Monoaminergic Modulation of the Na\(^+\)-Activated K\(^+\) Channel in Kenyon Cells Isolated From the Mushroom Body of the Cricket (Gryllus bimaculatus) Brain

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Aoki K, Kosakai K, Yoshino M. Monoaminergic modulation of the Na\(^+\)-activated K\(^+\) channel in Kenyon cells isolated from the mushroom body of the cricket (Gryllus bimaculatus) brain. J Neurophysiol 100: 1211–1222, 2008. First published June 8, 2008; doi:10.1152/jn.90459.2008. Recent studies have suggested that octopamine (OA) and dopamine (DA) play important roles in mediating the reward and punishment signals, respectively, in olfactory learning in insect. However, their target molecules and the signaling mechanisms are not fully understood. In this study, we showed for the first time that OA and DA modulate the Na\(^+\)-activated K\(^+\) (K\(_{Na}\)) channels in an opposite way in Kenyon cells isolated from the mushroom body of the cricket, Gryllus bimaculatus. Patch-clamp recordings showed that the single-channel conductance of the K\(_{Na}\) channel was about 122 pS with high K\(^+\) in the patch pipettes. The channel was found to be activated by intracellular Na\(^+\) but less activated by Li\(^+\). K\(^+\) channel blockers TEA and quinidine reduced the open probability (Po) of this channel. Bath application of OA and DA respectively increased and decreased the Po of K\(_{Na}\) channel currents. An increase and a decrease in Po of K\(_{Na}\) channels were also observed by applying the membrane-permeable analogs 8-Br-cyclic-AMP and 8-Br-cGMP, respectively. Furthermore, it was revealed that cAMP-induced increase and cGMP-induced decrease in Po were attenuated by the specific protein kinase A (PKA) inhibitor H-89 and protein kinase G (PKG) inhibitor KT5823, respectively. These results indicate that the K\(_{Na}\) channel is a target molecule for OA and DA and that cAMP/PKA and cGMP/PKG signaling pathways are also involved in the modulation of K\(_{Na}\) channels.

INTRODUCTION

The biogenic amines—dopamine (DA) and octopamine (OA), a monophenolic amine, structurally related to noradrenaline—are known to have prominent cellular functions in both the peripheral nervous system and the CNS. In vertebrate, DA is widely recognized as a neurotransmitter that mediates the reward signals from a wide variety of sensory modalities such as visual, auditory, and somatosensory sensations (Schultz 1998; Schultz et al. 1993; Wise 2004). On the other hand, OA is found only in trace amounts and its function as a true neurotransmitter is unclear in vertebrate. In insect, however, there have been increasing evidences showing that OA mediates unconditioning stimulus (US) or reward (appetitive) signals, whereas DA mediates punishment (aversive) signals in olfactory learning. OA has been widely distributed in the insect nervous system and its roles are often compared with adrenergic receptors of vertebrates (Evans 1985). In honeybee, OA acts as a mediator that conveys sugar information, whereas DA acts as a mediator that conveys electrical shock information. Behavioral and pharmacological experiments with honeybee further revealed that OA injected into the mushroom bodies facilitates olfactory learning and memory retrieval (Bicker and Menzel 1989; Menzel 1990). OA has been shown to be released by ventral unpaired median (VUM) neuron onto antennal lobe and also mushroom body neurons and electrical stimulation of VUM neuron could substitute the US in the proboscis reflex (Kreissl et al. 1994). Hammer and Menzel (1998) provided further evidence that local injection of OA into the mushroom body calyces produces a lasting pairing-specific enhancement of proboscis reflex. Recently, Matsumoto and Mizunami (2000, 2002, 2004) and Matsumoto et al. (2006) have shown that crickets (Gryllus bimaculatus) have high capacities to form olfactory long-term memory. By using both the behavioral and pharmacological techniques, Unoki et al. (2005, 2006) have demonstrated that OA and DA could mediate the reward and punishment signals, respectively, in both olfactory and visual learning. It has also been suggested that the mushroom body intrinsic neurons, called Kenyon cells, are the site of association of conditioning stimulus (CS) and US for both aversive and appetitive conditioning (Schwarzzel et al. 2003) and acetylcholine (ACh) has been postulated to convey CS (olfactory information) from the antennal lobes to the mushroom body Kenyon cell (Kreissl and Bicker 1989). Despite the presence of many studies describing that mushroom body Kenyon cells are the site of CS–US association, few studies have been carried out to investigate the action of putative neurotransmitters mediating CS and US signals and their signaling mechanisms in Kenyon cells. To understand the molecular basis of CS–US association underlying the olfactory learning, it is first necessary to characterize the target molecules—including receptors and ion channels expressed in native Kenyon cells—and then to investigate their modulation by putative neurotransmitters that convey US and CS information. The voltage-dependent ionic channels of Kenyon cells have been previously described (Cayre et al. 1998; Goldberg et al. 1999; Grünewald 2003; Grünewald et al. 2004; Schäfer et al. 1994; Wright and Zhong 1995). Iontropic receptors such as ACh and γ-aminobutyric acid receptors have also been identified in cultured Kenyon cells (Grünewald et al. 2004; Su and Dowd 2003; Wright and Zhong 1995). However, the modulation of those functional molecules has not yet been studied, although the modulatory role of cyclic adenosine monophosphate/protein kinase A (cAMP/PKA) signaling pathways are also involved in the modulation of K\(_{Na}\) channels.
signaling cascade on nicotinic acetylcholine receptor current has been reported (Courjaret and Lapied 2001). Here we describe that $K_{Na}$ channel is an important target molecule for OA and DA and cAMP/PKA and cyclic guanosine 3',5'-monophosphate/protein kinase G (cGMP/PKG) signal cascades are also involved in the modulation of $K_{Na}$ channels.

