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EFFECT OF 6-HYDROXY-DOPAMINE ON THE FLUOROGENIC MONOAMINE CONTAINING NERVE ELEMENTS IN THE FORE-GUT MUSCULATURE OF *HELIX POMATIA*

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Abstract

Using glyoxylic acid induced fluorescence method an intensive green fluorescence characteristic to catecholamines was observed on nerve fibres with varicosities, nerve bundles and perykaryons on wholemounts stretch preparates of snail's gut.

On the effect of 6-hydroxy-dopamine (6-OHDA) administered intracardially (2.5 mg/animal) the amount of fluorescent nerve elements were significantly reduced. 48 hours after the injection of 6-OHDA practically no fluorescent fibres were seen. However several cell bodies showing intensive green fluorescence were detectable during the 72 hours of experimental periode.

Key words: fluorescence, catecholamines, 6-hydroxy-dopamine, snail

Introduction

Glyoxylic acid (GA) has been found to be suitable for the histochemical demonstrations of biogenic monoamines (AXELSSON et al. 1973). It was shown that GA induced fluorescence (GIF) is specially very sensitive for the demonstration of catecholamines in peripheral adrenergic nerves (FURNESS and COSTA, 1974). The use of a different combination of GIF techniques the wholemounts preparates of intact ganglia (MARSDEN and KERKUT, 1970) and a number of peripheral tissues such as the heart, prostate gland, salivary gland (BARBER, 1983) provided valuable information on the localization of nerves, cell bodies and axons having associated biogenic monoamines. By the use of sucrose-phosphate-glyoxylic acid (SPG) technique (DE LA TORRE and SURGEON, 1976) on wholemount stretch preparates of snail's gut, the distribution of fluorogenic monoamines containing nerve elements were followed in the whole length of snail alimentary tract (FEKETE, 1984), and three segments were revealed concerning the amount of fluorescent nerve elements and also the fluorescence intensity. It is likely from pharmacological studies (TRIMBLE et al., 1984) that among catecholamines in Mollusc only dopamine acts as neurotransmitter. However preliminary biochemical (HALASY et al. 1985) and some physiological (KAZACHENKO et al. 1978) investigations demonstrated that both adrenaline and noradrenaline are present in snail's gut and may act as neurotransmitters or neuromodulators. However noradrenergic axons have often been identified by their ability to take up noradrenaline analogues, such as 5-, or 6-hydroxydopamine (COSTA et al. 1976, DREYFUS et al. 1977, FURNESS and COSTA, 1974), the literature concerning

the selectivity and the mode of action of 6-OHDA is very contraversary. It is well documented that 6-OHDA causes degeneration in the mammalian adrenergically innervated organs (PORTER et al. 1963, THOENEN and TRANZER, 1968), due to degeneration of their noradrenaline terminals. The typical degenerative changes seen in mammalian adrenergic neurons after 6-OHDA treatment could not be detected in Octopus peripheral nerves (MARTIN and BARLOW, 1975). An investigation on different Mussel neurons (ELEKES et al. 1977) showed that different part of mussel's neurons have different sensitivity to 6-OHDA. In the central nervous system of *Planorbis corneus* a giant dopaminergic cell (GDC) was identified, in which 6-OHDA caused an increased catecholamine-specific fluorescence (BERRY et al. 1974).

To clarify the effect of 6-OHDA in the enteric nerves of *Helix pomatia* we followed the nerve elements surrounding blood vessels in the fore-gut of the snail and evaluated the differences in the fluorescence intensity and the number of fluorescent nerve elements before and after intracardially administered 6-OHDA.

Materials and Methods

Helix pomatia used in these studies were collected over the period of April to October. Seasonal changes in the intensity of histofluorescence were not observed during this period.

