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EC NEUROLOGY
Research Article

Pharmacological Dissection of Potassium Currents in Cultured, Identified, Molluscan Neurons

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Received: March 11, 2019; **Published:** May 04, 2019

Abstract

The potassium currents in neurons of gastropod molluscs are quite complex and here we have investigated those of cultured, isolated pedal I cluster neurons of *Lymnaea stagnalis* under whole-cell patch clamp conditions using physiological and pharmacological techniques. Four types of K+ currents were clearly identified in cultured pedal I cluster neurons: transient or A current (I_A), ATP dependent K^+ current ($I_{(KATP)}$), Ca^{2+} dependent K^+ current ($I_{(Kca)}$) and the delayed rectifier current (I_K).

Keywords: *Potassium Currents; Pharmacological Dissection; Molluscan Neurons, Whole Cell Patch Clamp, Lymnaea stagnalis*

Introduction

In a previous paper [1] we pharmacologically determined that only a single type of high voltage-activated current, resembling a classical L-type calcium current [2] was found in cultured, isolated pedal I cluster neurons of the pond snail *Lymnaea stagnalis*, using the whole cell patch clamp technique. Here we consider the potassium channels of these same cells and separate them physiologically and pharmacologically.

Molluscan neurons are known to have a complicated pattern of outward currents [3] which can be separated either kinetically or pharmacologically [4-6], using patch clamp methodology [7]. The membrane potential of most cells is stabilized by high K^+ conductance, which generates a relatively negative membrane potential [8]. Over the decades K^+ channels have been purified, sequenced and cloned [9]. They appear to be conserved in both eukaryotes and prokaryotes and are divided into two families: the voltage-gated (K_V) and ligand-gated K^+ channels [10], but many variants exist and their structures [11] gating [12] and activation mechanisms [13] are gradually being better understood and modelled at a molecular level [14]. Here we restrict ourselves to considering the types of potassium currents found in neurons of *Lymnaea stagnalis* as part of a larger study on the actions of general anesthetics on this excellent model preparation [15]. We identify, for the first time, four separate K^+ currents occurring in cultured, isolated pedal I cluster neurons of this animal.

Materials and Methods

Animals and cell culture techniques

Lymnaea stagnalis were obtained from suppliers (Blades Biological Ltd, Cowden, Edenbridge, Kent TN8 7DX) and were maintained and dissected as previously described [16,17]. Identified pedal I culture neurons were cultured in isolation according to the methods of Yar and Winlow [18].

Whole cell patch clamp

The whole cell patch clamp is basically as described by Yar and Winlow [18]. It is based on the methods of Hamill., *et al.* [19], but in this case we used it to dissect K^+ currents. We controlled the composition of the solutions on both sides of the membrane and used different bath and pipette solutions as appropriate (see below). Voltage steps were applied every 10s and three different protocols were employed to identify different K^+ currents.

Protocol 1: This protocol was a holding potential of -30, -50 or -70 mV, delivered every 10 ms with 10 mV voltage steps from -110 mV to +110 mV (Figure 1B).

Protocol 2: This protocol is designed to study the reversal potential of pedal I cluster neurons using bath solution and pipette solution D. The holding potential was -50 and two pulses were applied. The prepulse was 170 mV and was followed by a second pulse of 140 mV and thereafter pulses declined by 20 mV steps. The duration of every pulse was 500 ms, similar to other protocols used to study K^+ currents (Figure 2B).

Protocol 3: In this protocol, K^+ current was triggered at 10s intervals for at least 30 minutes. The membrane potential was held at -50 mV with a step depolarization potential of +80 mV and a duration of 500 ms, applied every 10 s (Figure 4B).

K^+ currents were dissected in 6 cells for each protocol using 1 mM concentrations, of tetraethyl ammonium (TEA), 4-aminopyridine (4-AP), $BaCl_2$ and $CdCl_2$. Different types of pipette solutions were employed. The basic bath solution for K^+ current was (mM): NMDG (N-Methyl-D-Glucamine, Sigma, M-2004), 51.6; $CaCl_2$ 4.0; $MgCl_2$, 1.5; Hepes, 10; Glucose, 10. NMDG is an impermeable positive charge carrier, used instead of Na^+, thus avoiding its effects on the membrane potential. Pipette solution A contained KCl, 50; NaCl, 1.6; $MgCl_2$, 1.5; HEPES, 10; Mannitol, 10; EGTA, 0.5; and $CaCl_2$, 4.7 (in mM); pipette solution B (Ca^{2+} and ATP) contained KCl, 50; NaCl, 1.6; $MgCl_2$, 1.5; Hepes, 10; Mannitol, 10; EGTA, 0.5; $CaCl_2$, 4.7 and ATP, 3 (in mM); pipette solution C (ATP) contained (in mM) KCl, 50; NaCl, 1.6; $MgCl_2$, 1.5; HEPES, 10; Mannitol, 10; EGTA, 5; ATP, 3 without Ca^{2+} and pipette solution D contained KCl 50, NaCl 1.6, $MgCl_2$ 1.5 Hepes 10, Mannitol 10, EGTA 5, $CaCl_2$ 4.7, ATP, 3 mM.

