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Dopamine as a Strong Candidate for a Neurotransmitter in a Hydrozoan Jellyfish

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Abstract: Recent studies have shown that dopamine applied to cultured swimming motor neurons of *Polyorchis penicillatus* produces an inhibitory action by opening potassium channels through D_2-like receptors. In this study, it was demonstrated that dopamine found in the hydromedusa was not from exogenous sources and the content of dopamine depended on the Ca^{2+} content of the dissecting media. In addition, a combination of thin layer chromatography and high performance liquid chromatography showed the presence of DOPA and DOPAC-like compounds in the jellyfish. The glyoxylic acid method for catecholamines suggested that a population of small cells, neither swimming motor neurons nor B-like neurons, had dopaminergic systems. From all these results, it is suggested here that DA synthesized from DOPA in some cells is released, being dependent on calcium concentrations, into a synaptic cleft and degraded into DOPAC after acting as an inhibitory transmitter to swimming motor neurons.

Key words: crumpling, dopamine, glyoxylic acid, HPLC, jellyfish.

The cnidarians are believed to be the most primitive organisms with a true nervous system (Anderson and Schwab, 1982a). They have attracted considerable attention because of their evolutionary position and the relative simplicity of their neuronal organization. Any information about chemical functioning of the cnidarian nervous system should be useful for reconstructing the evolution of interneuronal communication.

Several cnidarian model systems have been developed with these goals in mind. These include the hydrozoan jellyfish *Polyorchis penicillatus* (Anderson and Mackie, 1977; Spencer, 1978; Spencer and Arkett, 1984), *Aglantha digitale* (Roberts and Mackie, 1980) and a scyphozoan jellyfish *Cyanea capillata* (Anderson and Schwab, 1982b). In *Polyorchis penicillatus* among them, the neuro-neuronal and neuromuscular synapses have been more extensively studied than any other cnidarian synapes, since the *in vivo* preparation of *Polyorchis* is amenable to conventional intracellular recording techniques. Electrophysiological studies show that the hydrozoan synapes have conventional properties: i) Excitatory postsynaptic potentials follow the presynaptic spikes with a constant delay of about 7 ms between 'B' neurons (bursting neurons carrying photic information) and swimming motor neurons (Spencer and Arkett, 1984), and of 3 ms between swimming motor neurons (SMNs) and overlying epithelial cells (Spencer, 1982). ii) This transmission is blocked by high concentrations of Mg^{2+} (Spencer, 1982), suggesting that Ca^{2+}-dependent release may be the mechanism of synaptic transmission in *P. penicillatus* as in the frog neuromuscular junction (del Castillo and Katz, 1954). iii) Injecting current into SMNs does not alter the membrane potential of postsynaptic epithelial cells (Spencer, 1982). Ultrastructural studies also show that there are dense or clear vesicles on one side of a relatively short length of apposed, parallel, electron-dense membrane separated by a synaptic cleft in neuro-neuronal and neuromuscular synapses in *P. penicillatus* (Spencer, 1979). However, the identity of neurotransmitters in the hydromedusae remains enigmatic, despite considerable efforts over the past decade.

Dopamine (DA), one of the best-known neurotransmitters in the mammalian nervous system, was found in the bell margin of the hydrozoan *Polyorchis penicilliatus* where most neurons are concentrated (Chung *et al.*, 1989). It suggests that DA may play a role as a neurotransmitter even in this "primitive" nervous system. However, the mere presence of a chemical substance gives no indication of neuroeffectiveness nor releasibility which are important criteria in determining a chemical substance as a neurotransmitter. Recently, it was found that DA applied to cultured SMNs of this

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jellyfish produced hyperpolarization accompanied by a decrease in firing rate or complete inhibition of spiking induced by anodal break excitation (Chung and Spencer, 1991a). Pharmacological studies also showed that such an inhibitory action of DA was mediated by a D_2-like receptor (Chung and Spencer, 1991b). Here, the releasibility of DA in the jellyfish nervous system and the presence of dopaminergic cells were examined using a biochemical and a histochemical method, respectively.

Materials and Methods

Materials

Dopamine (DA), epinephrine (EN), norepinephrine (NE), epinine, 5-hydroxytryptamine (5HT), tryptamine, octopamine hydrochloride 3,4-dihydroxybenzylamine (DHBA), 3,4-dihydroxyphenylalanine (DOPA), 3,4-dihydroxyphenylacetic acid (DOPAC), 3,4-dihydroxyphenylglycol (DHPG), homovanillic acid (HVA), 5-hydroxyindoleacetic acid (HIA), tyrosine (Tyr), and tris(hydroxymethyl)aminomethane (TRIS) were obtained from Sigma (St. Louis, MO, USA): sodium octyl sulfate (SOS) was from Kodak (Rochester, NY, USA): Other chemicals were HPLC or reagent grade and were obtained from local suppliers.

