J. Biochem. Mol. Biol. Vol. 29, No. 2 pp. 151~155 March 31, 1996

Effect of Dopamine on a Voltage-Gated Potassium Channel in a Jellyfish Motor Neuron

Jun-mo Chung* and Andrew N. Spencer[1]

Department of Biology, College of Natural Sciences, Ewha Womans University, Seoul 120-750, Korea
[1]Department of Biological Sciences, The University of Alberta, Edmonton, ALTA, Canada T6G 2E9 and Bamfield Marine Station, Bamfield, BC, Canada V0R 1B0
(Received December 15, 1995)

Abstract: To swimming motor neurons (SMNs) of *Polyorchis penicillatus*, a hydrozoan medusae, dopamine (DA) acts as an inhibitory neurotransmitter by hyperpolarizing its membrane potential and decreasing its firing rate as well. Such an inhibitory action of DA is caused by an increased permeability to potassium (K) ions. To investigate whether voltage-gated K channels are directly responsible for the membrane hyperpolarization induced by DA, we employed whole-cell voltage clamp configuration. One μM DA applied to SMNs increased the peak and rear values of voltage-gated K currents by 37 and 54%, respectively, in a reversible manner. Combined with subtraction analysis, this result suggests that the outflux of K ions by DA in SMNs occurs mainly through rectifier-like K channels.
Key words: catecholamines, cnidaria, K rectifier, voltage-sensitive potassium channel, whole-cell voltage clamp.

Previous studies (Chung and Spencer, 1991a and 1991b) have shown that dopamine (DA) has an inhibitory action on cultured swimming motor neurons (SMNs) of *Polyorchis penicillatus* through a D_2-like receptor. Applied DA caused membrane hyperpolarization due to increased potassium (K) conductance. This resulted in total inhibition or reduction of spiking frequency after anodal break excitation. Inhibition was associated with decreased spike duration. These observations on the physiological actions of DA combined with an HPLC, GC/MS spectrometric study (Chung *et al.*, 1989) which demonstrated the presence of DA in nerve-rich tissues of *P. penicillatus*, suggest that DA may act as a neurotransmitter and/or neuromodulator in this jellyfish.

Although inhibitory actions of DA in this animal are surely mediated through the changes of K conductance, the exact nature of the channel responsible for the DA-induced K current is not clear. There are several ways to explain the underlying mechanism of DA responses related to the channel properties. *First*, DA may increase the conductance of ligand-gated K channels, resulting in membrane hyperpolarization. The membrane hyperpolarization may then remove steady-state inactivation of a purely voltage-activated K channel, and as a result, spike duration decreases. *Second*, DA may alter cellular activity by acting directly on voltage-dependent K channels. For example, DA increases delayed rectifying and transient K currents in rat lactotroph cells (Lledo *et al.*, 1989) and transient K currents in the MMQ clonal pituitary cells (Login *et al.*, 1990). The observation that action potential duration was decreased during exposure to DA could also suggest modulation of voltage-sensitive currents. *Third*, it is possible that voltage-sensitive, ligand-gated K channels may be present in jellyfish motor neurons. We examined the effect of DA on voltage-gated K channels as the first step to find out the candidate mechanism which may describe best the nature of the DA-induced K current in this animal. We here represent the evidence that a voltage-gated K channel is directly modified by DA.

*To whom correspondence should be addressed.
Tel: 82-2-360-2395/2944, Fax: 82-2-360-2385/3110.
Email: jmchung@mm.ewha.ac.kr

Materials and Methods

Cell dissociation and culture

The method for isolating SMNs from *P. penicillatus* has been described elsewhere (Przysiezniak and Spencer, 1989). These isolated SMNs were plated to Petri dishes (Falcon 1008, 35×10 mm) coated with homogenized mesoglea and maintained for up to 6 days in artificial sea water (ASW) containing gentamycin sulfate (0.005%, w/v). The ASW contained (in mM): 378 NaCl, 9.5 $CaCl_2$, 5.7 Na_2SO_4, 13.4 KCl, 29 $MgCl_2$, 42 choline chloride, 10 HEPES, 5 NaOH; and had a pH

of 7.5.

