Biochimica et Biophysica Acta, 1153 (1993) 249–256

BBAMEM 76152

A voltage-dependent and a voltage-independent potassium channel in brown adipocytes of the rat

Ulrich Ruß, Thomas Ringer and Detlef Siemen *

Institut für Zoologie, Universität Regensburg, D-93040 Regensburg (Germany)

(Received 5 April 1993)
(Revised manuscript received 12 July 1993)

Key words: Brown adipose tissue; Potassium ion channel; Potassium ion conductance; Delayed rectifier; Ion channel selectivity; Tetraethylammonium; (Rat)

Single-channel recordings of a voltage-dependent potassium channel in brown adipocytes of the rat confirm recordings of macroscopic currents. Single-channel conductance (γ) is 8 pS at 20°C in KF solution inside vs. a modified Ringer's solution outside. With KCl solution outside, γ is 17 pS for outward currents and 21 pS for inward currents. The majority of the channels inactivate with a time constant around 200 ms; deactivation occurs within milliseconds. The channel is blocked by tetraethylammonium (TEA) with an inhibiting constant of 1.8 mM. The type of block is fast. Selectivity sequence for monovalent cations is $K^+ > Rb^+ \gg NH_4^+ \gg Li^+ \geq Na^+ \approx Cs^+$. Cs^+ at the outside causes a voltage-dependent block of inward currents. This channel is remarkably similar to the delayed rectifier of the F-type in the node of Ranvier. Occasionally, an additional K^+ channel was found. This channel is voltage-insensitive, not blocked by 10 mM TEA, and has not been recorded in brown adipocytes before. Physiological relevance of this channel could be the steady-state membrane potential.

Introduction

In small mammals brown adipose tissue is the main site of thermoregulatory heat production by nonshivering thermogenesis [1] due to its ability to uncouple the mitochondrial respiratory chain from ATP-generation. Nonshivering thermogenesis is activated by β-adrenergic stimulation. Apart from the pathway leading to uncoupling of the mitochondria, norepinephrine induces multiple conductance changes in the plasma membrane [2,3]. Na^+-influx and membrane depolarization can probably be explained by a nonselective channel for monovalent cations [4–7]. Flux measurements and patch clamp experiments in the whole-cell configuration have shown that the potassium efflux is due to a calcium-activated potassium channel [8,9] and/or to a voltage-dependent potassium channel [10]. It is not known if the observed increase in Ca^{2+} is caused by opening of Ca^{2+} channels in the plasma membrane [11]. So far no Ca^{2+} channels have been identified at the single-channel level.

In this paper we report details of the potassium fluxes based on the recordings of single-channel currents. These currents flow through (i) an inactivating voltage-dependent potassium channel which can be blocked by TEA, and (ii) a voltage-independent channel that is insensitive to TEA. There are widely diverse types of voltage-dependent potassium channels of the delayed-rectifier type. Jonas et al. [12] have shown that, in the amphibian node of Ranvier, a classical preparation for studying this type of channel, there are at least three types termed I, F, and S for 'intermediate', 'fast', and 'slow' with respect to their deactivation kinetics after a depolarizing test pulse. In the rat node, I- and F-channels show inactivation with time constants of about 7.4 s and 143 ms, respectively, at +40 mV [13]. Like most potassium channels, they may be blocked by external tetraethylammonium ions, although with different sensitivities.

A previous study has shown clearly that a voltage-dependent potassium current is the main carrier of charge during hyperpolarization of the brown adipocyte plasma membrane [10]. Almost all the data in that study were measured in the whole-cell configuration. Only a few cell-attached recordings demonstrated the channel which is responsible for the current. Here we examine the characteristics of this channel in excised patch recordings in order to establish detailed information under well defined conditions to compare it with delayed rectifiers from other preparations.

* Corresponding author. Fax: + 49 941 9433304.

We have also recorded a voltage-insensitive potassium channel in brown adipocytes but find it far less frequently than the delayed rectifier. This channel has not been observed previously. Preliminary results have appeared elsewhere [14,15].

Materials and Methods

Preparation and experimental conditions. Brown adipocytes were dissected from interscapular and cervical tissue of 6–8-week-old male or female Sprague-Dawley rats as described previously [5]. After collagenase treatment (type II, Sigma, St. Louis, MO) for 25 min, cells were centrifuged at $1000 \times g$, leaving most of the precursor cells with little or no fat content at the bottom and the mature, filled cells in a thin layer at the top of the vial just below the uppermost layer of fat from destroyed cells. Neither sex nor developmental stage of the rat made any difference to the occurrence of the channels described below. Mature cells were grown on Heraeus 'Biofolie' and kept up to 10 days under tissue culture conditions in a CO_2 incubator [5]. Cells growing at the bottom of 35-mm Petri dishes out of precursers were kept up to 4 weeks and used for some control experiments. As no significant differences of the single-channel parameter were obvious, only mature cells were used for the experiments described in this paper. For channel recordings we used the patch-clamp technique as described by Hamill et al. [16]. During whole-cell experiments 80–90% of the series resistance was compensated for. Pipette resistance was about 4 MΩ for experiments in the whole-cell configuration and 13 MΩ for the other experiments.