HPLC-ED system

Apparatus : A Waters M-45 solvent delivering system was equipped with a U6K injector with a biophase ODS RP-18 column (5 μm spheres, 250×4.7 mm I.D.) and a BAS LC-4A electrochemical detector (ED) with an applied potential of 0.8 V. For inejction into both liquid chromatographs, Hamilton 801 or 825 syringes were used.

Mobile phase : The components of this mobile phase consisted of 50 mM monobasic sodium phosphate, 30 mM citrate buffer, 0.1 mM disodium EDTA, and 25% methanol. pH adjustments were made using conc. phosphoric acid. Both mobile phases were finally vacuum-filtered through a 0.22 μm membrane filter (Millipore). The flow rate was 0.5 ml per minute.

Preparation of standard solutions : Working standard amine solutions of 80 nM were prepared by dilution of stock solutions immediately prior to use. The stock solutions of reference amines and internal standards (1 mM) were prepared monthly in 0.02 N HCl containing 0.54 mM EDTA sodium salt. These solutions were stored in dark bottles at about 4℃.

Sample preparation : The determination of catecholamines in tissue samples is often plagued by the problems associated with post-mortem changes. The extent of these changes depends upon the time that elapses between death and sample preparation (Carlsson and Winblad, 1976), as well as the temperature at which samples were stored prior to processing (Wilk and Stanley, 1978). The optimal conditions for assessment of catecholamine levels in a sample requires storage of the tissue sample at 4℃ and immediate homogenization after dissection.

The jellyfish, *Polyorchis penicillatus*, were kept in cooled, recirculated artificial sea water (10~12℃). They were sometimes fed with brine shrimp. All animals were starved for a minimum of 2 days before use.

The jellyfish were dissected by removing the apex and radially bissecting the bell. Single hemispheres were then pinned, subuumbrellar-side uppermost, in an anesthetic solution of 1 : 1 isotonic $MgCl_2$ and sea water to a Sylgard (Dow-Corning) base in a large Petri-dish using stainless steel pins and spines of the cactus *Opunita*. Nerve rings were dissected out from the preparation illuminated obliquely from below by a fiber-optic lamp. The tissue samples were pooled from 5 to 8 animals as required to give sufficient tissue (100~200 mg wet mass), then the sulface water was remove and the sample weighed. After weighing, the tissue samples were immediately frozen at −25℃. One ml of freshly prepared, ice-cold 0.1 N perchloric acid containing 1% sodium metabisulphite, and internal standard (DHBA; dihydroxybenzylamine) were added to 100~200 mg of the frozen tissue. The samples were homo-homogenized, placed in an ice bath for 10 min, and then centrifuged at 3600×g for 1 h at 4℃. The catecholamines were extracted with acid-activated alumina, baked at 120℃ for 2 h, following the procedure described by Cyril (1985). The supernatant was transferred to another tube containing 50 mg of activated alumina. After adding 1.5 M TRIS buffer containing 54 mM EDTA (pH 8.6), the tube was vortexed for 5 min and then centrifuged for 30s at 1000×g. The solution was removed by vacuum aspiration and the alumina was washed twice with double-distilled, deionized water (pH to 7.0). Catecholamines were eluted with 100 μl of 0.1 M or 0.5 M HCl.

Calculations of catecholamine concentrations : Sample concentrations were calculated by comparing peak height ratios (relative to DHBA) of samples with peak height ratios of the unextracted standards. For example,

$$\text{Concentration of DA in sample} = \frac{\text{peak height ratio DA/DHBA for sample}}{\text{peak height ratio DA/DHBA for standard}} \times [\text{I.S.}] \times K$$

where [I.S.] is the concentration of the internal standard DHBA added to the sample and K is the calibration factor due to the matrix effect.

Data are presented as mean values and error bars as S.E.M. The significance of the results was analyzed by Student's *t*-test with $p<0.05$.

TLC analysis

One ml of freshly prepared, ice-cold 0.1 N perchloric acid containing 1% sodium metabisulphite was added to 0.05 g of the dried tissue. The samples were homogenized, placed in an ice bath for 10 min, and then centrifuged at $3000\times g$ for 30 min at 4℃. The supernatants were used in TLC analysis.