Electrophysiological recording

Whole-cell voltage-clamp recordings of DA-induced or voltage-gated K currents were obtained from cultured SMNs with a List L/M-EPC 7 amplifier (Medical Systems Corp., Greenvale, NY, USA) at room temperature (18~20℃). Patch electrodes (1.0~3.0 MΩ) were pulled on a Narishige electrode puller PP-83 (Narishige, Tokyo, Japan) from non-heparinized capillary tubing (I.D. 1.1 mm, O.D. 1.2 mm). The electrode solution contained (in mM) 1 $CaCl_2$, 105 KCl, 2 $MgCl_2$, 10 HEPES, 11 EGTA, 35 KOH, 640 glucose and had a pH of 7.5. The total osmolarity of the electrode solution was 950 mosmol. The ASW was used as an external solution for the recordings of DA-induced current. For the recordings of voltage-gated K currents, a Na /Ca-free ASW with high K (HKSW) was adopted as an external saline to remove inward currents; NaCl and Na_2SO_4 were replaced with choline chloride since voltage-gated Na channels of SMNs are not sensitive at all to tetrodotoxin (Przysiezniak and Spencer, 1992), a well-known Na channel blocker; $CoCl_2$ was added in order to avoid possible actions of residual Ca during saline exchanges. To minimize the error due to series resistance, K concentration was also increased here from 13.4 to 55.4 mM, resulting in a reduction in the amplitude of the voltage-gated K currents. It contained (in mM) 55.4 KCl, 29 $MgCl_2$, 386.5 choline chloride, 10 HEPES, 5 KOH, 9.5 $CoCl_2$ and had a pH of 7.5. Its osmolarity was approximately 1020 mosmol.

For a proper space clamping, SMNs were chosen that were relatively compact with a few processes with lengths shorter than 20 μm. Note that recordings were obtained from cells which were approximately 150 μm in length and 50 μm in width. One mV liquid junction potential between the electrode and bath solutions was corrected electronically with the electrode in the bath. Voltage pulses were produced and data were acquired through a Lab-master TL-1 interface (Axon Instruments, Forster city, CA, USA) connected to a PC, using pClamp software (Axon Instruments). Leakage and capacitive currents were subtracted on-line from active responses using a P/4 protocol (Benzanilla and Armstrong, 1977). DA-induced currents were recorded continuously on a Gould Brush 2400 pen recorder and stored on VCR tapes using a MTS VCP (model MCR-220R) and ADC VCR recorder adapter (PCM-2, Medical Systems Corp.). Recorded signals were displayed on a Tektronix 5223 digitizing oscilloscope.

The stock solutions of DA (0.1 M) were prepared in deionized double-distilled water and were frozen at −20℃ after being divided into aliquots of 500 μl. The pH of the stock solution was 6.8 and the pH of the working DA solution was 7.5. The working DA solution in vehicles oxidized easily. However, antioxidants such as ascorbic acid were not used in these experiments to avoid any possible interfering effect of pH (Anderson and McKay, 1985; Spencer, 1988). Instead, the working DA solutions were kept in a dark box at 4℃ and were prepared freshly every 4h during the experiment. DA solutions were introduced using home-made plastic fine tubings (O.D. < 0.3 mm) into capillary pipets which were pulled from aluminosilicate glass (AM Systems; O.D. 1.5 mm, I.D. 0.6 mm). A fluid stream from the pipet was obtained by applying 69~138 KPa pressure to the pipet using a Picospritzer (General Valve Corp.). A rapid perfusion system similar to Johnson and Ascher's (1987) consisted of 5 polyethylene tubes (PE intramedic #7401, I.D. 0.28 mm) arranged in parallel in one plane. Changes of solution were accomplished by aligning various barrels with the cell (For further details see Chung and Spencer, 1991b).

Results and Discussion

Characteristics of DA-induced outward currents

Typical DA-induced outward currents evoked by 1 mM DA from a SMN which was voltage-clamped at −20 and 20 mV are shown in the inset of Fig. 1. The DA-induced current (0.80 nA) at 20 mV of holding potential (V_h) was much larger than that (0.18 nA) at −20 mV of V_h, showing that the DA responses