Analysis. Data were digitized by a Labmaster TM-40 AD/DA board (Scientific Solutions, Solon, OH). PCLAMP-software (version 5.5, Axon Instruments, Foster City, CA) on an IBM-compatible 486 AT computer was used for the analysis. Leakage current and capacitive current were subtracted by the p/4-method in whole-cell experiments. In single-channel analysis, currents were corrected by subtraction of silent traces.

Kinetics of whole-cell currents and averaged single-channel currents were determined by least square fit using the function:

$$I = I_0 - I_a\, e^{-t/\tau_a} + I_{i,1}\, e^{-t/\tau_{i,1}} + I_{i,2}\, e^{-t/\tau_{i,2}} \quad (1)$$

where I_0, I_a, $I_{i,1}$, and $I_{i,2}$ are the current components of the total K^+-current (I) at $t = 0$, τ_a is the activation time constant and $\tau_{i,1}$, and $\tau_{i,2}$ are the inactivation time constants.

Single-channel current vs. voltage curves were adapted to data by optical fit using a modified Goldman-Hodgkin-Katz (GHK) current equation in order to determine the relative permeabilities. To account for inward rectification and for presence of the test ion, two more current terms were added, assuming independence of the different current components:

$$i = P_{K_o} \frac{F^2E}{RT} \frac{[K]_i - [K]_o\, e^{-FE/RT}}{1 - e^{-FE/RT}} + P_{K_i} \frac{F^2E}{RT} \frac{[K]_o - [K]_i\, e^{-FE/RT}}{1 - e^{-FE/RT}} + P_X z_X^2 \frac{F^2E}{RT} \frac{[X]_o - [X]_i\, e^{-z_X FE/RT}}{1 - e^{-z_X FE/RT}} \quad (2)$$

where i is the single-channel current, E the applied membrane potential, z_X the valence of the test ion, $[K]_i$, $[K]_o$, $[X]_i$, and $[X]_o$ the internal and the external concentrations of potassium ions and of the test ion, respectively (cf. Ref. 17). F, R, and T are the usual thermodynamic terms. P_X/P_{K_i} gives the relative permeability for the test ion.

Blocking characteristics were determined by least square fitting of the data with the modified Hill equation:

$$B = \frac{C^n}{C^n + IC_{50}^n} \quad (3)$$

where B is the fractional block, C is the blocker concentration, IC_{50} is the half-maximal inhibition, and n is the Hill coefficient.

Solutions. Test solutions were applied by a peristaltic pump driven 'sewer pipe' system as described by Yellen [18]. Solutions were composed as follows (concentrations in mM). Bath solution (modified Ringer's): 134 NaCl, 6 KCl, 10 Na-Hepes, 1.2 $CaCl_2$, 1.2 $MgCl_2$, 11 Glucose (pH 7.4). Pipette solution: 139 KF, 4 KCl, 10 K-Hepes, 5 EGTA (pH 7.4). Potassium fluoride was used to avoid unphysiologically high chloride concentrations at the cytoplasmic surface of the cell membrane. As all recordings were made without free Ca^{2+} in the pipette, i.e., at the internal side of the membrane, the nonselective cation channel was inactive [5,7]. NaCl solution: 153 NaCl, 10 Na-Hepes, 1.2 $CaCl_2$, 1.2 $MgCl_2$ (pH 7.4). KCl solution: 153 KCl, 10 K-Hepes, 1.2 $CaCl_2$, 1.2 $MgCl_2$ (pH 7.4). Temperature was kept constant at 20 ± 0.4°C.

Results

Whole-cell measurements

Fig. 1A shows current-families from whole-cell measurements in mature brown adipocytes with KF solution in the pipette and NaCl solution in the bath. Together with recordings in symmetrical potassium concentrations in both pipette and flow system (Fig. 1B), they demonstrate the presence of 'macroscopic' potassium currents similar to those described by Lucero

