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Short Communication

Few cultured rat primary sensory neurons express a tolbutamide-sensitive K^+ current

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Abstract

The response of dorsal root ganglion (DRG) neurons to metabolic inhibition is known to involve calcium-activated K^+ channels; in most neuronal types ATP-sensitive K^+ channels (K_{ATP}) also contribute, but this is not yet established in the DRG. We have investigated the presence of a K_{ATP} current using whole-cell recordings from rat DRG neurons, classifying the neurons functionally by their "current signature" (Petruska *et al*., *J. Neurophysiol.,* **84**:2365-2379, 2000). We clearly identified a K_{ATP} current in only 1 out of 62 neurons, whose current signature indicated that it was probably a nociceptor. The current was activated by cyanide (2 mM NaCN) and was sensitive to 100 μM tolbutamide; the relation between reversal potential and external K^+ concentration indicated it was a K^+ current. In a further two neurons, cyanide activated a K^+ current that was only partially blocked by tolbutamide, which may also be an atypical K_{ATP} current. We conclude that K_{ATP} channels are expressed in normal DRG, but in very few neurons and only in nociceptors.

Keywords: tolbutamide • ATP-sensitive K^+ (K_{ATP}) channel • metabolic inhibition • primary sensory neuron • dorsal root ganglion • nociceptor • current signature

Introduction

The early response of mouse dorsal root ganglion (DRG) neurons to metabolic inhibition has been shown to involve Ca^{2+}-dependent K^+ channels [1]; no evidence was found in that study for the expression of an ATP-sensitive K^+ channel (K_{ATP}) in DRG neurons. However, an ATP-sensitive K^+ channel has been described in peripheral axons [2] and the antinociceptive effect of morphine on axon terminals appears to involve K_{ATP} channels [3]. Since DRG neurons in culture express properties characteristic of their axon terminals [4, 5], absence of K_{ATP} channels in cultured DRG neurons would be surprising. This study was designated to search specifically for the presence of a whole-cell K_{ATP} current in cultured DRG neurons, using metabolic inhibition with cyanide to activate it, and tolbutamide to identify whether any current due to metabolic inhibition was through K_{ATP} channels. In order to identify the cellular subtypes, we used the "current signature"

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method [6]; this method allows a classification of DRG neurons based on the pattern of expression of voltage-gated ionic currents, which is correlated with peptide expression, reactivity to the plant lectin IB4 and sensitivity to capsaicin, ATP and protons.

Materials and methods

Adult male Wistar rats (150-250 g) were killed with 100 % CO_2 inhalation followed by decapitation. DRGs from all spinal levels were dissociated with collagenase (0.6 - 1 mg/ml) and Dispase (3 mg/ml), plated on poly-D-lysine coated Petri dishes and cultured for 2-3 days (37 °C, 5% CO_2 in air) in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium with 50 μg/ml gentamicin and 10 % horse serum (all items from Sigma).

Whole-cell patch recordings were made from small DRG neurons (<32 μm diameter) using an EPC-7 amplifier (HEKA, Lambrecht, Germany). After filtering at 3 kHz (-3 dB, 3 pole Bessel), data were acquired with pClamp 5.5.1 and analyzed with Clampfit 8.1 software (both from Axon Instruments, Union City, CA). All values are given as mean ± SD. Borosilicate glass pipettes (GC150TF, Harvard Apparatus, Edenbridge, Kent, UK) were heat-polished to a resistance of 3-5 MΩ. Neurons with membrane potential more positive than -40 mV or more negative than -70 mV were excluded from further analysis. All experiments took place at 25°C in a Ca-free bath solution, chosen to reduce any contribution from Ca^{2+}-activated K^+ currents, containing (in mM): NaCl 140, KCl 1.6 or 4, $MgCl_2$ 3, HEPES 10, NaOH 4.54 (pH 7.4 at 25°C).

The pipette solution contained (in mM): NaCl 10, KCl 120, $CaCl_2$ 1, $MgCl_2$ 3.45, EGTA 10, KOH 25.25, HEPES 10 (pH 7.2 at 25 °C). NaCN 2 mM (prepared as a 200 mM stock solution in water) was used as a metabolic inhibitor, and tolbutamide 100 μM (prepared as a 100 mM stock solution in DMSO) was used to block the current. Test solutions were gravity-fed with a flow of 40 μl/min; the outlet of the application system (internal diameter 120 μm) was positioned 20-40 μm from the cell.

To assess the functional characteristics of the neurons recorded, "current signatures" were derived from voltage-activated currents, based on the approach of Petruska *et al.* [6]. These authors matched the pattern of expression of voltage-gated currents to the chemosensitivity (capsaicin, ATP and proton-activated currents) of dissociated DRG neurons, to the action potential time course and to the expression of histochemical markers known to be correlated with nociceptive phenotype; the correlation between these properties and the "current signature" was found to be consistent, allowing the current signature to be used to predict reliably the phenotype of a neuron [6]. In this study we have based the classification of neurons as nociceptive or non-nociceptive on current signatures found by these authors to correlate with nociceptive or non-nociceptive properties.

