J. exp. Biol. 156, 433–451 (1991)
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DOPAMINE AS A NEUROACTIVE SUBSTANCE IN THE JELLYFISH *POLYORCHIS PENICILLATUS*

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Accepted 22 October 1990

Summary

Recent studies have shown that nerve-rich tissues in the margin of *Polyorchis penicillatus* (Eschscholtz), one of the hydromedusae, contain dopamine. The present experiments were conducted to determine the physiological action of dopamine at the cellular level. In the current-clamp mode, dopamine, ranging from 10^{-8} to 10^{-3} mol l^{-1}, applied to cultured swimming motor neurons of this jellyfish produced hyperpolarizations accompanied by a decrease of firing rate or complete inhibition of spiking produced by anodal break excitation. Dopamine in the voltage-clamp mode elicited outward currents at more positive levels than −55 mV, which is the reversal potential of the response. The results of a series of ionic experiments suggest that the inhibitory effect of dopamine is caused by an increased permeability to potassium ions.

Introduction

The cnidarians are believed to be the most primitive organisms with a true nervous system and attract considerable attention because of their evolutionary position and relative simplicity. Neurochemical studies of cnidarian nervous systems should lead to a better understanding of the evolution of chemical transmission. Both morphological and physiological evidence (Martin and Spencer, 1983; Anderson and Spencer, 1989) from various cnidarian species indicate that chemical transmission occurs at many synapses in the nervous system of cnidarians. However, the nature of the neurotransmitters used by cnidarians has remained enigmatic.

Over the past decade, information has accumulated to support the role of peptides as neurotransmitters or neuromodulators in this phylum. Peptides with the carboxy-terminus Arg-Phe-amide are present in neuronal populations within all classes of cnidarians (Grimmelikhuijzen, 1983, 1985) and the physiological role of such peptides has been demonstrated in some cnidarians (McFarlane *et al.* 1987; Spencer, 1988). However, the evidence for the existence and functional significance of the conventional transmitters, such as biogenic amines, acetylcholine and

Key words: cnidarian neurones, catecholamine, Hydrozoa, potassium conductance, whole-cell recording, *Polyorchis penicillatus*.

amino acids, is still ambiguous and vague. Most of our knowledge of the biogenic amine systems in cnidarians comes from biochemical and histological studies; however, the precise functions served by the catecholamines are unknown. Several studies have used extracellular recording techniques to examine the action of biogenic amines, but none has allowed us to understand these actions at the cellular level (Ross, 1960*a*,*b*; Lentz and Barrnett, 1963; Schwab, 1977; Anctil *et al.* 1982; Anctil, 1989).

Recently, Chung *et al.* (1989), using high performance liquid chromatography and gas chromatography/mass spectrometry, demonstrated that biogenic amines are present in nerve-rich tissues of the hydrozoan *Polyorchis penicillatus*. Dopamine was found to be the major catecholamine present in the nerve-rich tissues of the margin of this animal. The aim of the present study was to elucidate the possible electrophysiological effects of dopamine on an identified type of neuron from *P. penicillatus* and to determine the underlying ionic mechanisms of its action. Although there was no reason to suppose that swimming motor neurons (SMNs) were any more likely to be target neurons for dopamine than other neuronal types in the cultures, SMNs were used because their properties have been well characterised from *in vivo* studies and they were readily identified in culture. The epithelia overlying neurons (King and Spencer, 1979) in *P. penicillatus* form a diffusion barrier, preventing chemicals applied exogenously from reaching target neurons. A primary culture technique for jellyfish neurons was adopted in this study to circumvent this problem. The whole-cell recording (WCR) technique (rather than intracellular microelectrode recording) was used to obtain more stable recordings.

Materials and methods

Medusae of *Polyorchis penicillatus* (Eschscholtz) were collected from Bamfield Inlet, British Columbia, Canada, by scuba divers, and held in running sea water (10–12 °C) for up to 5 days.

Cell dissociation and culture

Neuronal cultures were prepared, as previously described (Przysiezniak and Spencer, 1989) with some modification. After removal of the apex, the bell was radially bisected. Jellyfish were then pinned, subumbrella-side uppermost, in an anaesthetic solution of 1:1 isotonic $MgCl_2$ and sea water to a Sylgard (Dow-Corning) base in a large plastic Petri dish using stainless-steel pins. Strips of velum (approximately 30 mg wet mass) containing the nerve-rings were then removed, cut into small pieces, and placed in a borosilicate glass test tube containing normal artificial sea water (NASW; 2 ml) for about 5 min. The NASW was replaced with double-distilled deionized water for 30 s to 1 min, which removed many vacuolated epithelial cells as a result of hypo-osmotic shock. The tissue was rinsed again with NASW, and then exposed to divalent cation-free ASW (DFASW) for 10 min. This was followed by digestion in fresh collagenase solution, 1000 units ml^{-1} in NASW

After 6–8 h of enzyme treatment at room temperature (18–20°C), the enzyme solution was removed using a Pasteur pipet, and 2–5 ml of NASW was added. The tissue was triturated 10–30 times with a fire-polished Pasteur pipet and the cell suspension divided among 10 culture dishes coated with homogenized, dried mesoglea. One hour after the cells had been plated, the cultures were rinsed twice, covered with NASW and then kept at 10–12°C.

Recording methods

Recordings from cultured neurons were made with a List L/M-EPC 7 amplifier (Medical Systems Corp., Greenvale, NY) and were obtained in either voltage- or current-clamp mode using the whole-cell recording (WCR) configuration (Fenwick *et al.* 1982).

Patch pipets were made on a Narishige electrode puller PP-83 (Narishige, Tokyo) using non-heparinized hematocrit capillary tubes (Fisher; length 75 mm, i.d. 1.1–1.2 mm, thickness 0.2 mm). Their resistances were 1.0–3.0 MΩ when patch solution (PS) and NASW (listed in Table 1) were used as the patch pipet solution and bath solution, respectively.

