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OCCURRENCE OF DOPAMINE AND NORADRENALINE IN THE NERVOUS TISSUE OF SOME INVERTEBRATE SPECIES

BY

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Östlund (1954) extensively investigated the distribution of catecholamines in invertebrate tissues. He found that both noradrenaline and adrenaline are constituents of the earthworm ganglionic chain and that insects contain appreciable quantities of certain catecholamines. However, he was unable to detect adrenaline or noradrenaline in whole tissue extracts of species from the following groups: *Protozoa, Coelenterata, Urochordata, Echinodermata, Crustacea,* and also *Mollusca.* Similarly, von Euler (1961) was unable to detect noradrenaline or adrenaline in extracts of the latter three groups. More recent studies by Sweeney (1963) have shown that dopamine (3,4-dihydroxy-phenylethylamine) is a constituent of molluscan nervous tissue. The amine was found in appreciable quantities in the ganglia of several gastropods and lamellibranchs but it was shown to be particularly abundant in the nervous tissue of the clam, *Mercenaria mercenaria.* However, noradrenaline was not detected in any of the species investigated.

This communication describes studies on the occurrence of both noradrenaline and dopamine in the nervous tissue of species of *Echinodermata, Mollusca* and *Crustacea.* In addition, it includes some studies on the effect of reserpine on the catecholamine content of nervous tissue of two species of these groups.

METHODS

The animals used in this study were as follows: sea urchins *Echinus esculentus* (*Echinoidea, Echinodermata*), starfish *Asterias rubens* (*Asteroidea, Echinodermata*), clams *Spisula solida* (*Lamellibranchia, Mollusca*), octopods *Eledone moschata* (*Cephalopoda, Mollusca*), and crabs *Hyas araneus* and *Carcinus maenas* (*Crustacea, Arthropoda*).

Freshly dissected tissue was homogenized in ice-cold 50% acetone/0.01N HCl and then centrifuged for 20 min at 6,500 *g*. The supernatant solution was concentrated under reduced pressure for chromatography. Chromatograms (Whatman 3 mm) were developed in an ascending fashion. Two solvents were used: (1) phenol/HCl/KCN (Waalkes, Sjoerdsma, Creveling, Weissbach & Udenfriend, 1958), (2) n-butanol/HCl (Bertler & Rosengren, 1959). Noradrenaline and dopamine spots were localized by the highly sensitive fluorescence method, using paraformaldehyde, which has recently been developed for paper chromatography by Bell & Somerville (1966). Bell and Somerville showed that the fluorescence of dopamine on paper is linear over the range 0.2–4 μg of amine. We have also observed that there is a linear relationship between fluorescence and concentration of noradrenaline over this range.

The catecholamine content of chromatographed spots was estimated by comparison with known amounts of pure substance, ranging in amount from 0.5 to 3 μg amine, by direct vision under ultraviolet lamps (peak emission 254 mμ and 366 mμ) and/or spectrophotofluorometrically on a Baird-Atomic Fluorispec. The concentration of extracts was arranged so that their content of noradrenaline, or dopamine, or both, fell within the range 0.5 to 3 μg of amine. The fluorescence characteristics of extract spots were routinely compared with pure chromatographed dopamine and noradrenaline. Of all the methods tested, chromatographic and spectrophotofluorometric, the procedure outlined above gave the most accurate and reproducible results in our hands with the comparatively small amounts of tissue available.

In order to determine the effect of reserpine on the catecholamine content of *Spisula* and *Asterias* nervous tissue, batches of animals were immersed for five days in sea water containing 2 μg/ml. (*Spisula*) or 3 μg/ml. (*Asterias*) of soluble reserpine phosphate (kindly supplied by Ciba Laboratories, Ltd., Horsham, Sussex). The temperature of the medium was maintained at 15–16° C. These particular conditions were chosen bearing in mind the studies of Mirolli & Welsh (1964) on the effect of temperature and length of period of administration of reserpine on the depletion of 5-hydroxytryptamine from molluscan ganglia. Controls were kept at the same temperature. The bathing solution of the animals was changed daily.

