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Action of Dopamine as Inhibitory Neuromodulator in Jellyfish Synapse

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Dopamine (DA) acts on swimming motor neurons (SMNs) of *Polyorchis penicillatus* as an inhibitory neurotransmitter by hyperpolarizing their membrane potentials, which results from the activation of voltage-sensitive potassium channels mediated through a D_2-type receptor. In addition, DA, and not the hyperpolarized membrane potential, directly decreased the input resistance of SMNs by ca. 50% from 1.42 to 0.68 GΩ. It strongly indicates that DA can shunt other excitatory synaptic signals onto SMNs where DA usually elicited much greater responses in their neurites than soma. All these evidences suggest that DA may operate in this primitive nervous system in dual modes as an inhibitory neurotransmitter and neuromodulator as well.

Keywords: Catecholamines, Cnidarian neurons, Input resistance, Shunt, Synaptic efficacy.

Introduction

Although there has been extensive effort by neurobiologists for the past four decades to determine the identity of neurotransmitters in the cnidarian nervous system because of the importance of their evolutionary position, we have to admit that no complete picture has yet been drawn. For the last decade, however, a series of studies have shown that dopamine (DA), and not other biogenic amines, may act as an inhibitory neurotransmitter in the hydromedusa *Polyorchis penicillatus*: (1) DA is present in the bell margin of the jellyfish where most neurons are concentrated (Chung *et al.*, 1989); (2) Dopaminergic cells are present in the bell margin (Chung, 1995); (3) Exogenously applied DA to swimming motor neurons (SMNs) of this animal produces membrane hyperpolarization by increasing potassium ion (K) permeability (Chung and Spencer, 1991a); (4) This inhibitory action of DA is mainly mediated through D_2-type receptors (Chung and Spencer, 1991b); (5) K-rectifier, one of the voltage-gated K-channels present in this animal (Przysiezniak and Spencer, 1994), is directly responsible for the membrane hyperpolarization induced by DA (Chung and Spencer, 1996).

In addition to its action as a neurotransmitter, DA is well-known to exert a number of effects which are considered to be neuromodulatory, i.e., they modify the efficacy of another process (Kupfermann, 1979). Among its modulatory actions, DA has been shown to decrease glutamate release at the excitatory synapse in crustaceans (Miller *et al.*, 1985), reduce junctional conductance in horizontal cell electrical synapses (McMahon and Mattson, 1996), and weaken the GABAergic inhibition of substantia nigra pars reticulata neurons (Martin and Waszczak, 1996). Very recently, Kemnitz (1997) also reported that DA modulates the cycle period of fictive swimming of lamprey by reducing the late after-hyperpolarization of spinal neurons. In this study, DA appears not to have any effect on the input resistance of the motor neurons in the lamprey spinal cord. However, interestingly, DA is known to modulate neuromuscular synapses by increasing muscle fiber input resistance in crustaceans (Lingle, 1981). This difference in DA action may reflect one of the variations between invertebral and vertebral neurons. Therefore, we examined whether or not DA acts as a neuromodulator by affecting the input conductance of SMNs of *P. penicillatus*, one of the primitive invertebrates.

Here, we present the results, obtained from using a very easy, direct, and simple way to determine the exact action of DA on the input resistance, showing that DA may play

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a role as a synaptic shunter in jellyfish synapses by decreasing the input resistance of a target neuron.

Materials and Methods

Cell dissociation and culture The method for isolating SMNs from *P. penicillatus* has been described elsewhere (Przysiezniak and Spencer, 1989). Isolated SMNs were plated to Petri-dishes (Falcon 1008, 35 × 10 mm) coated with homogenized mesoglea and maintained for up to 6 d in artificial sea water (ASW) containing gentamycin sulfate (0.005%, w/v). The ASW contained: 378 mM NaCl, 9.5 mM $CaCl_2$, 5.7 mM Na_2SO_4, 13.4 mM KCl, 29 mM $MgCl_2$, 42 mM choline chloride, 10 mM HEPES, 5 mM NaOH, and had a pH of 7.5.