The series resistance of the pipet tip was measured by two methods: (i) the amplitude of a voltage step was divided by the peak current recorded at the onset of the capacitative transient and (ii) the time constant of the transient was divided by the membrane capacitance. Membrane capacitance was obtained from time constants of either depolarizing or hyperpolarizing responses to current pulses in the current-clamp mode. Series resistance values from 5 to 10 MΩ were routinely obtained, and 20–50 % of series resistance could be cancelled using the slow capacity compensation of the patch-clamp amplifier. With series resistances of 3–5 MΩ, currents of 1 nA [the largest that applied dopamine (DA) provoked in this study] would have caused no more than a 5 mV voltage drop across this resistance. We therefore chose to neglect series resistance errors in the analyses of the results.

Junctional potentials were nulled by an offset sufficient to make the output current zero when the pipet was in the bath. The maximum potential drift observed when junctional potential was checked after recording from cells was 2 mV. The junctional potentials did not vary by more than 1 mV when the bath was perfused with the various test solutions listed in Table 1. We did not correct for this error.

The following criteria for whole-cell recordings were used: (1) the amplitude of the rebound action potentials had to reach at least 80 mV; (2) the resting potential had to exceed −25 mV (usually −30 mV) initially and the change in the resting potential had to be less than 5 mV during the experiment; (3) leak resistance had to be larger than 0.8 GΩ (usually 1 GΩ; maximum 2.5 GΩ). The recordings were accepted for analysis when all three criteria were satisfied.

Intracellular voltages and currents were recorded continuously on a Gould Brush 2400 pen recorder and stored for later analysis on video tapes using a video recorder (MTS, model MCR-220R) and an A/D video recorder adapter (PCM-2,

Table 1. *Ionic composition of salines*

	NASW	DFASW	High K^+	Very high K^+	Low Cl^-	PS
NaCl ($mmol\,l^{-1}$)	378	378	378	328.4	378*	–
$CaCl_2$ ($mmol\,l^{-1}$)	9.5	–	9.5	9.5	9.5	1
Na_2SO_4 ($mmol\,l^{-1}$)	5.7	5.7	5.7	5.7	5.7	–
KCl ($mmol\,l^{-1}$)	13.4	13.4	55.4	105	13.4*	105
$MgCl_2$ ($mmol\,l^{-1}$)	29	–	29	29	29	2
Choline chloride ($mmol\,l^{-1}$)	42	99	–	–	–	–
Hepes ($mmol\,l^{-1}$)	10	10	10	10	10	10
NaOH ($mmol\,l^{-1}$)	5	6	5	5	4	–
EGTA ($mmol\,l^{-1}$)	–	1	–	–	–	11
Glucose ($mmol\,l^{-1}$)	–	–	–	–	93.5	700
KOH ($mmol\,l^{-1}$)	–	–	–	–	–	35
Osmolality ($mosmol\,kg^{-1}$)	1019.4	1019.4	1019.4	1019.4	1026.9	1010

* These salts were replaced with sodium and potassium gluconate, respectively.

Gentamycin sulfate was added to all saline solutions (0.005 %, w/v) except patch solution (PS).

NASW, normal artificial sea water (K^+ 13.4 $mmol\,l^{-1}$, Cl^- 510.4 $mmol\,l^{-1}$); DFASW, divalent cation-free artificial sea water; high K^+, high-potassium (55.4 $mmol\,l^{-1}$) saline; very high K^+, very high-potassium (105 $mmol\,l^{-1}$) saline; low Cl^-, low-chloride (77 $mmol\,l^{-1}$) saline.

Medical Systems Corp.). Recorded signals were displayed on a Tektronix 5223 digitizing oscilloscope.

Preparation of solutions

The compositions of the bathing solutions and a patch solution are listed in Table 1. Dopamine solutions of 10 $nmol\,l^{-1}$ to 1 $mmol\,l^{-1}$ in NASW were prepared daily by dilution of stock solutions prior to use. The stock solutions of dopamine (0.1 $mol\,l^{-1}$) were prepared in deionized double-distilled water and were frozen at −20 °C after being divided into samples of 500 μl. The pH of the stock solution was 6.8, and the pH of the working dopamine solutions was 7.5. The working dopamine solution in NASW oxidized relatively easily, becoming a brownish pink colour. Such autoxidation of dopamine could have been prevented by adding ascorbic acid. However, it was not used as an antioxidant in these experiments to avoid any possible interfering effect of pH (Anderson and McKay, 1987; Spencer, 1988). Instead, the working dopamine solutions were enclosed in aluminum foil, kept in a dark box at 4 °C, and prepared freshly every 4 h during the experiment. Dopamine solutions were introduced into pipets using a stainless-steel hypodermic needle. Dopamine was purchased from Sigma Chemical Co. (St Louis, MO); other chemicals were of reagent grade and were obtained from local suppliers.

Dopamine application

Dopamine dissolved in NASW was delivered to isolated cells *via* capillary pipets. Pipets were pulled in two stages from aluminosilicate glass (AM Systems

o.d. 1.5 mm, i.d. 0.58 mm) in the same manner as the pipets used for the WCR. Pipets obtained when the heat was reduced to about the breaking point were used (their bubble numbers were 6.2–6.5). The pipet was positioned 10–50 μm from the target soma, with the tip at an angle of about 45° to the perfusion path. A fluid stream from the pipet was obtained by applying 69–138 kPa pressure to the pipet using a Picospritzer (General Valve Corp., model 2). The concentration of dopamine in the vicinity of the cell body depended on many factors: thus, the dopamine concentration in the pipet represents only an upper limit for the concentration that could have reached the surface of the cell. The amount of dopamine ejected was calculated by determining the ejected volume at the end of experiments (Sakai *et al.* 1979).