RESULTS

A summary of the results is presented in Table 1. The positive identification of dopamine and noradrenaline was based on their Rf values in two solvent systems and the colour of the fluorescent spots on paper. Furthermore, when sufficient quantities were available, the fluorescent spots were eluted with 0.1N HCl, and the activation and fluorescent spectra of the eluted materials determined.

Nervous tissue from both species of echinoderms contained dopamine and noradrenaline. The fluorescence spectra of these compounds from the radial nerve of *Echinus* and dilutions of pure dopamine and noradrenaline are shown in Fig. 1. With both *Echinus* and *Asterias*, the content of dopamine in nerves exceeded that of noradrenaline.

Immersion of *Asterias* in sea water containing reserpine caused a pronounced reduction in the dopamine content of the radial nerves ; the depletion of noradrenaline was less. After starfish had been bathed in reserpinized sea water for four or five days they took considerably longer to right themselves, after being placed oral side uppermost, than control animals.

Chromatographic studies showed that ganglia from the lamellibranch *Spisula* contained a large amount of dopamine and also some noradrenaline. Further evidence that noradrenaline is present in *Spisula* ganglia was obtained by bioassay. Extracts, each prepared from the ganglia of 70 clams were chromatographed in the phenol/HCl solvent system, which completely separates noradrenaline from 5-hydroxytryptamine. After thorough drying, the chromatograms were cut into small segments, which were eluted with distilled water. The eluates were tested on the isolated *Eledone* heart. Speed and force of beat of this preparation are increased with amounts of noradrenaline of 50 ng or greater and also by very small amounts of 5-hydroxytryptamine. Only two areas of the chomatograms had these excitatory effects on the heartbeat. One of these areas corresponded precisely with the Rf value of noradrenaline and the other with that of 5-hydroxytryptamine. The degree of excitation produced by the noradrenaline region of the chromatogram was consistent with the amount of noradrenaline estimated fluorometrically.

TABLE 1

APPROXIMATE CONCENTRATIONS OF DOPAMINE AND NORADRENALINE IN TISSUES OF SEVERAL INVERTEBRATES AND THE EFFECT OF RESERPINE ON NERVOUS TISSUE CONTENT OF THESE CATECHOLAMINES

The figures represent the range of values obtained from different batches of tissue dissected at different times. The values have not been corrected for recovery, which varied between 50% and 30% depending largely on the presence of substances in the extracts which interfered with the detection of the fluorescence spots on paper. Thus values for dopamine and noradrenaline in molluscan and echinoderm nervous tissues should be multiplied by a factor of two and those of crustacean tissues by a factor of three, to account for loss and imperfections in the technique

Species	Catecholamine content in μg/g wet tissue		Percentage Depletion of catecholamines with reserpine		Total number of experiments, and number of quantitative estimations in brackets
	Dopamine	Noradrenaline	Dopamine	Noradrenaline	
Echinodermata					
Echinus esculentus					
radial nerve	2·5–7·0	1·5–3·5			5(3)
Asterias rubens					
radial nerve	3·0–8·0	0·5–2·0			8(3)
3 μg soluble reserpine/ml. in sea water at 15° C for 5 days			50–70	20–50	(2)
Mollusca					
Spisula solida ganglia	40–50	5·0–6·0			12(6)
2 μg soluble reserpine/ml. in sea water at 15° C for 5 days			40–50	?	(4)
Eledone cirrhosa brain	8·0–13	2·0–5·0			6(5)
Crustacea					
Hyas araneus ganglia	<0·5	<0·5			(2)
heart	<0·5	<0·5			(2)
Carcinus maenas ganglia	0·5–1·0	<0·5			9(3)

Reserpine depleted the stores of dopamine in *Spisula* ganglia. However, under the conditions used, less dopamine than 5-hydroxytryptamine was lost from the tissue. In several experiments we have found that immersion of clams in sea water containing reserpine depletes some 50% of the total dopamine, and 80% of the total 5-hydroxytryptamine. The 5-hydroxytryptamine was estimated spectrophotofluorometrically. It is worth noting that *Spisula* ganglia also contain very high levels of 5-hydroxytryptamine. An average value from five estimations was 58 μg of 5-hydroxytryptamine/g wet tissue.