Electrophysiological recording Intracellular recordings from cultured SMNs were obtained by switching from the voltage-clamp mode to the current-clamp mode in the whole-cell recording configuration, using a List L/M-EPC 7 amplifier (Medical Systems Corp., Greenvale, USA). Patch electrodes (1.0–3.0 MΩ) were pulled on a Narishige electrode puller PP-83 (Narishige, Tokyo, Japan) from nonheparinized capillary tubing (I.D. 1.1 mm, O.D. 0.2 mm). The electrode solution contained: 1 mM $CaCl_2$, 105 mM KCl, 2 mM $MgCl_2$, 10 mM HEPES, 11 mM EGTA, 35 mM KOH, 640 mM glucose, and had a pH of 7.5.

Current pulses were produced and data were acquired through a Lab-master TL-1 interface (Axon Instruments, Forster City, USA) connected to a PC, using pClamp software (Axon Instruments). In addition, all electrical signals displayed on a Tektronix 5223 digitizing oscilloscope were recorded continuously on a Gould Brush 2400 pen recorder and stored on VCR tapes using an MTS VCP (model MCR-220R) and ADC VCR recorder adapter (PCM-2, Medical Systems Corp., Greenvale, USA).

The stock solutions of DA (0.1 M) were prepared in deionized double-distilled water and frozen at −20°C after being divided into aliquots of 500 μl. The pH values of the stock and working solutions were 6.8 and 7.5, respectively. The working DA solution in vehicles oxidized easily. However, antioxidants such as ascorbic acid were not used in these experiments. Instead, the working DA solutions were kept in a dark box at 4°C and were prepared freshly every 4 h during the experiment. DA solutions were introduced using home-made plastic fine tubings (O.D. < 0.3 mm) into capillary pipettes which were pulled from aluminosilicate glass (AM Systems, Toledo, USA; O.D. 1.5 mm, I.D. 0.6 mm). A fluid stream from the pipette was obtained by applying 69–138 KPa pressure to the pipette using a Picospritzer (General Valve Corp., Fairfield, USA). A rapid perfusion system 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.

Results and Discussion

Membrane hyperpolarization induced by DA through the D_2-like receptor Switching from voltage-clamp to current-clamp mode evoked "anodal break" spikes when swimming motor neurons (SMNs) were held at −60 mV in the voltage-clamp mode (Fig. 1). Pulses (10 μM, 5 s) of DA applied to SMNs during the anodal break excitation produced hyperpolarizations of 12.5 mV which were associated with a complete inhibition of firings (Fig. 1A). Control applications of ASW, which was used as a DA vehicle, did not normally produce any responses (data not shown). Haloperidol (10 nM), one of the well-known D_2-receptor blockers, reduced the dopamine-induced hyperpolarization by 20% (Fig. 1B), indicating that the DA-induced hyperpolarization was mediated by a D_2-type receptor in mammals as previously shown in other studies (Chung and Spencer, 1991b). Removal of haloperidol only partially restored the excitability of the cell (Fig. 1C). In most cases (N = 7), cells did not recover their