METHODS

Animals

Experiments were carried out on adult male crickets, *Gryllus bimaculatus*, maintained in a colony in the Department of Biology at 25–30°C with a relative humidity of 65–85% under a 12-h/12-h light/dark photoperiod. Crickets were fed on an artificial insect diet (Oriental Yeast) and supplied with water. The present experiments were performed under the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences, recommended by the Physiological Society of Japan.

Kenyon cell isolation

Adult male crickets were anesthetized using CO₂ before dissection. The brain was carefully removed from the head capsule, taking care not to tear the alimentary tract. The mushroom bodies were then dissected out of the head capsule, taking care not to tear the alimentary tract. The mushroom bodies were then dissected out and placed in a silicone chamber (volume of 3 ml) filled with Ca²⁺-free normal saline and incubated for 15 min. The mushroom bodies were then transferred to the vial tube containing dissociation solution (Sumitomo nerve-cell culture medium, Sumitomo Bakelite). The isolated mushroom bodies were incubated in this culture medium for 30 min at 25°C. After incubation, the pooled mushroom bodies were rinsed with normal saline and dissociated by gentle trituration through a fire-polished pipette with an inner diameter of about 100 μm.

Patch-clamp recording

Patch pipettes were pulled from capillary tubes (G-1.5, Narishige) with a two-stage pipette puller (PC-10, Narishige) and had a tip resistance of about 5 MΩ when filled with a solution for each experiment. Freshly dispersed cells were allowed to settle on the flat-bottom glass slide of a silicone chamber mounted on the stage of an inverted microscope (IX70, Olympus) and the patch electrode was mounted on the cell surface with a three-dimensional hydraulic micromanipulator (MHW-3, Narishige). Single-channel currents were recorded in cell-attached patch configuration, as described in Hamill et al. (1981), through a patch-clamp amplifier (Axopatch 200B). Some experiments were performed in the inside-out configuration by pulling the electrodes from the cell-attached membranes. Current signals were sampled at 5 kHz and were low-pass filtered at 1 kHz (six-pole Bessel). Digitized signals were further analyzed by personal computer using pClamp 9.2 software (Axon Instruments). All experiments were performed at room temperature (20–25°C).

Analysis of single-channel currents

Amplitudes of single-channel currents were measured by eye or a cursor on Clampfit at different holding potentials and the single-channel conductance was measured from the slope of the current-voltage (I–V) relationship. Membrane potentials and reversal potentials are defined as the potential at the cytosolic face of membrane with respect to the potential at the external face of the membrane. Multiple channels were present in every membrane patch. As a consequence, open probability (Po) was expressed as NPo, where N represents the number of single channels, and calculated using the following expression: $N\cdot Po = A_0 + 2A_2 + 3A_3 + \ldots + nA_n$, where $A_n$ is the area under the curve of an all-point amplitude histogram corresponding to the current in the closed state, and $A_0 = A_0$ represents the histogram areas reflecting the different open-state current levels for 1 to $n$ channels present in the patch. Histogram parameters were obtained from multiple least-squares Gaussian fits of the data using Clampfit 9.2 software. The single $K_{Na}$ channel currents recorded from the cell-attached patch membranes often showed subconductance levels of various amplitudes between the closed and the full-open levels. Therefore we focused only on the $K_{Na}$ channel activity with full-open level. Averaged data are expressed as the means ± SE, where $n$ equals the number of patches (cells).