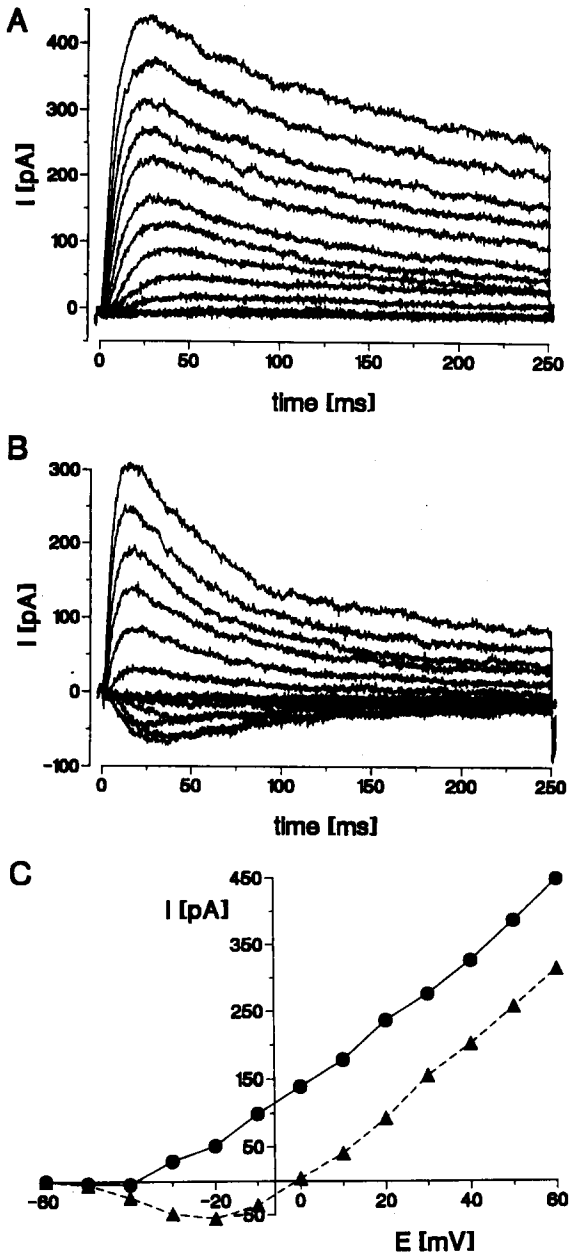

Fig. 1. Whole-cell currents of mature brown adipocytes in NaCl and in KCl solution. Pulse program: holding potential of −60 mV, prepulse of −100 mV for 1 s, test pulse variable from −60 mV to +60 mV, 250 ms duration. (A) Pipette solution at the membrane inside vs. NaCl solution at the outside. (B) Pipette solution at the membrane inside vs. KCl solution. (C) Peak-current–voltage relations for currents shown in (A, •) as well as in (B, ▲). Same cell in (A) and (B), 20°C.

and Pappone [10]. Related peak current voltage curves are shown in Fig. 1C. Inward currents in symmetrical K^+ solutions pass through a minimum at −20 mV. The activation time constant decreased slightly from about 15 ms to 8 ms with increasing depolarizations to different potentials from 0 mV to +40 mV, as expected for channels of the delayed-rectifier type [19]. Inactivation kinetics of the whole-cell currents were best described by a single exponential ($I_{i,2} = 0$ in Eqn. 1). The time constant for test potentials between 0 mV and +60 mV, scattered in the range from 35 ms to 350 ms (approx. 100 ms in Fig. 1A and 55 ms in Fig. 1B) almost independently of the holding potential. Furthermore, we found a component of the whole-cell current which did not inactivate within 500 ms. Its contribution differed from patch to patch.

Outside-out patches

Density. Single channels were recorded from outside-out patches under the same conditions used for whole-cell measurements (Fig. 2). Generally, every second reliable patch showed events of the voltage-dependent, inactivating channel described here, that could be blocked by TEA. The maximum number of current levels per patch was 5, the mean was 1.5. Assuming a maximum patch area of 5 μm^2 it follows a minimum density of 0.3 channels per μm^2. With a mean cell diameter of a brown adipocyte of 30 μm, a minimum number of 850 channels per cell can be calculated. This value is in agreement with the values calculated by dividing the macroscopic K^+-conductance by the single-channel conductance and with the value found by Lucero and Pappone [10].

Kinetics. Current traces from 192 depolarizing test pulses applied to outside-out patches were averaged for comparison of kinetic data with those obtained from the whole-cell recordings (Fig. 3). Using Eqn. 1 two exponentials for inactivation yielded a better fit for the time course of the single-channel data, but as the slow time constant was only 4–10-times slower than the

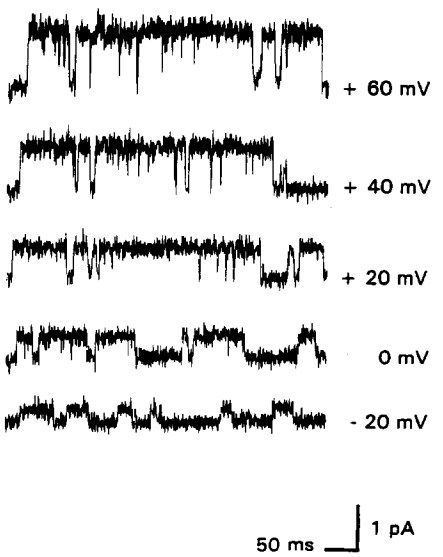

Fig. 2. Single-channel recordings of delayed-rectifier potassium channels. Test pulse potential indicated at the right. Holding potential: −40 mV, prepulse of −120 mV for 2 s. Outside-out patch, pipette solution with 153 K^+ at the membrane inside, bath solution with 144 Na^+ and 6 K^+ at the outside of the patch. Lower current level is always the closed-level.