Data analysis

Voltage-clamp data was analysed with the help of CED Patch and Voltage clamp software. Parametric statistical analysis of significance, standard for this type of work [50,51], was done with Student's paired or unpaired t-tests using the 'Oxstat' program. Data is either expressed as mean ± S.D. or mean ± S.E.M.

Results

Gross K^+ current: The membrane potential of cultured pedal I cluster neurons using bath and pipette solutions mentioned in the legend of figure 1 were measured at -43 ± 5 mV (n = 8). To enable us to find the best holding potentials, three holding potentials of -70, -50 and -30 mV were examined (Figure 1). The A-current usually occurs at the holding potential of -70 mV but other K^+ currents which have been studied here (K_{ca}, K_{ATP} and the delayed rectifier) are generated at holding potentials of -50 mV. There are significant differences in recordings made between holding potentials of -70 mV and -30 mV but no significant differences were found at holding potentials between -70 and -50 or -50 and -30 mV. Thus, the holding potential of -50 mV was chosen because it is close to the membrane potential of pedal I cluster neurons and was used in previous studies [1,18,20].

The reversal potential was recorded using pipette solution D to ensure that the current recorded was K^+ current. Figure 2 shows that the reversal potential of the recorded gross current is -75 mV which is close to the reversal potential of K^+ as predicted by the Nernst equation (Figure 2). There should be no K^+ current when the membrane is at the potassium equilibrium potential [4,21]. GABA at the concentration of 10^{-3} M was also applied to these neurons to activate GABA receptors or chloride (Cl^-) conductance and was found to have no effect.

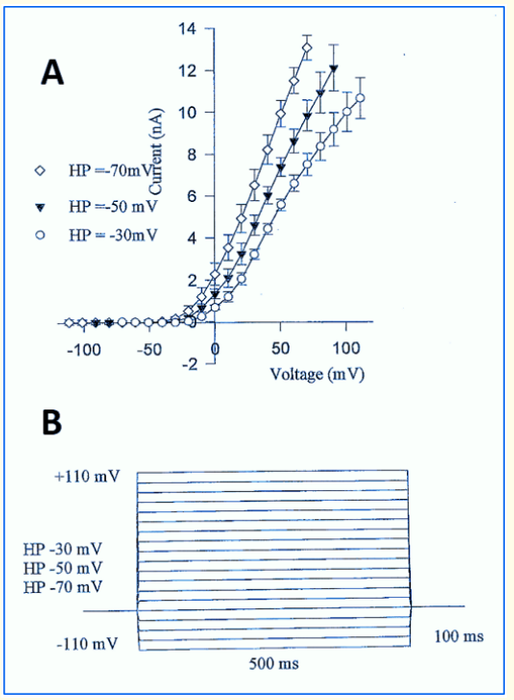

Figure 1: *Gross K^+ current recorded at three different holding potentials. A) As the figure shows the amplitude of the K^+ current increased using the more negative holding potentials. There is no significant difference between traces up to +10 mV depolarization steps, but at higher potentials significantly different currents were recorded (P value ≤ 0.005). The bath solution contained (in mM): NMDG, 51.6; $CaCl_2$, 4; $MgCl_2$, 1.5; HEPES, 10 and Glucose 10. Pipettes had a resistance of 2 - 5 MΩ and contained KCl, 50; NaCl, 1.6; $MgCl_2$, 1.5; HEPES, 10; Mannitol, 10; EGTA, 5; $CaCl_2$, 4.7 and ATP, 3 mM. B: The stimulus protocol showing the amplitude and duration of the command pulse*