Backfill procedure

In some experiments, Cl⁻ channel blockers [niflumic acid (50 μM), 9-antracencarboxylic acid (9-AC, 50 μM), and 4,4‘-diisothiocyanatoestilbene-2,2‘-disulfonic acid (DIDS, 100 μM)] and K⁺ channel blockers [tetraethylammonium chloride (TEA, 1 or 10 mM) and iberiotoxin (100 nM)] were applied from the outside of the patch membrane by using the backfill procedure. Briefly, tips of freshly pulled pipettes were filled by being placed in a 1-ml sample vial containing a filtered solution (140 mM K⁺) for 2–3 min and then the shanks were backfilled with solutions containing those chemicals. In this experiment, the effects of these chemicals became visible 3–5 min after the backfill. Therefore we measured the effect of these chemicals on a single channel 5 min after the backfill.

Solution and chemicals

Patch-clamp recordings of single K⁺ channel activity were made from cell-attached patches by using pipettes filled with (in mM): 140 KCl and 5 HEPES, buffered to pH 7.4 (Tris). The cell resting potential was zeroed with an external solution containing (in mM): 140 KCl, 10 NaCl, 44 glucose, and 2 HEPES, buffered to pH 7.4 (Tris). In excised inside-out patch recordings, the pipette solution contained (in mM): 140 KCl and 5 HEPES, buffered to pH 7.4 (Tris) and the bath solution contained (in mM): 140 KCl, 10 NaCl, 44 glucose, and 2 HEPES, buffered to pH 7.4 (Tris). In some experiments, extracellular NaCl (10 mM) was replaced with NaCl (0 mM), NaCl (5 mM), NaCl (30 mM), and LiCl (30 mM). Niflumic acid, 9-AC, DIDS, KT5823, N-[2-(p-bromocinnamylamino)ethyl]-5-isouquinoline sulfonamide (H-89), and quinidine were dissolved in dimethylsulfoxide to make stock solutions.

RESULTS

Identification of the K⁺ channel

Single-channel currents were recorded in cell-attached patch with high K⁺ (140 mM) in the patch pipettes at both positive and negative membrane potentials (Fig. 1, A and B). At membrane potentials of +50 and +60 mV, the unitary currents in the outward direction were recorded and they displayed slower open–closed kinetics. On the other hand, the unitary currents in the inward direction were recorded and they displayed burst-like activity with short open times interrupted by very brief closed times at membrane potentials of −50 and −60 mV (Fig. 1B). The amplitudes of single-channel currents were calculated from all-point amplitude histograms at each holding potential and the I–V relationship was obtained by plotting the current amplitude against the membrane potentials (Fig. 1C). The average slope conductance was calculated to be $127 ± 6$ pS ($n = 4–6$) in the potential range −40 to +60 mV.

Ion selectivity

To examine the selectivity of this channel for K⁺, single-channel currents were measured under different external K⁺ concentrations between the closed and the full-open levels. Therefore we focused only on the $K_{Na}$ channel activity with full-open level. Averaged data are expressed as the means ± SE, where $n$ equals the number of patches (cells).
concentrations ([K\textsubscript{o}]) and the I–V relationship was obtained. With 70 mM [K\textsubscript{o}], the slope conductance was 122 ± 5 pS (n = 4–6) in the potential range −70 to +60 mV and the reversal potential obtained by extrapolating the regression line was −15 ± 2 mV (Fig. 2, A\textsubscript{1} and A\textsubscript{2}). With 20 mM [K\textsubscript{o}], the slope conductance was 84 ± 7 pS (n = 4–5) in the potential range −30 to +60 mV and the reversal potential was −40 ± 3 mV (Fig. 2, A\textsubscript{2} and B\textsubscript{2}).