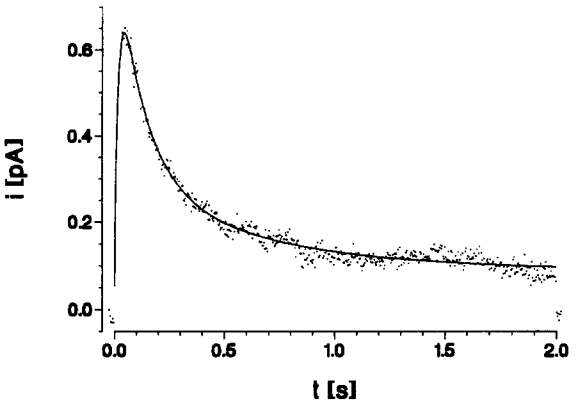

Fig. 3. Kinetics of a voltage-dependent potassium current reconstructed from single-channel data of outside-out patches by averaging 192 records. Prepulse potential −120 mV; test pulse potential 20 mV. Pipette solution at membrane inside, bath solution at the outside of the patch. Continuous curve fitted by the least-squares method using Eqn. 1. Best fit was obtained with $\tau_a = 15.5$ ms, $\tau_{i,1} = 130$ ms, and $\tau_{i,2} = 608$ ms. The corresponding I values were $I_0 = 0.09$ pA, $I_a = 0.8$ pA, $I_{i,1} = 0.55$ pA, and $I_{i,2} = 0.22$ pA.

fast, the functional background of a second time constant seems to be uncertain. Single-channel activity was reduced by decreasing the prepulse duration of 2 s to less than 1 s. Prepulse durations of less than 250 ms led to current traces without any current events. Deactivation after a brief depolarizing pulse is completed within milliseconds.

Conductance. A single-channel conductance $\gamma = 8$ pS ($n = 9$ patches) was observed in outside-out patches with a physiological distribution of Na^+- and K^+-ions, i.e., pipette solution vs. modified Ringer's. Extrapolation of the related i–E curve to negative potentials by means of the GHK current equation (see Materials and Methods) yielded a reversal potential of −63 mV (data not shown). Switching to solutions with symmetrical K^+-concentrations increased γ to 20.5 pS for inward currents and to 17 pS for outward currents ($n = 29$ patches), thus showing a weak inward rectification (see also ▼ in Fig. 4). In two inside-out patches, channel activity with about the same mean γ (7.5 pS) appeared under physiological conditions.

Inactivation curve. When the amplitude of a 2-s prepulse is stepped from −170 mV to −50 mV, the K-single-channel events during a 500 ms test pulse at +40 mV vanish completely (pipette solution vs. modified Ringer's). To obtain the inactivation curve, the currents during 32 test pulses at each prepulse potential were summed, integrated and normalized to the maximum value. In two experiments, the half-maximum of this curve was found at a prepulse potential of −94 mV. This is a surprisingly low value compared with other voltage-dependent ion channels.

Selectivity. Experiments using outside-out patches always started with pipette solution at the inner side of

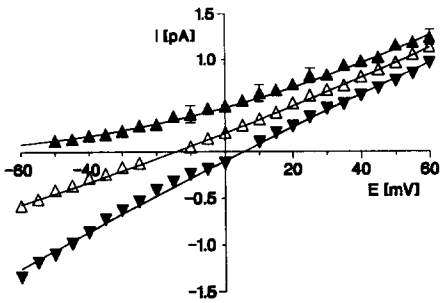

Fig 4. Selectivity for Na^+ and K^+ ions. i–E relations for the voltage-dependent K^+ channel before (▼) and after exchanging 50% (△) and 100% (▲), respectively, of the K^+-ions at the outside by Na^+. Inward current reduced in 50% Na^+ and missing in 100% Na^+. Membrane inside: pipette solution; membrane outside: NaCl solution, KCl solution, or mixtures of equal parts of these solutions. Continuous curves calculated by means of Eqn. 2. Standard deviation is only given if it exceeds the size of the symbol.

the membrane and KCl solution in the flow system. We then exchanged 50% and 100% of the potassium at the outside for a test ion. Each trial, thus consisting of three runs, was performed repeatedly. Test ions were four different alkali metal ions, two earth alkali metal ions or NH_4^+ ions. A concentration of 102 mM was used for divalents instead of 153 mM for the monovalents in order to maintain osmolarity as well as isoelectricity. Data from three experiments with Na^+ as test ion are shown in Fig. 4 as an example. Inward conductances and, to smaller extent, also outward conductances were reduced as compared with the control in plain K^+. The reversal potential was shifted to the left.

The ion permeability was determined by fitting the data using an extended form of the GHK current equation (Eqn. 2, see Materials and Methods). The resulting permeabilities relative to that for potassium are summarized in Table I. A voltage-dependent block

TABLE I

Results of ion exchange experiments for determination of relative permeabilities (P_X / P_K)

Several control experiments were performed for each ion tested. Mean values of control experiments are given in the line for K^+. Right column gives number of trials, each consisting of three subsequent i–E curves as described in Results. Number of outside-out patches in brackets.