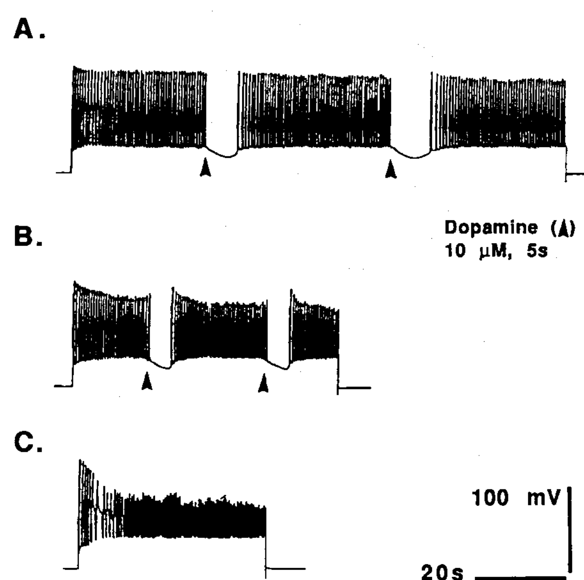

Fig. 1. The effects of haloperidol on DA-induced inhibition. A. The first application of DA (10 μM) applied for 5 s using a Picospritzer (pressure, 138 KPa) resulted in membrane hyperpolarization (12.5 mV and 7.5 s). The time (τ_p) to reach the peak amplitude was 5 s. The second application of DA resulted in a membrane hyperpolarization of 12.5 mV and 10 s. τ_p was 6 s. During this experiment, the cell was continuously perfused with ASW by a rapid solution exchange system consisting of 5 barrels; the first to third barrels contained ASW and the fourth and fifth barrels contained 10 nM of haloperidol solution. B. After the change of solutions from the ASW to haloperidol, the first application of DA generated a smaller and shorter membrane hyperpolarization of 10 mV and 5 s. τ_p was 4.5 s. The second application of DA also generated a smaller hyperpolarization of 10 mV and 6 s; its τ_p was 5 s. C. Even 10 min after changing the solution from haloperidol to ASW, the cell did not recover its excitability completely on an anodal break stimulation which was obtained by switching from the voltage clamp mode (holding potential −60 mV) to current clamp. [Note: during the washing step, the fourth and fifth barrels delivering haloperidol solutions were blocked using a three-way stopcock.]

responsiveness to DA after they were exposed to haloperidol. This lack of reversibility was more noticeable when higher concentrations of haloperidol were used (data not shown). Even after prolonged washing, cells did not recover their activity (Fig. 1C). Considering the high hydrophobicity of haloperidols, it is probable that high concentrations of haloperidols elicited a variety of nonspecific actions, such as fluidization of the membrane, which might destroy the membrane's excitability.

Direct action of DA on the input resistance of SMNs DA also elicited a decrease in the input resistance of SMNs. A series of current pulses of 2 s duration and 10 pA incrementing-steps from −30 to 0 pA produced anodal break spikes following passive hyperpolarizing responses and spontaneous spikes from a SMN in ASW (Fig. 2A). However, current pulses from −40 to 10 pA failed to generate a spike and the hyperpolarizing steps were of decreased amplitude in the presence of DA (Fig. 2B). After washing, the cell recovered its excitability (Fig. 2C). From the relationship of the current stimulations to the voltage traces, the input resistance of the cell was calculated to be 1.4 GΩ (Fig. 2D). The input resistance changed from 1.42 to 0.68 GΩ in the presence of 10 μM of DA and returned to its control value after washing.