Results

Identification of swimming motor neurons

Swimming motor neurons (SMNs) could be distinguished morphologically and electrophysiologically from other subsets of neurons *in vitro* (Przysiezniak and Spencer, 1989). The morphological criteria used to identify SMNs in primary culture were: a clear cytoplasm, short and wide processes, and membranous inclusions around the nucleus (Fig. 1A). Neurons identified as SMNs maintained a distinctively shaped action potential in culture (Fig. 1B). The amplitudes of action potentials in culture (80–100 mV) recorded using WCR were similar to those recorded *in vivo* using intracellular recording techniques (Satterlie and Spencer, 1983). Spike duration at half amplitude ranged from 20 to 50 ms. Afterhyperpolarizations with amplitudes of −10 to −15 mV followed each spike. Neurons exhibiting spikes with these characteristics were generally larger (somata larger than 30 μm) than other neurons; however, some were as small as 10–20 μm in diameter. Only cells showing these distinctive characteristics were used in this study.

Whole-cell recording

Intracellular recordings were obtained by switching from the voltage-clamp mode to the current-clamp mode in the WCR configuration. Measurements of the resting membrane potential with standard solutions gave a mean of −30.0±1.2 mV (N=30, mean±S.E.M.; range from −48 to −20 mV). These values are different from those obtained from either *in vivo* or *in vitro* preparations using intracellular microelectrodes. Anderson and Mackie (1977) measured an average *in vivo* resting potential for SMNs of −60±5 mV, Spencer (1981) measured a mean of −57 mV and J.-M. Chung and A. N. Spencer (unpublished results) measured a mean of −48±3 mV (N=10) using intracellular microelectrodes. Such values may not correspond to the true cell resting membrane potential because, in the *in vivo* preparation, all excitatory and inhibitory inputs to the SMN network might not be completely blocked, resulting in fluctuations of the resting potential. Measurement of resting potentials from an isolated neuron should circumvent such a

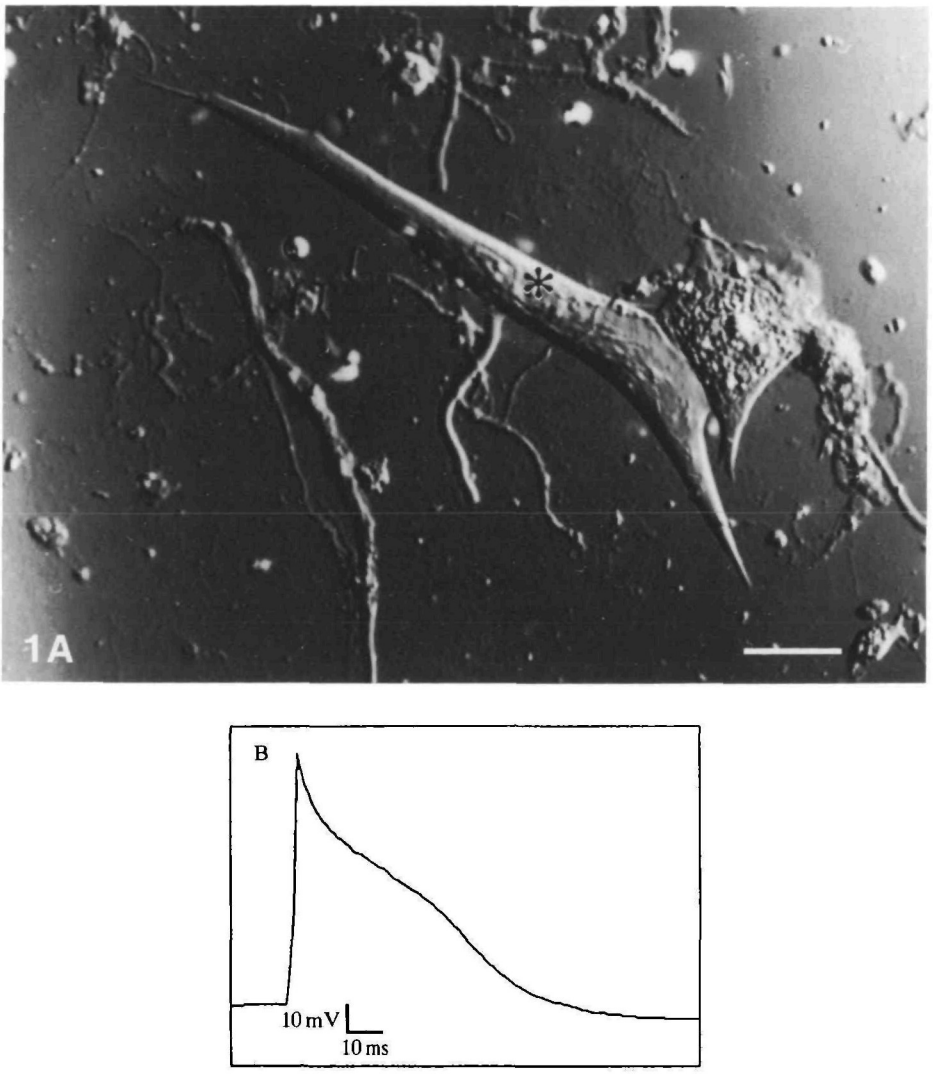

Fig. 1. Identification of swimming motor neurons (SMNs) in cell culture. (A) A cell, marked with an asterisk, viewed by Nomarski differential-interference-contrast microscopy shows the clear cytoplasm and perinuclear membranous structures typical of SMNs. Scale bar, 50 μm. (B) An action potential elicited by current stimulation (2.5 ms, 999 pA) from the cell shown in A while the resting membrane potential was held at −35 mV. The bath contained normal artificial sea water and the patch pipet contained patch solution (Table 1), both at pH 7.5.

problem. Przysiezniak and Spencer (1989) measured a mean resting potential of -53 ± 20 mV from isolated SMNs using intracellular microelectrodes. It is apparent that cell membrane damage may be more frequently associated with intracellular microelectrode recording from *in vitro* preparations than from *in viv*

preparations. For example, the amplitudes of spontaneous spikes in isolated SMNs (Przysiezniak and Spencer, 1989) were reported to be lower than those from neurons *in vivo*, with mean values of 50 mV *in vitro* and 90 mV *in vivo*. Also, the measured resting potentials from the *in vitro* preparation have far greater variance than those from cells *in vivo*.