Ion	P_X / P_K (inward)	No. of trials (No. of patches)
K^+	1	80 (29)
Rb^+	0.79	10 (1)
NH_4^+	0.23	9 (6)
Li^+	0.037	10 (5)
Na^+	0.030	10 (3)
Cs^+	0.030	10 (2)
Ba^{2+}	0.015	18 (6)
Ca^{2+}	0	13 (6)

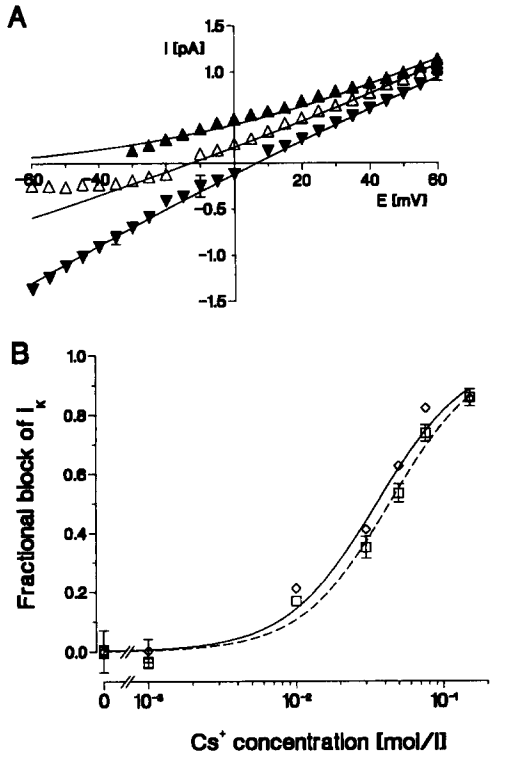

Fig. 5. (A) $i-E$ relations for the voltage-dependent K^+ channels before (▼) and after exchanging 50% (△) and 100% (▲) of the K^+ ions at the outside by Cs^+. Solutions as in Fig. 4 but Cs^+ instead of Na^+. Analysis as in Fig. 4. Note voltage-dependent block of the inward current with 50% Cs^+ at the membrane outside. Relative permeability given in Table I. (B) Dose–response relation of voltage-sensitive Cs^+-block. Reduction of single-channel current at different Cs^+ concentrations normalized by control current in plain K^+ solution (fractional block) is shown. □: normalized block at −40 mV. ◇: normalized block at −60 mV. Bars show standard deviation and are only given if they exceed the size of the symbol. Data were fitted by Eqn. 3 with a Hill coefficient of 1.4. IC_{50} values were 44.9 mM and 35.8 mM at −40 mV (— — —) and −60 mV (———), respectively.

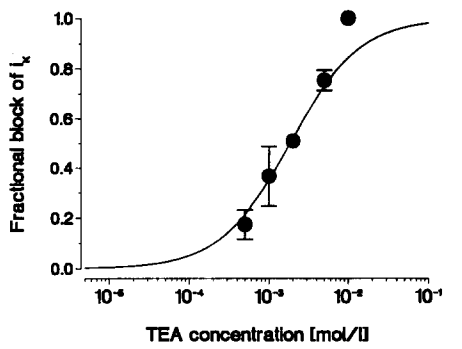

Fig. 6. Dose–response relation for the block of the voltage-dependent K^+ channel by external TEA. Pulse program: holding potential −40 mV, prepulse −120 mV for 2 s, test pulse +20 mV for 500 ms. Membrane inside: pipette solution, membrane outside: substitution of part of the NaCl from bath solution against the TEA concentration tested. Data were collected from four experiments in which two TEA concentrations were tested and from five experiments with one TEA concentration. Reduction of single-channel conductance was normalized to control. The point at 10 mM TEA was not included in the determination of the IC_{50} as it was impossible to determine whether single-channel events of small amplitude were hidden in the noise. Continuous curve was calculated with Eqn. 3 and a Hill coefficient of 1, yielding an IC_{50} of 1.84 mM.

was observed using Cs^+ (Fig. 5A) and Ca^{2+} as test ions; this bent the currents towards the x-axis at more negative potentials. The voltage dependence and the concentration dependence of the Cs^+-block are shown in Fig. 5B. When fitting data by Eqn. 3 with a variable Hill coefficient, a value of 1.4 proved to be optimal.

The i–E-curves with Ba^{2+} or Ca^{2+} as test ions were shifted by about −10 mV. The shifts are comparable to those due to the Gouy-Chapman-Stern effect (cf. Ref. 19) observed by Vogel [20] for sodium and potassium channels in the node of Ranvier.