So far we have been unable to determine the effect of reserpine on the noradrenaline content of *Spisula* ganglia, because of the prohibitively large numbers of animals and large amounts of reserpine which would have to be used for such studies with the present methods.

Dopamine and noradrenaline were also detected in the brain of the small octopod *Eledone cirrhosa*. Again the content of dopamine exceeded that of noradrenaline.

Heart and nervous tissue of the spider crab *Hyas* was analysed for catecholamines. However, none was definitely detected, although on one chromatogram of ganglia extract a weakly fluorescent spot with the Rf value of noradrenaline was observed. Chromatograms of ganglia showed strongly fluorescent blue spots which may have obscured very small amounts of noradrenaline.

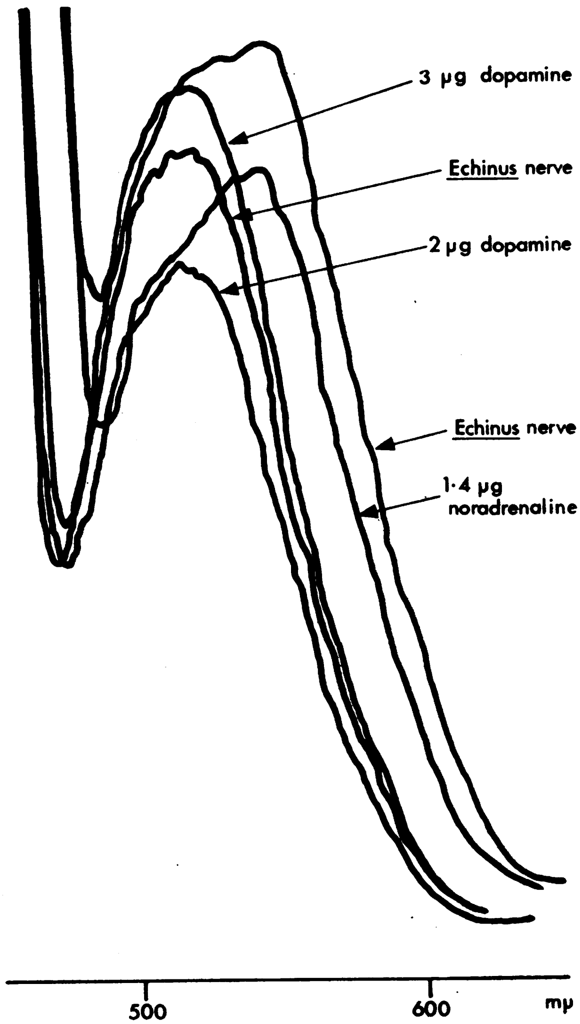

Fig. 1. Fluorescence spectra of eluates of strips, corresponding to the Rf of noradrenaline and of dopamine of *Echinus* nerve extract and samples of pure noradrenaline and dopamine, chromatographed at the same time. The chromatogram had been sprayed with glycine and exposed to paraformaldehyde (Bell & Somerville, 1966). The excitation wavelength was 430 mµ.

Many chromatograms of *Carcinus* nervous tissue extracts were developed in the two solvent systems. With some of the chromatograms, blue fluorescent spots and streaks obscured any yellow fluorescence in the positions corresponding to noradrenaline and dopamine. However, a faint yellow spot with an Rf value corresponding to that of dopamine was observed in some chromatograms of each solvent. None of the blue fluorescent spots has yet been definitely identified. However, Bell & Somerville (1966) have shown that certain methoxy derivatives of dopamine give intense blue fluorescence when treated with paraformaldehyde under the conditions used in this study.

DISCUSSION

As far as is known, this is the first report on the occurrence of dopamine and noradrenaline in the radial nerves of the starfish and the sea urchin. These observations are of interest in relation to the finding of Boltt & Ewer (1963) that noradrenaline has a potent depressant action on the lantern retractor muscle of the echinoid *Parechinus*. The fact that dopamine was more readily lost than noradrenaline from the radial nerve of starfish following treatment with reserpine could possibly be explained by a more rapid turnover of dopamine than noradrenaline in the tissue. The possibility that these amines are particle bound in the radial nerves has not yet been investigated.