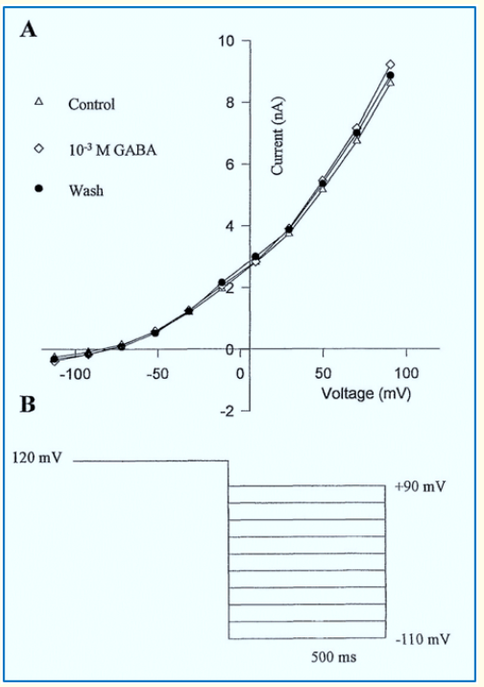

Figure 2: *The reversal potential of pedal I neurons. A) Effect of GABA on the reversal potential of pedal I cluster neurons (n = 6). The current was delivered from a -50 mV holding potential at 10 s intervals. A 170 mV prepulse applied for 500 mS, was followed with a command potential starting with a step of 140 mV depolarization which was repeated every 10s with -20 depolarization reversal potential step. Bath and pipette solutions were the same as in figure 1 except for the addition of 1 mM GABA dissolved in the bath. The figure shows that the reversal potential of this current is -75 mV and GABA has no significant effect on it. Below -100 mV what seems to be inward rectifier current appears and this needs further study. B: The stimulus protocol showing the amplitude and duration of the command pulse.*

The gross K^+ current was reduced (Figure 3), using 1 mM $CdCl_2$, by 35%, but a mixture of 1 mM $CdCl_2$ and $BaCl_2$ in the bath solution decreased the gross K^+ current by 80% at +90 mV membrane potential depolarization, strongly supporting the supposition that it was a K^+ current. Barium is commonly used to block K^+ channels but it also carries large inward currents through some Ca^{2+} channels [21]. Similar results were obtained when the above experiment was carried out using a -30 mV holding potential.

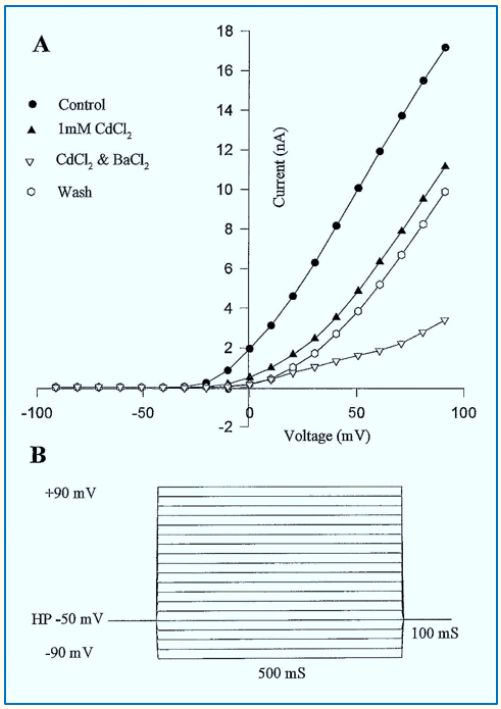

Figure 3: *$CdCl_2$ and $BaCl_2$ decrease I_k of pedal I cluster neurons at HP -50 mV (n = 6). A) The membrane was held at -50 mV and bath solution contained (in mM): NMDG, 51.6; $CaCl_2$, 4; $MgCl_2$, 1.5; HEPES, 10 and Glucose, 10. Pipettes had a resistance of 2 - 5 MΩ and contained KCl, 50; NaCl, 1.6; $MgCl_2$, 1.5; HEPES, 10; Mannitol, 10; EGTA, 0.5; $CaCl_2$, 4.7 and ATP, 3 mM. The sequence of drug application is shown mentioned from top to bottom of the figure. The control curve was constructed in normal bath solution. Another bath solution contained 1 mM $CdCl_2$ and decreased K^+ current by 35% at the +90 mV depolarization step. Application of a mixture of $CdCl_2$ and $BaCl_2$ decreased I_K by 80%. Finally, after continuous wash-out, the preparations significantly increased K^+ current but did not fully restore it to control levels. B) The stimulus protocol showing the amplitude and duration of the command pulse.*

Delayed rectifier current, I_k: Kandel and Tauc [22] have reported an anomalously rectifying K^+ current and a slow K^+ current has also been described [23,24]. Barium is a potent divalent metal which can interact with the delayed rectifier from both sides of the membrane [25]. When barium was applied externally, under the continuous recording protocol it blocked 60% of the sustained K^+ currents (Figure 4) and this was not reversed when 1 mM caffeine was applied simultaneously with barium. This suggests that it was blocking the delayed rectifier current. Caffeine which is known to release Ca^{2+} from internal stores and activate Ca^{2+} dependent K^+ current was not able to alter K^+ current in the presence of Ba^{2+} (Figure 4). The effect of Ba^{2+} was partially reversible after continuous, prolonged washing with normal bath solution.

