Innervation of the ovarian interstitial cell of the chick embryo

A. Amanuma and K. Yamada

Department of Biology, Gifu College of Dentistry, Hozumi-cho, Gifu-ken (Japan), 19 September 1978

Summary. Electron microscopic examination of the developing ovary of 15–20-day chick embryos revealed that the interstitial cells were well innervated. Nerve fibres and nerve endings were observed in close contact with steroid-producing cells.

The fine structure of the developing gonad of the chick embryo has been described by several authors[1–8]. Dahl[9] studied the fine structure of the nerves of the ovarian stroma of the adult hen. There is no report of the fine structural studies of the innervation of embryonic ovarian tissues of chick. This study outlines the ultrastructure of nerves adjacent to the embryonic ovarian interstitial cells.

Materials and methods. White Leghorn chicken embryos at 15–20 days of incubation were used in this study. The left gonads dissected from the embryos were fixed in 5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3 at 4 °C for 5 h. The gonads were rinsed for 4 h in the same buffer and postfixed in 1% osmium tetroxide at 4 °C for 3 h. The fixed materials were stained with uranyl acetate for 3 h, dehydrated in graded series of ethanol and embedded in Epon 812. Ultrathin sections were prepared and restained with lead nitrate, examined with the JEM 100U electron microscope.

Observations and discussion. In the ovary of 15-day chick embryo interstitial cells were observed only in the medullary region and not in the cortical region. The cells were irregular in shape and lying in small groups. In their cytoplasm there were many lipid droplets, mitochondria with tubular cristae, well developed Golgi apparatus and abundant smooth-endoplasmic reticulum. Dense bodies and coated vesicles were also found frequently (figure 1). These characteristics are in accordance with the general features of steroid-producing cells accepted in many vertebrates[10–12]. The cells bearing these features described above were also observed in the ovarian medulla at the later stages. Small nerve fibres were merely seen in the vicinity of capillaries in the ovary of the 15-day embryo. At 17 days of incubation, the innervation of the interstitial cells were first observed in the ovary. As the development proceeded, the nerve fibres and nerve endings were found more frequently. Nerve fibres were seen as a bundle around the interstitial cell-cluster. Occasionally, some fibres were observed throughout the interspace of interstitial cells in a cluster, and seemed to be in close contact with the cells. Sometimes, the nerve endings adjacent to the interstitial cells with a narrow gap (20 nm) were noticed. The endings contained mitochondria, microtubules and vesicles of size usually recognized as synaptic vesicles (figure 2)[12,13]. Two kinds of vesicles were distinguished. One type was small (30–50 nm in diameter) with clear content, another was larger (50–100 nm) and characterized by the central osmiophylic granule. Moreover, the nerve endings were found in contact with enclosing cells which surrounded a cluster of interstitial cells.

The present study indicates that the embryonic ovarian interstitial cells in postembryonic stages are well innervated. No biochemical, histochemical or physiological investigations were carried out. So, the nature of the contents of vesicles and the function of nervous systems described here is unknown. Dahl[9] reported the fine structure of the nervous systems of the ovarian stroma of the domestic fowl, and he suggested that the steroid-producing cells in the theca interna might be subjected to some nervous control. He found 2 kinds of efferent nerve endings and discussed a possibility of antagonistic function, stimulation and inhibi-

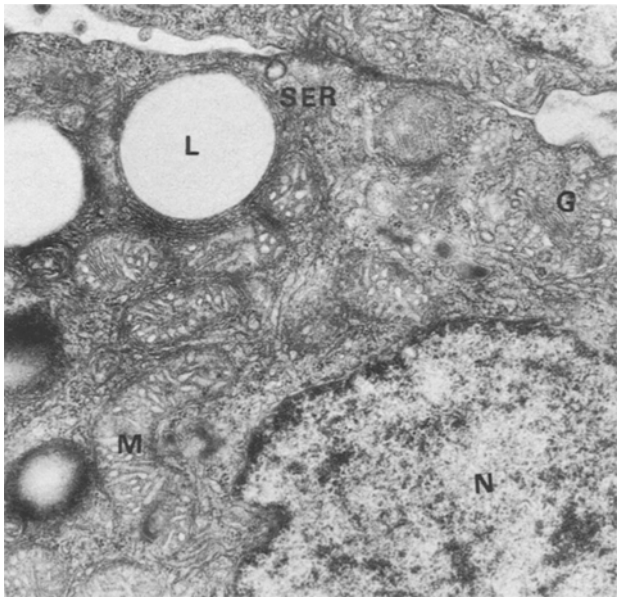

Fig. 1. The ovarian interstitial cell from a 15-day embryo. Many mitochondria (M) with tubular cristae, lipid droplets (L) surrounded by smooth-endoplasmic reticulum (SER) and Golgi apparatus (G) are indicated. N: nucleus. × 16,500.