Results

From 62 cells, the following cellular types have been identified [see ref. 6]: type 1 (n=15, 24%), type 2 (n=11, 18%), type 3 (n= 4, 6%), type 4 (n=5, 8%), type 6 (n=8, 13%), type 7 (n= 1, 2%), type 8 (n=3, 5%) and type 9 (n=4, 6%). In all, 32 neurones (52 %) were of types 1, 2, 4 or 7 which correspond most likely to nociceptors. In addition 11 cells (18%) were of a type not previously described, distinguished by a combination of I_h with a transient outward A-type K^+ current on returning to the -60mV holding potential after a hyperpolarizing pulse. Currents were recorded during voltage ramps before and during application of 2 mM NaCN. From a holding potential of -80 mV, a 5 s prepulse to -120 mV was followed by voltage ramps rising from -120 mV to -40 mV in 160 ms, then falling back to -120 mV in 160 ms. The same ramps were also applied without the prepulse to eliminate any contribution from activation of I_h. As we show below, I_h was absent in neurons expressing a K_{ATP} current so in these neurons the prepulse had no effect; for simplicity, only responses recorded without the prepulse are shown. There was no clear difference between the currents in response to rising and falling voltage ramps, and we have shown only currents during falling ramps.

In one type 1 neuron and two type 2 neurons, 20 s application of 2 mM NaCN activated a current which was blocked by 15 s application of 100 μM tolbutamide. These three neurons comprise only 5 % of the total (3/62), or 9 % of types 1, 2, 4 and 7 (3/32). The concentration of tolbutamide was chosen to ensure maximal inhibition of the K_{ATP} current (IC_{50} = 7 μM in pancreatic β-cells; ref. [7]). In one neuron recorded in 1.6 mM K^+ the cyanide-activated current reversed at -100 mV and tolbutamide blocked it by about 78% (at -40 mV; Fig. 1A). The tolbutamide-sensitive current reversed at -105 mV, which is close to the estimated potassium equilibrium potential (E_K) of -110mV. In two neurons in 4 mM K^+ the cyanide-activated current reversed at around -70 mV and tolbutamide blocked the outward current by 76 % -

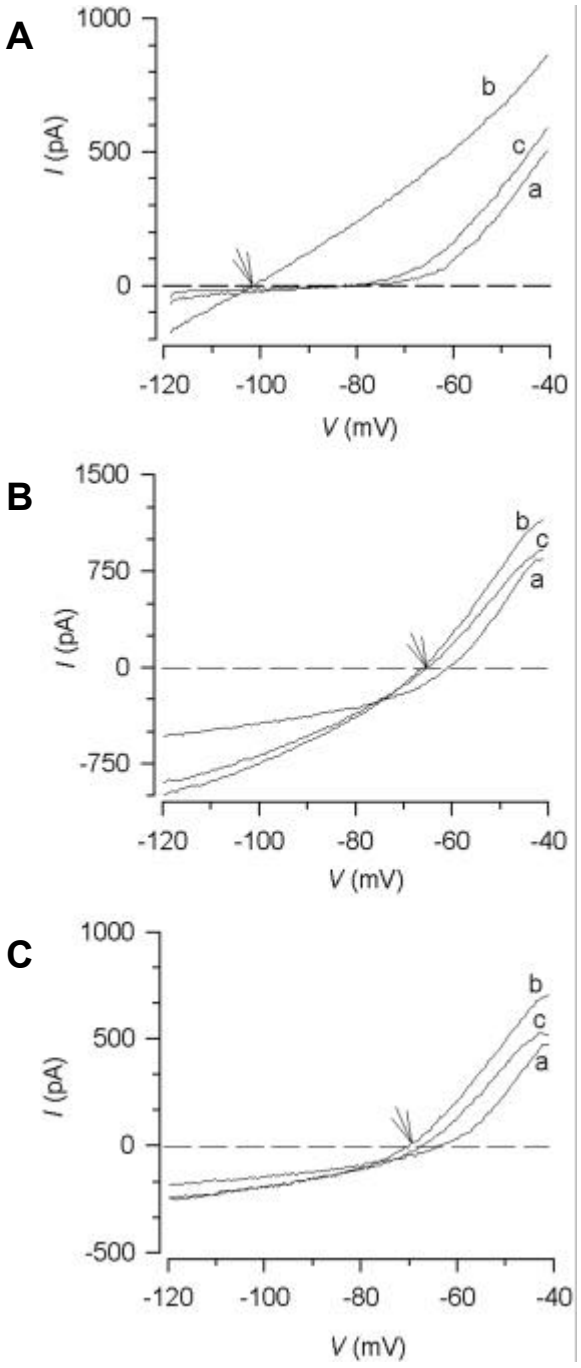

Fig. 1. Current activated by 2mM NaCN and blocked by tolbutamide in DRG neurons of types 1 and 2. (A) In 1.6 mM K^+, the cyanide-activated current reverses at -110 mV (arrow). Application of 100 μM tolbutamide blocks it by 78% at -40 mV. In 4 mM K^+, the cyanide-activated current reverses at -70 mV (arrow) and is blocked by 76 % by tolbutamide (B), or at -85 mV (arrow) and is blocked by tolbutamide by 78 % (C). The blocking effect of tolbutamide leaves unaffected a tolbutamide insensitive current with a mean reversal potential of about -70 mV. a) control; b) 2 mM NaCN; c) 2 mM NaCN + 100 μM tolbutamide.