The theoretical values of the reversal potential under these conditions were calculated by using the Nernst equation, assuming that [K\textsubscript{i}] is 140 mM. The formula is: $E = \frac{RT}{zF} \ln \left( \frac{[K\textsubscript{o}]}{[K\textsubscript{i}]} \right)$, where R is the universal gas constant, T is the absolute temperature, F is the Faraday (electric charge per gram equivalent of univalent ions), z is the atomic number, [K\textsubscript{o}] and [K\textsubscript{i}] are the potassium concentrations on each side, and ln is the natural logarithm. The calculated values of reversal potential are as follows: 0 mV at 140 mM [K\textsubscript{o}], −17 mV at 70 mM [K\textsubscript{o}], and −49 mV at 20 mM [K\textsubscript{o}]. These values are close to those obtained by extrapolation of the regression lines at each [K\textsubscript{o}], indicating a high selectivity of this channel for K\textsuperscript{+}.

### Channel properties in inside-out configuration

To determine whether the unitary current activities recorded in cell-attached patch configuration are K\textsuperscript{+} currents passing through Na\textsuperscript{+}-activated K\textsuperscript{+} channels, single-channel currents were recorded in inside-out patch configuration. After excising the membrane into the inside-out configuration, the dependence of K\textsuperscript{+} channel activation on internal Na\textsuperscript{+} concentration was examined under the conditions with the same ionic strength and with the same [K\textsubscript{o}] (120 mM) (Fig. 3, A\textsubscript{1}, A\textsubscript{2}, and A\textsubscript{3}). When the cell-attached recordings had been obtained, the patches were excised and transferred into bath solution containing high Na\textsuperscript{+}. As shown in Fig. 3A, there was little or no channel activity in a nominally Na\textsuperscript{+}-free bathing solution (120 mM KCl, 30 mM choline chloride). When the bathing solution was replaced with a solution containing high Na\textsuperscript{+} (5 mM NaCl, 120 mM KCl, 25 mM choline chloride), channel activity increased (Fig. 3A\textsubscript{2}). Further increase in NaCl (30 mM NaCl, 120 mM choline chloride) in bath solution greatly enhanced the Po of the single-channel currents (Fig. 3A\textsubscript{3}). The single-channel conductance, how-
ever, remained unchanged; the average slope conductance was $130 \pm 8$ pS ($n = 8-10$) with 5 mM NaCl (Fig. 3B1) and $143 \pm 7$ pS ($n = 12-15$) with 30 mM NaCl (Fig. 3B2).

Effects of intracellular Li$^+$ and quinidine

It has been suggested that K$_{Na}$ channels are inhibited by intracellular Li$^+$ (Bishoff et al. 1998). Therefore we investigated the effects of the replacement of intracellular Na$^+$ with an equimolar Li$^+$. Records were obtained from an inside-out patch membrane in a solution containing high Na$^+$ (30 mM NaCl, 120 mM KCl) showing high activities (Fig. 4A1). When all intracellular Na$^+$ was replaced by Li$^+$, the single-channel current activities were drastically reduced (Fig. 4A2). The Po of the K$_{Na}$ channel was 0.693 with 30 mM Na$^+$ and 0.131 with 30 mM Li$^+$. Average values of Po examined from seven different cells were as follows: 0.66 (control) and 0.09 (30 mM Li$^+$). Next we examined the action of quinidine, which is widely used as a K$_{Na}$ channel blocker. Figure 4B1 shows the control record showing unitary activities of K$_{Na}$ channel under the inside-out patch configuration. Intracellular Na$^+$ was 30 mM and the membrane potential was set to +30 mV. When 100 $\mu$M quinidine was applied to the bath solution, the Po of K$_{Na}$ channel currents decreased by 54% ($p = 0.08$) (Fig. 4B2), indicating that the K$_{Na}$ channel in cricket Kenyon cells is quinidine sensitive.

Effects of intracellular Ca$^{2+}$

Figure 5A shows single K$_{Na}$ channel activity obtained from the inside-out patch membrane in the high K$^+$ bath solution containing 30 mM Na$^+$. When this high K$^+$ bath solution was replaced with a high K$^+$ solution that contained 30 mM Na$^+$ and 10 $\mu$M Ca$^{2+}$, the Po of K$_{Na}$ channel was drastically reduced from 0.638 (control) to 0.102 ($p = 0.05$). Average values of Po examined from eight different cells were as follows: 0.65 (control) and 0.09 ($p = 0.05$). As a result of the decrease in Po of K$_{Na}$ channel current by intracellular Ca$^{2+}$, it was difficult to resolve the unitary currents.
Effects of TEA and iberiotoxin

