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Research Report

Characterisation of large-conductance calcium-activated potassium channels (BK_{Ca}) in human NT2-N cells

H. Chapman[a,1], *C. Piggot*[b], *P.W. Andrews*[b], *K.T. Wann*[a,*]

[a]*Welsh School of Pharmacy, Cardiff University, King Edward VII Avenue, Cardiff CF10 3XF, UK*
[b]*Department of Biomedical Science, The University of Sheffield, Western Bank, Sheffield S10 2TN, UK*

ARTICLE INFO

Article history:
Accepted 26 October 2006
Available online 6 December 2006

Keywords:
Calcium-activated potassium channel
Patch-clamp
Paxilline
ATP
Nucleotide

ABSTRACT

Large-conductance calcium-activated potassium (BK_{Ca}) channels were studied in inside-out patches of human NTERA2 neuronal cells (NT2-N). In symmetrical (140 mM) K^+ the channel mean conductance was 265 pS, the current reversing at ~0 mV. It was selective (P_K/P_{Na} = 20:1) and blocked by internal paxilline and TEA. The open probability–voltage relationship for BK_{Ca} was fitted with a Boltzmann function, the $V_{½}$ being 76.3 mV, 33.6 mV and –14.1 mV at 0.1 μM, 3.3 μM and 10 μM $[Ca^{2+}]_i$, respectively. The relationship between open probability and $[Ca^{2+}]_i$ was fitted by the Hill equation (Hill coefficient 2.7, half maximal activation at 2.0 μM $[Ca^{2+}]_i$). Open and closed dwell time histograms were fitted by the sum of two and three voltage-dependent exponentials, respectively. Increasing $[Ca^{2+}]_i$ produced both an increase in the longer open time constant and a decrease in the longest closed time constant, so increasing mean open time. "Intracellular" ATP evoked a concentration-dependent increase in NT2-N BK_{Ca} activity. At +40 mV half-maximum activation occurred at an $[ATP]_i$ of 3 mM (30 nM $[Ca^{2+}]_i$). ADP and GTP were less potent, and AMP-PNP was inactive. This is the first characterisation of a potassium channel in NT2-N cells showing that it is similar to the BK_{Ca} channel of other preparations.

1. Introduction

Large-conductance calcium-activated potassium (BK_{Ca}) channels are found in many cell types (Marty, 1981; McManus and Magleby, 1988; Tanaka et al., 1997) with the importance of their role in physiology increasingly evident (Brenner et al., 2000; Ahluwalia et al., 2004; Sausbier et al., 2004). In neurones, the membrane depolarisation and calcium influx that occur during the action potential activate BK_{Ca} channels which then contribute to the final half of spike repolarisation and to the subsequent fast after-hyperpolarisation (Lancaster and Nicoll, 1987). The intimate coupling of BK_{Ca} channels with voltage-dependent calcium channels (VDCC) (Marrion and Tavalin, 1998) consequently provides a negative feedback mechanism limiting VDCC activation and calcium entry. In nerve terminals, this BK_{Ca} channel action serves to regulate the amount of transmitter release (Robitaille and Charlton, 1992).

Numerous single-channel patch-clamp studies of BK_{Ca} channel have been carried out using various rodent neuronal preparations (e.g. Smart, 1987; Egan et al., 1993; Lee et al., 1995; Scholz et al., 1998; Dopico et al., 1999; Womack and Khodakhah, 2002). Few equivalent studies exist in human neurones (Simard et al., 1993; Jiang and Haddad, 1997). Using the human embryonal carcinoma (EC) cell line, NTERA-2/

* *Corresponding author*. Fax: +44 29 20874149.
E-mail address: Wann@cardiff.ac.uk (K.T. Wann).
[1] Present address: Department of General Pharmacology, Orion Pharma, FIN-20101, Turku, Finland.

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doi:10.1016/j.brainres.2006.10.060

clone D1, stable postmitotic neuronal cells (NT2-N) can be obtained by treatment with retinoic acid (Andrews, 1984; Pleasure et al., 1992). NT2-N neurones survive, mature and integrate when transplanted into the CNS of rodents (Kleppner et al., 1995; Lee et al., 2000; Watson et al., 2003), implantation of NT2-N cells into the human brain in a phase I clinical trial for stroke has been conducted and positive effects have been demonstrated in animal models of stroke (Borlongan et al., 1998), brain and spinal cord injury (Saporta et al., 2002; Watson et al., 2003), Parkinson's Disease (Baker et al., 2000) and familial amyotrophic lateral sclerosis (Garbuzova-Davis et al., 2002).

NT2-N cells express multiple phenotypes (Guillemain et al., 2000) including functional dopaminergic (Sodja et al., 2002) and muscarinic acetylcholine (Squires et al., 1996) receptors as well as $GABA_A$ (Matsuoka et al., 1997), NMDA and non-NMDA glutamate receptor channels (Younkin et al., 1993; Squires et al., 1996; Matsuoka et al., 1997). NT2-N cells extend axonal and dendritic processes, exhibit tetrodotoxin-sensitive sodium currents and the capacity for regenerative action potentials (Rendt et al., 1989). In addition various VDCC subtypes are also expressed by NT2-N cells (Neelands et al., 2000). However, little characterisation of NT2-N potassium channels has been undertaken. In this study, we describe for the first time the presence of BK_{Ca} channels in human NT2-N cells, their single-channel properties and modulation by nucleotides.