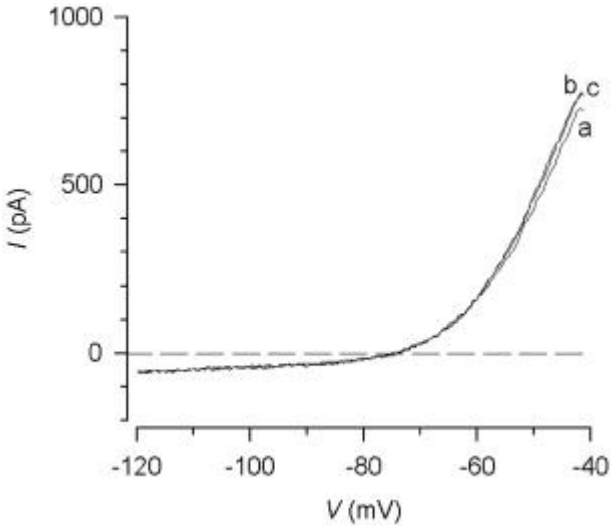

Fig. 2. Lack of effect of cyanide and tolbutamide in a type 1 neuron. a) control; b) 2 mM NaCN; c) 2 mM NaCN + 100 μM tolbutamide.

78 % at -40 mV (Fig. 1B, C); the reversal potential of the tolbutamide-sensitive current in these two neurons was -73 and -85 mV, close to the estimated E_K of -85mV. The difference in reversal potential on changing K^+ concentration indicates that the current activated by metabolic inhibition is principally carried by K^+ ions. Tolbutamide blocked the inward component of the current to a small degree in one neuron and not at all in the other (Fig. 1B, C), in contrast to the neuron shown in Fig. 1A where the block of the inward current was strong. In the remaining 55 neurons, there was no cyanide-activated and tolbutamide-sensitive current (Fig. 2); it was noticeable that the tolbutamide-sensitive current was never found in any neuron type containing I_h.

Discussion

In the 3 neurons where cyanide activated a current blocked by tolbutamide, only the response in 1.6 mM K^+ is consistent with K_{ATP} currents described in other neuronal preparations [8]; its identity is confirmed by the strong blocking effect of tolbutamide on the inward component of the current, as well as by the -105 mV reversal potential, very close to the E_K of -110 mV. In the other two neurons where cyanide activated a current which was blocked by tolbutamide, the identity of the current is

uncertain. Although we observed a 76 % - 78 % block of the outward current by tolbutamide at -40 mV, and the reversal potential was close to E_K, the inward component of the current was very little affected in one neuron (Fig. 1B) and not affected in the other neuron (Fig. 1C). Because of this characteristic, it cannot be concluded that this is a typical K_{ATP} blocked by tolbutamide, although it is clearly a K^+ current.

As a conclusion, we have shown that the proportion of DRG neurons displaying a typical KATP current (a cyanide-activated K^+ current blocked by 100 μM tolbutamide) is very small, and it is restricted to neurons whose current signature indicates they are likely to be nociceptors (see Materials and Methods and [6]). Patch-clamp studies have shown that the sulphonylureas (glibenclamide, tolbutamide) are selective inhibitors of K_{ATP} channels in pancreatic β-cells, cardiac myocytes, skeletal muscle, peripheral axons and central neurons; this sensitivity to sulphonylureas is commonly used to characterize the K_{ATP} [8, 9]. The specific sensitivity to tolbutamide of the current described here, and its K^+ selectivity, indicate that it is likely to be a K_{ATP} current similar to that previously identified in pancreatic β-cells, cardiac myocytes, skeletal muscle cells, central neurons and pituitary cells [9]. The K_{ATP} channel has been previously identified in peripheral axons [2] and suggested to exist in nociceptive terminals [3]; however, in a previous study no evidence was found for its existence in the soma, but this conclusion was based only on the observation of a lack of an effect of ATP in the pipette [1]. Using blocking with tolbutamide, which is a much clearer test of whether K_{ATP} is activated, we show that a very small proportion of cultured rat DRG neurons, probable nociceptors, express a K_{ATP} current in the soma. This observation is consistent with the earlier conclusion that the increased K^+ current in DRG neurons in response to metabolic inhibition is primarily mediated by Ca^{2+} activated K^+ channels and not by K_{ATP} channels [1], while suggesting that K_{ATP} channels also have a role in some nociceptors. The cultured DRG soma is a useful model because it takes on many of the properties of its own former receptor terminal [4, 5]. However, the sparse expression of KATP channels in the cultured DRG soma observed in this study appears to be inconsistent with their expression at nociceptive terminals observed *in vivo* [3]. This paradox could be resolved if the *in vivo* expression is also confined to a few nociceptors.

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