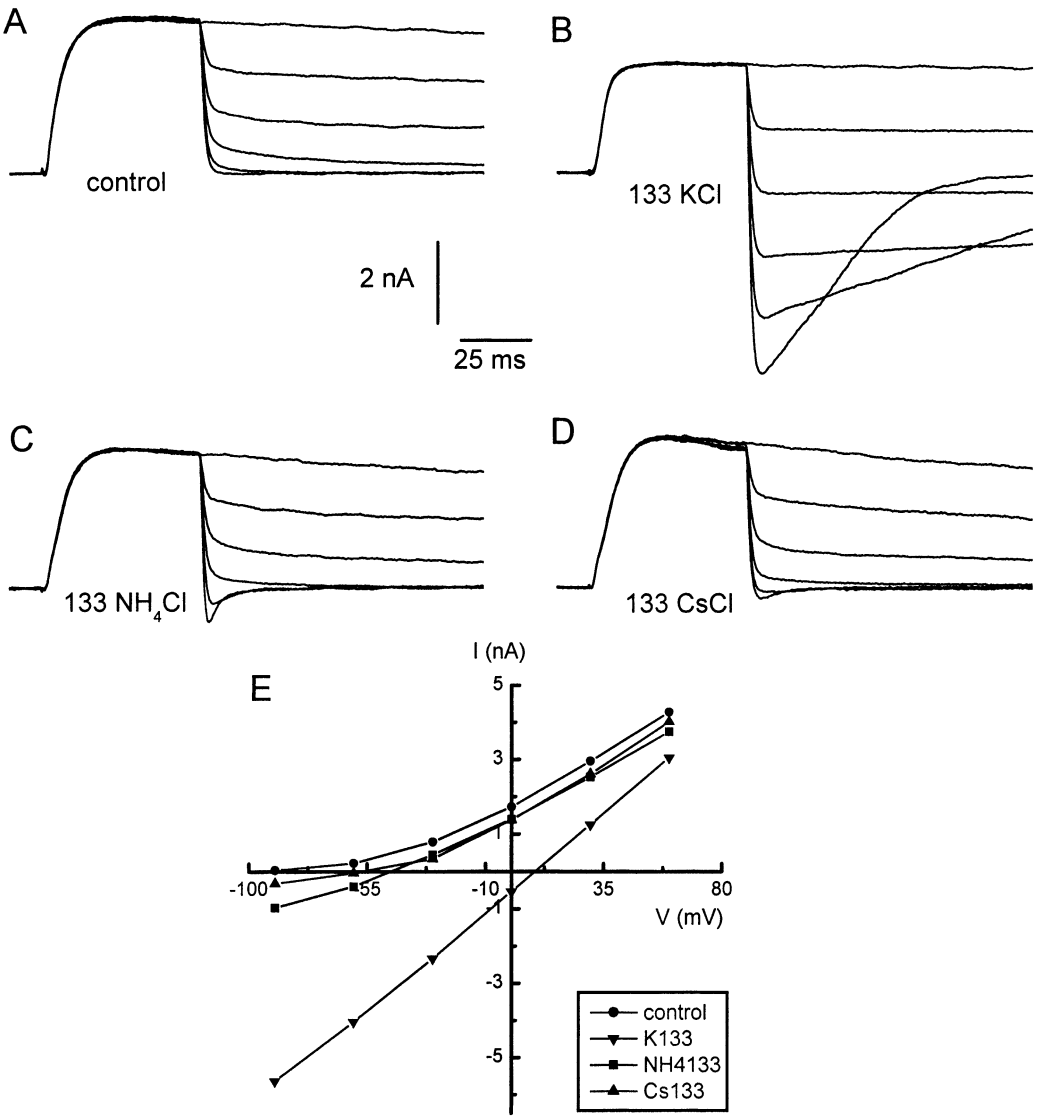

Fig. 4. Selectivity of the potassium channels for monovalent cations. Tail currents were recorded every 2 s during 60 ms voltage steps in 15 mV increments from −90 mV to +60 mV after pulses to +60 mV of 50 ms duration, applied from a holding potential of −70 mV. (*A*) Whole-cell currents recorded in external control solution, and (*B–D*) when external Na^+ was replaced by (in mM) 133 K^+, 133 NH_4^+ or 133 Cs^+, respectively. The internal control solution was as described in Material and Methods. (*E*) Current-voltage relations measured by the peak amplitude of the tail currents.

