ELECTRON MICROSCOPIC AND PHARMACOLOGICAL STUDIES
ON THE RAT MEDIAN EMINENCE*

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Received August 6, 1965

Summary. The rat median eminence contains at least three kinds of granules or vesicles: 1. large electron-dense granules (perhaps carriers of neurohypophysial hormones), 2. small electron-dense granules with or without haloes (perhaps carriers of catecholamines) and 3. synaptic vesicle-like structures (perhaps carriers of acetylcholine). The former two electron-dense granules exist in separate axons but they coexist with the latter vesicles in the same axons.

The pars nervosa shows basically a similar structure to the median eminence. However, the axons containing the small electron-dense granules are very few. In the pars tuberalis, there are at least two types of cells: the cells of one type contain much cytoplasm with large round nuclei and those of the other type contain a small amount of cytoplasm with polymorphic nuclei. The cells of the former include multivesicular bodies and secretory granules, but those of the latter do not. Some of capillaries of the primary plexus are surrounded by the cells of the pars tuberalis on one side and by neurosecretory axon endings on the other side.

The median eminence contains high concentration of acetylcholine or an acetylcholine-like substance and shows neurohypophysial hormone activity.

Although there are many physiological and anatomical investigations showing the importance of the median eminence of the neurohypophysis in the regulation of the adenohypophysial function (see Wingstrand, 1951; Harris, 1955; Benoit, 1959; Farner and Oksche, 1962; Everett, 1964; Etkin, 1963), electronmicroscopy of the median eminence had been limited until a few years ago. In 1958, Brettelschneider investigated the rat infundibulum. In 1961, two reports appeared on the fine structure of the median eminence of the parakeet (Kobayashi et al., 1961) and of the guinea pig (BARRY and COTTE, 1961). They reported the presence of neurosecretory and non-neurosecretory axon endings in the external layer of the median eminence. The ultrastructure of the median eminence has since been systematically studied in our laboratory (see Kobayashi and Oota, 1964; Kobayashi, 1965; Kobayashi, Hirano and Oota, 1965). Histochemical studies on enzymes in the median eminence have been recently started. Uemura (1964) and Kobayashi and Farner (1964) reported the distribution of acetylcholinesterase (AChE) in the avian median eminence. Further, Uemura (1965) has systematically examined the distribution of AChE in the median eminence and the pars nervosa of the tetrapod. All these investigations have revealed that AChE is present in the external layer of the median eminence, especially in tissue around the capillaries of the primary plexus of the hypophysial portal vein. As to the adrenergic mechanism in the median eminence, Fuxe (1964) found catecholamines condensed in the mammalian median eminence and Matsui and Kobayashi (1965) demonstrated a strong monoamine oxidase reaction in the external layer of the median eminence.

* Aided by Grant A-3678 from the United States National Institute of Arthritis and Metabolic Diseases. The authors are indebted to Dr. Welsh, Harvard University, for the kind gift of Mytolon.

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of the tree sparrow and of the rat. Pharmacological studies on neurohypophysial hormones in the median eminence of vertebrates have been systematically attempted in our laboratory since 1962, and the presence of the neurohypophysial hormones in the median eminence of the vertebrate has been reported (ISHII et al., 1962; HIRANO, 1964; KOBAYASHI, HIRANO and OOTA, 1965; KOBAYASHI, 1965).

Thus, information of the median eminence is rapidly accumulating. The purpose of our investigation of the median eminence is to further explore the anatomical and functional relationship between the median eminence and the adenohypophysis. In the present study, the rat median eminence was examined with an electron microscope, the amount of acetylcholine (ACh) or an ACh-like substance in the median eminence was measured and neurohypophysial hormones in the median eminence were bioassayed.

Material and Methods

For the electron microscopic studies, the hypothalamic regions were removed from adult rats of the Wistar strain immediately after decapitation, and were placed for two hours in cold DALTON's (1955) osmium tetroxide potassium bichromate fixative (pH 7.4). Following fixation, the tissues were dehydrated by passing through a series of ethanol and embedded in Epon 812. Sections were cut with a Porter-Blum microtome, and mounted on Formvar-coated grids and examined with a JEM-T6 electron microscope. To compare fine structure, the pars nervosa was also examined in the same manner. For light microscopic studies, tissues were fixed in Bouin's solution for 24—28 hours, sectioned in paraffin at 10 μ, and were stained by the paraldehyde-fuchs in staining method.

Acetylcholine or an ACh-like substance was assayed on isolated hearts of the bivalve, Schizothaerus nuttalli, principally according to WELSH's method (1943). The median eminence was removed from adult rats of the Wistar strain (weighing 150 to 300 g) immediately after decapitation and was weighed rapidly. They were then homogenized in eserinized deionized water adjusted to pH 3.6 with 0.1 N HCl. Average weight of 136 median eminences was 1.11 ± 0.02 mg. About 1.5 min were required from decapitation to transfer of the median eminence into the solution. The homogenate was boiled for 3 to 4 min, cooled, and neutralized with 0.2 N NaOH just before assay. Thus total (free plus bound) ACh or an ACh-like substance was determined. Usually 10 to 30 median eminences were pooled and used for 2 to 4 determinations. Several known concentrations of acetylcholine chloride (AChCl) were used as standards. In a previous experiment using AChCl, we have shown that the log-dose (x)-response(y) curves of individual hearts fit the regression equation y = a + b-log x between 20 and 80 percent inhibition in the amplitude by AChCl (UEMURA, 1965). In early summer the heart becomes 10 times as much sensitive to AChCl as in spring. This was true in the case of an extract of the rat median eminence. Testing the statistical hypothesis on fitting the line revealed that the ratios of variances were far above 99.9 percent in the case of AChCl and above 99 percent in the median eminence extract. There was no significant difference in the regression coefficient (b) between AChCl (—56.2 ± 3.15) and the extract (—69.5 ± 8.81). Before each assay of the samples, sensitivity and linearity of log-dose-response curve of each heart were always checked with AChCl. The intervals between two assays were 10 to 15 min when AChCl was used as standards, and 20 to 30 min when the extract was used, so that the after-effect was avoided. As will be discussed later, neurohypophysial hormones, l-noradrenalin and serotonin which might be present in the median eminence were found to be negligible for determination of ACh or an ACh-like substance in our assay method. The titer of ACh or an ACh-like substance in the tissues was expressed as the amount of AChCl which produces an identical heart response. As antagonist of ACh, Metyolon (2:5-bis[3'diethylaminopropylamino]-benzoquinone bis-benzyl chloride, Sterling-Winthrop) was used. The heart was treated with Metyolon (5 × 10⁻⁶ g/ml) for 15 min before the assay.

Neurohypophysial hormone activity was assayed by the superfused rat uterus method (GUDDUM, 1953) and by the rat vasopressor assay method (DEKANSKI, 1962). The determination of activity was done by the four-point assay method. Extracts of the median eminence and of

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