TLC was performed on commercially pre-coated 20 ×20 cm, 0.25 mm silica gel TLC plates (Whatman Co.), using n-butanol-acetic acid-water (volume ratio, 60 : 20 : 20) as the solvent. The elution procedure lasted 4 h at room temperature. The front was allowed to advance 16 cm from the base line. Two development procedures were used: i) The bands were developed by spraying the plates with a solution of 2% paraformaldehyde in absolute ethanol and then baking them in an oven at 150℃ for 20 min. Fluorescent bands were generated by UV light. ii) Control plates, which were not treated with paraformaldehyde solution, were completely dried under an air-stream. Samples of silica gels at specific regions were obtained by scratching them off with a razor. They were then placed in 0.5 N HCl or methanol solution. After removal of the silica gel from these solutions with centrifugation at $3000\times g$ for 30 min, the supernatants were introduced to the HPLC.

Cytochemistry

Neurons were cultured from the bell margin of the jellyfish *P. penicillatus* using the method described by Chung and Spencer (1991a), with some modification. Strips of velum (approximately 30 mg wet weight) were removed from the bell margin of the jellyfish, cut into small pieces, and placed for 5 min in a borosilicate test-tube containing normal artificial sea water (NASW : NaCl 378, $CaCl_2$ 9.5, Na_2SO_4 5.7, KCl 55.4, $MgCl_2$ 29, HEPES 10 in mM; pH adjusted to 7.4 using 1 N NaOH at 18℃). The NASW was replaced with double-distilled, deionized water for 30s to 1 min, which removed many vacuolated epithelial cells due to hypoosmotic shock. The tissue was rinsed again with NASW, and then exposed for 10 mins to divalent cation-free ASW. Then followed a digestion in fresh collagenase solution (Sigma, Type I), 1200~1500 U/ml in NASW. After 6 to 8 h of enzyme treatment at room temperature (18~20℃) the enzyme solution was removed using a Pasteur pipitte and then NASW was added. After rinsing twice with NASW, the tissues were triturated with a fire-polished Pasteur pipette. The cell suspension was plated to Petri-dishes coated with homogenized, dried mesoglea. One hour after the cells were plated, the cultures were rinsed twice and covered with NASW, and then kept at 10~12℃ until use.

The modified glyoxylic acid (GA) method of de la Torre and Surgeon (1976) was followed with a small modification for the cultured cells; HEPES buffer was used instead of phosphate buffer solutions for GA solutions. Cultures were incubated at 12℃ in an incubator with precursors (0.1 mM of DOPA or tyrosine solutions) for 30 mins and then washed twice with NASW. The prepared cultures were washed twice with sucrose-HEPES solution and incubated at 4℃ with SHG solution (contained 1% GA, 20 mM HEPES, and 0.6 M sucrose; pH adjusted to 7.4 with 1 N NaOH) for 1 to 5 min. Cultures were then air-dried at room temperature and baked at 80℃ in an incubator for 90s. A drop of mineral oil was placed over the cultures and then the dish was coverslipped. They were examined under a UV microscope. Controls were not incubated in precursors solutions and/or SHG solutions but were otherwise treated the same way.

Results

Endogenous or exogenous ?

Any substance existing as a neurotransmitter can be expected to be found in relatively higher concentrations in nerve-rich tissues than tissues having few or no neurons. In this respect, the higher concentrations of DA detected in tissue samples containing nerve-rings are probably significant (Chung *et al.*, 1989). Then it was important to establish whether any of the detected DA came from an exogenous source such as food. Jellyfish were kept in a tank recirculating artificial sea water and starved for various time intervals. The residence time of food in the hydrozoan gastrovascular system was assumed to be 24 to 48 h, since pseudofaeces were usually found in the tank 1 day after jellyfish were fed with brine shrimp. Starvation for 1 week apparently made jellyfish less active. The level of DA in the tissues examined increased ($p<0.05$) from 150 fmol ($N=10$) to 180 fmol ($N=9$) per mg wet mass after 3 days of starvation and then decreased ($p<0.005$) to 130 fmol ($N=10$) which was close ($p=0.12$) to the control level (Fig. 1). The level of DA would have decreased continuously with starvation if the DA were exogenous. It is, thus, highly probable that DA is an endogenous compound in this hydromedusa.