Ca^{2+} dependent K^+ current, $I_{(Kca)}$: Current has been reported in gastropod neurons by Meech and Standen [4] in *Helix aspersa* and in *Helix pomatia* by Heyer and Lux [5] and "ensures that any depolarizing trend is countered by an efflux of K^+" [26]. TEA is a simple quaternary ammonium compound used experimentally to block K^+ channels. Pedal I cluster neurons are partially sensitive to TEA as shown in figures 5 and 6A. 40 mM TEA decreased sustained gross K^+ currents in pedal I cluster neurons (n = 6) by no more than 40% (Figure 5) similar to its effect in figure 6A, where continuous wash out of TEA with normal bath solution increased K^+ currents significantly, but they never reached the control levels. The action of caffeine in overcoming the effects of TEA indicates the presence of $I_{(Kca)}$.

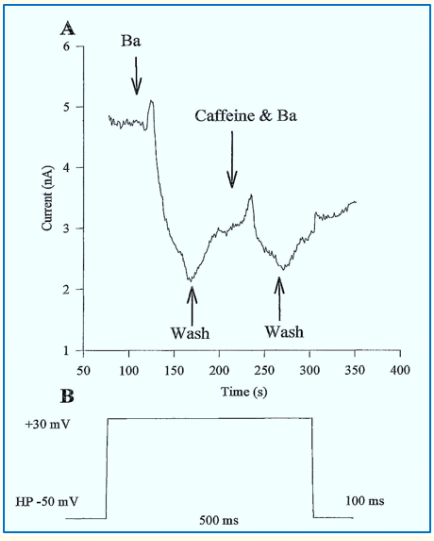

Figure 4: *The effect of Ba^{2+} and Caffeine on I_K in a continuous recording from a pedal I neuron. The normal bath solution contained (in mM): NMDG, 51.6; $CaCl_2$, 4; $MgCl_2$, 1.5; HEPES, 10 and Glucose, 10, pipettes had a resistance of 2 - 5 MΩ and contained KCl, 50; NaCl, 1.6; $MgCl_2$, 1.5; HEPES, 10; Mannitol, 10; EGTA, 5; $CaCl_2$, 4.7 and ATP, 3 mM. A) Ba^{2+} (1mM) decreases I_K which is then washed out in normal bath solution; so that I_k partially recovers. Application of a mixture of Ba^{2+} and caffeine (1 mM) decreases I_K to the same level, which indicates that Ba^{2+} suppressed the delayed rectifier K^+ current and can prevent $I_{k(ca)}$ which is normally stimulated with caffeine via Ca^{2+} released from internal stores. The sequence of chemical application is illustrated in figure. B) The stimulus protocol showing the amplitude and duration of the command pulse.*

