Acta Biologica Szeged 17 (1—4), pp. 153—158 (1971)

ZINC-IODIDE-OSMIUM IMPREGNATED CELLS IN THE PARAVERTEBRAL BUNDLE OF THE MARSH-FROG (RANA RIDIBUNDA PALL.)

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(Received December 1st, 1969)

In connection with the vesicles containing stimulation-transmitting materials there are several problems to be solved. According to certain investigators, the clear synaptic vesicles contain acetylcholine and amino acids (SJÖSTRAND, 1953; PALADE and PALAY, 1954; AKERT and SANDRI, 1968), and the dense-core vesicles catechol-amine and serotonin (DE ROBERTIS and PELLEGRINO DE IRALDI, 1961).

The separation of mediator material with monoamine content was completed by fluorescence histochemical investigations elaborated by FALCK (1962), ANGELAKOS and KING (1967). And the mediator materials of the synaptic vesicles of different forms (UCHIZONO, 1966) are connected with zinc-iodide-osmium impregnation (AKERT and SANDRI, 1968; MAILLET, 1962). With the latter method there was described the impregnation of other cell components, apart from the vesicles (JABONERO, 1964; PELLEGRINO DE IRALDI and GUEUDET, 1968; MADARÁSZ, 1969).

As in the paravertebral bundle and the adrenal gland of the marsh-frog I have found some cells similarly impregnated with zinc-iodide-osmium, I see some possibility to supply information about the function of the different kinds of paravertebral nerve cells.

Materials and Methods

The investigations have been carried out on eight full-grown specimens of marsh-frog (*Rana ridibunda* PALL.). I have treated a part of the paravertebral ganglia as well as the cells of adrenal glands, exposed from the animal anaesthetized with urethan and kept cold, with a mixture of CHAMPY-MAILLET's (1962) zinc-iodide-osmium mixture at 4°C for one and half hours. The other part of neurons and of the adrenal glands were fixed in BOUIN's and CARNOY's fixing mixtures, resp. in neutral formalin of 10 p.c. After dehydration, the 5—7 μ sections prepared from tissues embedded in paraffin were deparaffinized and then stained with MALLORY's mixture and with alkali tetrasolium modified by FINDLEY (KISZELY et al., 1958).

The ganglia used for the electron microscopic investigations have been fixed in a 1 p.c. osmium-tetroxide solution of pH 7,4 buffered according to MILLONIG (1961) on 4°C for one hour. The tissue pieces were treated with uranil-acetate and then embedded in araldit. The sections were cut with an ultramicrotome Tesla BS 478. The sections contrasted with lead-citrate have been investigated with an electron microscope of type Tesla BS 242 D.

Results

The vegetative nerve cells of frogs had been studied by several investigators (DOGIEL, 1882; SMIRNOW, 1890; KRAUSE, 1923; PICK, 1960, 1963; TAXI, 1965; HORVÁTH, 1966). Some problems still remained unsolved which is proved by our investigations carried out on the vegetative ganglia and the adrenal gland of the marsh-frog with a zinc-iodide-osmium (ZIO) impregnation.

Among the nerve cells some cells or minor groups of them occur, which stain darker or lighter, primarily in the ganglia of the abdominal part *(ggl. sympathicum* IV—VII; Table I, Fig. 1*)*, most of them being in the vicinity of the coeliac ganglion beside the intestinal artery after leaving the dorsal aorta (Table I, Fig. 2). The average size of the neurons is 5—15 μ, their 4—6 μ nucleus being mostly centrally situated.

I have observed the neurons of different staining and sizes both in the ganglia of younger and in those of older frogs. According to my supposition, the ZIO-positive neurons may be those containing in their cytoplasm electron-microscopically, too, more or less dense-core vesicles and osmiophilic granules of different sizes (Table II, Fig. 1). Besides them, in the larger neurons (25—35 μ) that form the majority of ganglia, we can only observe smaller or larger granules stained with ZIO.