Although the purpose of the starvation experiments was to determine if the DA found in neve-rich tissue was endogenous, it was surprising that the levels of DA increased after 3 days starvation. There is no ob-

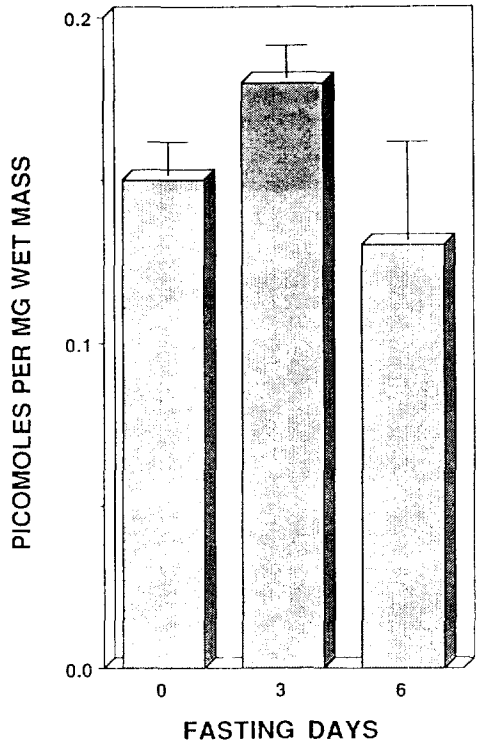

Fig. 1. Changes in the levels of DA in nerve-rich tissue during fasting. The amount of DA in nerve-rich tissue was 150 fmol/mg wet mass ($N=10$) from animals which were sacrificed immediately after they were collected. Three days later the DA levels increased to 180 fmol/mg wet mass ($N=9$; $p<0.05$, *t*-test) and after six days returned to a level of 130 fmol/mg wet mass ($N=10$) which was not significantly different from the control's. During this experiment, jellyfish were not fed with brine shrimp and tissues were collected under an anesthetizing saline (Mg^{2+}-containing saline). Error bars, S.E.M.

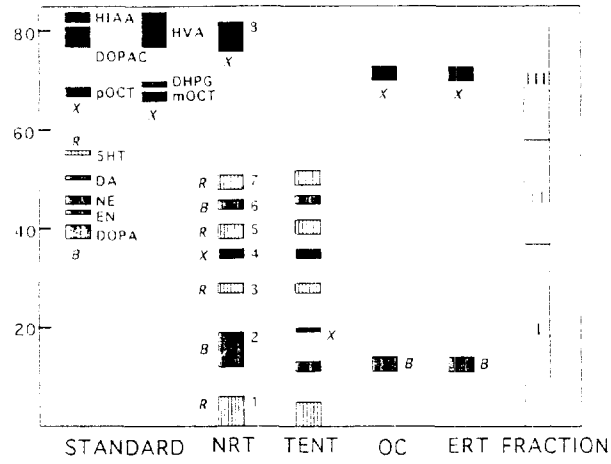

Fig. 2. Thin layer chromatogram of standard amines, their metabolites and dried tissue samples from *P. penicillatus*. Each of 10 μl of 1 mM standard catecholamines and DOPA solutions (10 nmol in 0.1 N perchloric acid) was applied to a TLC plate. They were clearly separated on the TLC plate and generated their characteristic fluorescence when they reacted with aldehyde: Whitish-blue fluorescence (B) from DOPA and catecholamines and red one (R) from serotonin (5HT). Octopamine, acidic metabolites of monoamines and DHPG did not produce any fluorescence. However, they were easily spotted on a TLC plate since they remained in the plate as dark brown scorch marks (X) after baking in an oven. Homogenates (in 0.1 N perchloric acid) of dried tissue samples were used in TLC analysis. Several fluorescent bands appeared from tissue samples, especially from NRT and TENT. Their R_f values and fluorescent colours (i.e., excitation wavelength) did not match those of standard compounds. Therefore, the compounds in the NRT, producing fluorescent bands 5, 6 and 7, are unlikely to be catecholamines. Fraction II which was supposed to include these compounds was introduced into HPLC system (see the following Fig. 3). The R_f value of band 8 appearing in only NRT was similar to that of DOPAC. [a DOPAC-like compound appeared in the HPLC chromatogram for Fraction III which was supposed to include a compound corresponding to band 8. See Fig. 3.] NRT, nerve-rich tissue; TENT, tentacle; OC, ocelli; ERT, endoderm-rich tissue.

vious explanation for this finding. However, it is possible that the amounts of DA present in nerve-rich tissue varies in a cyclical manner (e.g. diurnal cycle) and the differences measured during starvation were merely due to samples being taken at different times during this cycle.

