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K^+ channels activated by L-alanine transport in isolated *Necturus* enterocytes

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Using the patch-clamp technique, we demonstrate here the opening of K^+ channels evoked by the actively transported amino acid L-alanine in isolated *Necturus* enterocytes. These channels had a conductance of about 30 pS and their activation was dependent on transmembrane electrical potential and cytosolic Ca^{2+}.

K^+ channel; Ca^{2+}; L-Alanine; Patch-clamp; (*Necturus* enterocyte)

1. INTRODUCTION

Sodium-coupled nutrient transport by enterocytes activates homeostatic adjustments which avert gross changes in cell volume and membrane potential [1–3]. A component of these regulatory mechanisms is a basolaterally located K^+ permeability pathway, the basis of which has been proposed to be a Ca^{2+}-activated K^+ channel sensitive to Ba^{2+} [1,4].

The patch-clamp technique has shown Ca^{2+}-activated K^+ channels to be present in a variety of cell types [5]. Both large and small conductance Ca^{2+}-activated K^+ channels have been reported in enterocytes [6–8], but no studies have yet been undertaken to discern which channels might have a physiological significance during Na^+-coupled nutrient transport. Here, we address this issue in *Necturus* enterocytes and directly demonstrate that L-alanine can evoke the opening of a Ca^{2+}-dependent K^+ channel of small unitary conductance.

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2. MATERIALS AND METHODS

Necturi maintained in tap water at 4°C were anaesthetised and pithed. The intestinal vasculature was perfused with normal Ringer and then with 5 ml of 1 mg/ml of both collagenase type I and pronase type XIV (both from Sigma, Poole, Dorset) made up in normal Ringer containing 1 mM DL-dithiothreitol (DTT) at 22°C. The proximal third of the intestine was then removed from the animal and the underlying musculature stripped before incubation at 22°C for 3 h in normal Ringer containing 1 mg/ml of both collagenase type I and pronase type XIV on an orbital mixer.

Enterocytes released by gentle agitation were washed twice in normal Ringer containing 1% bovine serum albumin (BSA) and once in normal Ringer containing 1 mM DTT before being stored at 4°C in antibiotic-containing Dulbecco's modified Eagle's medium, buffered with 10 mM Hepes-Tris (pH 7.2) and diluted to a final osmolality of 260 mosmolal. Antibiotics used were 0.4 mg/ml penicillin, 0.58 mg/ml streptomycin and 0.2 mg/ml kanamycin. Stored cells, used for experiments up to 48 h after isolation, were plated onto plastic Petri dishes previously coated with Cell-Tak (Biopolymers, Farmington, CT) and bathed in a Ringer of the following composition (mM): 105 NaCl, 2.5 KCl, 1.3 $CaCl_2$, 0.5 $MgCl_2$, 10 Hepes and 20 mannitol. Patch pipettes used in these experiments were filled with either (mM): 100 KCl, 1.3 $CaCl_2$, 0.5 $MgCl_2$ and 10 Hepes; 100 NaCl, 1.3 $CaCl_2$, 0.5 $MgCl_2$ and 10 Hepes or 20 KCl, 80 NaCl, 1.3 $CaCl_2$, 0.5 $MgCl_2$ and 10 Hepes. All solutions were titrated to pH 7.2 with Tris.

Single-channel currents were recorded in the cell-attached and excised inside/out patch configurations using an LM-EPC7 patch-clamp amplifier as described elsewhere [9]. The signal was simultaneously displayed on a storage oscilloscope and

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recorded on videotape for subsequent analysis using a patch-clamp analysis programme (Strathclyde Electrophysiology Software, J. Dempster, Department of Physiology and Pharmacology, University of Strathclyde, Glasgow, Scotland).