Whole-cell recording may eliminate these errors. However, this does not necessarily mean that the resting potentials measured by WCR represent the actual resting membrane potential. It is worth noting that the calculated equilibrium potential for potassium is −59.1 mV and that for chloride is −38.4 mV at 20°C, on the basis of the concentrations of each ion in the bath and patch solutions. The measured resting potential should thus have been close to the calculated equilibrium potential for potassium, assuming that complete solution exchange occurred between the patch solution and the cytoplasm, and that the resting potential was determined by the potassium concentration gradient across the membrane. This suggests that resting potentials in SMNs may not be based simply on the potassium ion gradient. Also, since the normal intracellular milieu may be significantly altered by cytoplasmic dialysis, measurements of resting membrane potential using patch electrodes would be in error if other cellular components as well as ions participated in determining the resting potential of a cell.

Characteristics of the dopamine response in swimming motor neurons

Current-clamp mode

Switching from voltage-clamp to current-clamp evoked 'anodal break' spikes when neurons were held at −60±0.2 mV (N=64) in the voltage-clamp mode. The presence of long-duration spikes (more than 20 ms) confirmed that the neurons were SMNs.

Pulses of dopamine applied to SMNs during the anodal break excitation produced hyperpolarizations associated with a decrease of the firing rate or complete inhibition of firing (N=15; Fig. 2A,B). Control applications of normal artificial sea water (NASW), which was used as a dopamine vehicle, did not normally produce any responses (N=5). In some cases when the leak resistance was less than 500 MΩ, NASW caused depolarization (less than 3 mV) with a slight increase in firing frequency.

The shapes of action potentials before and after dopamine-induced hyperpolarization are shown in Fig. 2C. Spikes just following the hyperpolarization usually had slightly greater amplitudes and shorter durations than spikes before and tens of seconds after the dopamine-induced hyperpolarization. It is interesting that dopamine-induced increases in spike amplitude were greater in recordings from cells in which the initial amplitudes of the action potentials were low, possibly as a result of cell damage by the electrode. These 'poor' action potentials were transformed by dopamine into 'healthy' looking action potentials of large

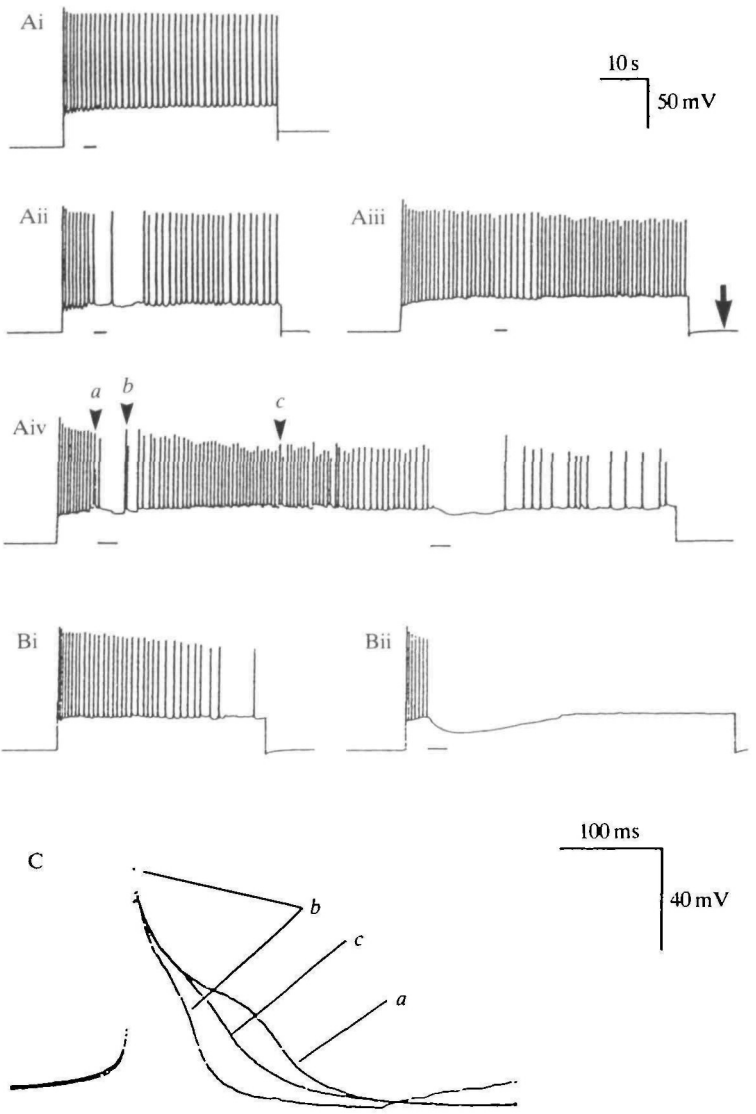

Fig. 2

amplitude and short duration. The rising phases of action potentials were not altered by the application of dopamine.