The presence of DA precursor and metabolite

The family of DA should be present in the nerve-rich tissue if DA is an endogenous compound. To find out whether or not DOPA and DA metabolites were present in the jellyfish, TLC combined with HPLC was employed because the alumina extraction method used for quantitation of DA in sample tissues (Chung *et al.*, 1989) are highly selective for catecholamines.

Five clearly separated fluorescent bands were observed on the TLC plates using standard catecholamines, DOPA and serotonin, after elution with butanol-aceticacid-water (Fig. 2). The white-blue fluorescence of four of the bands corresponded to DOPA and catecholamines while the red fluorescence of the fifth band corresponded to serotonin. Para- and meta-octopamine and the acidic metabolites of amines such as DOPAC, HVA, DHPG, and HIAA were clearly separated and distinguished from catecholamines and serotonin. Their R_f values were distinctly greater than those of the catecholamines and serotonin and these compounds did not produce any fluorescence after reacting with paraformaldehyde. The low sensitivity of the detection method used here may be one of the reasons that octopamine at 10 nmol, both para- and meta-form, did not produce under UV light after reacting with 4% paraformaldehyde solution. The tissue samples increased the developing time of amines on the plates but had no effect on their R_f values. The values of $R_f \times 100$ are presented in Fig. 2.

Each of 0.05 g of dried nerve-rich tissues and tentacles produced TLC chromatograms having 8 bands,

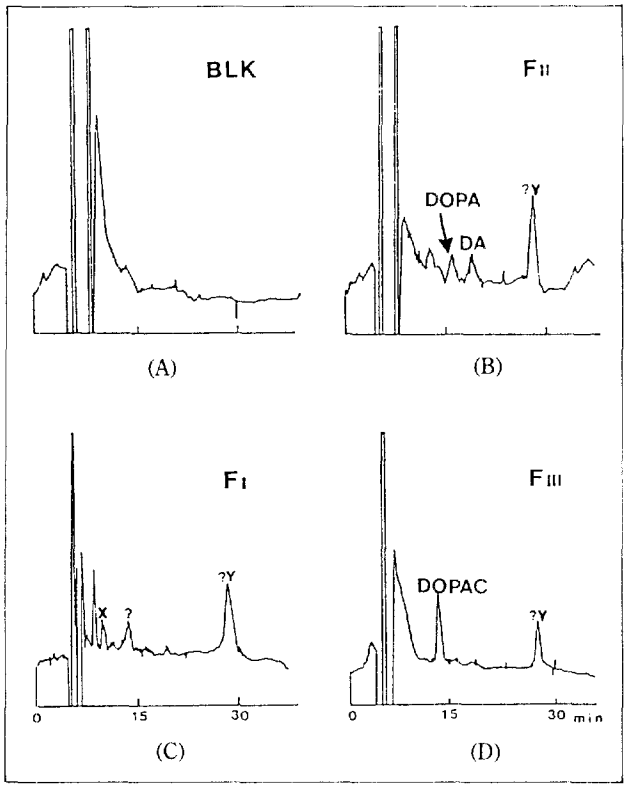

Fig. 3. Liquid chromatograms of TLC fractions of nerve-rich tissue of *Polyorchis*. The acidic eluate of each fraction from the TLC plate (refer to the previous Fig. 2) was introduced to HPLC. A shows a blank chromatogram (BLK) for an acidic eluate of the TLC plate itself. The other panels, B, C and D show respectively the chromatograms for acidic eluates of the TLC fractions I, II and III of nerve-rich tissue.