3. RESULTS AND DISCUSSION

The activation of K^+ channels by L-alanine is illustrated in fig.1A. Ionic currents were recorded from a cell-attached patch using a pipette containing 100 mM NaCl. Pipette voltage was set at a command potential of 30 mV at which no single-channel currents were observed under control conditions. Addition of 20 mM L-alanine to the bath produced bursts of outward currents. Channel activity appeared within 1 min of addition and remained elevated throughout the period the cell was bathed in L-alanine. The *I/V* curve of this channel is shown in fig.1B. Single-channel currents were plotted vs different clamp potentials used between −40 and 80 mV. Only outward currents were recorded within this range of potentials. Given the composition of the pipette solution (100 mM NaCl) and the configuration of the patch (cell-attached), only K^+ can account for this current. The slope of the line gives a conductance of 30 pS at positive potentials. Fig.1B also shows values obtained prior to the addition of L-alanine (circles) showing that the channel was present then but only active at extremely depolarized potentials. Channel mean open probability (P_o) was close to zero before addition of L-alanine, the highest value being 0.04 at 120 mV clamp potential. After L-alanine, however, it increased to about 0.4 and 0.6 at 0 and 80 mV clamp potential, respectively. Five channels examined in this way were activated in a similar manner.

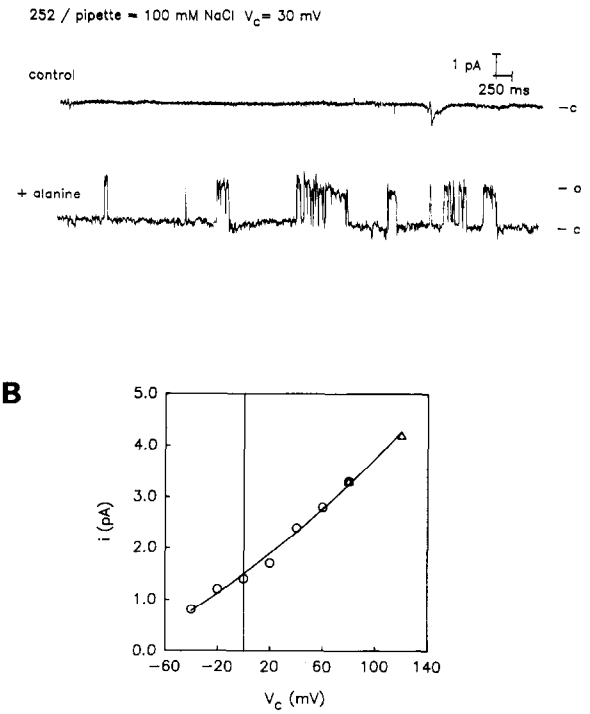

Fig.1.(A) L-Alanine-evoked single-channel activity. Two traces of a continuous recording from a single cell-attached membrane patch from a *Necturus* enterocyte are shown. The upper trace illustrates the situation before L-alanine addition, the lower trace being taken 30 s following 20 mM L-alanine addition to the solution bathing the enterocyte. In each instance, the clamp potential (V_c) was 30 mV. The pipette contained (mM) 100 NaCl, 1.3 $CaCl_2$ and 0.5 $MgCl_2$ but all K^+ had been omitted. Upward deflections of the trace indicate outward current. The current records have been filtered at 0.5 kHz. (B) Single-channel current-voltage relationship for the experiment shown in panel A before (△) and after (○) the addition of 20 mM L-alanine to the bath. Inset: channel opening in the presence of L-alanine at a potential of 80 mV (V_c). The current record was filtered at 0.5 kHz.

Inwardly directed K^+ currents were also recorded in cell-attached patches with KCl-filled electrodes. With a 100 mM KCl pipette solution and at zero clamp potential inward currents could be observed. Hyperpolarisation genetared larger currents and currents reverted at clamp potentials above 60 mV. Unitary channel conductance measured under these conditions at 0 mV clamp potential was 28 ± 2 pS (n = 6). Fig.2A (circles) shows the *I/V* relationship of a single K^+ channel recorded in cell-attached mode in the presence of 20 mM L-alanine with the patch pipette containing a 20 mM KCl solution. Under these conditions current reversal occurred at a V_c value of approx. −20 mV which for an intracellular K^+ activity of 90 mM corresponds to a value for the membrane potential of −25 mV, a figure which is within the range of potentials recorded in the intact tissue [10]. Excision of the patch into normal Ringer (triangles) shows that the channel is highly selective for K^+ over Na^+ and Cl^-, since outward currents were now absent and instead a strong rectification was observed.