For the histochemical detection of biogenic monoamines the SPG method (DE LA TORRE and SURGEON, 1976) was applied to wholemount stretch preparates of snail's gut. The digestive tract were quickly dissected and incubated in a reaction mixture containing 6.8 g sucrose, 3.2 g KH_2PO_4 and 1 g glyoxylic acid (GA) in 100 ml of distilled water, at 4 °C for 15 minutes. The muscular layer of the gut wall was then stretched on microscope slides, blotted with blotting paper and dried under cool air for about 1/2 hour. Finally the specimens were placed in an oven at 95 °C for 4 min. and mounted with liquid paraffin. The preparations were viewed through a Leitz Ortoplan microscope equipped with indirect illumination and an HBO 50W super pressure mercury lamp. An E-3 filter block was used to observe induced fluorescence. Black and white photographs were taken on FORTE-PAN 400 film. To follow the effect of 6-OHDA, 6-OHDA HCl (Sigma) was administered intracardially. The 6-OHDA was dissolved in 0.1 ml of saline (NaCl 0.65%; KCl 0.015%) and was administered as one dose of 2.5 mg/animal. The animals were killed and processed for histochemical detection of fluorogenic monoamines after 2, 24, 48 and 72 h of injection.

Results

In the untreated controls (Fig.1–3., Fig.8.) the fluorescence was bright and well localized in the fore-gut. Fluorescent fibres showed a characteristic arrangement around blood vessels and these part of the specimens were the most comparable in the whole length of the fore-gut. Fluorescent fibres occurred singly or in bundles and were concentrated in several areas (Fig.1., 8.). Most of the single fibres were varicous (Fig. 1–2., arrowheads). Fibres running along the vessels divided at some places and made connections with neighboring nerve trunks. Single cells with bright fluorescence were also seen. Most of them were in close association with nerve trunks (Fig.1., 2. arrows). Processes of these cells could be followed on the surface of the