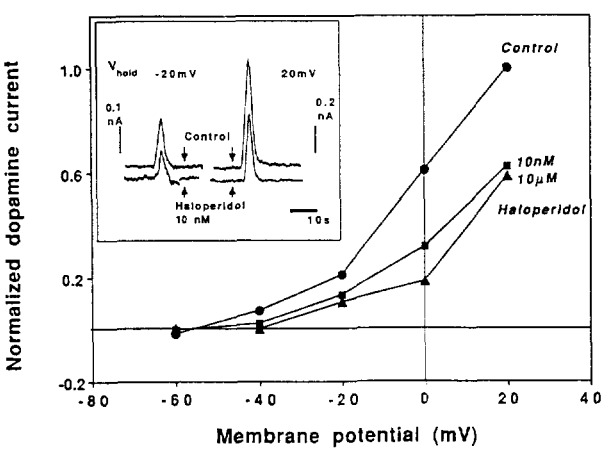

Fig. 1. Current-voltage plot showing the effect of haloperidol on the DA-induced current. The inset shows the effects of haloperidol on the DA-induced current occurring at two different holding potentials. [n.b. Different amplitude scales were used; 0.1 & 0.2 nA scales were for the outward currents at −20 mV & 20 mV, respectively.] One μM of DA was applied for 2s to the cell, using a Picospritzer (pressure, 138 KPa), to evoke DA-induced responses. Changing the solutions from NASW (●) to 10 nM (■) and 10 μM (▲) of haloperidol was accomplished using a rapid exchange system. N=2.

Table 1. The percentage reduction of DA-induced currents by haloperidol

Holding potential (V_h, mV)	Concentrations of haloperidol	
	10 nM	10 μM
−20	39	59
0	49	70
20	35	45
Mean[a]/S.E.M.	41/4	58/7

[a]$p<0.05$, *t*-test.

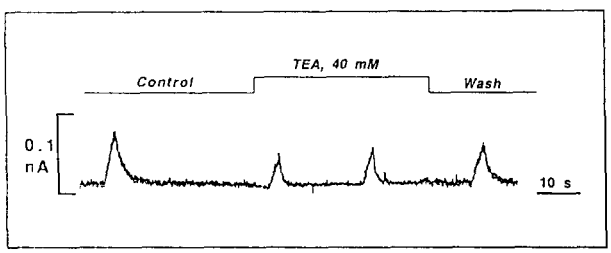

Fig. 2. TEA reduces the DA-induced outward currents. One mM of DA applied for 2s using a Picospritzer (pressure, 138 KPa) evoked an outward current from a SMN which was held at −20 mV. When the neuron was exposed to 40 mM tetraethylammonium chloride (TEA) solution using a rapid exchange perfusion system, the amplitudes of the DA-induced outward currents were reduced by 40%.

are voltage-sensitive as seen in the previous study (Chung and Spencer, 1991a). Reversal potentials of the DA-induced currents were about −55 mV, which were close to the K equilibrium potential (Fig. 1). Ionic substitution experiments previously showed that DA-induced currents in SMNs are carried by K ions (Chung and Spencer, 1991a). Haloperidol, a D_2-receptor blocker, decreased the DA-induced current in a concentration-dependent manner; at V_h of −20 mV, 10 nM and 10 mM haloperidols reduced the DA responses by 39 and 59%, respectively (the inset of Fig. 1). Since DA responses were voltage-sensitive, we examined whether haloperidol would block the DA response in a voltage-dependent manner. No significant voltage-dependency of haloperidol blockade was observed (Table 1).

The effect of DA on voltage-gated K currents

A portion of the DA-induced current was sensitive to tetraethylammonium (TEA). Fig. 2 shows that DA-induced currents from a SMN were reduced drastically by 40 mM TEA. It does not necessarily mean that the TEA-sensitive portions of DA-induced currents are voltage-dependent. However, it intrigued us to examine the possible effect of DA on voltage-gated K channels in SMNs because many voltage-dependent K currents are known to be TEA-sensitive (Hille, 1984) and to

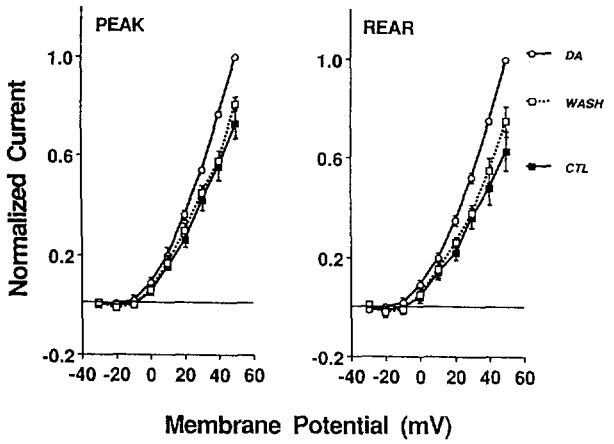

Fig. 3. Current-voltage relations of voltage-gated K currents. Step depolarizing pulses of −30 to 50 mV in 10-mV increments, 450 ms duration, were applied from a holding potential of −40 mV. DA (1 μM) was applied for 6 min using a Picospritzer (pressure 138 KPa) and immediately followed by a wash. Current-voltage relations for the peak and the rear values measured at 400 ms after the pulse of the elicited-K currents appear in the left and right panels, respectively. Note that DA appears to increase the rear portions of the currents more than the peaks. N=10; error bars, SEM; ■, control; ○, in the presence of DA; □, wash.

be increased by DA in several other preparations (Lledo *et al.*, 1989; Login *et al.*, 1990).