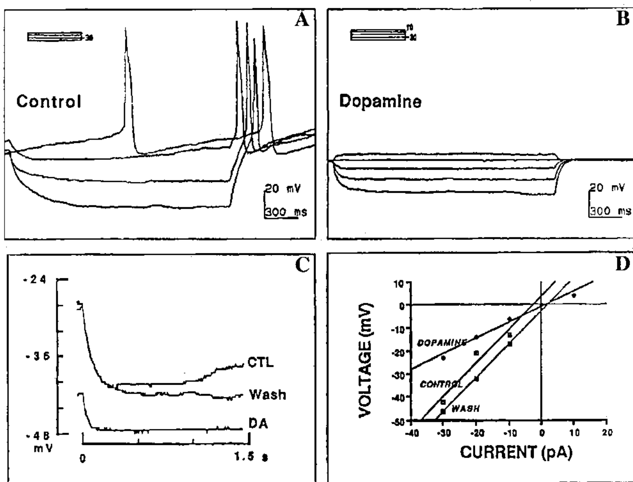

Fig. 2. The effects of DA on the input resistance of SMNs. A. Control voltage traces produced by a series of current pulses of 2 s duration starting at −30 pA, increasing in 10 pA steps. The responses to negative current stimulations of 10 pA or more produce anodal break action potentials. B. In the presence of DA (10 μM), the same current pulses produced smaller voltage deflections and did not generate any anodal break discharges. DA also hyperpolarized the membrane potential. C. Voltage-traces to current pulses of −10 pA under control conditions, with DA, and after washing. Digitized sweeps for the control (CTL) and dopamine (DA) were reconstructed using the hyperpolarizing responses to a current step of –10 pA that appeared in A and B. In the presence of DA, the base membrane potential was drastically hyperpolarized from −28 to −42 mV and the hyperpolarizing response to the current pulse was decreased, implying that the input resistance decreased. D. Voltage values at 500 ms after the start of current pulses were selected to measure the input resistance. The input resistance changed from 1.42 to 0.68 GΩ by DA and returned to its control value after washing (1.42 GΩ).

The decrease of input resistance could be due to a direct action of DA or indirectly by altering the membrane potential. To examine this, the membrane potential was manually manipulated by injecting direct current into a cell in order to simulate hyperpolarization by DA (Fig. 3). The hyperpolarization due to DC current did not decrease the input resistance, while DA at 10 nM elicited membrane hyperpolarization and a decreased input resistance. Thus, the decrease of input resistance in the presence of DA was due to DA itself, and not to the change in membrane potential.

Possible role of DA as a synaptic shunter The characters of synaptic interactions between neurons are either excitatory or inhibitory. Activity in one neuron either enhances or reduces activity in the target neuron. Even though the mechanisms responsible for the postsynaptic effect are variable, the typical synaptic action is to open ion channels, causing excitatory current to flow inward or inhibitory current to flow outward (Katz, 1966). In *P. penicillatus*, DA definitely modulates the firing patterns of SMN by increasing voltage-gated outward current (Chung and Spencer, 1989; 1996), indicating that DA participates in forming inhibitory synapses in this animal as an inhibitory neurotransmitter. However, there may also be a more subtle consequence of DA action in this animal. In addition to generating inhibitory postsynaptic potentials by activating voltage-gated K-channels, DA may also exert inhibitory actions by increasing the postsynaptic

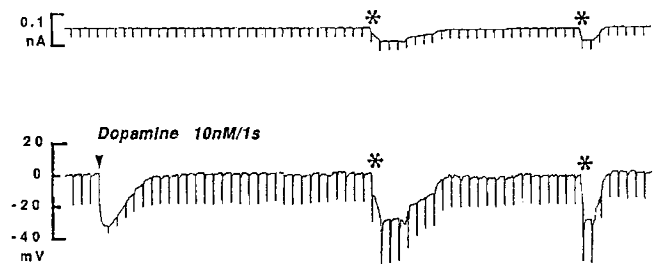

Fig. 3. The decrease of input resistance when DA is applied is a direct effect and is not due to membrane hyperpolarization. Ten nM DA generated a 34 mV hyperpolarization and a marked decrease of input resistance in a cell which was held at 0 mV (lower trace). Direct current was intracellularly injected (asterisks) to mimic the membrane hyperpolarization (upper trace). The hyperpolarization due to injected DC current did not decrease the input resistance. This indicates that the change of input resistance in the presence of DA was not a consequence of the change of membrane potential but was due to DA itself. Current pulses were applied at 0.35 Hz, 30 pA, and 200 ms durations.

conductance (Figs. 2 and 3). Such a shunting effect of DA in jellyfish is not observed in other preparations such as crustacean muscle fibers (Lingle, 1981) and motor neurons of lamprey spinal cord (Kemnitz, 1997). The significance of these differences is unclear but perhaps extrapolation of results between species should be treated with caution.