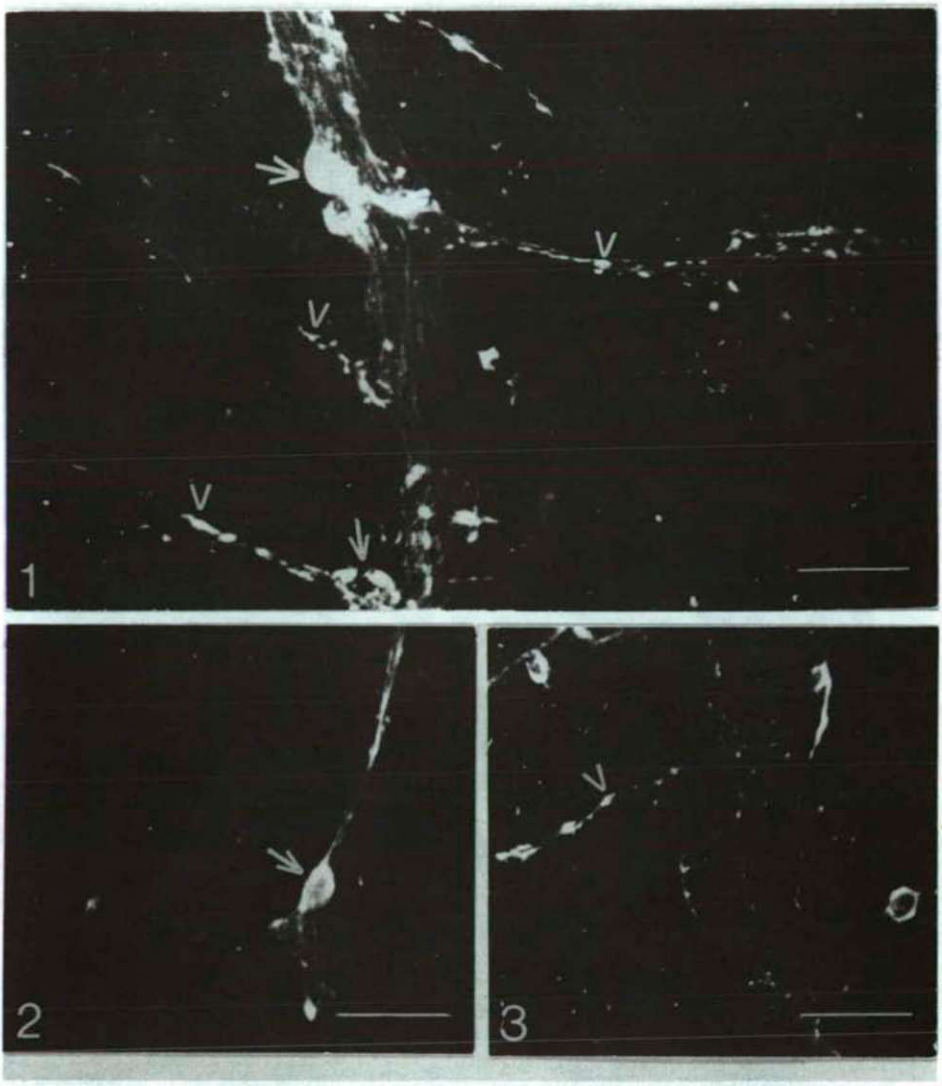


Fig. 1. **Glyoxylic acid-induced fluorescence in whole-mount stretch preparates in the snail's fore-gut musculature. Arrows indicates cell bodies, arrow-heads varicosities. Scale bar: 50 μm**

Fig. 2. Fluorescent cell bodie in the fore-gut (arrow) being in close connection to nerve trunks. Scale bar: 50 μm

Fig. 3. Single cells of different sizes and shapes in the fore-gut with no visible connection to the bundles. In the neighborhood of these cells randomly oriented varicous fibres (arrows-head) are running. Scale bar: 50 μm

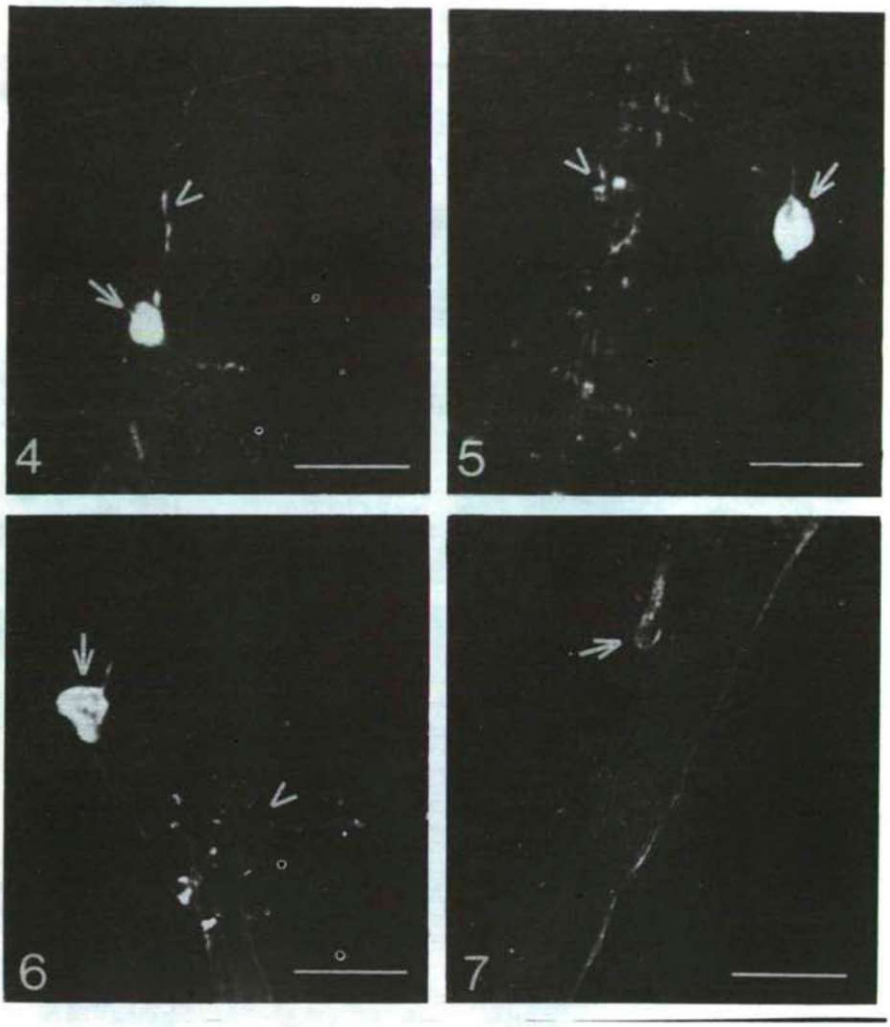
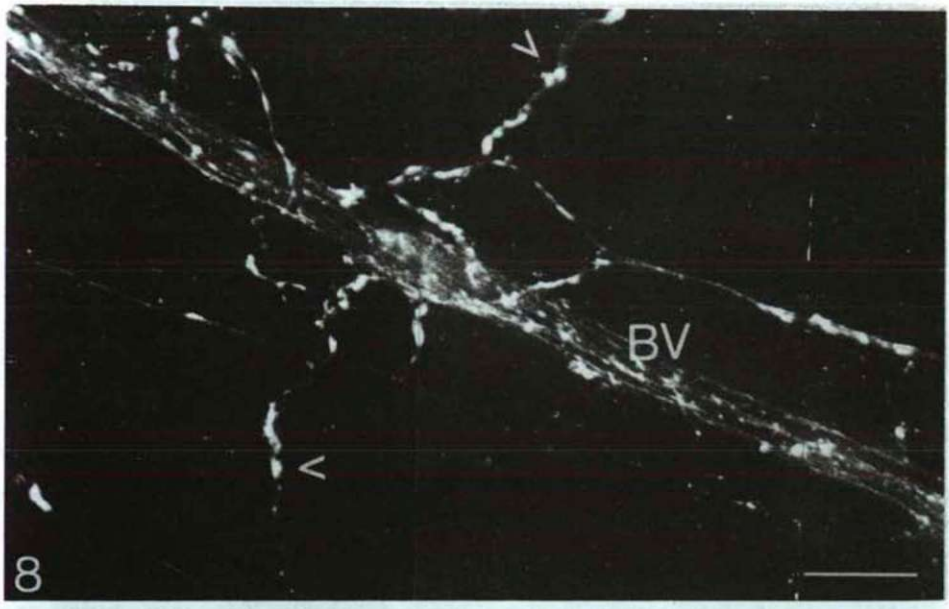