Dopamine also induced a decrease in the input resistance of neurons (R_{in}). Hyperpolarizing current pulses of 30 pA at a frequency of 0.35 Hz were injected through the electrode into cells in which the membrane potential was varied from −50 to 0 mV. When cells were held at −50 mV, which was close to the potassium equilibrium potential (−59 mV), changes of R_{in} due to dopamine application were negligible (N=3). However, when a cell was held at −30, −20 or 0 mV, 10 nmol l^{-1} dopamine drastically decreased R_{in} by 80, 85 or 93 %, respectively, at the peak of the DA-induced hyperpolarization (Fig. 3). It was apparent that the inpu

Fig. 2. The effects of dopamine on rebound action potential trains from cultured swimming motor neurons (SMNs). (A) Either saline or 10 μmol l^{-1} dopamine in saline was applied (shown by the bar) through a micropipet (bubble number 6.2) using a Picospritzer (pressure 138 kPa). (i) Control experiment in which saline was applied for 2 s. (ii) Dopamine applied for 2 s resulted in membrane hyperpolarization and a dramatic reduction in the firing frequency. (iii) A second application of dopamine 3.2 min later for 2 s produced only a slight reduction in firing frequency as a result of desensitization. Saline perfusion started at the arrow. (iv) Dopamine applied for 4 s evoked distinct membrane hyperpolarization and a reduction or cessation of firing. Note that the first spike to appear after dopamine application had a higher amplitude than the spike prior to dopamine application. (Bi) Control experiment in which saline was applied for 4 s. (Bii) Application of dopamine at 10 μmol l^{-1} for 4 s provoked a long hyperpolarization (peak amplitude, 15 mV; duration, 26 s) from this cell. (C) Digitized single sweeps obtained from the rebound spike trains in Aiv. *a* shows a spike just before dopamine application; *b* shows a spike appearing immediately after dopamine application and *c* shows a spike 2.8 min after dopamine application.

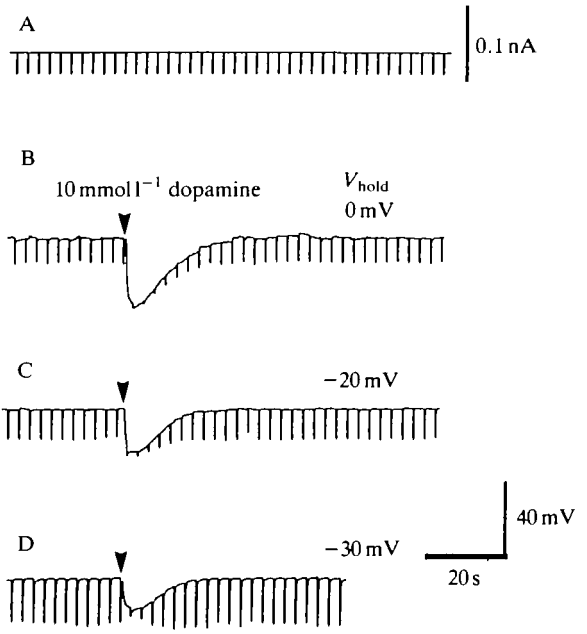

Fig. 3. The effect of dopamine on the input resistance of swimming motor neurons. 30 pA hyperpolarizing current pulses of 200 ms were applied at a frequency of 0.35 Hz to a cell in which the holding potential was varied from −50 to 0 mV. 10 nmol l^{-1} dopamine was applied for 1 s using a Picospritzer (pressure 138 kPa). (A) The stimulating current traces. (B, C, D) The changes in the input resistance in the presence of dopamine at holding potentials (V_{hold}) of 0, −20 and −30 mV, respectively. Note that, as the holding membrane potential is more depolarized, the hyperpolarizations increase and the input resistances decrease.

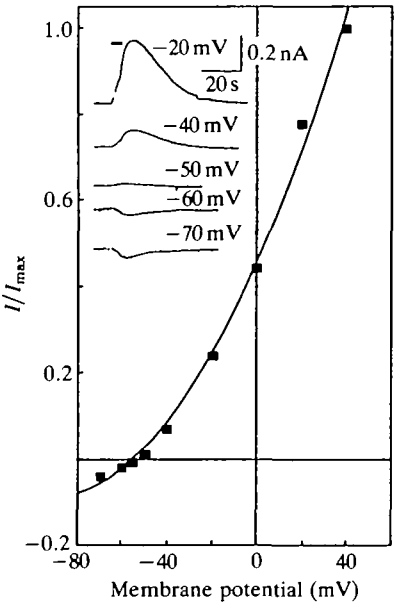

Fig. 4. Current–voltage plot of the dopamine response. Dopamine was applied at 0.1 mmol l^{-1}. Each application at a different membrane holding potential consisted of five consecutive ejections of 900 ms each at 138 kPa. The inset shows selected current responses close to the reversal potential of −53 mV.

resistance change associated with dopamine-induced membrane hyperpolarization was voltage-dependent.

Voltage-clamp mode

Applications of dopamine at concentrations ranging from 10^{-8} to 10^{-3} mol l^{-1} evoked monophasic outward currents when the membrane potential was held at levels more depolarized than −50 mV (inset of Fig. 4). All SMNs (N=56) responded to applied dopamine by producing outward currents when the recording criteria were met and the pressure-ejection pipets were positioned within 100 μm of the soma. Neurons that gave short-duration spikes and were provisionally identified as 'B' neurons (Spencer and Arkett, 1984) did not respond to dopamine (N=3). Control applications of saline to SMNs either did not produce a response or produced responses that did not resemble those due to dopamine (N=8). Three such control applications gave small inward currents (<20 pA) whose amplitudes were not dependent on membrane holding potential.

Voltage dependence of dopamine-induced outward currents

The response evoked by dopamine was strongly dependent on membrane potential, showing a current–voltage plot with outward rectification as the membrane was depolarized (Fig. 4). The amplitude of the response to dopamine

varied non-linearly with the clamped membrane potential in all cells studied. The reversal potential of the response was -55.0 ± 1.1 mV (mean±S.E.M.; $N=11$).