Voltage-sensitive outward currents were elicited from SMNs when step pulses were applied in 10-mV increments from a V_h of −40 mV. The outward currents reached peaks within 50 to 60-ms after the initiation of stimulus pulse and declined to a steady-state after 380-ms of the pulses (refer to current traces in Fig. 4). It suggests that the outward current consist of more than 2 kinetically different components. Current-voltage (I-V) relations of the currents were, therefore, obtained from 10 different cells using either the peak values or the rear ones measured at the end of the 400-ms step pulse (Fig. 3). Reversal potentials of the I-V curves obtained from either peak or rear values of the currents were very close to the calculated K equilibrium potential (−16 mV) at 18℃, indicating that the outward currents were mediated by K ions. Fig. 3 clearly shows the enhancing effect of DA on the voltage-gated K currents. When 1 mM of DA solution was continuously delivered to the cell using a Picospritzer, the outward currents increased significantly by 37 and 54% at the peak and the rear respectively, which was voltage-independent (Table 2). The completion of DA application was immediately followed by perfusion of the bath with a fast perfusion system. Even though separation of the outward currents, either pharmacologically and kinetically, were not attempted in this study, it was most likely that the voltage-gated K currents arose from more than 2 different K channels such as a transient

Table 2. The percentage increase of volage-gated K currents by DA

The amplitude of step pulse, mV	The percentage of increase	
	Peak	Rear
90	40	61
80	42	58
70	30	47
60	42	61
50	33	42
Mean[a]/S.E.M.	37/3	54/4

[a]$p<0.001$, *t*-test.

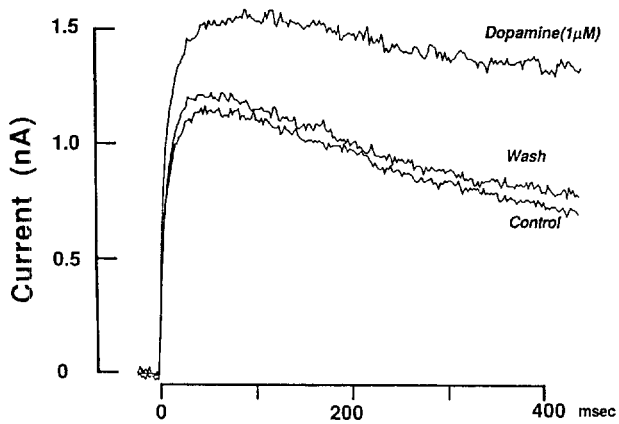

Fig. 4. The effect of DA on voltage-gated K current. Voltage-gated K currents elicited by 80-mV step pulses from −40 mV of V_h as described in Fig. 3. Superimposed are current traces obtained in either the presence or absence of DA, which clearly shows the increase of voltage-gated K currents by DA.

K (A-like) channel and K rectifier-like one. Then the simple question is which ion channels are mainly affected by DA. If DA enhanced only the K current (I_{fast}) which was mediated by A-like channels, voltage-gated K currents should have been much more enhanced by DA at the peak than the rear where A-like channels would be completely inactivated. Our results cannot be comparable with this prediction. The partial decrease of DA-induced K currents by TEA suggests that the DA-induced currents contain voltage-gated K rectifier-like current (I_{slow}), although pharmacological properties of I_{slow} were not examined in detail. The idea that DA enhances I_{slow} was further supported by the obvious DA-induced increases of voltage-gated K currents at the rear. The smaller increases of the current at the peak than at the rear might be due to a simple addition of the increased I_{slow} to voltage-gated K currents. However, the smaller increase of the peak current would also happen if DA were less effective to I_{fast} than I_{slow}. A subtraction analysis was employed to solve this problem.