Fig. 4. Fluorescent nerve cell bodie after 24 hours of 6-OHDA treatment in snail's fore-gut. The cell processes show varicosities (arrow-head). Scale bar: 50 μm

Fig. 5. Varicouse fibres are concentrated at several areas after 48 hours of 6-OHDA treatment. Intensively fluorescent cell bodie (arrow) running parallel to the fibres. Scale bar: 50 μm

Fig. 6. After 72 hours of 6-OHDA treatment varicous fibres (arrow-heads) and intensively fluorescent cell bodie (arrow) still can be seen at several areas in the fore-gut. Scale bar: 50 μm

Fig. 7. After 72 hours of 6-OHDA treatment some of the remaining cells (arrow) show much reduced fluorescence. Scale bar: 50 μm



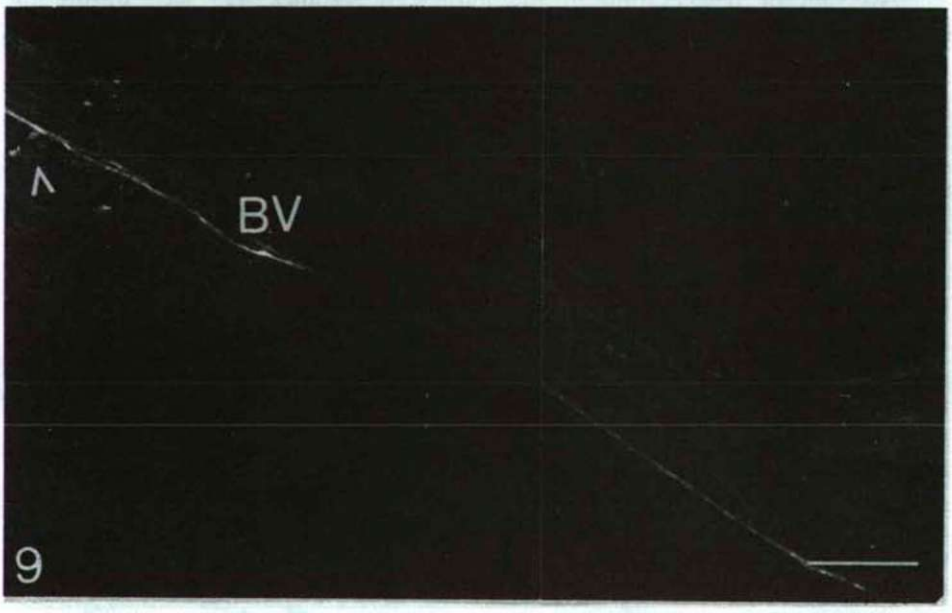


Fig. 8. Whole mount of fore-gut showing the characteristic pattern of cathecolamine containing nerve fibres along the blood vessels (BV) before 6-OHDA treatment. Scale bar: 50 μm