and controlling obesity. Purinergic stimulation of white adipocytes in rats also produced a significant modulation of the voltage-gated K^+ current (Lee & Pappone, 1997). P2 purinergic and adenosine (A1 and A2) receptors have been identified in human adipose cells (Schmidt & Loffler, 1998, Borglum et al., 1996). Changes have been described in adenosine A1- and A2-receptor expression during adipose cell differentiation. A1 and A2 are associated with either stimulation (A2) or inhibition (A1) of adenylate cyclase. Northern blot analysis revealed that A1-receptor mRNA is exclusively expressed in the mature adipocytes, whereas A2-receptor mRNA gradually diminished during the differentiation process. These results may be related to our proposal that cAMP reduces the expression of K^+ channels in preadipocytes and in consequence provokes a decrease in the differentiation of these cells (Ramírez-Ponce et al., 2002). Although further studies will be necessary to clarify this, these antecedents constitute data that lead us to think that I_{KV} plays a very important role in the physiology of human adipose tissue.

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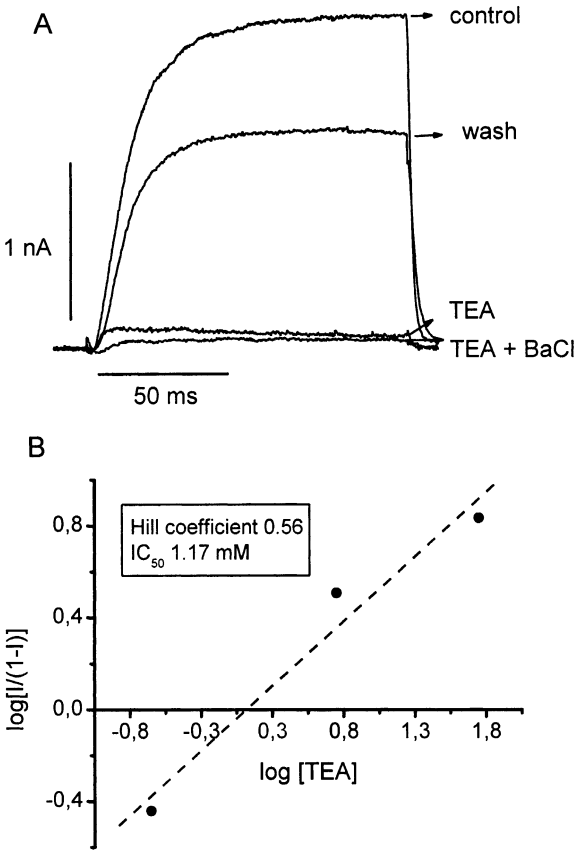

Fig. 5. Effects of TEA and barium on potassium currents. (*A*) Ionic currents from a cell during a 125 ms voltage pulse from −70 mV to +40 mV, applied from a holding potential of −70 mV, in control solution, with 50 mM TEA, 50 mM TEA + 25 mM Ba^{2+}, and after washing TEA and Ba^{2+} from the bath. The control internal solution was as described in Material and Methods. (*B*) Dose-response relationship for blocking potassium currents. *I* was determined from peak currents with the blocker relative to control currents during depolarization to +70 mV. Each symbol represents the average value from measurements in 4–7 cells. The data were fitted to the Hill equation. IC_{50} value and Hill coefficient were 0.56 mM and 1.17, respectively.

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