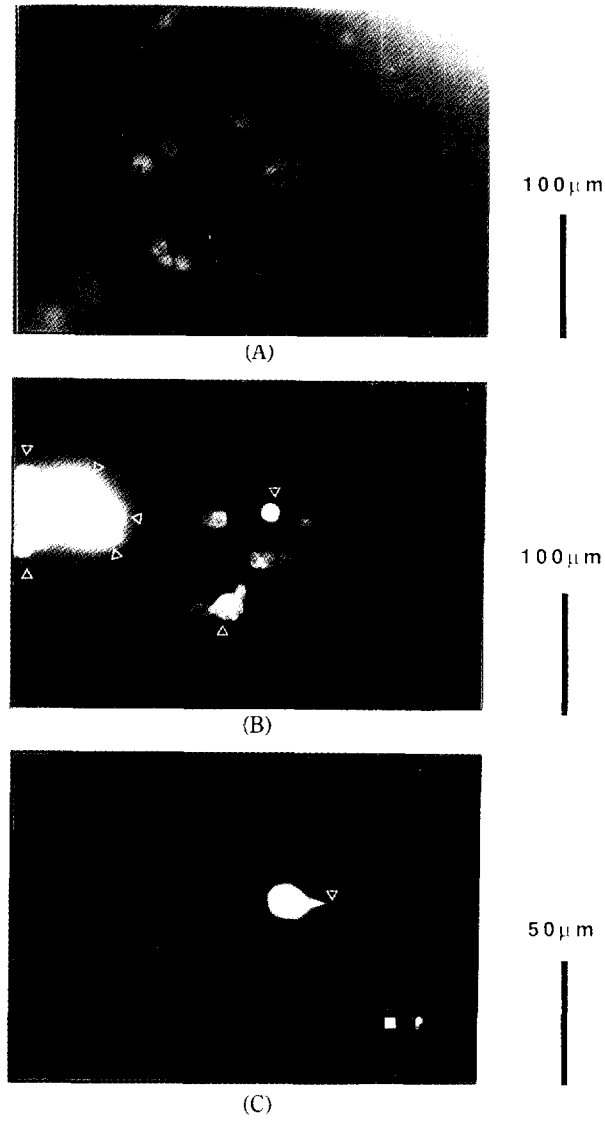

Fig. 4. Glyoxylic acid (GA) induced fluorescence in primary cultures of nerve-ring tissue of *Polyorchis penicillatus* which were pre-incubated with DOPA. A) A control in which cells were not incubated with sucrose-HEPES-glyoxylic acid (SHG) solution. B) Some cells treated with DOPA for 30min prior to processing with SHG exhibited a strong blue-white fluorescence. Triangles (△) denote cells producing specific fluorescence. C) One of the fluorescent cells which were fluorescent is shown at higher magnification. The cell had a thin and long process (△). [n.b. another cell (■) seen in the panel did not show specific fluorecence.]

while the same amount of ocelli and radial canal tissue produced chromatograms having only two bands (Fig. 2). The yellow fluorescence of band 8 in the nerve-rich tissue sample had a high R_f value. This, however, gave little information as to whether or not the compound of band 8 corresponded to the catechol-4, with a high R_f value on a paper chromatogram, which Östlund (1954) identified in a sea anemone *Metridium dianthus*. Fluorescent bands 5, 6, 7 (red, blue and red, respectively) of nerve-rich tissue appeared on regions of the plate where standard monoamines had located but their R_f values and colors were different from those of standard amines. Furthermore, all these fluorescent bands were not observed after extracting with alumina (data not shown). Approximately 0.05 g of dry sample of nerve-rich tissue corresponded to around 0.45 g wet weight since the ratio of dry samples to wet was approximately 9 and DA content in nerve-rich tissue was 120 fmol/mg wet mass (Chung *et al.*, 1989). Thus, dry samples of nerve-rich tissues used in this experiment should contain DA at concentrations less than one nanomole which is below the detected level of the method used. When larger quantities of dry sample were used, the fluorescent bands on the plate overlapped and could not be distinguished from one another.

An HPLC analysis on the TLC fractions I, II and III (Fig. 3) was run in order to 1) confirm the previous HPLC observations that neither NE nor EN were present in the nerve-rich tissue and that only DA was present, 2) determine the R_f values of TLC for the unknown compounds X and Y appearing on the previous LC chromatograms (see Chung *et al.*, 1989), and 3) examine a metabolic pathway for the DA present in

the jellyfish. This was not entirely satisfactory because of the presence of the unknown peaks from the TLC fractions appearing on the LC chromatograms (Fig. 3) and the extremely low recovery of amines from silica gel when acidic metanol was used as the extracting solvent (less than 5% yield in the case of DA). Also, the concentrations of either DOPA or DOPAC-like compounds were so capriciously variable, from sample to sample, that their levels in tissue could not be quantitatively determined. These results (Fig. 3), however, suggest the following; 1) DOPA and DA are present in Fraction II and correspond to the catecholamine region of the TLC, 2) NE and EN are absent from the nerve-rich tissue, 3) a DOPAC-like compound, presumably one of the DA metabolites, is present in Fraction III corresponding to the DOPAC region of the TLC, 4) the unknown compound X is present in the TLC Fraction I. Because both catechol-4 and the unknown compound X have low R_f values on open chromatography, vz. paper and thin layer chromatography respectively, and probably are present in fairly large amount in the tissues used, the unknown compound X may be catechol-4.

