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AUTORADIOGRAPHIC LOCALIZATION OF 5-HYDROXYTRYPTAMINE AND NORADRENALINE IN THE CENTRAL NERVOUS SYSTEM OF *LITHOBIUS FORFICATUS* L. (MYRIAPODA; CHILOPODA)

by

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ABSTRACT

Using the ability of selective uptake by the neurons of their own secreted amines, two ^3H labeled neurotransmitters were used: 5-hydroxytryptamine (5 HT, serotonin) and noradrenaline (NA). Autoradiographic study was conducted on semithin and on ultrathin sections.

In the brain, ^3H-5 HT labeling is observed in the frontal lobes of the protocerebrum (two pairs of neurons) and in the lateral areas of the deutocerebrum and tritocerebrum (three or four pairs of neurons). A pair of labeled neurons is also present in each ganglion of the posterior part of the ventral nerve cord.

In the protocerebrum, ^3H-NA is taken up by a pair of neurons located in the frontal lobes; two pairs of noradrenergic neurons can be found in each ganglion of the nerve cord.

Labeled axons (5-HT and NA) are present in the brain and in the neuropils of the abdominal ganglions. The pathway of some cerebral axons can be followed in the perioesophageal connectives and in the cerebral glands.

These results are in agreement with the effects of injected neurotransmitters on (1) the brain electrical activity and (2) the stimulated activity of some of the protocerebral neurons (ultrastructural study by Jamault-Navarro in preparation).

INTRODUCTION

Catecholamines and indoleamines are present in the central nervous system of Arthropoda, e.g. in Insecta (for references see Evans, 1980), in Crustacea (Aramant & Elofsson, 1976; Elofsson et al., 1966, 1982), in Xiphosura (Walker & James, 1980; O'Connor et al., 1982; Roberts et al., 1983) and in Arachnida (Binnington & Stone, 1977; Meyer & Jehnen, 1980). Biogenic amines are also present in the neurohaemal organs of Insecta (for references see Lafon-Cazal, 1978) and Crustacea (Fingerman et al., 1974; Hanumante & Fingerman, 1982). These amines are able to act as neurotransmitters or neurohormones (Evans et al., 1976; Kravitz et al., 1980); however, little is known about the physiological role of these products.

In Chilopoda only one pharmacological study has been performed on the action of putative neurotransmitters and agonistic and antagonistic drugs on the electrical activity of the brain (Descamps & Lassalle, 1983). It appeared that a wide range of neurotransmitters has an electrical action on brain activity. So, it was interesting to study the localization of the neuromessengers in centipedes.

Most of the results concerning the localization of biogenic amines have been obtained by fluorescence methods (Falck-Hillarp or derived methods). Autoradiographic localization methods are less numerous. Yet autoradiography has the advantages of: (1) a more accurate result concerning the localization of neurons or axons in the nervous tissue and (2) a possibility of electron microscope study for cytological characteristics. Nevertheless, the use of specific antibodies has the same advantages.

In the present study the first results, concerning 5-hydroxytryptamine and noradrenaline localizations in the nervous system and cerebral glands of *Lithobius forficatus* (Linnaeus), are reported.

MATERIAL AND METHODS

The study was conducted on adults of *Lithobius forficatus* collected in the North of France.

5 Hydroxytryptamine (5-HT, serotonin)

5 Hydroxy ^{3}H tryptamine creatinine sulfate (specific activity 15.9 Ci/mM) was used, both in in vitro and in vivo labeling procedures.

In the in vitro series, the anterior part of the nervous system (brain and sub-oesophageal ganglion) and the posterior part of the nerve cord (ganglions no. 13, 14, 15 and terminal ganglion) were dissected and placed in 10^{-6}M ^{3}H 5-HT in Ringer solution at room temperature for one hour. Pieces were rinsed in Ringer prior to fixation.

In the in vivo series, each animal was injected with 5 μCi of ^{3}H-5-HT (estimated dilution in the animal: 10^{-6} M). After about 40 minutes, the anterior and posterior parts of the nervous system were dissected and fixed. The cerebral glands (paired cephalic neurohaemal organs) were also removed and fixed.