In connection with the action of DA as a synaptic shunter in inhibitory synapses, it should be important to know where DA receptors are localized on SMNs since all the neuro-neuronal chemical synapses of *P. penicillatus* are only observed between neurites, and not between neurite and soma (Singla, 1978; Spencer, 1979). There are no obvious experimental data showing that the DA-receptors are localized to specific areas of the SMN. However, some cells (N = 3) gave larger currents when DA was applied to processes rather than soma. For example, a tip of neurite (2) gave a 60% larger response than the soma (4) to applied DA (Fig. 4). Also, the more distal to soma, the bigger responses from neurites were elicited. Since the pipette delivering DA was positioned close to the cell, the concentration of DA was significantly greater at the cell surface immediately under the pipette than anywhere else on the cell. This was confirmed by adding a phenol red solution to the pipette when a distinct ejection plume was seen with little diffusion or mixing. For this experiment, we chose SMNs with relatively 'long processes' that we did not usually use for voltage-clamp experiments to avoid space clamping errors. The differences between responses from neurites and those from soma seemed likely to be underestimated because the resting membrane potentials of SMNs are usually −30 to −40 mV in our experimental conditions. Much bigger responses from neurites would thus occur than those shown in Fig. 4. Combining this evidence with the effect of denervation on redistribution of either ion channels (Gustafsson, 1979; Gilly and Brismar, 1989) or receptors (Kuffler *et al.*, 1971), it is highly probable that DA receptors on SMNs *in vivo* may also localize on the area of distal neurites. Therefore, DA signal occurring through D_2-like receptors on neurites of SMNs presumably plays an important role as a shunter for neuro-neuronal communications in generating a tonic inhibition of SMNs which is responsible for either 'shadow' responses or 'crumpling' behaviors of this animal.

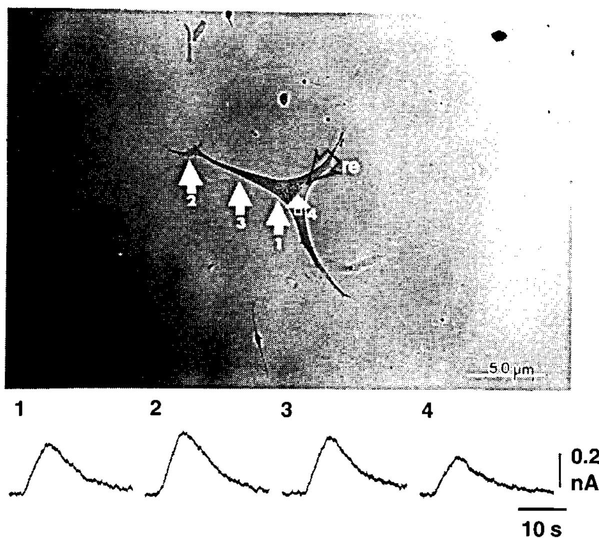

Fig. 4. Distribution of DA receptors. Ten μM of DA was applied for 5 s using a Picospritzer (pressure, 138 Kpa) successively to the base (1), the tip (2), the middle (3) of a process, and the centre of a soma (4) of a SMN. The arrows point to the direction that the opening of the micropipette which delivered DA was facing. During the experiment, the cell was continuously perfused with ASW. The photograph was taken before recording. Holding potential, −20 mV; *re*, the position of a recording electrode.

Depolarizing stimuli, such as those that might be produced by rapid synaptic inputs, normally produce rapidly reversible changes in the activity. However, after the input resistance of SMNs have been reduced by DA, the depolarizing stimulus produces smaller and shorter lasting modulation of bursting activity, because there is less of the voltage-dependent current to be turned on by the small voltage changes. Thus, the very obvious actions of DA on firing patterns of an SMN are accompanied by a less conspicuous but nonetheless profound alteration in the sensitivity to synaptic input. Although the effects of DA as a single modulator on resting membrane properties of SMN may appear to be minor, its true influence on the excitability of a SMN may be evident when it is coupled with stimulation of another synaptic input.

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