LETTER

UPTAKE OF RADIOACTIVE LEUCINE AND URIDINE BY THE CAUDAL NEUROSECRETORY CELL OF THE LOACH (*MISGURNUS ANGUILLICAUDATUS*)

To the Editors:

It is generally accepted that the synthetic activity of the nerve cell occurs only at the perikaryon and that the substance therein produced is conveyed through the nerve fiber to the terminal. This conception is based on such observations as the accumulation of axoplasmic substance at the proximal site to the construction of the nerve fiber, chronological changes in diffusion of the labelled substance in the nerve cell after the administration of radioactive amino-acids or phosphorus and the biochemical gradient along the neurons (Lubińska, 1964; Gutmann, 1964). A similar concept has been accepted in regard to the hypothalamo-hypophysial neurosecretory system, and the neurosecretion is produced at the perikaryon and conveyed through the fiber (Hild and Zetler, 1953; Scharrer, and Scharrer, 1954; Bargmann, 1954).

The neurosecretory cell of the caudal neurosecretory system which is localized at the caudal aspect of the spinal cord in most of the fishes (Enami and Imai, 1955, 1956a and b; Sano, 1958; Sano *et al.*, 1962), show also proximo-distal movement of neurosecretion (Enami, 1956; Inoue, 1959).

In the present experiment the authors have attempted to examine the incorporation of labelled amino-acids in the secretory substance of the caudal neurosecretory cell and also to observe the migration of the labelled substance along the axon of the neurosecretory cell. The caudal neurosecretory system of fishes consists of large neurosecretory cells with nerve fibers terminating in a bulbous depot. This histological characteristic of the system is expected to facilitate the observation by autoradiography of the distribution of labelled substance in each cell including nerve fiber and bulbous ending. The loach, *Misgurnus anguillicaudatus* was chosen because this species was reported to show rapid response of neurosecretory activity when injected with hypertonic sodium chloride solution (Enami, 1956).

The loaches supplied from a commercial source, 10 to 12 grams in body weight, were kept in a room maintained at a temperature of about 22°C. In the first series of experiments, H³DL-leucine (15μC/g) and H³ uridine (10μC/g) were injected intramuscularly into intact animals. The excised caudal parts of the animals were fixed in toto at the time of 10 and 40 mins., 3 and 24 hrs. and 2, 4, 8 and 17 days after H³ leucine injection, and 30 mins., 1, 3, 8, 16, 24 hrs. and 2, 4 and 11 days after injection in the case of H³ uridine. In the second series, loaches which had previously received intramuscular injection of 0.5 cc of 2% sodium chloride solution daily for 3 doses were injected with H³ leucine (10μC/g)

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at 2 hrs. after the final injection of the sodium chloride solution. The recipient animals of the two groups were fixed at 2 and 48 hrs. after the injection of the isotope, respectively. Animals given the isotope injection alone served as control.

The specimens used for autoradiography were fixed in Bouin's solution and those for cytological work in Helly's fixatives. Strip-film autoradiographs for counting silver grains, and stained preparations with Gomori's C.H.P., Mallory's triple stains and toluidine blue for cytological observations were made after the routine paraffin embedding procedure. Relative uptake of isotopes by different parts of the neurosecretory cells was evaluated quantitatively by counting the

Fig. 1. Uptake of H^3 DL-leucine by the neurosecretory cells 3 hrs. after injection. Perikaryon is labelled to a considerable measure and labelled substance has already reached the fiber. X 870.

Fig. 2. Weak uptake of H^3 DL-leucine by the bulbous depot in an area adjacent to the central canal, and moderate uptake by the ependymal cells. Three hrs. after the injection. X 870

Fig. 3. Uptake of H^3 DL-leucine by the bulbous depot with profiles of neurosecretory cells 2 days after the injection. Note pronounced uptake by the small neurosecretory cells and nerve endings of neurosecretory cells. X 870

Fig. 4. Uptake of H^3 uridine by the storage depot 24 hrs. after injection. Note the absence of uptake by the nerve endings of neurosecretory cells and fair amount of incorporation into cellular elements other than nerve terminals. X 870

Arrows indicate caudal direction.

number of grains in 7μ square microns, and the mean grain counts per $7\mu^2$ corrected for background are given in parentheses. The following observations are based on 70 loaches for autoradiography and 23 loaches for cytology.