Block. It was not possible to block the voltage-dependent K^+ channel with 1.0 μM tetrodotoxin (TTX), 0.3 mM verapamil or by 0.5 μM apamin as would have been expected for voltage-dependent Na^+ channels, Ca^{2+} channels of the L-type or Ca^{2+}-sensitive SK-channels, respectively. 1.0 mM Ba^{2+} and 0.01 mM dendrotoxin (DTX), two well known blockers of voltage-dependent K^+ channels, did not effect the chan-

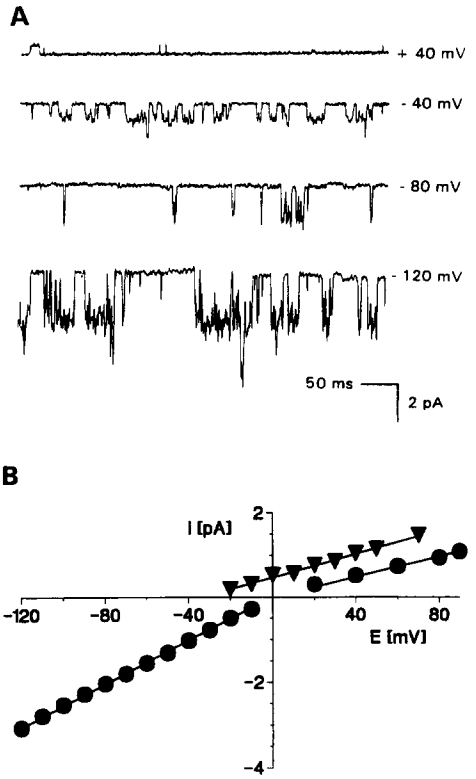

Fig. 7. Single-channel current traces of the voltage-insensitive K^+ channel at the indicated membrane potentials (A) and related current–voltage relationship (B). Membrane inside: pipette solution; membrane outside: 148 KCl, 10 K-Hepes, 1.2 $CaCl_2$, 1.2 $MgCl_2$, pH 7.4 (A and ● in B) or modified Ringer's (▼ in B). ▼: mean of two experiments; ●: mean of three experiments; standard deviation bars smaller than symbols.

nel. 50% Cs^+ and 100% Ca^{2+} at the membrane outside caused voltage-dependent block of the inward current (Fig. 5), but did not alter the outward current. Tetraethylammonium (TEA) is a common blocker of many K^+ channels. In brown adipocytes, when given from the membrane outside, it blocks the voltage-dependent K^+ channel by reducing its single-channel current amplitude. Fig. 6 shows a dose–response curve with an IC_{50} of 1.84 mM.

Voltage-independent potassium channel

In 13 patches we found an almost voltage-insensitive K^+ channel that has not been reported before (Fig. 7A). In 7 of these patches, this channel appeared together with the voltage-sensitive channel. The voltage-insensitive channel showed inward rectification in symmetrical K^+-concentrations with a single-channel outward conductance of 12 pS and an inward conductance of 25 pS (Fig. 7B). No inward currents could be measured with modified Ringer's at the outside. The outward conductance increased only insignificantly to 14 pS. The channel showed fast open and closed kinetics, sometimes giving the single-channel events a flickering appearance (Fig. 7A, lower trace). Occasionally, periods of lower activity occurred for some seconds as shown in Fig. 7A (first and third trace). Neither 0.5 μM apamin nor 10 mM TEA could block the channel.

Discussion

Voltage-dependent potassium channel

Classification of voltage-dependent K^+ channels of the delayed-rectifier type depends mostly on single-channel conductance, voltage dependence of activation, time course of inactivation and deactivation and, finally, on the ability of different agents to block the channel. At the node of Ranvier, three distinct classes, called I, F, and S, can be distinguished using these criteria [12,13]. The voltage-dependent K^+ channel, described here, is similar to the F-channel (time constants, TEA blocking constant, low DTX-sensitivity) but also shows a difference (smaller single-channel conductance resembling the I-type).

Inactivation. Schwarz and Vogel [21] first described inactivation of voltage-dependent K^+-currents in frog nodes during long depolarizing pulses. They found two time constants, a fast one of 600 ms and a slow one of several seconds (voltage-dependent). K^+ channel inactivation always remained incomplete and the amplitude of the noninactivating component varied considerably. In our whole-cell experiments (pulse duration 250 ms or 500 ms), one time-constant yields optimal fits. We detected a second time-constant for inactivation in averaged single-channel data when using longer pulse duration (1 s or 2 s). Lucero and Pappone [10] found a fast component of the order of hundreds of milliseconds and a slow component of the order of 2 s in the potential range of 0 to + 40 mV. In contrast to their results, our fast component of about 200 ms is always the dominating one. This difference becomes most prominent when comparing their Fig. 2 with our Fig. 1A. Our fast inactivation time constant, as well as the presence of a noninactivating component which may hide further slow inactivation, are in good agreement with the data of Safronov et al. [13] for the K_F channel.

50%-inactivation appeared at −46 mV [10] or at −62 mV (slow component found by Schwarz and Vogel [21]). In our single-channel experiments, however, we reconstructed the total K^+-current from single-channel data and found a 50% inactivation at −94 mV, i.e., a 10–20% availability at rest. The reason for this difference in voltage dependence is unknown but it should be noted that the preparations and, in the case of Schwarz and Vogel [21], also temperature differed. It is possible that excising a patch changes protein conformation in a way which influences voltage dependence of the gating mechanism [22]. Only A-channels are known to be almost completely inactivated at resting potential [23,24].