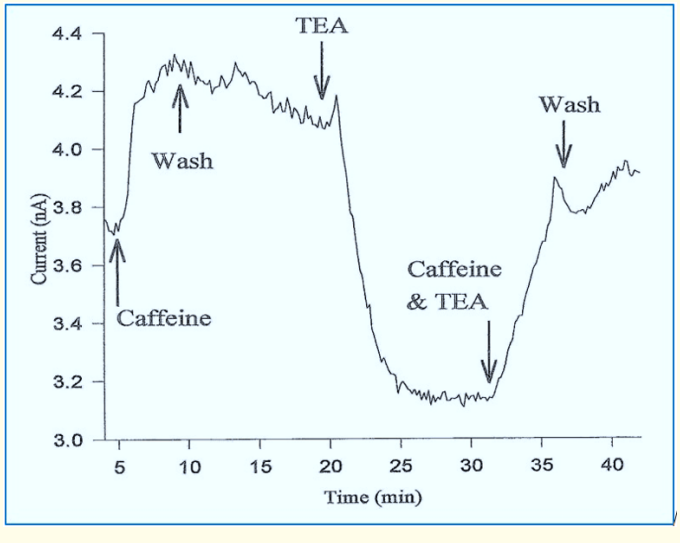

Figure 5: *The effect of caffeine and TEA on I_K in a continuous recording from a pedal I neuron. A) Caffeine, which releases intracellular free Ca^{2+}, increases I_K, whilst TEA decreases it. This implies the presence of K_{ca} amongst other K^+ currents. The bath solution contained (in mM) NMDG, 51.6; $CaCl_2$, 4; $MgCl_2$, 1.5; HEPES, 10 and Glucose, 10, and the pipette solution contained (in mM) The pipette solution contained KCl, 50; NaCl, 1.6; $MgCl_2$, 1.5; HEPES, 10; Mannitol, 10; EGTA, 5; $CaCl_2$, 4.7 and ATP, 3. Sequence of application of solutions: 1) 1 mM caffeine; 2) Wash in normal bath solution; 3) 40 mM TEA; 4) A mixture of 40 mM TEA and Caffeine; 5) Wash in normal bath solution. B) The holding potential was -50 mV with the potential duration 500 ms. Depolarization steps from -50 to +30 mV were delivered at 10s intervals as in figure 5.*

Because the delayed rectifier is not very sensitive to aminopyridines, the application of 4-AP slightly decreased K^+ current at a high level of depolarization (+60 to +90 mV), but not lower than +60 mV (Figure 6B). The second wash with 5 - 10 minutes slightly increases K^+ current (Figure 6A and 6B).

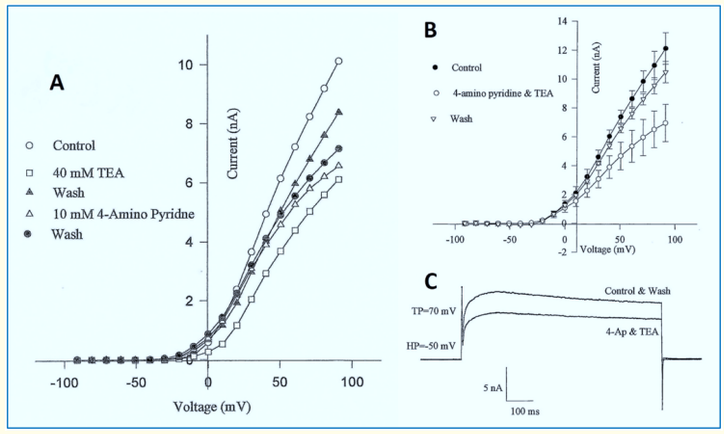

Figure 6: *A) Effects of TEA and 4-AP on gross I_K of pedal I cluster neurons (n = 6). The Bath solution contained (in mM): NMDG, 51.6; $CaCl_2$, 4; $MgCl_2$, 1.5; HEPES, 10 and Glucose, 10. TEA, 1 mM or 4-AP was added to the bath solution as appropriate TEA or 4-AP. The pipette solution contained KCl, 50; NaCl, 1.6; $MgCl_2$, 1.5; HEPES, 10; Mannitol, 10; EGTA, 5; $CaCl_2$, 4.7 and ATP, 3 mM (in mM). Sequence of application of solutions: 1) Pre-control in normal bath solution; 2) 40 mM TEA; 3) Wash in normal bath solution; 4) 10 mM 4-AP; 5) As 3. TEA decreased sustained K^+ currents by 40% which might be the delayed rectifier K^+ current. Except for certain depolarization steps 4-AP has no significant effect. The stimulus protocol was as in figure 3. B) A mixture of 1 mM TEA and 4-aminopyridine was applied to another pedal I cell and produced a significant decrease in the gross K^+ current (n = 6). Then the preparation was washed in normal bath solution and K^+ current partially recovered. Sequence of application of solutions: 1) Pre-control in normal bath solution; 2) 1 mM TEA and 1 mM 4-AP; 3) Wash in normal bath solution. C) The membrane potential was stepped to test potential (TP) +70 mV from a holding potential of -50 mV and current traces recorded under control and application of 4-AP and TEA. The current trace with continuous, prolonged wash out of drugs returns to the control position.*

Transient current (A current): The transient current (A current) is a fast transient outward current [27-29]. It is both activated and inactivated at negative potentials [28]. Here we were able to generate it at -70 mV holding potential between command potentials from -30 to +20 mV in 10 mV depolarizing steps (Figure 7). 40 mM TEA had no effect on the transient current but it was completely blocked when 1 mM 4-AP was applied to the bath solution. Other sustained K^+ currents were also blocked by application of either TEA or 4-AP.