The amplitudes of the responses seen in different cells varied between 150 and 500 pA at 0 mV. This variation was related neither to cell size nor to the age of the culture. Conductances were calculated from cells whose peak amplitudes were between 150 and 200 pA at 0 mV. Slope conductance measurements, obtained from tangents to the current–voltage plots at -60 mV and 0 mV, increased by a factor of 5.1 ± 0.8 (mean±S.E.M., $N=8$; range 2.2–8.8) as the membrane potential was depolarized. In some cases, the current–voltage relationship of responses was of nearly zero slope conductance over the membrane potential range -40 to -60 mV. When neurons were depolarized beyond -20 mV the slope conductance increased rapidly.

Changes in the slope of current–voltage relationships highlight the voltage sensitivity of the response, but reveal little about the underlying conductance mechanism. For a parallel conductance model (Ginsborg, 1967), the agonist-evoked current I_X is related to the agonist-activated ionic (chord) conductance G_X by the equation:

$$I_X = G_X(E_m - E_X),$$

where E_m is the membrane potential and E_X is the reversal potential of the agonist-activated current. We were able to record dopamine-evoked currents on both sides of the reversal potential in many cases and could therefore estimate the driving force ($E_m - E_X$) either by direct measurement or from the intersection of the current–voltage curve on the voltage axis. The dopamine-activated ionic conductance was then calculated and plotted against the membrane potential (Fig. 5). The conductance activated by dopamine was an outward rectifier and increased as the membrane was depolarized. The outward rectification ratio obtained by dividing the chord conductance at $+40$ mV by that at -40 mV was about 4.

Ionic dependence of the inhibitory current

The reversal potential of the dopamine-evoked response suggested that the inhibitory effects evoked by dopamine were caused by an increased permeability to potassium. To determine the underlying ionic mechanism of the response, the effects of ionic substitutions on the reversal potential and the amplitude of the dopamine response were examined.

Fig. 6A,B shows that the reversal potential of the outward current was not altered by changing the sodium ($N=5$) or chloride ($N=3$) equilibrium potential. However, the amplitude of the current induced by dopamine declined when the external chloride concentration was reduced from 510 to 77 mmol l^{-1} (Fig. 6B). This reduction in the amplitude of the response was not reversible: neurons did not recover their responsiveness to dopamine even after changing the bath solution from low-Cl^- saline to NASW. It is unlikely that this reduction of the current was due to desensitization of receptors or run-down of the cell since this

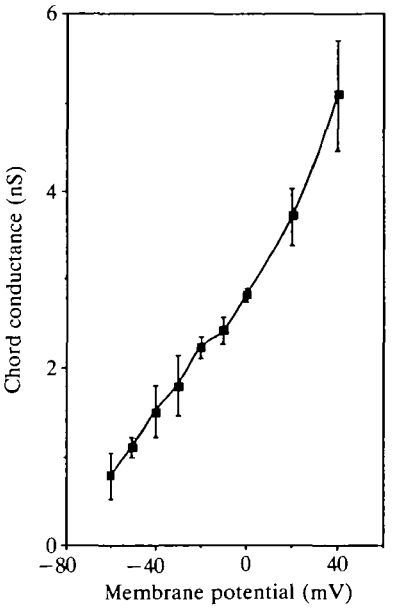

Fig. 5. Chord conductance/membrane potential relationship of the dopamine response. Chord conductances were calculated by dividing the current response amplitude at a given membrane potential by the driving force for dopamine-activated ionic current. The conductance mechanism activated by dopamine behaves as an outward rectifier, increasing in amplitude as the membrane is depolarized. Values are mean±S.E.M. (N=10).

effect was not seen in either sodium or potassium ionic substitution experiments. It is clear that the reversal potential of the dopamine response is insensitive to changes in extracellular sodium and chloride concentration. The dopamine inhibitory response reversed around −55 mV, which is close to the calculated equilibrium potential for potassium (−59.1 mV). Thus, it appeared likely that the dopamine effect was mostly due to an increase in K^+ permeability. The dopamine reversal potential followed the equilibrium potential for potassium when the concentration of extracellular potassium was varied. Fig. 6C illustrates the effect of an increase in extracellular potassium concentration from 13.4 to 55.4 and 105 mmol l^{-1}. The reversal potential of the dopamine response decreased from −55.0 to −27.2 and −6.6 mV (N=3), respectively, values that approximate those predicted by the Nernst equation (Fig. 6D). The amplitude of the response also decreased as the concentration of extracellular potassium increased, which could be explained by the decreased driving force on potassium.

Discussion

Although there is considerable electrophysiological evidence that chemical neurotransmission in the Cnidaria (Anderson, 1985; Mackie and Meech, 1985)