Noradrenaline (NA, norepinephrine)

Since with 5-HT the results were similar after in vitro and in vivo labeling, with NA, we have just used in vivo procedures.

Per animal 40 μCi of ^{3}H Noradrenaline chlorhydrate (specific activity: 39 Ci/mM) were injected (estimated final dilution 10^{-5} M). The nervous system and the cerebral glands were dissected and fixed 1 or 2 hours after injection.

In all experimental series the pieces were fixed in 6.25 % glutaraldehyde in a 0.1 M Na monophosphate/Na diphosphate buffer at pH 7.2 and postfixed with 1% OsO_4 in the same buffer. After acetone dehydration the pieces were embedded in Araldite.

Autoradiography was conducted both on semithin and ultrathin sections, covered by Ilford K5 and Ilford L4 emulsions, respectively. Kodak D 19 b and Kodak Microdol X, respectively, were used as developers. Thin sections were observed in a Jeol JEM 100 CX electron microscope.

RESULTS

5 Hydroxytryptamine

In the brain protocerebrum, two pairs of neurons, located in the neurosecretory area of the frontal lobes, are labeled (fig. 1). These cells are rather small (15 × 7 μm), smaller than the neighbouring neurosecretory cells. The beginning of the axon is strongly labeled. The cytoplasm of these cells has no peculiar characteristics (fig. 2). Another 3 or 4 pairs of labeled cells are located in the deutocerebrum (fig. 3).

Most of the labeled axons in the protocerebrum are located in the pars intercerebralis, at the level of the origin of the nervus glandulae cerebralis.

In the other parts of the brain (i.e. in the deuto- and tritocerebrum) and in the perioesophageal collar, labeled axons are more numerous (fig. 3). Labeled brain axons are of two types: (1) axons with small clear vesicles (diameter: 50-60 nm) and slightly dense granules (50-120 nm) (fig. 4) and (2) axons with only small clear vesicles (50-60 nm) (fig. 5).

Labeling can also be observed in the nervus glandulae cerebralis and the cerebral gland.

Fig. 1. Localization of serotoninergic protocerebral cells (1, 2) in the frontal lobes area. The arrow points to a neurosecretory cell. Autoradiography on semithin section (300 ×).

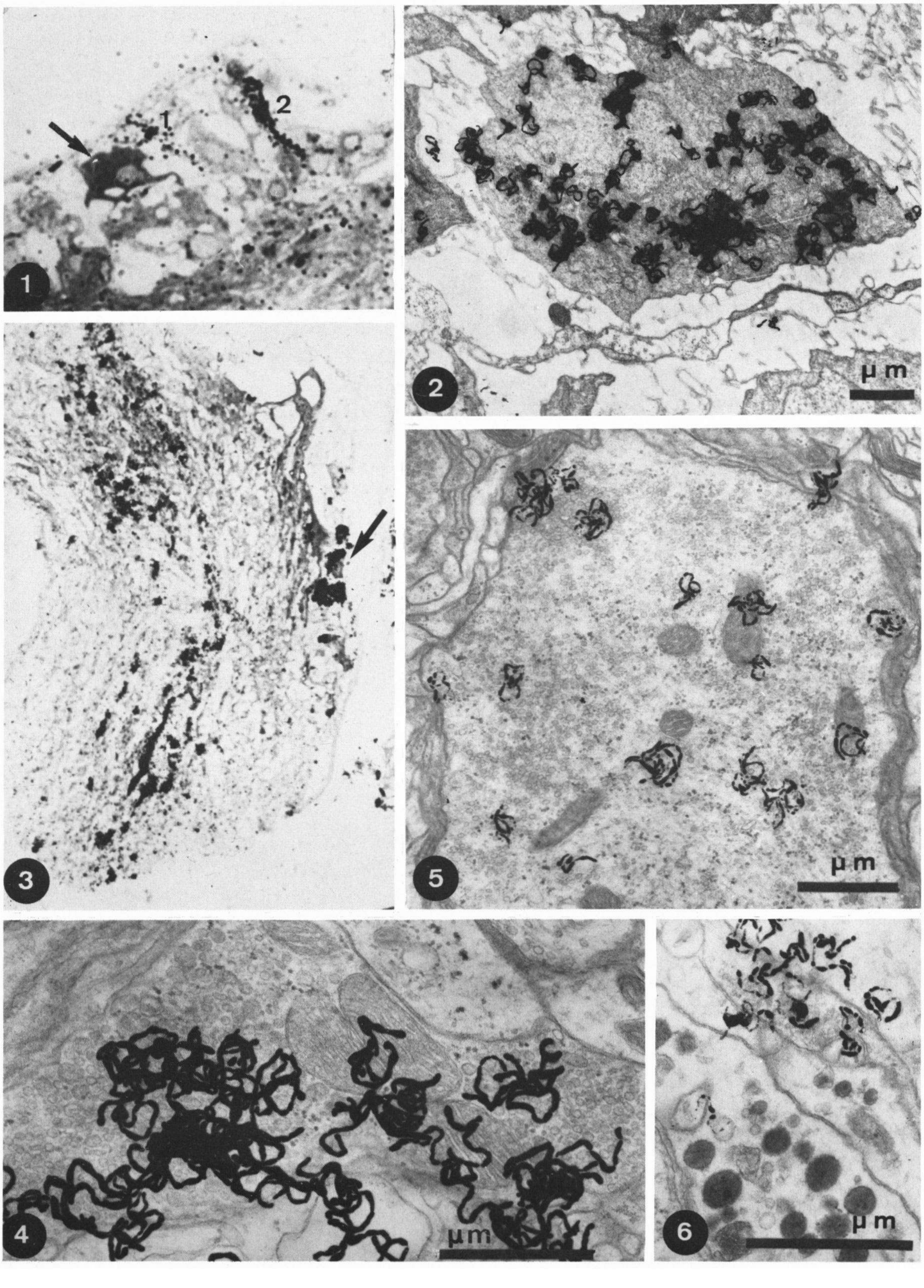
Fig. 2. Fine structure of serotoninergic protocerebral cell.