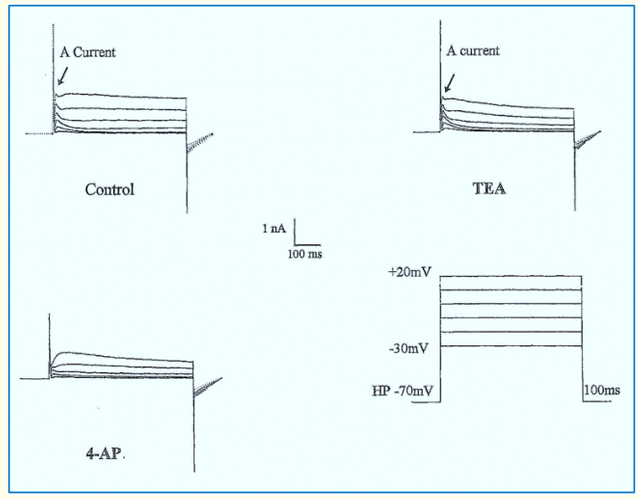

Figure 7: *The A current in pedal I cluster neurons. Transient current (A current) was recorded at -70 mV holding potential, with 10 mV command potential steps, from -30 to +20 mV. Transient K^+ current is shown in the control traces. Application of TEA 40 mM has no effect on the transient current, but it reduced the delayed rectifier and other TEA sensitive sustained currents. Finally, 1 mM 4-AP was applied to the bath solution and blocked the transient currents.*

The K_{ATP} channels: Have a low open state probability and are regulated by intracellular ATP and other metabolites [30,31]. Many physiological (peptides and hormones) and pathological conditions, as well as pharmacological agents, are able to open K_{ATP} channels [32-34]. High concentrations of ATP are known to inhibit open K_{ATP} channels. In figure 8 we demonstrate the interactions of $I_{(KATP)}$ and $I_{(Kca)}$, where, the existence of at least two K^+ currents is clearly shown. Three different pipette solutions were employed. Using pipette solution A (Ca^{2+} without ATP), the upper curve was produced. Using pipette solution B (Ca^{2+} and ATP) middle curve was recorded and finally using pipette solution C (ATP) the bottom curve was generated. The figure shows a bigger current when there is high Ca^{2+} in the pipette to stimulate K_{ca}. The current is even larger when there is no ATP in the pipette solution and will thus stimulate K_{ATP} (Top curve). In the middle curve the pipette solution is similar to pipette solution A except that it has ATP to suppress K_{ATP}. The difference between the two top curves are significant (P value ≤ 0.005). The bottom curve with pipette solution C has no Ca^{2+} to stimulate K_{ca} but it is rich in ATP which suppresses K_{ATP} as well. The difference between the two lower curves is due to the presence or absence Ca^{2+} in the pipette solution and the differences between curves are significant (P value ≤ 0.005).