To examine whether $K_{Na}$ channels are inhibited by blockers known to block $K^+$ channels, TEA and iberiotoxin were added to the patch pipettes by the backfill procedure. Recordings of $K^+$ channel activity were made in a cell-attached patch. As shown in Fig. 6A1, the amplitude of the single-channel currents was reduced by adding 1 mM TEA. On increasing the concentration of TEA from 1 to 10 mM, the amplitude of the single-channel currents was further reduced. The average values of relative inhibition of the single-channel current amplitude were 49$\pm$14% ($n$ = 5; 1 mM TEA) and 82$\pm$11% ($n$ = 5; 10 mM TEA) (Fig. 6A2). Next we examined the effect of iberiotoxin, known as a specific blocker for the large-conductance Ca$^{2+}$-activated $K^+$ channel on the unitary current activities (Fig. 6, B1, B2, and B3). All-point amplitude histograms obtained in the absence and presence of iberiotoxin (100 nM; shown in the bottom of each current trace) clearly showed that there is no obvious change in either the amplitude or the Po of single-channel currents.

Effects of OA and DA

We investigated the effect of OA and DA on the unitary current activities of the $K_{Na}$ channel under the cell-attached patch configuration. Figure 7A1 shows control records in the absence of OA at a holding potential of +50 mV. When 1 $\mu$M OA was applied to the bath solution, the channel activities gradually increased (Fig. 7, A2 and A3). As a result, the Po of the $K_{Na}$ channel increased from 0.088 (control) to 0.374 (210 s after) and 0.713 (295 s after) (Fig. 7, B1 and B2). The average values of Po examined from six different cells were as follows: 0.11 (control), 0.39 (200 s after), and 0.68 (300 s after). Next we examined the effect of DA on the unitary current activities of the $K_{Na}$ channel. Figure 7B1 shows control records in the absence of DA, illustrating the activity of the unitary currents at a holding potential of +30 mV. When 1 $\mu$M DA was applied to the bath solution, the Po of the $K_{Na}$ channel currents decreased from 0.389 (control) to 0.158 (1 $\mu$M DA). The average values of Po examined from six different cells were as follows: 0.47 (control) and 0.20 (1 $\mu$M DA).

Effects of 8-Br-cAMP and 8-Br-cGMP

To investigate whether the cAMP and cGMP signaling pathways are involved in the modulation of $K_{Na}$ channel, we examined the effect of 8-bromoguanosine-3',5'-cyclic adenosine (8-Br-cAMP) and 8-bromoguanosine-3',5'-cyclomonophosphate (8-Br-cGMP) on the $K_{Na}$ channel currents. Figure 8A1 shows...
control records illustrating the activity of the unitary currents at +50 mV. When 1 mM 8-Br-cAMP was applied to the bath solution, the Po of KNa channel increased drastically from 0.113 to 0.586 (Fig. 8A2). Next to examine whether the downstream pathway of cAMP involves PKA activation, we investigated the effects of the PKA inhibitor H-89. As shown in Fig. 8A3, the addition of H-89 (1 μM) reduced the Po from 0.586 to 0.155, indicating that the excitatory action of cAMP on the KNa channel is via phosphorylation by PKA. The average values of Po examined from eight different cells were as follows: 0.13 (control), 0.61 (1 mM 8-Br-cAMP), and 0.16 (1 mM 8-Br-cAMP plus H-89).

Next we examined the effect of 8-Br-cGMP on the unitary currents. Figure 8B2 shows control records in the absence of 8-Br-cGMP at a holding potential of +30 mV. When 1 mM 8-Br-cGMP was applied to the bath solution, the Po of KNa channel drastically decreased from 0.483 to 0.088 (Fig. 8B2). To determine whether the inhibition by cGMP involves PKG activation, we investigated the effect of the PKG inhibitor KT5823. As shown in Fig. 8B3, the addition of KT5823 (1 μM) increased the Po from 0.09 to 0.42, indicating that the inhibitory action of cGMP on KNa channels is via phosphorylation by PKG. The average values of Po examined from five different cells were as follows: 0.55 (control), 0.11 (1 mM 8-Br-cGMP), and 0.51 (1 mM 8-Br-cGMP plus KT5823).