Fig. 3. Serotoninergic pathways in the perioesophageal collar. Autoradiography on semithin section (120 ×). The arrow points to labeled cell bodies.

Fig. 4. ^{3}H-5HT labeled protocerebral axon. Type 1: granules and vesicles.

Fig. 5. ^{3}H-5HT labeled protocerebral axon. Type 2: vesicles. Note the labeling over the patches of peripheric small vesicles.

Fig. 6. ^{3}H-5HT labeled axonal sections in the cerebral gland.


1
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Labeled axons contain small vesicles (50-60 nm) and slightly dense granules (about 100 nm in diameter) (fig. 6).

In each ganglion of the ventral nerve cord that was studied there is an anteroventral pair of labeled serotoninergic neurons (fig. 7). The cytoplasm of the cell bodies has no peculiar characteristics, as is true for the brain serotoninergic neurons.

Axons labeled are of 2 types. The more abundant type contains dense cored vesicles (fig. 8) of about 50 nm in diameter. Axons only containing small vesicles (50-70 nm) (fig. 9) are less strongly labeled.

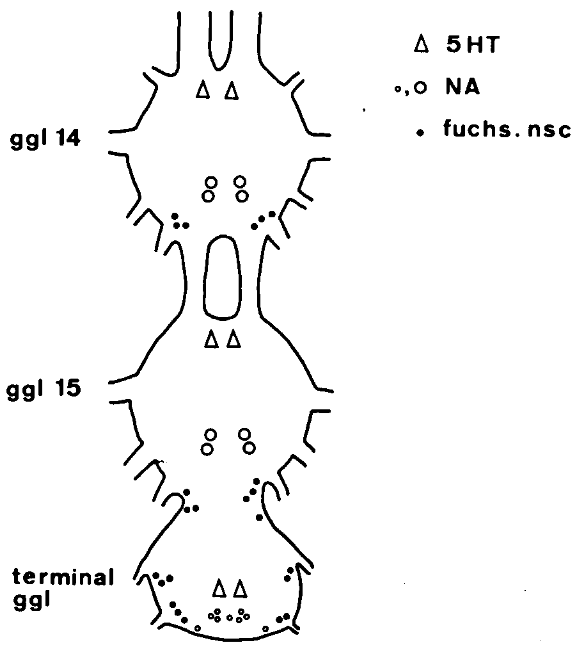


Fig. 7. Localization of serotoninergic and noradrenergic cell bodies in the posterior part of the ventral cord; fuchs. nsc = fuchsin positive neurosecretory cells, ggl = ganglion.