I have observed also some of the chromaffine cells in the adrenal gland of frogs that are, concerning impregnation and size, similar to the ZIO-positive neurons of the vegetative ganglia (Table I, Figs. 3, 4). There, the greater number of cells contain purple-red formasane granules of large diameter with alkali tetrasolium. Apart from these cells, there could be observed also minor groups of round, projectionless cells of changing shape and painted paler, with black ZIO-impregnation (Table I, Fig. 4). These cells show, like those in the ganglia of the boundary bundle, acidic staining.

Discussion

CHAMPY-MAILLET's procedure of zinc-iodide-osmium impregnation had earlier been applied at light microscopic examinations and recently also at electron microscopic ones. JABONERO (1964) observed in the course of his light microscopic examinations that the pigments of the vegetative nerve cells of the paravertebral trunk in cat and rabbit, the GOLGI's apparatuses of very different kind of cells and the sensory neurons of the vagus nerve become black impregnated by ZIO. According to MADARÁSZ's (1969) electron microscopic examinations, only the material occurring inside the endoplasmatic reticular cisterns is stained by ZIO.

Table I

Fig. 1. ZIO-positive neurons of two sizes and two kinds of staining in Ggl. sympathicum VII. CHAMPY—MAILLET's procedure. x 600.

Fig. 2. A group of strongly stainable large nerve cells beside the intestinal artery. CHAMPY—MAILLET's procedure. x 600.

Fig. 3. Chromaffine cells of the adrenal gland stained with alkali tetrasolium. PEARSE's procedure modified by Findlay. x 300.

Fig. 4. ZIO-positive cells in the adrenal gland, stainable rather pale and rather dark. CHAMPY—MAILLET's procedure. x 300.