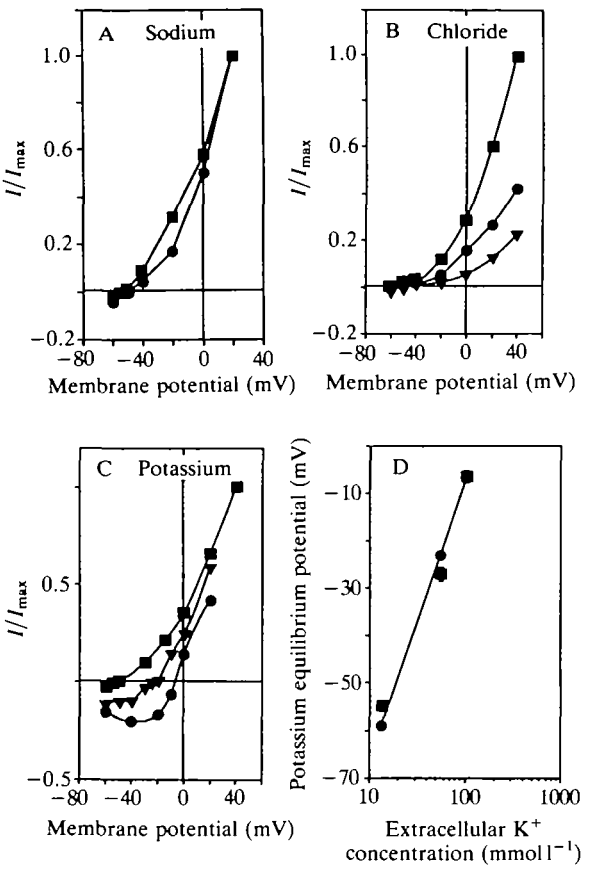

Fig. 6. (A) Current–voltage plot showing the effect of sodium ion substitution on the dopamine response of swimming motor neurons (SMNs) to 1 μmol l^{-1} dopamine. Changing the bath solution from NASW (■) to Na^+-free saline (●) did not cause any significant changes in either reversal potentials or amplitudes of dopamine responses. (B) Current–voltage plot showing the effect of chloride substitution on the dopamine response. Dopamine responses were obtained from an SMN continuously perfused with NASW (■), low-Cl^- (●) and low-Cl^- saline followed by NASW (▼). In all cases the reversal potentials remained constant whereas the responses were drastically reduced after ionic substitution and did not recover their amplitudes after washing with NASW. (C) Current–voltage plot showing the effect of potassium ion substitution on the dopamine response. Dopamine responses were obtained from an SMN continuously perfused with NASW (13.4 mmol l^{-1}, ■), very high-K^+ (105 mmol l^{-1}, ●) and high-K^+ saline (55.4 mmol l^{-1}, ▼). As the extracellular K^+ concentration increased, the reversal potential of the dopamine response decreased, so that it approximated the K^+ equilibrium potential, and the dopamine response was reduced. The decreasing amplitude of the response could not be due simply to run-down of the cell, since the dopamine responses increased when the K^+ concentration of the perfusion solution decreased from 105 to 55.4 mmol l^{-1}. (D) The relationship between the reversal potential of the dopamine response at different extracellular potassium concentrations and the calculated equilibrium potentials (●). The measured values (■) are given as mean reversal potential±S.D. (N=4). T=293 K.

Satterlie, 1984; Spencer, 1982) is similar to that in higher phyla (such as annelids, molluscs, arthropods and chordates), there have been no cellular studies that give definitive data on the mechanisms of a transmitter's actions. Additionally, we have only fragmented information as to the nature of the transmitters (Martin and Spencer, 1983). These types of information are needed for reconstructing the early evolution of interneuronal chemical communication, since it is presumed that it was in this group, or a common ancestor, that such mechanisms first evolved. In this study we show that dopamine is neuroactive: it gates an increase in potassium conductance that leads to a transitory hyperpolarization and inhibition of spontaneous spiking in swimming motor neurons.

Dopamine was chosen as a likely neurotransmitter candidate as it had been found in extracts from the nerve-rich tissues of the margin of this jellyfish by HPLC and gas chromatography/mass spectrometry (Chung *et al.* 1989). Epinephrine, norepinephrine, serotonin and octopamine were not detected, though an unidentified catecholamine with a mobility similar to that of norepinephrine eluted from the column.

Inhibitory actions of dopamine

The current-clamp experiments showed that brief (2–4 s) pulses of dopamine at $10\,\mu mol\,l^{-1}$ had an inhibitory effect on swimming motor neurons by eliciting immediate hyperpolarizations of 5–15 mV which lasted as long as 15–30 s. These hyperpolarizations were sufficient either to slow or to arrest spiking produced by anodal-break stimulation (Fig. 2A,B). The threshold concentration for producing hyperpolarizations has not been precisely determined; however, voltage-clamp data showed that dopamine at $10^{-8}\,mol\,l^{-1}$ elicited an outward current at a holding potential of −20 mV.

The membrane hyperpolarizations seen after application of dopamine were shown to be due to a conductance increase which was voltage-dependent. Dopamine responses associated with a conductance increase have been reported in several other preparations, such as rat lactotrophs (Israel *et al.* 1987; Castelletti *et al.* 1989), human prolactinoma cells (Israel *et al.* 1985) and identified neurons of the snail *Helix aspersa* (Bokisch and Walker, 1986; Cox and Walker, 1988). Dopamine caused a larger decrease in membrane resistance at more depolarized membrane potentials (Fig. 3). The voltage-sensitivity of the 'dopamine response' could be clearly seen in voltage-clamp recordings where dopamine elicited outward currents that increased with membrane depolarization (Fig. 4). The slope conductance increased progressively as the membrane was depolarized. Such rectifying properties of current–voltage curves have been reported in identified *H. aspersa* neurons (Cox and Walker, 1988), while rectification was not seen in the growth-hormone-producing cells of the freshwater snail *Lymnaea stagnalis* (De Vlieger *et al.* 1986). The dopamine-activated chord conductance also increased with membrane depolarization (Fig. 5). The measured rectification ratio is 4. This is not as high as the ratio for *N*-methyl-D-aspartate (NMDA) receptors in cultured neurons of mouse embryo spinal cord (Mayer and Westbrook, 1984) but is three

times higher than that for non-NMDA receptors, which are known to be slightly sensitive to the membrane potential.

The reversal potential for this outward current was approximately −55 mV, which was close to the calculated E_K of −59 mV and, since altering the potassium gradient shifted the reversal potential in a Nernstian manner (Fig. 6C,D), we conclude that the dopamine-induced current is carried by potassium ions. Such a conclusion was further supported by the results of the ionic substitution experiments for sodium and chloride ions (Fig. 6A,B). The observation that the agonist responses were reduced in low-Cl^- saline may imply that there is a reduction in the driving force on K^+ because of a loss of intracellular potassium ions due to the contribution of a Donnan equilibrium (Boyle and Conway, 1941). However, this cannot explain why the cells (N=3) did not recover their responsiveness to DA even 30 min after replacement of low-Cl^- saline with NASW. It is likely that the WCR configuration lessens the importance of a Donnan equilibrium of the type described by Boyle and Conway, since the intracellular concentrations of these ions should remain constant during an experiment.