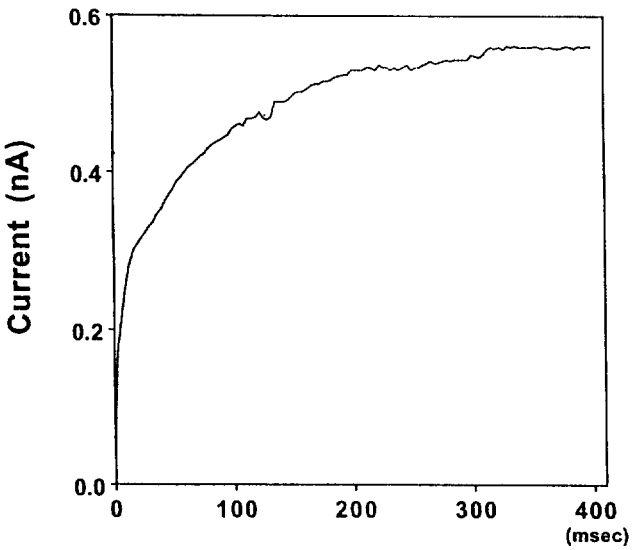

Fig. 5. Voltage-gated K current enhanced by DA. Reconstructed is a current trace by subtracting the control trace from the K current one obtained in the presence of DA in Fig. 4. This, corresponding to the portion of K current increased directly by DA, looks likely the well-known K rectifier. Sampled at 0.3 KHz, recorded at low-amplification.

A typical voltage-gated K current elicited by a 80-mV depolarizing step pulse (450-ms in duration) from a V_h of −40 mV appears as a control trace in Fig. 4. The K current reached a maximum of 1.13 nA at 50-ms after the onset of the step pulse and slowly decreased to a steady-state (0.75 nA at 400-ms). Superimposed in Fig. 4 are the outward currents obtained during DA application and after wash. The maximum (1.54 nA) of the outward current in the presence of DA appeared at 85-ms after the beginning of the pulse while the current was 1.47 nA at 50-ms which was the time to peak in the control. The control trace was subtracted from the outward current trace obtained in the presence of DA, remaining a DA-enhanced outward current (Fig. 5). The subtracted outward current was apparently comparable to a voltage-gated K rectifier. It was therefore concluded from these results that major contribution of DA-enhanced current comes from I_{slow}.

DA responses related to channel properties

Against a hydrozoan SMN, DA seems primarily to increase the conductance of I_{slow}, resulting in membrane hyperpolarization. Such a membrane hyperpolarization removes steady-state inactivation of I_{fast}. The disinactivation of I_{fast} indirectly induced by DA may be responsible for a shortening of spike duration, since it was suggested that unmasking of a fast, transient K current was responsible for duration decrease (Spencer *et al.*, 1989).

Now several questions concerning the modulation of K channels by DA remain unanswered. Does DA increase the conductance of I_{slow} directly or through a second messenger system? Are there any K channels which are gated by voltage as well as ligand such as DA? It is also possible that other K channels such as Ca-activated K ones are present and responsible for spike-shortening in SMNs. A thorough understanding of these questions will come with further knowledge of biophysical and pharmacological properties of K channels in the hydrozoan SMN.

Acknowledgement

We thank Bamfield Marine Station for providing its facilities. This study was supported mainly by National Science and Engineering Research Council of Canada grant to ANS and partly by Ewha Womans University grant to JMC.

References

Anderson, P. A. V. and McKay, M. C. (1985) *Biol. Bull. Mar. Biol. Lab. Woods Hole* **169**, 652.

Benzanilla, F. and Armstrong, C. M. (1977) *J. Gen. Physiol.* **70**, 549.

Chung, J. M. and Spencer, A. N. (1991a) *J. Exp. Biol.* **156**, 433.

Chung, J. M. and Spencer, A. N. (1991b) *J. Comp. Physiol. A* **169**, 599.

Chung, J. M., Spencer, A. N. and Gahm, K. H. (1989) *J. Comp. Physiol. B* **159**, 173.

Johnson, J. W. and Ascher, P. (1987) *Nature* **325**, 529.

Hille, B. (1984) in Ionic Channels of Excitable Membrane, pp. 280-285, Sinauer Associates Inc., Sunderland, Massachusetts.

Lledo, P. M., Legendre, P., Zhang, J., Israel, J. M. and Vincent, J. D. (1989) *Pflügers Arch.* **414** (Suppl.), 160.

Login, I. S., Pancrazio, J. J. and Kim, Y. I. (1990) *Brain Res.* **506**, 331.

Przysiezniak, J. and Spencer, A. N. (1989) *J. Exp. Biol.* **142**, 97.

Przysiezniak, J. and Spencer, A. N. (1992) *J. Neurosci.* **12**, 2065.

Spencer, A. N. (1988) *Can. J. Zool.* **66**, 639.

Spencer, A. N., Przysiezniak, J., Acosta-Urquidi, J. and Basarsky, T. A. (1989) *Nature* **340**, 636.