Table I

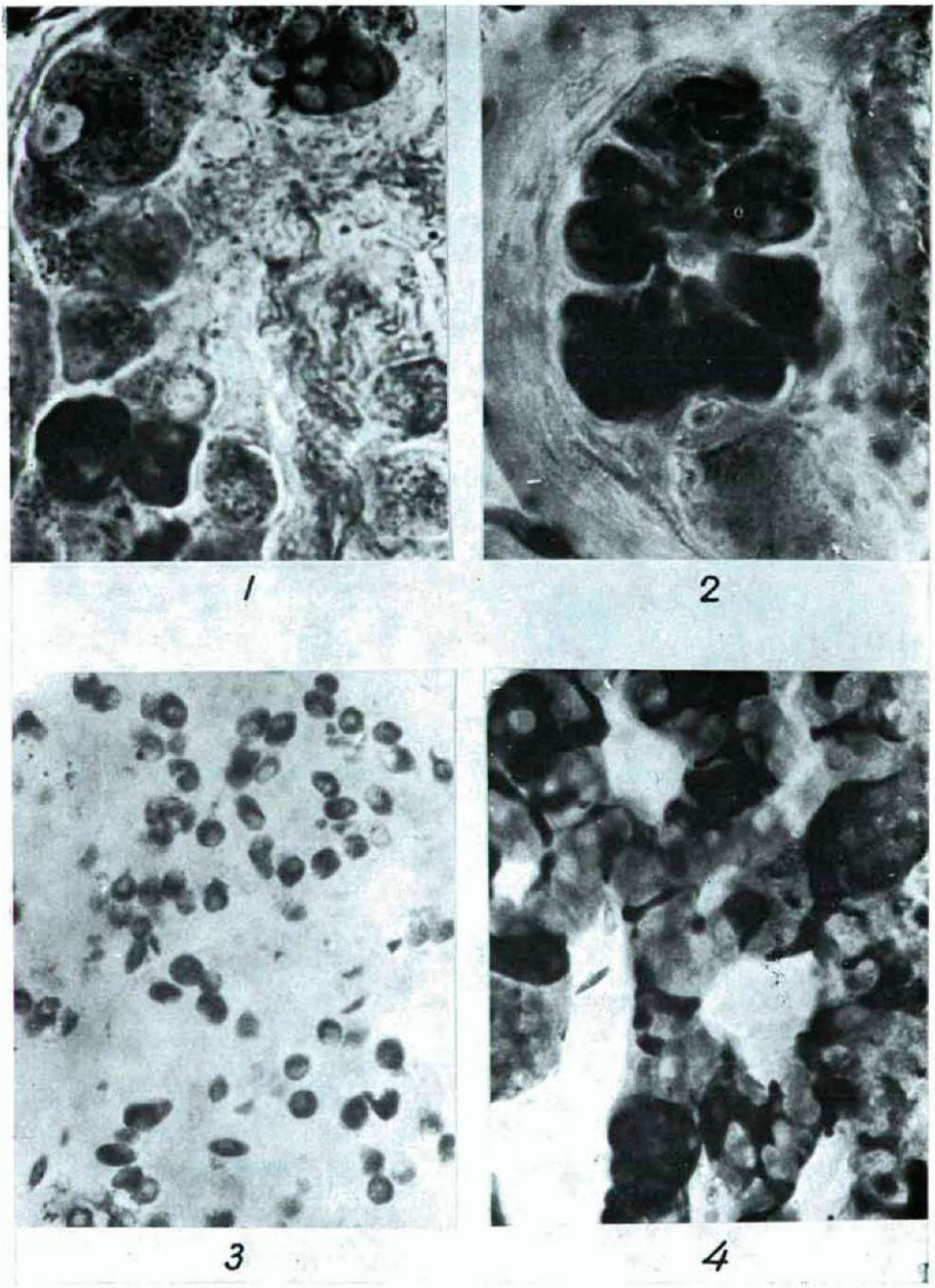
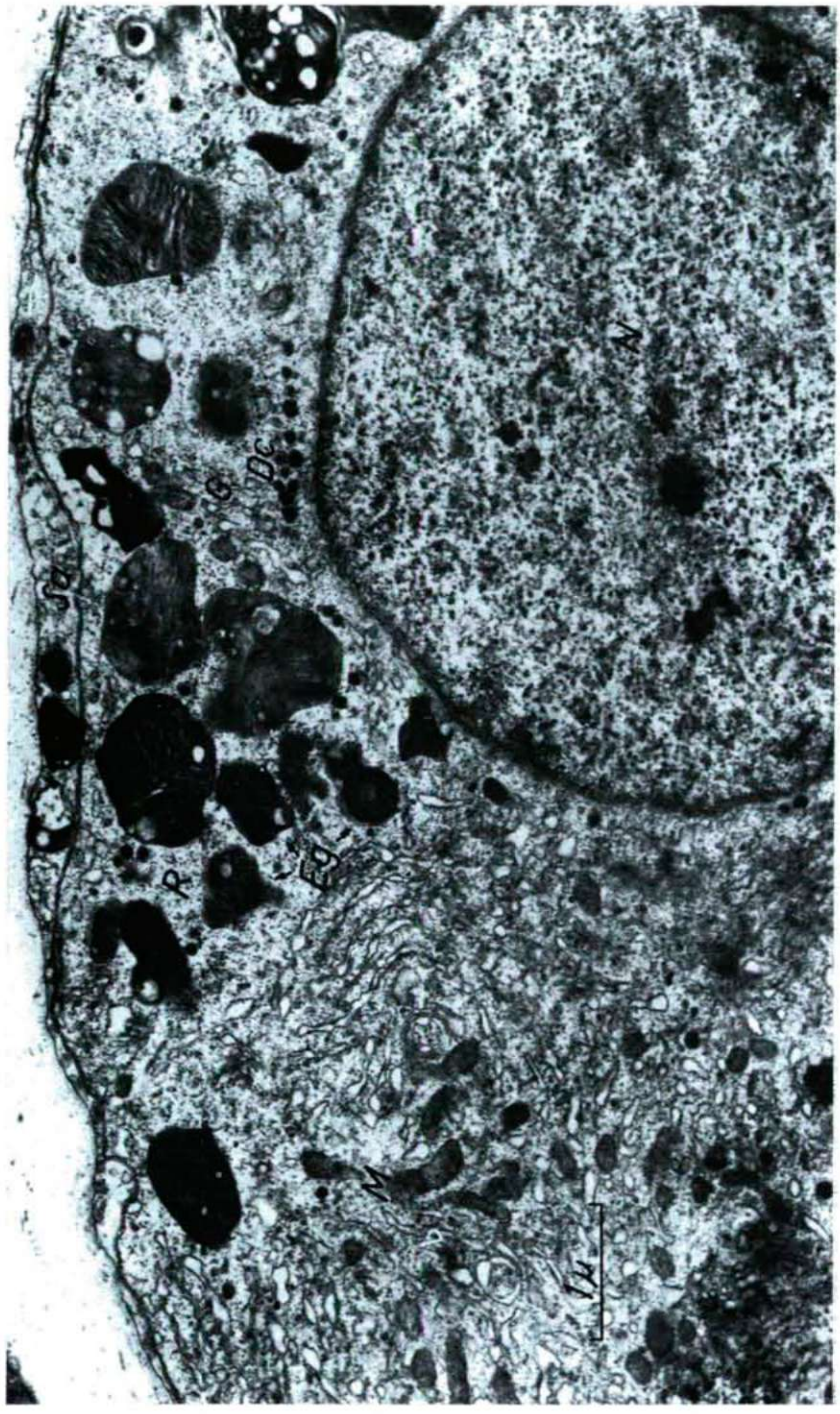