Noradrenaline

One pair of labeled neurons is located in the frontal lobe area of the protocerebrum. These cells, slightly labeled, have, on semithin sections, the cytological characteristics of the frontal lobe neurosecretory cells. Up to now, no more information on their structure is available; they were not included in the areas studied by electron microscope autoradiography.

Labeled brain axons are of at least two types: (1) axons with microtubules and only few microvesicles (diameter: 50 nm) (fig. 10) and (2) axons filled with small clear vesicles (50-80 nm) (fig. 11).

In the cerebral gland the labeling is also located over two types of axons: the first, with clear vesicles and small granules (50-120 nm) is a classical aminergic axon. The second type of axon shows granules with a diameter in the range of 100-200 nm (fig. 12); the diameter and the appearance of the granules allow to classify these axons as peptidergic.

Two pairs of labeled noradrenergic neurons are found in each ganglion of the ventral nerve cord (fig. 7). These neurons (about 30 μm × 15 μm) are characterized by a periphery indented by glial cells (fig. 13). In the ventral nerve cord only axons with microtubules and containing only few microvesicles are labeled (fig. 14).

DISCUSSION

As in other Arthropoda serotoninergic and noradrenergic neurons are present in the central nervous system of the centipede *Lithobius forficatus*.

The specificity of uptake of labeled amines is sometimes debated. Some experiments on vertebrate species showed that 5-HT was taken up by noradrenergic neurons (Taxi & Droz, 1969; Calas & Droz, 1971). Nevertheless, low concentrations lead to a specific uptake (Shaskan & Snyder, 1970). In the present study on *L. forficatus* uptake seems specific in spite of a rather high concentration in NA experiments (10^{-5} M) because (1) background labeling was relatively low, (2) different cells were labeled by different markers and (3) only few cells were labeled.

In *L. forficatus* aminergic neurons are more abundant in the deutocerebrum than in the protocerebrum and, under the experimental conditions used, there are more serotoninergic