The present studies on the occurrence of dopamine in *Spisula* ganglia with a new chromatographic method support the findings of Sweeney (1963). There is some evidence that dopamine is particle bound in molluscan nervous tissue (Cottrell, 1966). Furthermore it has been shown that the amine has marked inhibitory actions on certain molluscan neurones (Kerkut & Walker, 1961 ; Gerschenfeld, 1964). Thus the possibility is raised that this amine may act as a nerve impulse-mediating substance in molluscs.

Recently Kerkut, Sedden & Walker (1966) have also found that reserpine decreases the content of dopamine in the ganglia of the gastropod mollusc *Helix aspersa*. Furthermore, it was shown that injections of DOPA (3,4-dihydroxyphenylalanine) increased the level of dopamine in nervous tissue of this snail.

The detection of noradrenaline in *Spisula* ganglia is an interesting finding since previously it has been assumed that this substance is not present in molluscs (Östlund, 1954 ; von Euler, 1961 ; Kerkut *et al.*, 1966 ; Dahl, Falck, von Mecklenburg, Myhrberg & Rosengren, 1966). Kerkut & Walker (1961) have shown that noradrenaline inhibits some neurones and excites others in the snail brain. Could it be that noradrenaline acts both as an excitatory and inhibitory transmitter in the molluscan central nervous system as does acetylcholine ? The advantages of the molluscan central nervous system for studying certain fundamental problems concerned with chemical transmission between neurones have already been clearly demonstrated (Tauc & Gerschenfeld, 1962 ; Kerkut & Thomas, 1964). Kerkut & Walker (1961) showed that noradrenaline was active in even more dilute solutions than acetylcholine on *Helix* neurones.

The fluorescence-microscopical technique of Falck (1962) has been used to identify catecholamine-containing neurones in gastropod and bivalve molluscs (Dahl, 1963 ; Dahl *et al.*, 1966 ; Sweeney, 1965). Until the present time, fluorescence, characteristic of catecholamines in neurones of molluscan ganglia, has been assumed to represent the localization of dopamine. However, the results in the present communication suggest that noradrenaline must now also be considered in these histochemical studies, at least in bivalve and cephalopod molluscs.

Using bioassay methods, Bertaccini (1961) showed that the brain of *Eledone moschata* contains catecholamines and that they are released with reserpine. However, Bertaccini (1961) did not determine which particular catecholamines are present in octopus nervous tissue. The identification of dopamine and noradrenaline in *Eledone cirrhosa* brain helps to clarify this point.

In the echinoderm, molluscan and crustacean species studied, the content of dopamine in nervous tissues exceeded that of noradrenaline. This is the same condition as that found in certain areas of the mammalian brain—for example, the caudate nucleus of the cat, dog and man (Mclennan, 1963). The reason for the preponderance of dopamine over noradrenaline is unknown, but it would seem likely that dopamine may have a function other than being a precursor of noradrenaline in these situations. This view has been considered for some time both in invertebrates and also in vertebrates (Kerkut & Walker, 1961 ; Vogt, 1965).

Finally, it should be pointed out that, although the methods used in this study are suitable for detecting very small quantities of noradrenaline and dopamine, they are considerably less sensitive for adrenaline. The minimum amount of adrenaline that we could detect was of the order of one-twentieth of that of noradrenaline. The possibility, therefore, that the tissues studied contain small quantities of adrenaline cannot be excluded.

SUMMARY

1. The occurrence of noradrenaline and dopamine in nervous tissue of species of echinoid and asteroid echinoderms, bivalve and cephalopod molluscs and crustaceans was investigated.

2. A large amount of dopamine was detected in the bivalve mollusc *Spisula solida*, together with appreciable amounts of noradrenaline. Smaller quantities of these amines were also found in nervous tissue of the cephalopod mollusc *Eledone cirrhosa* and also in both echinoderm species studied. Dopamine alone was detected in the crustacean *Carcinus maenas*.

3. In all of these species, the concentration of dopamine in the nervous tissues greatly exceeded that of noradrenaline.

4. Reserpine depleted nervous tissue of dopamine and, to a lesser extent of noradrenaline, in the starfish, *Asterias rubens*. Reserpine also lowered the dopamine content of nervous tissue of the clam, *Spisula solida*.

5. The significance of these findings is briefly discussed.

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