Table II



According to AKERT's and SANDRI's (1968) electron microscopic examinations, the ZIO-positivity of synaptic vesicules is connected with a cholinergic mediator material. HALÁSZ and co-workers (1969) have not observed any significant change in the ZIO-positivity of synaptic vesicles in the superior cervical ganglion of cat after treating the nerve-terminations with haemicholinium and stimulating exhaustively the preganglionic fibres. On the other hand, MADARÁSZ (1969) noticed that after being stimulated electrically the preganglionic fibres, the impregnation inside the synaptic vesicles ceased. In addition, it is worth mentioning, too, that according to an experimental result, owing to the effect of reserpine, both the granules of amine content (PELLEGRINO DE IRALDI et al., 1961, 1963) and the ZIO-positive material disappear in the vesicles. The same was observed after a treatment with chloroform-metanol, as well. Consequently it is supposed that some lipid components may be responsible for the reaction (PELLEGRINO DE IRALDI and GUEUDET, 1968).

According to the data cited, several structures are impregnated by ZIO. We cannot conclude, therefore, an identical function from cells stained identically. Comparing my results with the data in literature, I came to the conclusion that the ZIO-positive cells found in the vegetative ganglia and in the adrenal gland of the marsh-frog may cary out, however, in different sites, similar functions.

ERÄNKÖ (1960) could separate two cell types in the adrenal gland of the rat with fluorescence and other histochemical methods, and BENEDECZKY (1967) separated three cell types in the adrenal gland of the edible frog (*Rana esculenta* L.) with electron microscopic examinations.

I could find two kinds of cells in the paravertebral bundle. According to my opinion, the less numerous ZIO-positive cells are the sympathetic nerve cells, producing adrenalin. The majority of the nerve cells in the paravertebral bundle — showing in the cytoplasm small black granules with ZIO-imprenation under light microscope, and containing no dense-core vesicles in the electron microscopic pictures, beling to the parasympathetic system.

Summary

Among the nerve cells of paravertebral bundle there can be separated two cell types in the mars-frog with a zinc-iodide-osmium staining procedure. These cells show differences probably depending on their functional state. In our opinion, the less or more stained 5—10 μ sized nerve cells similar with adrenal cells, function according to the adrenergic mechanism and the 25—35 μ neurons according to the cholinergic one.

References

AKERT, K. and SANDRI, C. (1968): An electron-microscopic study of zinc iodide-osmium impregnation of neurons. I. Staining of synaptic vesicles at cholinergic junctions. — Brain Res. 7, 286—295.

Table II

Fig. 1. Dense-core vesicles in one of the neurons of Ggl. sympathicum VI. Dc: dense-core vesicle, G: GOLGI's apparatus, M: mitochondrium, R: ribosoma, Eg: electron-dense granules, N: nucleus, Sa: satellite cell: Araldit embedding. x 18 000.