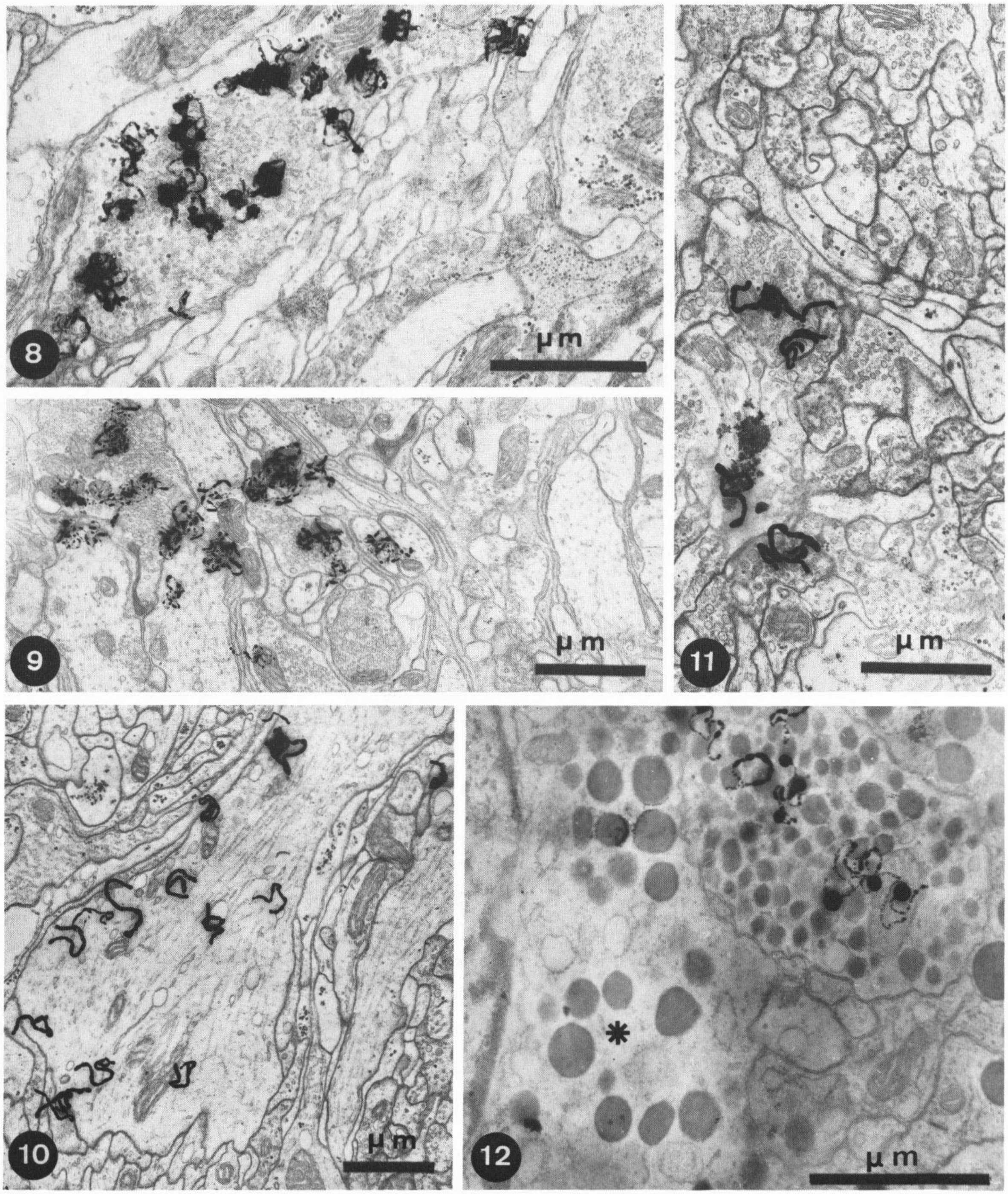
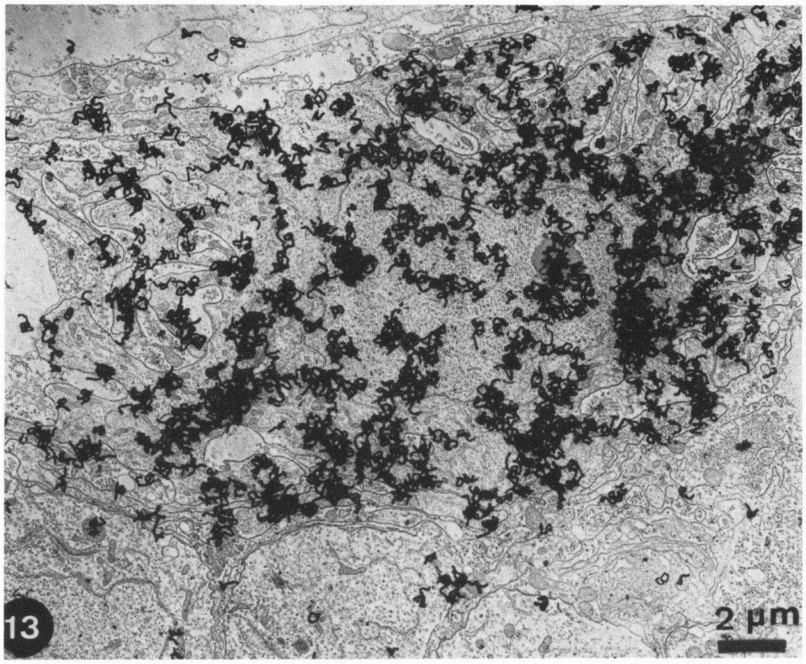


Fig. 8. Serotoninergic axon (dense cored and clear vesicles) in the ventral nerve cord.
Fig. 9. Serotoninergic axon (clear vesicles) in the ventral nerve cord.
Fig. 10. Noradrenergic axons (with neurotubules) in the protocerebral neuropil.
Fig. 11. Noradrenergic axons (with vesicles) in the protocerebral neuropil.
Fig. 12. ^{3}H-NA labeling in a peptidergic axon in the cerebral gland. The glandular cell (*) is not labeled.



