BK channel blockade alters the firing rate but not the gain of medial vestibular nucleus neurons *in vitro* 550.2

J.R.W. Menzies[1], S.R. Anderson[2], J. Porrill[2], P. Dean[2], M.B. Dutia[1].

[1]Centre for Integrative Physiology, University of Edinburgh, UK; [2]Neural Algorithms Research Group, Dept. of Psychology, University of Sheffield, UK.

Introduction

Alterations in the inherent properties of individual neurons can lead to functional plasticity in the adult brain. One well-studied example is changes in the intrinsic membrane properties of medial vestibular nucleus (MVN) neurons [1]. The molecular mechanisms which form the basis of these changes, characterised by an increase in resting spontaneous firing rate and an increase in gain, are, however, largely unknown.

An attractive candidate for a molecular substrate mediating changes in intrinsic excitability are Ca^{++}-dependent K^+ channels. A class of these channels, the large conductance Ca^{++}- and voltage-activated K^+ (BK) channels, have been implicated in controlling the spontaneous firing rate and firing dynamics of several neurons via their activation during spike depolarisation and the subsequent after-hyperpolarisation (AHP) [2, 3]. Here we examine the effect of BK channel blockade on the firing rate and response dynamics of mouse MVN neurons *in vitro*.

Methods

Brainstem slices (300 μm) containing the MVN were prepared from C57/B6 mice (either sex, aged P15-P24). Slices were cut horizontally in ice-cold sucrose aCSF (NaCl, 87 mM; sucrose 75 mM) then allowed to recover for at least 1 hr. Slices were bathed in normal aCSF (mM: NaCl, 140; KCl, 2.5; HEPES, 10; glucose, 11; $MgCl_2$, 1.3; $CaCl_2$, 2.4; NaH_2PO_4, 1.2) superfused at 2ml/min, bubbled with O_2 and maintained at 31°C. Some experiments were performed with lower (1.3 mM) $CaCl_2$, no differences were observed in firing rate or responses in these two conditions and data were pooled. Synaptic transmission was blocked with 2 mM kyneurenic acid and 200 μM picrotoxin. Neurons were visualised in the rostral part of the MVN using DIC-infrared microscopy and whole cell patch clamp recordings were made using electrodes of 8-10 MΩ resistance containing (mM) potassium gluconate, 145; HEPES, 5; EGTA, 0.1; K_2ATP, 5; $MgCl_2$, 2.

MVN neurons were subjected to step, ramp or "coloured noise" current injections in fast current clamp mode to assess some dynamics of their firing behaviour. 0.3-3 μM paxilline, a selective BK channel blocker [4], was superfused for 10 min and the current injection protocols were repeated to examine the effect of BK channel blockade on firing rate dynamics. Data were collected using Axon pClamp9 (Molecular Devices, CA, USA) and analysed using WinEDR (J. Dempster, University of Strathclyde, UK) and analytical software developed in our laboratories using Python (www.python.org) and MatLab (The Mathworks, MA, USA). Data are presented as mean±s.e.m.

Results

Electrophysiological classification

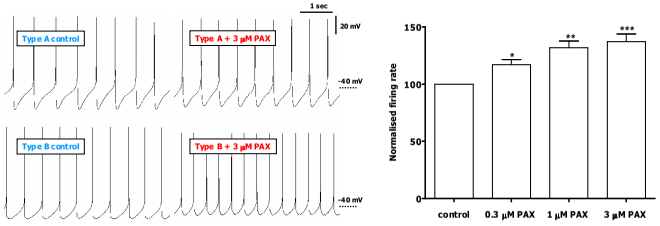
MVN neurons fire spontaneously *in vitro* and can be classified electrophysiologically as type A or type B cells according to their shape of their after-hyperpolarisation (AHP; figures 1 and 4). 90% (100/111) of mouse MVN cells were identified unambiguously as type B cells. The remainder (11/111; 10%) were identified unambiguously as A cells. The mean *in vitro* firing rate of type B cells was significantly higher then that of type A cells (14.4±1.0 Hz v. 7.8±1.0 Hz; p = 0.04; unpaired t-test) but there was no difference in the co-efficient of variation (type A cells, 0.09±0.03; type B cells, 0.09±0.01). No differences in cell capacitance or input resistance were observed between cell types (36.3±5.2 pA v. 38.0±2.4 pA; 650±129 MΩ v. 441±35 MΩ in type A v. type B cells respectively). Eighteen of these cells were used to assess the effect of BK channel blockade on firing rate, spike shape and gain.