The voltage dependence of this channel is analysed in fig.2B where channel open probability

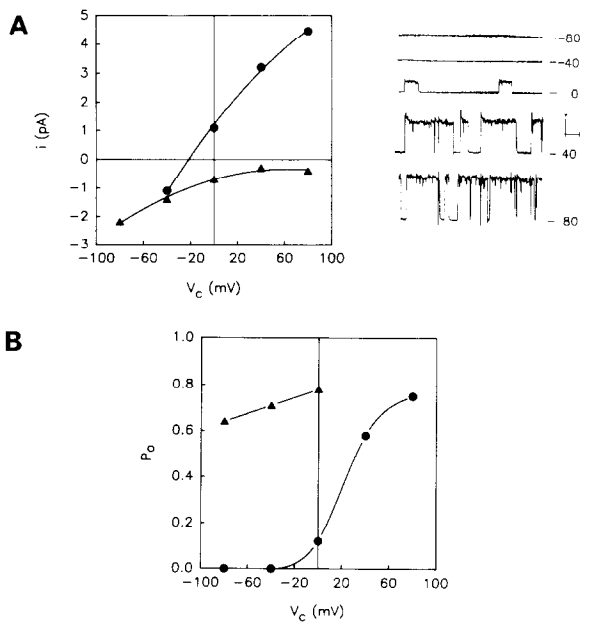

Fig.2. Channel selectivity and Ca^{2+} sensitivity. Upper panel: (A) single-channel current-voltage relationship in a cell-attached patch in the presence of 20 mM L-alanine (●) in the bath solution and from an excised inside/out patch (▲) exposed to asymmetric ion gradients. The pipette contained (mM): 20 KCl, 80 NaCl, 1.3 $CaCl_2$ and 0.5 $MgCl_2$. Inset: channel openings at the indicated clamp potentials (V_c). Vertical and horizontal calibration bars correspond to 2 pA and 500 ms, respectively. Current records were filtered at 0.5 kHz. Lower panel: (B) channel open probability as a function of (V_c) for the cell-attached patch, (●) used above, and following patch excision and exposure of the cytosolic membrane face to 10^{-3} M Ca^{2+} (▲).

(P_o) is plotted vs clamp potential. In the cell-attached mode (circles) P_o is proportional to V_c between −80 and 80 mV, the curve being steep around the cell membrane potential ($V_c = 0$ mV). Channel activity was also dependent on the Ca^{2+} concentration of the solution bathing the cytosolic side of the membrane patch. Excision of the patch into a solution containing 10^{-3} M Ca^{2+} (triangles in fig.2B) resulted in a dramatic increase in channel activity, P_o being elevated at all potentials. Note that since upon excision cell membrane potential is lost, the relationship between P_o and V_c is now displaced to the left. Excised inside/out patches containing K^+ channels became completely silent in EGTA-Ca^{2+}-free solutions (100 mM KCl) suggesting a strict dependence of channel activation on Ca^{2+}. Moreover, excised patches bathed in buffered solutions of intermediate Ca^{2+} concentrations (10^{-7}–10^{-6} M) showed intermediate activation profiles.

An increase in K^+ permeability has already been shown to occur during Na^+-coupled nutrient transport by small intestinal cells [1,2]. On the basis of flux measurements it has been proposed that Ca^{2+}-activated K^+ channels underlie this mechanism [1]. The present study utilises the patch-clamp technique to demonstrate that L-alanine induces the opening of a 30 pS Ca^{2+}-activated K^+ channel in *Necturus* enterocytes. At physiological levels of intracellular Ca^{2+} the channel was infrequently observed, channel open probability being low in the unstimulated cell.

It is premature to suppose that Ca^{2+} is the signal linking L-alanine uptake and K^+ channel activation, since with voltage-dependent channels the depolarisation caused by Na^+ entry with L-alanine would itself act as a stimulus to open K^+ channels. In hepatocytes it has been shown that L-alanine stimulates a 90 pS Ca^{2+}-activated K^+ channel [11]. The relationship between this channel and the one described here is not yet clear, nor is it known whether they both belong to a similar category.

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