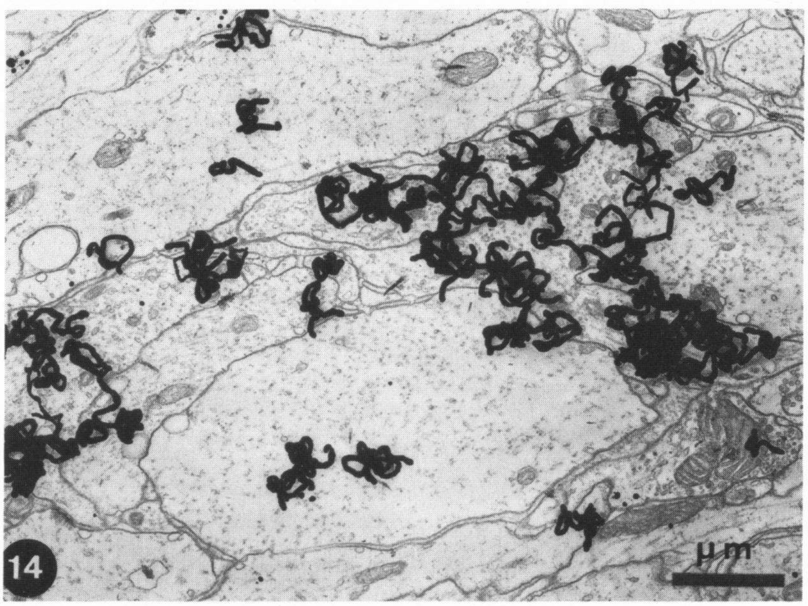


Fig. 13. ^{3}H-NA labeled cell body in a ganglion of the ventral nerve cord.
Fig. 14. Noradrenergic axons of the ventral nerve cord.

neurons than noradrenergic neurons in the whole brain. In other Arthropoda the levels of indoleamines (5-HT) are generally higher than those of the catecholamines. If this ratio is observed in the centipede brain, the opposite is the case in the ventral nerve cord (twice as many noradrenergic neurons than serotoninergic neurons). Brain aminergic neurons are less numerous in *L. forficatus* than in Insecta (Klemm & Sundler, 1983) and in Crustacea (Beltz & Kravitz, 1983). This fact may be related to the reduced central body and pons cerebralis of the centipede brain. In the ventral nerve cord the number of aminergic neurons is comparable to that described by Beltz & Kravitz (1983) in Crustacea.

Serotoninergic neurons are distinct from the frontal lobe neurosecretory cells by their size, their localization and cytological characteristics. Such a distinction is not possible for brain noradrenergic cells. It must be remembered that neurosecretory cells can be both peptidergic and aminergic (Vieillemaringe et al., 1982).

In *L. forficatus* various kinds of axon were found to be labeled by the same amine, as was observed in Insecta (Lafon-Cazal et al., 1973).

Biogenic amines are present in the cerebral gland of *L. forficatus* as well as in the corpora cardiaca of insects (first demonstration by Gersch et al., 1961), in which they are located mostly in axons and only sometimes in glandular cells. The labeling of peptidergic axons by ^{3}H-NA in the cerebral gland can be taken as indirect evidence for the coexistence of adrenergic and peptidergic secretions in the same cell (viz. neurosecretory cells of the frontal lobes).

Functions of monoamines are still unclear in centipedes, as in other Arthropoda. Nevertheless, biogenic amines are involved in the general physiological processes of the animals (for references see e.g. Lafon-Cazal, 1978). A role in hormonal release has also been demonstrated, in Insecta (Klemm, 1976; Samaranayaka, 1976), as well as in Crustacea (Fingerman et al., 1974; Hanumante & Fingerman, 1982). Classical neurotransmitters can also act as neurohormones (Evans et al., 1976).

So, the presence of monoamines in the cerebral gland, as well as in the cephalic arterial wall (Jamault-Navarro, unpublished) and of acetylcholine in the cerebral gland (Joly, unpublished) may be related to neuromodulation of hormone release and neurohormonal effects.

Finally, uptake of 5-HT and NA by some neurons are in agreement with the electrophysiological results obtained by Descamps & Lassalle (1983) and a study currently in progress on the effects of neurotransmitters on brain ultrastructure and on the release in the cerebral gland (Jamault-Navarro, unpublished).

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