Selectivity. Table I gives the selectivity sequence for the delayed rectifier of brown adipocytes. It is similar to the sequence found by Lucero and Pappone [10] for macroscopic currents: $K^+(1.00) > Rb^+(0.81) > NH_4^+(0.18) > Na^+(0.03) \approx Cs^+(0.02)$. In their sequence, the permeabilities for Na^+ and Cs^+ are reversed as compared with ours, but the last digit of these small numbers is difficult to determine. For delayed rectifiers in the node of Ranvier, Hille [25] determined: $K^+(1.00) > Rb^+(0.91) > NH_4^+(0.13) > Cs^+(< 0.077) > Li^+(< 0.018) \approx Na^+(< 0.010)$, again from macroscopic currents but close to our single-channel data. *i–E* curves with Cs^+, Ba^{2+}, Ca^{2+} at the outside differed from the GHK model, partly due to voltage-dependent block (Cs^+) or possibly due to the Gouy-Chapman-Stern effect (divalents).

Block. TEA, a blocker of many K^+ channels, may block at either side of the membrane in different concentrations [27]. While internal TEA receptors seem to show little variation, there is larger variation at the membrane outside [19]. In ATP-sensitive K^+ channels of frog skeletal muscle, TEA block shows an IC_{50} of 6–7 mM, clearly higher than at delayed rectifiers [28]. In the delayed rectifier of brown adipocytes TEA completely abolished the voltage-dependent current with a constant of 50% inhibition of 1.84 mM, if given from the outside. This value is closest to an IC_{50} of 1.7 found in a voltage-dependent K^+ channel (RCK2) derived from rat brain and expressed in *Xenopus* oocytes [29]. Whole-cell measurements at the delayed rectifier of brown adipocytes showed an IC_{50} between 1–2 mM both for TEA and for 4-aminopyridine [10].

Compared with S-, F-, or I-channels from peripheral nerves of the *Xenopus*-node of Ranvier [12], the brown adipocyte result corresponds best to that of F-channels (1 mM). In the peripheral node of rats the IC_{50} of S-channels (1.4 mM) and of F-channels (1.2 mM) show very little difference [13]. A-channels are generally less sensitive to TEA [30]. Hill coefficients of 1.0 are generally found in different K^+ channels of the node of Ranvier [13,31,32]. TEA-block in brown adipocytes belongs to the fast type, i.e., the amplitude of the single-channel events decreased with increasing TEA concentration. Kinetics of activation, inactivation, deactivation as well as single-channel kinetics were not influenced by TEA (data not shown).

Fitting our data at -40 and at -60 mV for the voltage-dependent Cs^+-block by the Hill equation (Eqn. 3) yielded Hill coefficients higher than 1, as it has been shown for K^+ channel in the squid axon by Adelman and French [26]. These authors found a 1:1 stoichiometry at a potential of 26 mV increasing to a 2:1 stoichiometry at -86 mV. 1 μM DTX, a toxin of the eastern green mamba (*Dendroaspis angusticeps*), blocks voltage-dependent K^+ channels of the I-type but not the F-type [12]. It did not show any effect on brown adipocytes.

Voltage-insensitive potassium channel

A potassium channel, insensitive to voltage, to Ca^{2+} and to 10 mM TEA was found for the first time in adipocytes. Such channels are suspected of being responsible for the resting membrane potential in peripheral nerves [32] or in sympathetic ganglia [33]. They obviously need not all be the same type of potassium channel. For example, an ATP-sensitive K^+ channel determines the resting potential in pancreatic β-cells [34]. It has been asked for some time why the resting potential as a potassium equilibrium potential has a very low sensitivity to TEA although nearly all potassium channels are blocked by this substance. The channel described here may explain this phenomenon for brown adipocytes. Unfortunately, the channel was observed only infrequently so that we were not able to test it systematically for blocking substances like Ba^{2+} [33]. Blocking it specifically could easily show whether the channel is functionally related to the resting potential or not.

So far, three ion channels have been shown on the single-channel level in brown adipocytes: (i) a nonselective channel for monovalent cations, activated by cytosolic Ca^{2+} but indirectly probably also by β-agonists [4,7], (ii) a potassium channel of the delayed-rectifier type, and (iii) a voltage-independent potassium channel. In perforated-patch whole-cell recordings, a Ca^{2+}-activated K^+ channel (apamin- and TEA-sensitive) has been described [9] and Ca^{2+}-influx has been reported on the basis of fura-2 measurements [11]. As a result of their stimulation by noradrenaline, brown adipocytes undergo a biphasic depolarization interrupted by a brief hyperpolarization [2]. The fast transient inward current and a slower K^+-carried outward current are α-mediated, while the sustained inward current follows β-receptor activation [3,9]. The physiological significance of the membrane fluxes induced by noradrenaline stimulation remains to be resolved. Potassium efflux together with Na^+-influx through the nonselective channel drive the Na^+/K^+-ATPase whose activity is thought to be responsible for about 5–15% of the heat production of an activated cell [6]. In contrast, Pappone and Lucero [35] found that blocking the TEA-sensitive channels did not reduce the maximum metabolic response to adrenergic stimulation. However, a response as small as 5% of total heat production is about of the size of their error bars, so it may have escaped detection. Nevertheless, brown adipocytes possess a much more powerful heat source by their uncoupling mechanism in the inner mitochondrial membrane and it is likely that there is a further and as yet unknown reason for developing such a complex electrical response to noradrenaline stimulation.