**DISCUSSION**

The present study provides the first evidence that OA and DA modulate KNa channels in an opposite way in Kenyon cells isolated from the mushroom body of the cricket *Gryllus bimaculatus*. OA and DA respectively increased and decreased the Po of KNa channels. cAMP/PKA and cGMP/PKG signaling pathways are also found to be involved in the modulation of KNa channels in an opposite way. Thus our results indicate that modulation of the KNa channel is involved in the process of olfactory learning.

**KNa channels in cricket Kenyon cells**

The results presented here provided strong evidence for the presence of KNa channels in Kenyon cells isolated from...
the mushroom body of the cricket *Gryllus bimaculatus*. They possess a large conductance of $143 \pm 7$ pS in 140 mM [K]/140 mM [K] solutions and show little voltage dependence. It was also found that for activation of the channel, intracellular Na is necessary, although Li could not substitute for Na in activation of $K_Na$ channels. The $K_Na$

The K Na channel currents were regulated by cytoplasmic Ca$^{2+}$. Fig. 5A: single-channel currents (Control), B: single-channel currents obtained by switching the bath solution into 10 µM Ca$^{2+}$-containing solution. At this Ca$^{2+}$ concentration there were few channel openings. The holding potential was set to $-50$ mV. Corresponding all-point amplitude histogram is shown in the bottom. C and O are the closed and complete opening levels, respectively.

Fig. 6. Effect of tetraethylammonium chloride (TEA) and iberiotoxin on $K_Na$ channel currents. A1: single-channel currents in the absence (Control) and presence of 1 and 10 mM TEA in the patch pipettes. The holding potential was set to $-30$ mV. A2: relative amplitude of the single-channel currents in the absence and presence of 1 and 10 mM TEA. The amplitude of single-channel currents reduced 49 and 82%, respectively. B1, B2, and B3: single-channel currents in the absence (control) and presence of 100 µM iberiotoxin in the patch pipette. Records were obtained 180 (middle) and 240 s (right) after starting the drug action. The holding potential was set to $-40$ mV. Corresponding all-point amplitude histogram is shown in the bottom. C and O are the closed and complete opening levels, respectively.
Channel currents activated by intracellular Na\(^+\) were first detected in mammalian cardiac cells (Kameyama et al. 1984) and in crayfish neuron (Hartung 1985). Subsequently, similar channels were reported in a variety of neuronal cells (Bader et al. 1985; Bischoff et al. 1998; Dale 1993; Dryer 1991; Dryer et al. 1989; Egan et al. 1992; Haimann et al. 1992; Safronov and Vogel 1996; Schwindt et al. 1989). K\(_{\text{Na}}\) channels are also found in cockroach DUM neurons (Grolleau and Lapied 1994) and in cultured Drosophila neurons (Saito and Wu 1991).

In cardiac myocytes, the K\(_{\text{Na}}\) channel has an EC\(_{50}\) for Na\(^+\) of 66 mM with Hill coefficient of about 3 (Kameyama et al. 1984). In brain stem neurons, the K\(_{\text{Na}}\) channel can be activated by 20 mM intracellular Na\(^+\) and shows no sign of saturation even at 150 mM (Dryer 1991; Dryer et al. 1989; Egan et al. 1992; Haimann et al. 1992; Safronov and Vogel 1996; Schwindt et al. 1989). K\(_{\text{Na}}\) channels are also found in cockroach DUM neurons (Grolleau and Lapied 1994) and in cultured Drosophila neurons (Saito and Wu 1991).

It has been shown that almost all neuronal K\(_{\text{Na}}\) channels so far described are insensitive to intracellular Li\(^+\) (Bischoff et al. 1998; Dryer et al. 1989; Dryer 1991; Haimann et al. 1990; Safronov and Vogel 1996). On the other hand, K\(_{\text{Na}}\) channels in crayfish motoneurons reported by Hartung (1985) were activated by intracellular Li\(^+\) as well as Na\(^+\). The present results showed that the replacement of intracellular Na\(^+\) with Li\(^+\) drastically reduced the Po of the channel currents but the currents still appeared. These results indicate that Li\(^+\) is actually an activator of the channel but a much less effective one than Na\(^+\). It has also been reported that vertebrate K\(_{\text{Na}}\) channels are blocked by intracellular Ca\(^{2+}\) (Dryer et al. 1989; Haimann et al. 1990, 1992). Similarly, the K\(_{\text{Na}}\) channel in cricket Kenyon cells is also found to be blocked by intracellular Ca\(^{2+}\), indicating that a common intracellular mechanism exists between the vertebrate and insect K\(_{\text{Na}}\) channel.