Present of dopaminergic cells in *Polyorchis penicillatus*

To localize dopaminergic cells, several histochemical techniques such as Falck-Hillarp technique (Corrodi and Jonsson, 1967), Faglu-PEG method (Scholer and Armstrong, 1982) were employed for the *in vivo* tissue preparations. The results were ambiguous and no clear demonstration of monoamines in nerve-rich tissues was possible. One possible explanation for the failure of these histochemical methods is that the autofluorescence that is often present in tissues of marine animals makes it difficult to distinguish the amine-induced fluorescence. In an attempt to circumvent this problem, I used a GA method with cultured cells which were pre-incubated in media containing a precursor, DOPA. When the cultured cells were pre-incubated with DOPA, specific fluorescence (blue-white) was seen in a population of small cells (Fig. 4B) which could not be identified. They might have been interstitial cells, epithelial cells, or small neurons. This result indicates that some cells, whether neurons or non-neuronal cells, have the ability to take up DOPA. In addition, these cells may have an aromatic aminoacid decarboxylase which converts DOPA into DA. However, it should be noted that DOPA as well as DA can produce a fluorophore as a product of the reaction with glyoxylic acid. Recently, some of the smallest cells with diameters less than 10 μm were excitable (unpublished data) and these cells had long and thin processes like dopaminergic cells (Fig. 4C). It is possible that these excitable cells contain DA. From the histochemical results, it is evident that SMNs do not contain DA (data not shown). Therefore, DA is probably not involved in neural transmission at the neuromuscular junction between SMNs and swimming muscle cells.

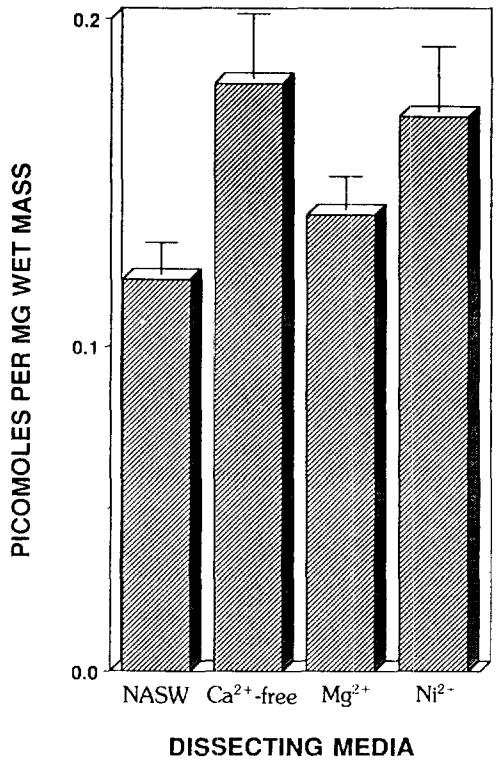

Fig. 5. Differences in the dopamine levels of nerve-rich tissue using different dissecting-media. When nerve-rich tissue (sample number N=14; 1 sample consisted of 3 animals) was dissected in normal artificial sea water (NASW), the concentration of DA was 120 fmol/mg wet mass. However, when dissected in Ca^{2+}-free saline or Ni^{2+}-containing saline, the DA levels were higher; 180 fmol/mg wet mass (N=10; $p<0.05$, *t*-test) and 170 fmol/mg wet mass (N=20; $p<0.05$) respectively. Tissue dissected under Mg^{2+}-containing saline showed a trend towards higher dopamine concentrations (140 fmol/mg wet mass, N=25) but the level was not significantly different from that in NASW. In this experiment, all animals were starved 2 days before use. Error bars, S.E.M.

Effect of divalent cations on the content of DA in nerve-rich tissues

Nerve-rich tissues were collected in different dissecting media; NASW, Ca^{2+}-free ASW, and ASW including Mg^{2+} or Ni^{2+} (obtained by mixing 0.33 M of $MgCl_2$ or $NiCl_2$ with ASW by 1 : 1 ratio). More DA was found in nerve-rich tissues collected in Ca^{2+}-free ASW or ASW including Mg^{2+} or Ni^{2+} than in tissues collected in NASW (Fig. 5), indicating that the content of DA in the tissues was higher in the absence of Ca^{2+} than in the presence of Ca^{2+}. Calcium ions are known to be necessary for the release of neurotransmitters (Katz,

1966). Therefore, one possible explanation for this observation is that, during dissection, less DA would be released from dopaminergic cells in the absence of Ca^{2+} ions.