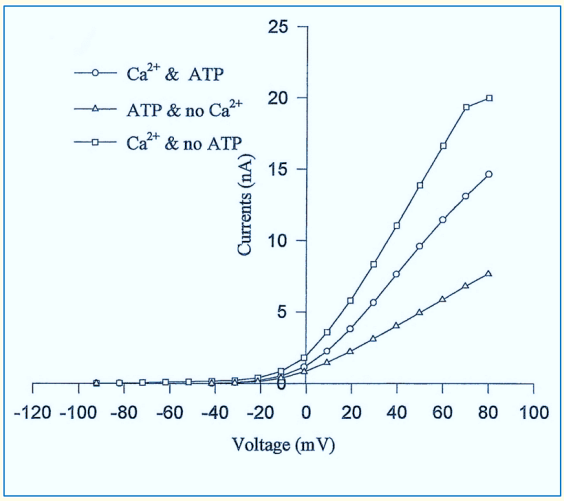

Figure 8: *Demonstration of K_{Ca} and K_{ATP} currents in pedal I cluster neurons recorded with different pipette solutions (n = 6). The membrane was held at -50 mV and the bath solution contained (in mM): NMDG, 51.6; $CaCl_2$, 4; $MgCl_2$, 1.5; HEPES, 10 and Glucose, 10. Three different pipette solutions were employed. Pipettes had a resistance of 2 - 5 MΩ. The sequence of curves is: the upper curve was produced using pipette solution A (Ca^{2+} without ATP) which contained KCl, 50; NaCl, 1.6; $MgCl_2$, 1.5; HEPES, 10; Mannitol, 10; EGTA, 0.5; and $CaCl_2$, 4.7 (in mM); pipette solution B (Ca^{2+} and ATP)(middle curve) contained KCl, 50; NaCl, 1.6; $MgCl_2$, 1.5; Hepes, 10; Mannitol, 10; EGTA, 0.5; $CaCl_2$, 4.7 and ATP, 3 (in mM) and pipette solution C (ATP)(bottom curve) contained (in mM) KCl, 50; NaCl, 1.6; $MgCl_2$, 1.5; HEPES, 10; Mannitol, 10; EGTA, 5; ATP, 3 (zero Ca^{2+}). The figure shows a bigger current when there is high Ca^{2+} in the pipette to stimulate K_{ca} (top two currents). It is even larger when there is no ATP in the pipette to inhibit K_{ATP} (Top curve). In the middle curve the pipette solution is similar to pipette solution A except that it has ATP to suppress K_{ATP}. The difference between the two top curves is significant (P value ≤ 0.005). The bottom curve with pipette solution C has no Ca^{2+} to stimulate K_{ca} but it is rich in ATP to suppress K_{ATP} as well. The difference between the two lower curves is due to the presence or absence Ca^{2+} in the pipette solution and the differences between curves are significant (P value ≤0.005). The stimulus protocol was as in figure 3.*

Discussion

Many physiological functions such as the conduction of nerve and muscle action potentials, the activity of cardiac pacemakers, secretory processes and spontaneous neuronal firing are strictly dependent on changes in K^+ conductances [10]. As our experiments demonstrate, the pedal I cluster neurons have at least four K^+ channels.

GABA has no effects on the gross current generated (Figure 2), which strongly supports the view that the gross recorded current is a K^+ current, and also that there are no GABA receptors in this type of neuron, although they are found on other *Lymnaea* neurons [35]. It

is known that GABA can enhance Cl^- currents. Using a mixture of 1 mM $CdCl_2$ and $BaCl_2$ the gross current was reduced by 85% which is further proof that the gross current was really a K^+ current (Figure 3). Cd^{2+} is able to block a portion of the K^+ current (36%) and Ba^{2+}, as a potent K^+ channel blocker, reduced the rest of the currents and is indicative of the delayed rectifier current (I_K).

Caffeine by itself can reversibly enhance the gross outward current due to release of stored calcium acting on I_{KCa} and this effect is blocked by TEA (tetraethyl ammonium) (Figure 5), which is a simple quaternary ammonium compound and is a classical, but rather nonspecific K^+ blocker with rapid kinetics [36]. TEA is the most potent blocker externally and 1 molecule of TEA appears to block one K^+ channel [37]. However, subsequent application of a mixture of caffeine and TEA can induce an increase in the amplitude of gross K^+ currents (Figure 5). Thus, these K^+ currents cannot be entirely suppressed by TEA when caffeine is present, supporting the presence of $I_{(Kca)}$. Caffeine is known to inhibit inositol triphosphate (IP_3) mediated liberation of calcium from intracellular stores [52], but at the same time it stimulates ryanodine receptor mediated calcium release [53]. Stimulation of adenosine A1 receptors rapidly mobilizes calcium release from intracellular stores in human bronchial muscle, but caffeine is known to antagonise all types of adenosine receptors (ARs) [54] and to inhibit phosphodiesterases (PDEs) [55] which promote calcium release from intracellular stores. Further work is required to determine the detail of TEA partial blockade of $I_{(Kca)}$ in the presence of caffeine.

The delayed rectifier K^+ channels are also blocked with 4-AP as is I_A (Figure 7). In these neurons we found that the K^+ outward current is a complicated current, which can be blocked by 85% with Ba^{2+} and Cd^{2+} cations at +90 mV command potential (Figure 3). However, in 40 mM TEA or 1 mM 4-AP the block does not exceed 43% at the same command potential. In fact the delayed rectifier K^+ current is blocked by TEA (Figure 5) and Ca^{2+} dependent K^+ current is blocked by Ba^{2+} (Figure 4) and Cd^{2+} (Figure 3).

4-AP (4-amino pyridine) is frequently used to block A-current in different preparations. 4-AP blocks I_A in many different tissues with an IC_{50} of around 2 mM [38]. It has also been reported that aminopyridines have little effect on the K_{ca} and delayed rectifier K^+ channels [39]. However, 4-AP is generally a more potent blocker of I_A rather than $I_{k(ca)}$ [40] and it is a useful tool in dissecting out the different K^+ currents in neuronal preparations [39].