Spontaneous firing rate

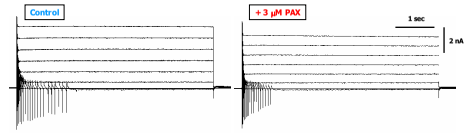
Paxilline (0.3-3 μM) increased reversibly the spontaneous firing rate in both type A and type B cells in a concentration-dependent fashion (figures 1 and 2). 3 μM paxilline increased significantly the spontaneous firing rate by 37.1±7.0% (Type A and B cells pooled; n = 14; p= 0.0004; paired t-test). The mean increase in rate in B cells (41.6±7.7%; n = 12) was greater than in A cells (9.7 and 11.3%; n = 2). Voltage clamp recordings were made to assess the effect of BK channel blockade on outward currents in five of these type B cells. Paxilline (3 μM) decreased the amplitude of outward currents by 12.6±7.1% (Figure 3). In 4/18 MVN neurons (all type B cells), 3 μM paxilline had no effect on, or decreased slightly (<15%), the spontaneous firing rate.

Spike shape

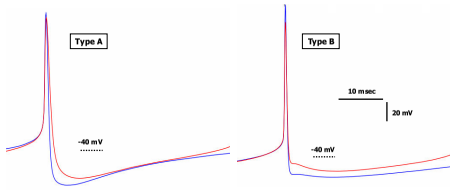
Figure 4 illustrates a typical type A and type B cell in control conditions and after BK channel blockade. Type A cells show a small decrease in spike amplitude of ~1.5 mV, an increase in spike width of ~0.5 msec and a decrease in AHP amplitude of ~5 mV. Type B cells also showed a concentration-dependent decrease in spike amplitude (control, 68.3±1.7 mV; 3 μM paxilline, 60.2±2.9 mV) and a trend to increased spike width (control, 0.89±0.07 msec; 3 μM paxilline, 1.01±0.11 msec). In 6/12 type B cells, paxilline had a predominant effect on the second component of the AHP. In the remainder of type B cells both components were affected.



1 Paxilline increases the spontaneous firing rate of mouse MVN neurons *In vitro* spontaneous firing activity in control type A (upper panel) and type B (lower panel) mouse MVN neurons. Firing rates are increased in the presence of the selective BK channel blocker paxilline (3 μM). **2 The effect of paxilline is concentration-dependent** Effect of paxilline on the *in vitro* spontaneous firing rate in mouse MVN neurons. Increases in rate were normalised to each cell's own control rate (100%). *p<0.02, **p<0. 02, ***p=0.0001 (paired t-test).



3 Paxilline inhibits outward currents Voltage clamp recordings (-70 to +90 mV in 20 mV steps) illustrating the inhibitory effect of 3 μM paxilline on outwards currents in a typical type B MVN neuron. Note a burst of action currents at -30 mV.



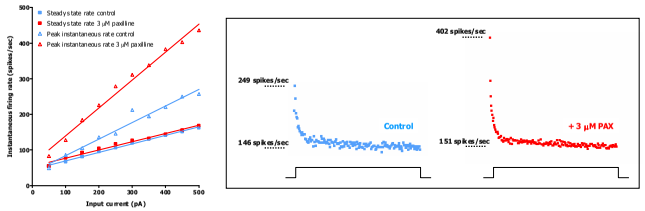
4 Paxilline alters the spike shape of MVN neurons Averaged spike shapes in control type A and type B MVN neurons and the effect of 3 μM paxilline. Note the decrease in AHP amplitude after BK channel blockade.