ANGELAKOS, E. T. and KING, M. P. (1967): Demonstration of nerve terminals containing adrenalin by a new histochemical techniques. — Nature *213*, 391—392.
BENEDECZKY, I. (1967): Electron-microscopic differentiation of cell types in the frog's adrenal medulla. — Acta Morph. Hung. *15*, 271—285.
DE ROBERTIS, E. and PELLEGRINO DE IRALDI, A. (1961): Plurivesicular secretory processes and nerve endings in the pineal gland of the rat. — J. biophys. biochem. Cyt. *10*, 361—372.
DOGIEL, J. (1882): Die Nervenzellen und Nerven des Herzventrikles beim Frosche. — Archiv. Mikr. Anat. *21*, 21.
ERÄNKÖ, O. (1960): Histochemical observations on the adrenal medulla of the rat. — Anat. Rec. *136*, 188—189.
FALCK, B. (1962): Observations on the possibilities of the cellular localization of monoamines by fluorescence method. — Acta physiol. scand. *56*, Suppl. *197*, 1—25.
HALÁSZ, N., PÁRDUCZ, Á. and JOÓ, F. (1969): Zinc iodide-osmium (ZIO) positivity of nerve terminals in exhausted sympathetic ganglia. — VI. Magyar Elektronmikr. Konf. Balatonszéplak.
HORVÁTH, I. (1966): Comparative cytological examinations of the paravertebral ganglia of tadpoles and full-grown frogs. — Acta Biol. Szeged *12*, 93—99.
JABONERO, V. (1964): Über die Brauchbarkeit der Osmiumtetroxid-Zinkjodid-Methode zur Analyse der vegetativen Peripheria. — Acta neuroveg. *26*, 184—210.
KISZELY, GY. and BARKA, T. (1958): Gyakorlati mikrotechnika és hisztokémia (Practical microtechnique and histochemistry). — Medicina, Budapest.
KRAUSE, R. (1923): Mikroskopische Anatomie der Wirbeltiere in Einzeldarstellungen. Walter de Gruyter Co. Berlin — Leipzig.
MADARÁSZ, B. (1969): Electron-microscopic study of the superior cervical ganglion of the cat after Zn—I. Osmic acid impregnation. — VI. Magyar Elektromikr. Konf. Balatonszéplak.
MAILLET, M. (1962): La technique de Champy à l'osmium ioduré de potassium et la modification de Maillet à l'osmium-ioduré de zinc. — Trab. Inst. Cajal Invest. biol. *54*, 1—36.
MILLONIG, G. (1961): A modified procedure for lead staining of thin sections. — J. biophys. biochem. Cytol. *11*, 736—739.
PALADE, G. E. and PALAY, S. L. (1954): Electron microscope observations of interneuronal and neuromuscular synapses. — Anat. Rec. *118*, 335—336.
PELLEGRINO DE IRALDI, A. and DE ROBERTIS, E. (1961): Action of reserpin on the submicroscopis morphology of the pineal gland. — Experientia. *17*, 122—123.
PELLEGRINO DE IRALDI, A. and DE ROBERTIS, E. (1963): Action of reserpin; iproniazid and pyrogallol on nerve endings of the pineal gland. — J. Neuropharmacol. *2*, 231—239.
PELLEGRINO DE IRALDI, A. and GUEUDET, R. (1968): Action of reserpin on the osmium tetroxide zinc iodide reactive sit of synaptic vesicles in the pineal nerves of the rat. — Z. Zellforsch. *91*, 178—185.
PICK, J. (1960): On the ultrastructure of sympathetic neurons in the frog (*Rana pipiens*). — Anat. Rec. *136*, 257— 258.
PICK, J. (1963): The submicroscopic organization of the sympathetic neurons in the frog (*Rana pipiens*). — J. Comp. Neur. *120*, 409—462.
SJÖSTRAND, F. S. (1953): The ultastructure of the retinal rod synapses of the guinea pig eye. — J. Appl. Phys. *24*, 1422.
SMIRNOW, A. (1890): Die Stuktur der Nervenzellen im Symphaticus der *Amphibien*. — Arch. mikr.-anat. *35*, 407—424.
TAXI, J. (1965): Contribution à l'étude des connexions des neurones moteurs du système nerveux autonome. — Ann. Sci. nat. Zool. *7*, 413—674.
UCHIZONO, K. (1966): Excitatory and inhibitory synapses in the cat spinal cord. — Jap. Jour. Phys. *16*, 570—575.

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