H³ leucine injection

It was noticed in this experiment that the radioactive count varied from animal to animal even killed at the same period. However, as a general rule, low but distinct uptake of the labelled leucine was recognized in the perikaryon between 10 and 40 mins. after the injection (1.06 and 2.43 respectively). Three hrs. later the amount of labelled leucine in the perikaryon increased in considerable measure and the maximum accumulation was seen in the animals 2 days after the injection (53.62 and Fig. 3). Thereafter the labelled leucine in the perikaryon gradually decreased. It also became evident that the amount of labelled leucine in the perikarya of the neurosecretory cells became 2 or 5 times as much as that in the ependymal cells of the spinal cord which were observed as a comparison.

The pattern of H³ leucine diffusion in bulbous ending was conspicuously different from that in the perikaryon; between 10 to 40 mins. after the injection, when the labelled leucine in the perikaryon gradually increased, there was scarcely a significant accumulation of the labelled leucine in bulbous nerve ending (0.02 and 0.31 respectively). After 3 hrs., however, the labelled leucine in the nerve ending gradually became evident (Fig. 2) and continued to increase until 2 days after the injection (Fig. 3), followed by gradual decrease in the subsequent periods. Intense labelling of the bulbous depot of 2 day specimens may indicate that the labelled leucine is incorporated into the neurosecretory substance synthesized in the perikaryon and the radioactive secretory substance migrates through the nerve fiber which terminates in the bulbous depot. Labelled leucine was still recognized in the perikarya as well as the depots at 17 days after the injection of isotope which seems to indicate that the newly produced protein retained in the neurosecretory cell for rather a longer period after being synthesized.

H³ uridine injection

As was seen in the case of H³ uridine injection, a great individual variation in uptake of H³ uridine by the perikarya was observed. Characteristic in this observation, however, was an appearance of fairly active uptake of the isotope by the perikarya at 30 mins. (1.84) and 60 mins. (2.91) after the injection, in which periods uptake of H³ leucine by the perikarya did not increase to the same extent. As far as the bulbous depots are concerned the diffusion of H³ uridine differed conspicuously from that of H³ leucine, and significant uptake of the isotope by them was hardly recognized in any period after the injection (Fig. 4).

Based on the present observations of H³ leucine uptake it may be mentioned that synthesis of proteinous substance occurs at the perikarya, and then the product is conveyed distad to the terminal depot. This assumption is in agreement with the opinion of many researchers on synthetic activity in nerve cells. Our observations with H³ leucine are coincident with the results by Sloper *et al.* (1960) in which they showed uptake of S³⁵ DL-cysteine and DL-methionine by the hypothalamus and pituitary of the rat. They showed that the uptake by the infundi-

bular process became greater than that in pars distalis or in the subjacent hypothalamus 9.5 hrs. and longer after injection of labelled cysteine, but not methionine. In the present experiment with H^3 uridine, it was observed that the uptake of H^3 uridine by the neurosecretory cells occurred exclusively at the perikarya and no significant uptake by the terminal depots was noted through the period of observation (11 days). This supports the view that there was no peripheral flow of nucleoprotein along nerve fiber (Samuel *et al.*, 1951) and no RNA granules in the axon of the intact neuron (see Lubińska, *op. cit.*).

Injection of hypertonic sodium chloride solution

After injecting sodium chloride solution in the same concentration and dosage as employed in the present experiment, Enami (1956) reported the occurrence of histological alterations in loach neurosecretory cells. As mentioned below, in the authors' experiments remarkable changes were observed especially in the perikarya of the neurosecretory cells in animals after repeated injection with sodium chloride solution. The peripheral disposition of the chromophile substance, which is a characteristic of the intact cell, was disarranged and scattered throughout the cytoplasm forming rough network or in droplet. Frequently the entire cytoplasm was acidophilic and dotted with chromophile remnants. Giant unclooli, which were vacuoles of various size, were occasionally observed within some of the cells.

In the group of animals injected with sodium chloride solution previously, the uptake of H^3 leucine by the perikarya was a little higher than that of the intact loaches, however, only when subjected to a single injection of salt solution and fixed 2 hrs. after injection of the isotope. However, the uptake of H^3 leucine in other cases was always lesser as compared with the intact loaches. Thus, the influence of injection of 2% sodium chloride solution on uptake of H^3 leucine by the perikaryon and migration of labelled substance to the bulbous ending in the loach caudal neurosecretory cell was inconclusive and needs further experimentation.

As regards the loach caudal neurosecretory cell, in summary, H^3 leucine injected intramuscularly was taken up first by the perikaryon, thereafter the labelled substance migrated to the terminal ending. The uptake of H^3 uridine by the neurosecretory cell was confined to the perikaryon and no significant uptake was observed at the terminal ending. The loaches which were injected with 2% sodium chloride solution showed inconsistent uptake of H^3 leucine in their caudal neurosecretory cell.

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