The exact nature of the channel responsible for the dopamine-induced potassium current is unclear. There are several ways to explain the underlying mechanism of dopamine responses related to the channel properties. First, dopamine may increase the conductance of ligand-gated potassium channels, resulting in membrane hyperpolarization. Membrane hyperpolarization may also remove steady-state inactivation of a purely voltage-activated potassium channel, resulting in a decrease of spike duration (Fig. 2C). For example, in current-clamp mode, the duration of action potentials varies with the membrane holding potential. Spencer *et al.* (1989) suggested that the unmasking of a fast, transient potassium current ($I_{K\text{-fast}}$) at hyperpolarized holding potentials might be responsible for duration decreases. Second, the observation that action potential duration was decreased during exposure to dopamine, as shown in Fig. 2C, could also suggest modulation of voltage-sensitive currents. Dopamine increases delayed rectifying (I_K) and transient (I_A) potassium currents in rat lactotroph cells (Lledo *et al.* 1989) and the I_A current in the MMQ clonal pituitary cells (Login *et al.* 1990). Dopamine may thus also alter cellular activity by acting on voltage-dependent potassium channels either directly or *via* a second messenger system. Third, it is possible that voltage-sensitive, ligand-gated potassium channels may be present in jellyfish neurons. Experiments are under way to examine the effects of dopamine on voltage-sensitive potassium currents.

Modulation of potassium channels would change the pattern of cell depolarization and thus alter the Ca^{2+} flux through voltage-gated calcium channels. There is also an influx of Ca^{2+} with each action potential in SMNs (Spencer *et al.* 1989). Therefore, two membrane events in the presence of dopamine, hyperpolarization and the absence of action potentials, may have a cumulative effect in decreasing the level of intracellular Ca^{2+}. In addition, catecholamines have been shown to decrease the duration of Ca^{2+} spikes in several invertebrate and vertebrate neurons. For example, in rat sympathetic neurons, norepinephrine decreases the

duration of the Ca^{2+} spike (Galvan and Adams, 1982) and similar effects were observed in cultured chick embryo dorsal root sensory neurons after application of several transmitters, including dopamine (Dunlap and Fishbach, 1980; Canfield and Dunlap, 1984). In both cases catecholamines decrease the Ca^{2+} conductance. Marchetti *et al.* (1986) demonstrated that dopamine and norepinephrine reversibly reduced calcium channel activity in outside-out membrane patches of cultured sensory and sympathetic neurons of the chick. Dopamine-induced Ca^{2+} conductance decreases have also been reported for identified neurons in the snail *Helix aspersa* (Paupardin-Tritsch *et al.* 1985). Although it is not known whether catecholamines can modulate Ca^{2+} conductances in jellyfish neurons, it is possible that the intracellular level of Ca^{2+} may be influenced by dopamine.

Does applied dopamine mimic IPSPs seen in vivo*?*

Two rather different sources of inhibitory input to swimming motor neurons have been identified. In the first, photoreceptive oscillatory or 'O' neurons, which descend into the nerve rings from the ocelli, appear to make inhibitory synapses onto the swimming motor neuron network. Shadowing the ocelli produces a graded hyperpolarization and inhibition of spontaneous membrane potential oscillations in the 'O' neurons. Since these hyperpolarizations are followed instantaneously by depolarizations of the SMNs, it was concluded that 'O' neurons tonically release an inhibitory transmitter onto SMNs (Arkett and Spencer, 1986*a*,*b*) and that the depolarization and consequent spiking of SMNs when jellyfish respond to a shadow is due to release from tonic inhibition. In this study, a rapid depolarization has not been recorded after application of dopamine. Therefore, it seems unlikely that dopamine is the substance responsible for the tonic inhibition of SMNs.

A second type of inhibitory postsynaptic potential (IPSP) can be recorded spontaneously by intracellular microelectrodes from the swimming motor neuron network. These IPSPs are associated with action potentials propagated in the surrounding epithelium (Spencer, 1981), and were assumed (Mackie, 1975) to result from an electric field effect or a temporary change in the concentration of an extracellular ion. The evidence for supposing that these IPSPs are not chemically mediated is that blockade does not occur with a 3:1 mixture of sea water and isotonic magnesium chloride solution. Nevertheless, it is possible that, had higher concentrations of Mg^{2+} anaesthesia been used, blockade would have been achieved. A 1:1 solution was needed to block the shadow response of SMNs (Arkett and Spencer, 1986*a*). Even so, some chemical synapses in hydrozoans are known to be insensitive to Mg^{2+} (Kerfoot *et al.* 1985). Thus, it is possible that the IPSPs seen in SMNs during 'crumpling' behavior (King and Spencer, 1981) are due to the actions of an inhibitory transmitter. The time courses of the spontaneous IPSPs recorded *in vivo* and those recorded after application of dopamine *in vitro* are both slow and sustained. Thus, of the two types of IPSP seen *in vivo,* that associated with epithelial impulses is more consistent with a mechanism involving mediation by dopamine. King and Spencer (1981) showed that neurons were

involved in transferring epithelial excitation to the effector muscles responsible for 'crumpling'. Therefore these same neurons, rather than the epithelial cells, might be responsible for releasing an inhibitory transmitter. The comparative sensitivities of these two synaptic events to Mg^{2+} need to be examined more carefully.

If the findings described here are considered in combination with the prior observation that dopamine is present in the nerve-rich tissues of the margin of this jellyfish (Chung *et al.* 1989), it is attractive to propose that dopamine could be an inhibitory neurotransmitter or modulator in the central nervous system of hydromedusae. It is premature to be more definite until it can be shown histochemically that dopamine is present in neurons or other cells located close to the swimming motor neurons and that these cells are able to release dopamine.

We thank Dr J. McInerney for providing facilities at Bamfield Marine Station. This study was supported by National Science and Engineering Research Council of Canada grant A0419 to ANS.

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