It has been revealed that the single-channel conductance of the K\(_{\text{Na}}\) channel is strongly dependent on [K\(^+\)]\(_{\text{o}}\) and [K\(^+\)]\(_{\text{i}}\) (Dryer et al. 1989; Mistry et al. 1997; Safronov and Vogel 1996).
Similarly, the conductance of the $K_{\text{Na}}$ channel in cricket Kenyon cells changes its value depending on the $[K^+]_o/[K^+]_i$; when $[K^+]_o$ was increased from 20 to 140 mM, the slope conductance increased from $84 \pm 7 \mu S(n=4-5)$ to $127 \pm 6 \mu S(n=4-6)$. The conductance value of $127 \mu S$ with a high $K$ symmetrical condition found in the present preparation is closely similar to the values obtained from that of vertebrate. Thus the properties of $K_{\text{Na}}$ channels in cricket Kenyon cells are quite similar to those reported in many other vertebrates with respect to their conductance value, $[K^+]_o/[K^+]_i$ dependence of single-channel conductance, and $Li^+$ and $Ca^{2+}$ sensitivities. Our results showed that the $K_{\text{Na}}$ channel current activities were most frequently recorded by the cell-attached membranes. This aspect is consistent with the notion reported by Grünewald (2003) that the main component of macroscopic outward current is not $Ca^{2+}$ activated.

**Inhibition by TEA and quinidine**

The present results showed that TEA blocks $K_{\text{Na}}$ channels even at low concentration (1 mM), whereas iberiotoxin had little effect even at high concentration (100 nM) in Kenyon cells isolated from the mushroom body of the cricket Gryllus bimaculatus. The recent molecular biological studies on $K_{\text{Na}}$ channels have revealed that there are two types of genes that encode $K_{\text{Na}}$ channels: the Slack (Slo2.2) and Slick (Slo2.1) genes (Bhattacharjee et al. 2002; Joiner et al. 1998; Yuan et al. 2003). Slick has been shown to be expressed in the nervous system and heart, whereas Slack has selectively been expressed in the nervous system. Single-channel conductance is similar between them and both channels show outward rectification and are activated by intracellular $Na^+$ (Bhattacharjee et al. 2003). In both the Slick and Slack channels, TEA is effective from the outside (Bhattacharjee et al. 2003), whereas 100 nM iberiotoxin is less effective (Bhattacharjee et al. 2003). In this respect, the $K_{\text{Na}}$ channel identified in the cricket Kenyon cells is basically similar to Slick and Slack channels except for the property of outward rectification.

One of the noteworthy properties of the $K_{\text{Na}}$ channels in cricket Kenyon cells is its blockade by quinidine. Quinidine has been shown to inhibit the delayed rectifier $K^+$ current (IK; Balser et al. 1991; Furukawa et al. 1989; Hiraoka et al. 1986; 1996).
Rodent and nonrodent studies have shown that the NO–cGMP pathway stimulates the cAMP pathway to induce long-term memory (LTM) in the cricket Kenyon cells (Matsuzaki et al. 2006). The involvement of cAMP and cGMP signaling pathways in Drosophila, Aplysia, and fruit flies has also been reported with the use of whole cell patch-clamp technique (Schafer et al. 1994). Therefore it seems possible that the quinidine-sensitive \( K_{\text{Na}} \) channel may underlie the major component of whole cell outward current in Kenyon cells.