As shown in figures 5 and 6, the pedal I cluster neurons are partially sensitive to TEA. TEA (6 mM), in a node of Ranvier under voltage clamp, causes complete block of K^+ current [21], but in our experiments 40 mM of TEA was not able to decrease I_k more than 40% (Figure 6A) in pedal I cluster neurons at the highest voltage step (+90 mV). The block may be quickly reversed by a rinse with bath solution. TEA (Figure 6B) even in a mixture with 1 mM 4-AP was not able to reduce gross K^+ current any further and had no effect on the transient A current (Figure 7). Aminopyridines tend to slow the activation of delayed rectifier currents as well as accelerating the inactivation of the transient K^+ current [6]. 1 mM 4-AP was able to decrease the transient A current (Figure 7) and the sustained current at certain depolarization voltages between +60 and +90 mV at which the delayed rectifier is strongly activated (P value ≤ 0.005), but at lower depolarization voltages it had no significant effect which is similar to a previous report [6].

It has been shown that Ca^{2+} increases the frequency and length of the opening of the individual Ca^{2+} dependent K^+ channels in different species [10]. Comparisons of the upper and middle curves with the lower curve of K^+ currents in figure 8 shows that, in the presence of $CaCl_2$, Ca^{2+} activated K^+ currents, were activated and increased K^+ currents significantly (P value ≤ 0.005), which supports previous findings [4] about the presence of I_{kca} in snail neurons. Comparisons of the upper curve and middle curve of figure 4. shows that in the presence of 3 mM ATP the K^+ currents are depressed by 33% at +70 mV command potential which is due to ATP suppression of $I_{(KATP)}$. K_{ATP} channels which exist in the neurons located in the ventromedial nucleus of mammalian hypothalamus have a very important role in feeding. This area is concerned with the regulation of food intake, which can be suppressed by glucose [41] or sulphonyl urea agents, K_{ATP} channel blockers [34]. $K_{(ATP)}$ channels are found in tissues of the cardiovascular system, muscle, pancreatic beta cells and many neurons [34], where they link membrane excitability to cellular metabolism. Thus, a rise in intracellular ATP inhibits open K_{ATP} channels [42]. Similar channels exist in *Lymnaea* neurons as well (Figure 8), because they can be blocked by 3 mM ATP via the patch electrode. It was shown by Kakei, *et al.* [43] that $K_{(ATP)}$ can be activated at ≤ 2 mM ATP in whole cell patches of rat ß pancreatic cells. Other purines (GTP, ITP and UTP) can also reduce K_{ATP} activity in different tissues [44]. $K_{(ATP)}$ channels have a low open state probability and are regulated by intracellular ATP and other metabolites [30,31]. Many physiological (peptides and hormones) and pathological conditions [34] as well as pharmacological agents are able to open $K_{(ATP)}$ [32,33]. Many types of K^+ currents have been identified in molluscan neurons.

The macroscopic current through BK channels (I_c) needs Ca^{2+} for activation and also it is a large, long-lasting outward current which is very voltage dependent, even at constant Ca^{2+} concentrations [40]. BK channels are involved in many physiological functions such as secretion. These channels are sensitive to glucose and close in the presence of small amounts of glucose. BK channels, which are distributed in a wide range of cells and tissues such as pancreatic ß cells and neurons increase action potential duration and increase secretion in response to glucose [45]. Ca^{2+} can be released from internal stores by various pharmacological agents. Caffeine is able to release Ca^{2+} from internal stores [46] and activates K_{ca} [47]. Ahmed [48] and Winlow., *et al.* [15] have shown, using ratio fluorescence microscopy, that caffeine is able to elevate the $[Ca^{2+}]_i$ in several identified cultured *Lymnaea* neurons. Thus, the result presented in figure 5 is consistent with previous reports which demonstrate K_{ca} in molluscan neurons [4]. It has been shown that some types of Ca^{2+} activated K^+ currents are sensitive to and blocked by TEA [40]. We have shown that this type of calcium dependent K^+ current is very sensitive to TEA (Figure 5). This current was also sensitive to Ba^{2+} (Figure 4) because BK and IK_{ca} can be inhibited by Ba^{2+}, but other types of $K_{ca,}$ such as small conductance Ca^{2+} activated K^+ channels (SK_{Ca}) are insensitive to Ba^{2+} [49] and could still be present. Application of a mixture of caffeine and Ba^{2+} (Figure 4) decreased K^+ currents which suggested that the type of K_{ca} in these neurons might be BK_{ca} and IK_{ca} (intermediate) channels.

Conclusions

Pharmacological dissection of the gross K^+ current recorded from cultured pedal I cluster neurons of *Lymnaea stagnalis* using the whole cell patch clamp technique indicate the presence of at least four potassium currents in these cells: I_A; $I_{(KATP)}$; $I_{(KCa)}$ and I_K.

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