Discussion

The case for a particular substance acting as a transmitter can only be built from a number of different lines of evidence. For technical reasons, it is rarely possible to satisfy all the following criteria (Leake and Walker, 1980; Schwartz, 1985): 1) *Presence*; it should be possible to localize the suspected transmitter histologically and biochemically, 2) *Release*; during stimulation of the presynaptic element, the suspected transmitter should be released from the nerve ending in amounts commensurate with its biologically effective concentration, 3) *Mimicry (biological activity)*; exogenous application of the putative transmitter should mimic the actions of the endogenous transmitter, 4) *Pharmacological properties*; the action of the putative transmitter should be altered by pharmacological agents in a predictable way, 5) *Inactivation*; a mechanism should be present for the removal of transmitter material from its site of action. Evidence is often collected in a piecemeal fashion until all the criteria are satisfied. Over the past several years, information has steadily accumulated to support the role of DA as a neurotransmitter in *P. penicillatus*.

DA as a neurotransmitter

According to the HPLC results, DA which is most abundant in nerve-rich tissues of *P. penicillatus* (Chung *et al.*, 1989) is endogenous (Fig. 1). The endogenous DA seems to be enzymatically synthesized from DOPA and degraded into DOPAC (Fig. 3). It suggests that DOPA decarboxylase and monoamine oxidase may be responsible for synthesizing and degrading DA, respectively. Indeed, some cells, not SMN, in nerve-rich tissues appear to take up DOPA and convert DOPA into DA (Fig. 4). Whether or not the dopaminergic cells are presynaptic to SMNs needs a histological experiment for the *in vivo* preparations. Tyrosine hydroxylase, one of the enzymes involved in catecholamine synthesis in mammals (Schwartz, 1985) and some invertebrates such as arthropod, mollusc and annelid (Leake and Walker, 1980), could not be demonstrated by immunohistochemical methods (unpublished data). Also, DA β-hydroxylase which converts DA into NE should not be present in *P. penicillatus* since neither NE nor EN appear in nerve-rich tissues. Therefore, DA synthetic pathway in this animal appears to be evolved differently than any other animals such as mammals. The idea that DA is involved in neural transmission in *P. penicillatus* comes from the observation that DA had specific electrophysiological effects on SMNs (Chung and Spencer, 1991a). DA apparently prevents a SMN from being excited by hyperpolarizing the cell membrane and inhibiting firing of the neuron. DA's actions are quite specific. DA affected only SMNs and was far more potent in exerting an inhibitory action than any other amine. In addition, D_2-like receptors were pharmacologically identified to be responsible for DA's action (Chung and Spencer, 1991b). The role of DA as a neurotransmitter in this animal is further supported by the observation that the DA content in the tissue is likely dependent on the concentration of calcium ions in the bathing medium (Fig. 5), suggesting that release of DA is calcium dependent. Calcium-dependent release of a transmitter appears to be common in many phyla (Linás, 1980; Zucker and Haydon, 1988), although there is some controversy (see Hochner *et al.*, 1989). All these results strongly suggest that DA may play an important role in neural transmission of the jellyfish as a neurotransmitter.

Crumpling : physiological significance of DA action

Crumpling, a contraction and shortening of the bell and an involution of the margin, usually initiated by mechanical stimulation of any part of the ectoderm, was observed when nerve-rich tissues were dissected out in NASW. On the other hand crumpling was rarely observed when tissues were dissected out in either Ca^{2+}-free saline or salines containing Ni^{2+} or Mg^{2+} ions (0.17 M). It is important to recall the HPLC results (Fig. 5): the DA content in tissues was greater in samples pooled in either Ca^{2+}-free saline or salines including Ni^{2+} ions than in those in NASW. This indicates that the residual content of DA was lower in tissues when crumpling occurred during the dissection procedures.

Intracellular recordings have identified two sources of inhibitory inputs to SMNs (Spencer, 1981; Arkett and Spencer, 1986). One of the inhibitory postsynaptic potentials (IPSPs) which accompany the phenomenon of crumpling is similar to the hyperpolarizaton induced by DA, being a slow, sustained membrane hyperpolarization followed by a spike with an afterhyperpolarization (AHP) greater than the AHPs of the previous spikes. These IPSPs were hypothesized to be electrically mediated since 0.08 M of Mg^{2+} ions could not block them (Spencer, 1981). However, it should be pointed out that a higher concentration (0.17 M instead of 0.08 M) of Mg^{2+} ions might have reduced or inhibited the IPSPs associated with crumpling. If so, then, the IPSPs associated with crumpling could be chemically mediated. Sensitivities of the synaptic events to Mg^{2+} and Ni^{2+} ions need to be examined more carefully.

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