Acknowledgements

We are indebted to Dr. Jenny Kien for reading the manuscript and to Magda Dietl and Ute Schmitt for expert technical assistance. Financial support by the Deutsche Forschungsgemeinschaft is gratefully acknowledged (Si 310/3-2).

References

1 Nicholls, D.G. and Locke, R.M. (1984) Physiol. Rev. 64, 1–64.
2 Horwitz, B.A. and Hamilton, J. (1984) Comp. Biochem. Physiol. 78C, 99–104.
3 Schneider-Picard, G., Coles, J.A. and Girardier, L. (1985) J. Gen. Physiol. 86, 169–188.
4 Siemen, D. and Reuhl, T. (1987) Pflügers Arch. 408, 534–536.
5 Weber, A. and Siemen, D. (1989) Pflügers Arch. 414, 564–570.
6 Siemen, D. and Weber, A. (1989) in Thermal Physiology 1989 (Mercer, J.B., ed.), pp. 241–246, Excerpta Medica, Amsterdam.
7 Koivisto, A., Dotzler, E., Ruß, U., Nedergaard, J. and Siemen, D. (1993) in Nonselective Cation Cannels – Pharmacology, Physiology and Biophysics (Siemen, D. and Hescheler, J., eds.), Birkhäuser, Basel, pp. 201–211.
8 Nånberg, E., Connolly, E. and Nedergaard, J. (1985) Biochim. Biophys. Acta 844, 42–49.
9 Lucero, M.T. and Pappone, P.A. (1990) J. Gen. Physiol. 95, 523–544.
10 Lucero, M.T. and Pappone, P.A. (1989) J. Gen. Physiol. 93, 451–472.
11 Wilcke, M. and Nedergaard, J. (1989) Biochem. Biophys. Res. Commun. 163, 292–300.
12 Jonas, P., Bräu, M.E., Hermsteiner, M. and Vogel, W. (1989) Proc. Natl. Acad. Sci. USA 86, 7238–7242.
13 Safronov, B.V., Kampe, K., and Vogel, W. (1993) J. Physiol. 460, 675–691.
14 Siemen, D. and Agari, B. (1991) Pflügers Arch. 418, R80.

15 Ruß, U., Ringer, T. and Siemen, D. (1992) in Rhythmogenesis in Neurons and Networks (Elsner, N. and Richter, D.W., eds.), p. 559, Georg Thieme, Stuttgart.
16 Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pflügers Arch. 391, 85–100.
17 Hodgkin, A.L. and Katz, B. (1949) J. Physiol. 108, 37–77.
18 Yellen, G. (1982) Nature 296, 357–359.
19 Hille, B. (1992) Ionic Channels of Excitable Membranes, 2nd Edn., Sinauer, Sunderland, MA.
20 Vogel, W. (1974) Pflügers Arch. 350, 25–39.
21 Schwarz, J.R. and Vogel, W. (1971) Pflügers Arch. 330, 61–73.
22 Kimitsuki, T., Mitsuiye, T. and Noma, A. (1990) Am. J. Physiol. 258, H247–H254.
23 Kolb, H.-A. (1990) Rev. Physiol. Biochem. Pharmacol. 115, 51–91.
24 Ficker, E. and Heinemann, U. (1992) J. Physiol. 445, 431–455.
25 Hille, B. (1973) J. Gen. Physiol. 61, 669–686.
26 Adelman, W.J. Jr. and French R.J. (1978) J. Physiol. 276, 13–25.
27 Im, W.B. and Quandt, F.N. (1992) J. Membr. Biol. 130, 115–124.
28 Davies, N.W., Spruce, A.E., Standen, N.B. and Stanfield, P.R. (1989) J. Physiol. 413, 31–48.
29 Kirsch, G.E. Taglialatela, M. and Brown, A.M. (1991) Am. J. Physiol. 261, C583–C590.
30 Adams, D.J. and Nonner, W. (1989) in Potassium Channels: Structure, Classification, Function and Therapeutic Potential (Cook, N.S., ed.), pp. 42–69, Ellis Horwood, Chichester, UK.
31 Jonas, P., Koh, D.-S., Kampe, K., Hermsteiner, M. and Vogel, W. (1991) Pflügers Arch. 418, 68–73.
32 Koh, D.-S., Jonas, P., Bräu, M.E. and Vogel, W. (1992) J. Membr. Biol. 130, 149–162.
33 Jones, S.W. (1989) Neuron 3, 153–161.
34 Ashcroft, F.M., Ashcroft, S.J.H. and Harrison, D.E. (1988) J. Physiol. 400, 501–527.
35 Pappone, P.A. and Lucero, M.T. (1992) Am. J. Physiol. 262, C678–C681.