2. Results

2.1. *Identification of BK_{Ca} channels*

Inside-out recordings in symmetrical 140 mM K^+ solution (30 nM Ca^{2+}) from the soma of NT2-N cells routinely contained active channels. In 63% (31/49) of patches, exposure to a raised $[Ca^{2+}]_i$ gave a reversible increase in channel activity. In symmetrical 140 mM K^+ solution, these channels exhibited a linear current–voltage relationship with a mean conductance of 264.5±8.7 pS (n=12). The reversal potential was −0.5± 0.5 mV (n=12). Representative single channel currents from a patch exposed to the symmetrical 140 mM K^+ gradient and then to an asymmetrical K^+ gradient, following substitution of the perfusate at the intracellular face with sodium Locke solution containing 3 mM K^+, are shown in Fig. 1A. Under this reverse physiological gradient, the current–voltage relationship displayed pronounced rectification (Fig. 1B), with extrapolation yielding a reversal potential of approximately 65 mV indicating a ~20-fold selectivity for potassium ions over sodium ions.

These characteristics correspond to those previously ascribed to BK_{Ca} channels. Furthermore, these channels were reversibly blocked by the specific BK_{Ca} channel inhibitor paxilline (3 patches), which is effective at the intracellular face (Knaus et al., 1994; Fig. 1C) and by TEA. TEA blocks BK_{Ca} channels with relatively low affinity when applied intracellularly, reducing the single channel current (see also Smart, 1987). Internal TEA of 20 mM decreased the amplitude of the channel openings by 29.8% (2 patches).

Occasionally subconductance levels and changes in the mode of activity to a type characterised by brief openings were observed as with BK_{Ca} channels in other preparations (McManus and Magleby, 1988; Wann and Richards, 1994). These were infrequent and in the main short-lived. In addition, a number of patches showed a spontaneous decline in activity after a period of time. Such run-down of BK_{Ca} channels has been attributed to exposure to an oxidative environment following patch excision (DiChiara and Reinhart, 1997). The data presented are confined to BK_{Ca} channel steady-state activity.

2.2. *Calcium and voltage sensitivity*

The calcium and voltage sensitivity of the BK_{Ca} channel was determined in excised patches in symmetrical 140 mM K^+. The calcium-sensitivity of the BK_{Ca} channel was assessed at a holding potential of +40 mV. As shown in Fig. 2 at this membrane potential, channel openings in response to 0.3 μM Ca^{2+} were few and brief, with activity increasing on exposure to 1 μM Ca^{2+} and further still with 10 μM Ca^{2+}. Channel open probability as a function of $[Ca^{2+}]_i$ was fitted by the Hill equation with a Hill coefficient of 2.7 and half maximal activation at $[Ca^{2+}]_i$ of 2.0 μM (Fig. 2B). The Hill coefficient would then suggest that at least three calcium ions are required to activate the channel.

The BK_{Ca} channel showed voltage dependence, with a fixed calcium concentration (1 μM); the open probability–voltage relationship could be fitted with a Boltzmann function yielding a $V_{½}$ of 58.5±3.9 mV (n=3). The voltage dependence of open probability could be dramatically altered by the $[Ca^{2+}]_i$. Fig. 3A shows the activity of an inside-out patch containing a single BK_{Ca} channel exposed to 10 μM Ca^{2+} at patch potentials of −50 and +60 mV. From the same patch, the open probability for three different Ca^{2+} concentrations is plotted over a wide range of patch potentials (Fig. 3B). An increase in $[Ca^{2+}]_i$ produced a hyperpolarising shift in the activation voltage ($V_{½}$) of the channel ($V_{½}$=76.3, 33.6 and −14.1 mV for 0.1, 3.3 and 10 μM Ca^{2+}, respectively). The apparent gating charge, as indicated by the slope factor (k) of the Boltzmann fit, varied less with $[Ca^{2+}]_i$, this being 2.4, 1.7 and 1.7 in 0.1, 3.3 and 10 μM Ca^{2+}, respectively.

2.3. *Single-channel kinetics*

Most patches had multiple BK_{Ca} channels, the mean number of channels per patch was 3.7±0.7 with a mean electrode resistance of 7.9±0.5 MΩ (n=11), precluding detailed kinetic analysis. Using patches (as in Fig. 3) with a single BK_{Ca} channel, the effect of $[Ca^{2+}]_i$ and voltage on channel kinetics was investigated. The open time histograms were best described by the sum of two exponential functions, whereas the closed time histograms were fitted by three exponentials. At a fixed voltage (+40 mV; Fig. 4A upper two panels), an increase in the $[Ca^{2+}]_i$ markedly increased the long open time constant and decreased the closed time constants, in particular the longest. This change in the magnitude of the time constants was also accompanied by a shift in the relative proportion of each. The net result was at +40 mV an increase in the mean open time from 1.2 ms in 0.1 μM $[Ca^{2+}]_i$ to 8.1 ms in 10 μM $[Ca^{2+}]_i$ whereas the mean closed time decreased from 295.8 ms to 0.5 ms (●—●, Figs. 4B and C).