Fig. 9. Blood vessel in the snail's fore-gut after 72 hours of 6-OHDA treatment. Some faintly fluorescent fibres (arrow-head) can only be seen in the biggest part of the fore-gut. Scale bar: 50 μm

vessels. Single cells showing a less intense fluorescence appeared a little farther with no visible connection to the nerve bundles (Fig.3.). The GIF in the perykarya of these cells was usually restricted to the cytoplasm.

Most of the speciemens prepared from 6-OHDA treated animals could be separated from the controls on a single blind bases (Fig. 8., 9.). However 2 hours after 6-OHDA treatment there was no visible changes neither in the intensity nor in the amount of fluorescent nerve elements. 24 hours after the drug treatment most of the fluorescence disappeared (Fig. 4–7., 9.). The rest of the fibres showed an irregular distribution and the fluorescence intensity was reduced comparing to the untreated samples. All of the remaining fibres were short and varicous in appearance (Fig. 4–6., arrowheads). After additional 24 hours practically there was no change in the GIF (Fig. 6.). While 72 hours after the 6-OHDA treatment a further decrease in the fluorescent nerve elements and fluorescence intensity was observable (Fig. 7., 9.). In these specimens a very few faintly fluorescent fibres were only seen on the surface of the vessels (Fig. 9.). Some of the cells with a very intense GIF having close connection to the vessels „survived" the 6-OHDA treatment and revealed a very intense catecholamine fluorescence within the whole experimental periode (Fig. 4–6.). In some cells the fluorescence intensity was dropped by 72 hours of treatment (Fig. 7.).

No sign of regeneration in the fluorescent profiles was seen during the experimental periode.

Discussion

The use of GIF technique on wholemount stretch preparates of gut musculature in *Helix pomatia* allowed us to map the distribution of aminergic nerve fibres and related cells in different segments of alimentary tract (FEKETE, 1984). Since the fluorescent network was most pronounced in the fore-gut (FEKETE, 1984) and was most comparable around the blood vessels this segment of the gut was used to follow the effect of 6-OHDA. The main finding of the present work was that 6-OHDA treatment resulted a significant decrease in the fluorescent intensity and the number of fluorescent nerve elements. Although a characteristic time-pattern appeared in the degeneration processe of CA nerves within the experimental period. Some of the cells being in close connection to vessels were not sensitive to drug treatment at all. The processes of these cells could be followed along the blood vessels and also several thin, varicous fibres were seen neighboring the cells. From these findings we assumed that different cells and connected fibres have different sensitivity to the neurotoxin 6-OHDA which is known to be specific for noradrenergic nerve elements (THOENEN and TRANZER, 1983). We have several alternative explanations for this phenomenom (HÖKFELT and UNGERSTEDT, 1973; FURNESS and COSTA, 1980). These cells may not contain noradrenalin but some other kind of monoamines which are not sensitive to the drug. This alternative is supported by data (BERRY et al. 1974) which showed up increased catecholamine-specific fluorescence in identified dopamine-containing cells after 6-OHDA treatment. We consider a possible higher

catecholamine level of these cells as an other explanation. This can cause a delayed exchange rate between the toxin and the fluorogen amine. This alternative is partly supported by data from the literature (LLEWELLYN et al. 1981), and also favored by our finding after 72 hours of 6-OHDA accumulation. Some of the cells showed very intense fluorescence after 48 hours of treatment but lost fluorescence intensity by 72 hours of treatment. The third possibility is that the selective amine pumpe present on adrenergic nerves and having an active role in monoamine and their antimetabolites uptake is missing from these cells.

Biochemical and ultrastructural investigations are in progress to decide the validity of these possible explanations.

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