**Involvement of cAMP and cGMP signaling pathways**

Santi et al. (2006) clarified that \( K_{\text{Na}} \) channels are important target molecules for neuromodulators through G-protein-coupled receptors (GqPCRs) and implicated their important role in the effect of long-lasting changes on neuronal excitability. Prevention studies on honey bees (Hammer and Menzel 1998; Kreissl et al. 1994; Menzel et al. 2001) and fruit flies Drosophila (Schwaerzl et al. 2003) suggest that acquisition with sugar depends on the octopaminergic system, whereas acquisition with electric shock depends on the dopaminergic system. The VUM neuron in the subesophageal ganglion has been shown to be octopaminergic and it carries the appetitive (sucrose) signals (Menzel et al. 1999). Direct injection of OA in termination areas of this neuron could substitute presentation of a sucrose reward in olfactory condition (Hammer and Menzel 1998). In cricket, it is also suggested that OA and DA act as neurotransmitters that convey the reward and punishment signals (Unoki et al. 2005). A molecular biological study using RNA interference of OA receptors further confirms this aspect (Farooqui et al. 2003). In many species, including mollusca Aplysia, fruit flies Drosophila, and mice, suggest that formation of long-term memory requires an increase in intracellular cAMP and recruitment of the PKA that phosphorylates the transcription factor, cAMP-responsive element-binding protein, CREB (Abel et al. 1998; Bartsch et al. 1995; Yin et al. 1995). Participation of mushroom bodies in learning and memory is considered to involve the cAMP signaling pathway because the affected genes of three Drosophila mutants—dance, rutabaga, and DCO—all defective in learning, encode for the enzymes cAMP phosphodiesterase, adenylyl cyclase, and protein kinase A (Davis 1996; Han et al. 1992; Nighorn et al. 1991; Skoulakis et al. 1993). The importance of cGMP in various physiological functions in brain is also implicated (Hofmann et al. 2000; Schmidt and Walter 1994). The most extensively studied cGMP signal transduction pathway is that triggered by nitric oxide (NO) (Bredt and Snyder 1990). Matsumoto et al. (2006) have shown that the NO–cGMP pathway stimulates the cAMP pathway to induce long-term memory (LTM) in the cricket Gryllus bimaculatus. They have implicated that the NO–cGMP pathway activates the adenyll cyclase (AC)–cAMP–PKA–CREB signaling pathway via cyclic nucleotide-gated channel and \( Ca^{2+} \)-CaM and thereby results in protein-synthesis-dependent LTM. The present studies show that 8-Br-cAMP increased the Po of \( K_{\text{Na}} \) channel currents, whereas 8-Br-cGMP decreased the Po of \( K_{\text{Na}} \) channel currents. It has to be determined whether OA and DA receptor activation triggers the signal cascade of cAMP/PKA and cGMP/PKG, respectively, or whether DA receptor activation triggers the signal cascade that leads to inhibition of AC.

**Physiological significance of the modulation of \( K_{\text{Na}} \) channel**

A physiological role for \( K_{\text{Na}} \) channels identified in Kenyon cells isolated from the cricket mushroom bodies is not yet clear. In other neuronal cells, it has been proposed that \( Na^{+} \) influx through voltage-gated \( Na^{+} \) channels during an action potential may produce a transient activation of \( K_{\text{Na}} \) channels, resulting in action potential repolarization (Bader et al. 1985; Dryer et al. 1989). The present studies have revealed that Po of \( K_{\text{Na}} \) channels is increased and decreased by OA and DA, respectively. Therefore it can be considered that increased Po by OA may result in a shortening of \( Na^{+} \)-dependent action potential duration, whereas DA results in a prolongation of action potential duration. Further studies are still necessary to clarify whether the possible change of action potential shape occurs when we associate CS and US in Kenyon cells.

In conclusion, we have shown that OA, which mediates reward information, increases the Po of \( K_{\text{Na}} \) channel, whereas DA, which mediates a punishment signal, decreases the Po of \( K_{\text{Na}} \) channel in cricket Kenyon cells. cAMP/PKA and cGMP/PKG signaling pathways are also found to be involved in the modulation of \( K_{\text{Na}} \) channel, probably via phosphorylation of target protein (\( K_{\text{Na}} \) channel) (Fig. 9).

![Diagram](J Neurophysiol • VOL 100 • SEPTEMBER 2008 • www.jn.org)

**FIG. 9.** Scheme illustrating the effects of OA, DA, cAMP, and cGMP on \( K_{\text{Na}} \) channel in cricket Kenyon cells. OA receptor (OAR) activation increases the Po of \( K_{\text{Na}} \) channel, whereas DA receptor (DAR) activation decreases the Po of \( K_{\text{Na}} \) channel. cAMP/PKA (protein kinase A) signaling pathway increase Po, whereas cGMP/PKG (protein kinase G) signaling pathway decreases the Po of \( K_{\text{Na}} \) channel probably via phosphorylation of target protein (\( K_{\text{Na}} \) channel). Arrowheads indicate the excitatory effect, whereas closed circles indicate the inhibitory effect on \( K_{\text{Na}} \) channels.
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REFERENCES