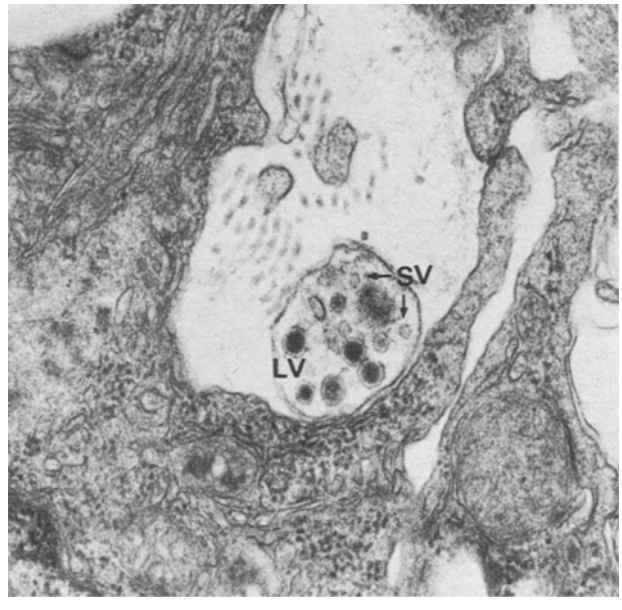

Fig. 2. The nerve ending in contact with a interstitial cell from a 18-day embryo. Large vesicles (LV) and small vesicles (SV) are seen. × 33,000.

tion, of these nerve endings. Furthermore, a baskettype axon terminal between steroid-producing cells and enclosing cells was described by the same author. In the present observation, 2 kinds of synaptic vesicle-like structure were discernible, but the existence of 2 kinds of nerve endings was uncertain as yet. Further observation on this is necessary.

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Neurosecretory products diversity in the pars intercerebralis of insects

M. Raabe, A.A. Panov, E.D. Davydova and D. Chervin

Neuroendocrinologie des Insectes, C.N.R.S. n° 24, Université Pierre et Marie Curie, Paris, 12 rue Cuvier, 75230 Paris Cedex 05 (France), and A.N. Severtzov Institute of Evolutionary Animal Morphology and Ecology, USSR Academy of Sciences, 33 Leninsky Prospekt, Moscow V-71 (USSR), 10 July 1978

Summary. The pars intercerebralis of insect brain, which has numerous physiological functions, contains more neurosecretory cell types than previously thought. There are 2 distinct types among the A cells. In addition to these cells, there are azocarminophilic cells, the C(r) cells, which are not apparent when using 'classical' staining methods.

The pars intercerebralis of insect brain regulates numerous phenomena, such as, for example, water content, trehalose metabolism, reproduction, etc. The origin of the neurohormones implicated in these regulations has been considered to be located in the neurosecretory cells, as demonstrated by the classical methods, chrome hematoxylin-phloxin and paraldehyde fuchsin. After oxidation, these methods allow 2 large categories of neurosecretory materials to be distinguished: neurosecretory materials stained by chrome hematoxylin and paraldehyde fuchsin (A type) and materials retaining acidic stains such as phloxin, light green (B type).

In this paper we attempted to study 2 problems. Firstly, whether there are several types of A cells and how to recognize them; secondly, to discuss another type of neurosecretory cell, which we will call C(r). In addition we have investigated in previous publications and by our own research whether these cells exist in various orders.

Materials and methods. In order to study A cells, we employed Bouin fixative and the following stains: chrome hematoxylin-phloxin (CHP), paraldehyde fuchsin (PF), alcian blue-alcian yellow, paraldehyde thionin-phloxin (PTh-Ph) using Panov's method and paraldehyde thionin-paraldehyde fuchsin (PTh-PF) according to the following method: oxidation under standard conditions, staining for 10 min by PTh, washing, dehydration, staining for 2 min by PF, washing with 95% alcohol, dehydration and mounting. The study of C(r) cells was carried out using Bouin and Helly fixatives, each being followed by both azan and CHP staining.

Results and discussion. The neurosecretion of A cells. The staining of the neurosecretory materials by CH or PF is based on the affinity of these stains for the acidic groups appearing after oxidation, but this affinity permits no clear distinction between them. However, the literature indicates the possibility that there are 2 categories of A cells. In particular, by staining with PTh followed by phloxin, Panov[1] noticed that among the secretions of A type, certain ones are strongly colored by the thionin, while others situated in cells of a similar aspect, retain thionin weakly and have at times a weak affinity for the phloxin.

On the basis of Panov's observation, we tried, using various staining methods, to characterize the substances in question. The best results were obtained with PTh-PF, certain cells containing substances stained by thionin (A1 cells) while others containing fuchsinophilic material (A2 cells). This latter material appears as weakly thionin positive in the staining by PTh-PH.

After applying Ravetto's alcian blue-alcian yellow method, which permits the recognition of strong and weak acids, we again observed a very fine distinction between 2 substances: blue blue-green ones and yellow yellow-green ones. When comparing, by the double staining method, sections treated alternately by PTh-PF and by alcian blue-alcian yellow, a correspondance appeared between the thionin and alcian blue positive material and between fuchsin and alcian yellow positive material. Thus the material stained by thionin is rich in strong acids, while that stained by fuchsin is rich in weak acids.

A cells containing 2 neurosecretory materials have already been reported in Orthoptera[2,3], in Neuroptera and Mecoptera[4], in Diptera cyclorrhapha[5], and in Heteroptera[6-8]. Our research has demonstrated their occurrence in Dictyoptera (figure 1, a), Orthoptera (figure 1, d, e), Hymenoptera (figure 2, f), Coleoptera (figure 2, c), Diptera cyclorrhapha (figure 2, d) and Nematocera (figure 2, e), Heteroptera (figure 2, g), which indicates the generality of this phenomenon.

The neurosecretion of C(r) cells. After using certain fixatives such as Helly, followed by azan staining, a particular neurosecretory material was demonstrated by Raabe in the tritocerebron of various insects[9], in ventral nerve cord ganglia and in the perisympathetic organs[10]. This neurosecretory material containing strongly basic proteins does not appear after Bouin fixative and retains neither chrome hematoxylin nor paraldehyde fuchsin. It has little affinity for phloxin under normal oxidation conditions; after weak oxidation, however, a certain phloxinophilia appears in some species. Previously named C type by Raabe[10], we suggest designating it C(r) in order to avoid any confusion with the C cells, having A type affinity, as described by