Gain and frequency response

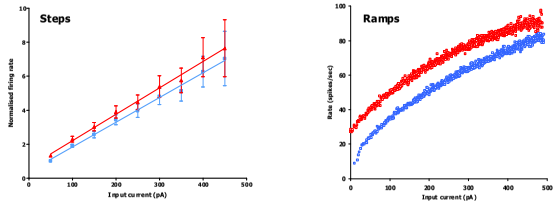
The gain of each neuron was assessed using a series of step input currents and a single ramping input current in the absence and presence of 3 μM paxilline. In all MVN neurons tested, the firing rate decayed to a steady state level during a stable step current input. Gain changes were examined using the steady state firing rate rather than the transient peak firing rate (Figure 5). Figure 6 shows the mean effect of paxilline on the steady state firing rate responses to a series of step inputs. Responses fitted well using linear regression (R^2 mean 0.985; range 0.96-0.99 in controls). Paxilline increased the underlying spontaneous firing rate but had no effect on the steady state gain (265±26 (spikes/sec)/nA in controls v. 275±27 (spikes/sec)/nA in the presence of 3 μM paxilline).

Figure 7 shows the response of a typical B cell to a ramp input current in the absence and presence of 3 μM paxilline. The underlying spontaneous firing rate was increased but there was no effect on the gain of the response. Responses fitted well using linear regression (R^2 mean 0.97; range 0.94-0.98 in controls). The mean slope of responses to control ramp inputs was 233±32 (spikes/sec)/nA. In the presence of 3 μM paxilline, this was unchanged (220±31 (spikes/sec)/nA; n = 14). There was no difference in the step or ramp input response slopes of type A and type B cells.

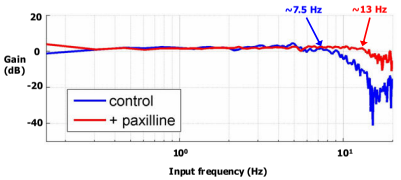
A further measure of gain was made using a coloured noise input (DC to 25 Hz input freqency, 10 Hz corner frequency, 60 sec duration). Analyses revealed no change in gain after BK channel blockade though the response corner frequency was increased by 32% from 8.5±0.9 spikes/sec to 11.1±0.9 spikes/sec (n = 9; p = 0.01; paired t-test; figure 8 shows a typical example). The phase of the control spike response relative to the input current was unchanged after BK channel blockade.



5 BK channel blockade potentiates peak firing rate but not steady state firing rate Left panel. Example control responses and responses in the presence of 3 μM paxilline to a step input currents in a type B cell. Measuring steady state firing rates reveals no change in gain during BK channel blockade. However, the gain measured with reference to the peak instantaneous rate is increased. Right panel. Examples of accomodation during a single step input (450 pA from silence (~-60 mV), 1 sec duration). Note that after BK channel blockade the transient peak firing rate is increased but the steady state rate is unchanged.



6 Paxilline has no effect on gain (step input currents) Mean steady state control responses and responses in the presence of 3 μM paxilline to a series of step input currents (50-450 pA in 50 pA steps; n = 5-11; type A and type B cells pooled). Data points for each neuron were normalised to the steady state firing rate observed during a 50 pA step in control conditions. **7 Paxilline has no effect on gain (ramp input currents)** Control response and response in the presence of 3 μM paxilline to a ramping input current (0-500 pA over 10 sec) in a typical B cell. The underlying spontaneous firing rate is increased but there is no change in the gain of the response (129 (spikes/sec)/nA v. 125 (spikes/sec)/nA in control conditions).



8 Paxilline has no effect on gain (coloured noise input currents) Plot of gain v. input frequency showing the control response and response in the presence of 3 μM paxilline. Note that the gain is unchanged but the frequency range over which the cell responds is increased.

Conclusions

i. All mouse MVN neurons recorded could be classified electrophysiologically as either type A or type B neurons. Type B cells are the predominant type (90%).

ii. Functional BK channels are present in mouse MVN neurons. BK channel blockade increased the *in vitro* spontaneous firing rate of MVN neurons in a reversible and concentration-dependent manner.

iii. BK channel blockade had no effect on the gain of MVN neurons assessed using steady state step, ramp or coloured noise current inputs. However, the dynamic response range of these neurons is increased.

iv. These data show that BK channels are likely to play an important role in setting the firing rate of mouse MVN neurons and that inhibition of their activity leads to an increase in the MVN neurons' input frequency-response range.

References

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