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Revaluation of neuronal contacts with pituicytes in the rat posterior pituitary

Jun-Qian Liu[1], Takanori Miki[1], Katsuhiko Warita[1], Ken-ichi Ohta[1], Tomiko Yakura[1], Yoshiki Matsumoto[2], Yoshiki Takeuchi[1]

[1]Department of Anatomy and neurobiology, Faculty of Medicine, Kagawa University, 1750-1 Ikenobe, Miki-cho, Kagawa 761-0793, Japan
[2]Laboratory of Animal Science, Faculty of Agriculture, Kagawa University, 2393 Ikenobe, Miki-cho, Kagawa 761-0795, Japan

Abstract

Synaptic structures of neuronal contacts with pituicytes in the posterior pituitary (PP) were investigated in detail after wheat germ agglutinin conjugated horseradish peroxidase (WGA-HRP) injection into the rat hypothalamus including the paraventricular, dorsomedial and arcuate nuclei. The injection resulted in heavy labeling of hypothalamic fibers in the PP. In the neuropil of the PP, there were a lot of terminals containing HRP-reaction product (RP) recognized as electron dense lysosomal-like structures. It was of particular interest that the terminals make synaptic contacts with pituicytes. Although the neuronal contacts were characterized by the structure of presynaptic thickening, occasionally two classes could be distinguished - asymmetrical and symmetrical synaptic contacts (Gray's type I and II). These findings provided the morphological evidence for tight structural relationship of neurons with pituicytes in the PP, as well as neuro-neuronal connections in the central nervous system, and additionally for pituicyte modulation of neurohormone output.

Key words: Neuronal contacts, Pituicytes, Synaptic thickening, Posterior pituitary, Hypothalamus, Rat

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Introduction

It has been well known that the posterior pituitary (PP) contains pituicytes and unmyelinated nerve fibers originating from the hypothalamus, and synaptic formation is made at the sites of neuronal contacts with pituicytes. Interestingly, the synaptic structure was characterized by presynaptic thickening in various animals [1-4]. This atypical synaptic structure, called synaptoid, is quite different from that of Gray's type I and II based on the theory of synaptic transmission in the central nervous system [5-8]. The contacts with pituicytes might imply to play an important role in the pituicyte regulation of neurohypophysial hormone output [9-11].

Therefore, in the present study, considering wheat germ agglutinin conjugated horseradish peroxidase (WGA-HRP) to be a valuable tool for neuronal tracer (12), the enzyme was injected into the hypothalamus including the paraventricular, dorsomedial and arcuate nuclei. The structure of synaptic contacts between hypothalamic terminals and pituicytes, particularly the zone of pre- or postsynaptic density, was observed in detail at the electron microscopic level.

Materials and Methods

The present experiments were performed on 19 male Wistar rats (SLC, Hamamatsu, Japan), weighing 230-300g. Animals were housed in separate cages and maintained under the standard laboratory conditions (temperature 24□, humidity 70%, 12h light: 12h dark cycle, water *ad libitum*). Experimental procedures were conducted in accordance with National Institute of Health (NIH) for Care and Use of Laboratory Animals. The Kagawa University Animal Care and Use Committee approved the procedures, and all efforts were made to minimize the number of animals used and their suffering.

Animals were anesthetized with intra-peritoneal injection of chloral hydrate (490 mg/kg) for all surgical procedures. 0.3-0.4 µl of WGA-HRP (Vector Laboratories, USA) were injected into the hypothalamus including the paraventricular, dorsomedial and arcuate nuclei using a 1-µl Hamilton microsyringe. After a survival period of 2-3 days, the animals were reanesthetized and sacrificed by perfusion through the ascending aorta with 0.1 M phos-

phate buffer (pH 7.4) followed by a fixative of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1M phosphate buffer.

For light microscopic observations (5 rats), the PP and cerebrum including the hypothalamus were removed from the skull and serial 30 µm-thick sections were cut transversely on a freezing microtome. Sections were processed for the demonstration of HRP reaction product (HRP-RP) according to the TMB method [13] or heavy metal-intensified DAB method [14].

For electron microscopic observations (11 rats), the PP was cut transversely into 200 µm-thick sections using a vibratome (Leica VT 1000S, Germany) and processed for demonstration of HRP-RP according to the DAB method. The sections were postfixed in buffer 1% osmium tetroxide for 2 hours, block-stained in saturated uranyl acetate for 1 hour, dehydrated in a graded ethanol series and embedded in epoxy resin mixture. The region of the PP was identified by examination of toluidine blue-stained 1 µm-thick sections. Ultrathin sections were cut and observed without further lead staining using a JEM 200 CX electron microscope. As a control case, animals with no treatment were also observed at the electron microscopic level (3 rats).

Results

In the light microscopic experiment, WGA-HRP was injected into the hypothalamus including the paraventricular, dorsomedial and arcuate nuclei. However, the injected enzyme spread to the thalamic nuclei (Fig. 1B). The injection resulted in heavy labeling of fibers which was extremely confined to the PP (Fig. 1A).

In the electron microspcopic experiment, axon terminals, light and dark pituicytes and capillaries were observed in the neuropil of the PP. Although the terminals usually contained small clear synaptic vesicles and large dense core vesicles with space, these were also characterized by containing HRP-reaction product (HRP-RP) recognized as electron dense lysosomal-like structures. In this osmium-fixed material, majority of unlabeled or labeled terminals showed heavy accumulation of small clear synaptic vesicles in vicinity to the presynaptic thickening which was much thicker than that on the postsynaptic side of light pituicytes (Figs. 2A and B). In contrast to these atypical neuronal synaptic contacts, labeled terminals occ-asionally made synaptic contacts with dark pituicytes. It was of particular interest that the contacts with dark pituicytes form much thicker zone of subsynaptic thickening (Fig. 1C) or two thinner zones of pre- and postsynaptic thickening (Fig. 1D) which are called “Gray’s type I or II” [8]. These characteristic morphological structures were not seen in a control case.

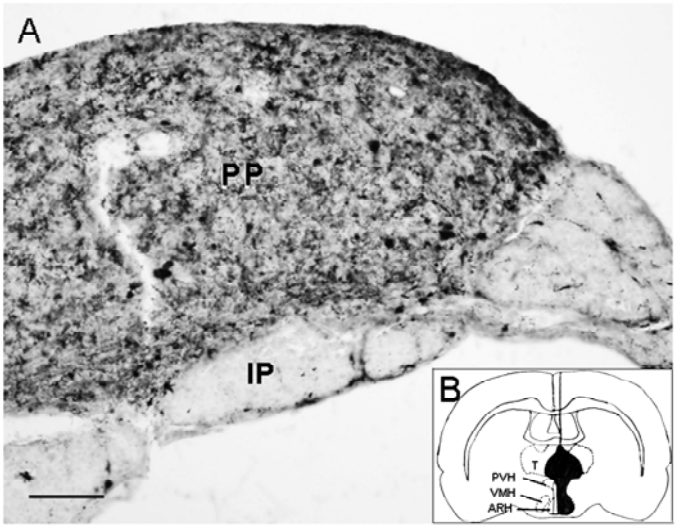

Figure 1: *Photomicrograph of the PP (A) after WGA-HRP injection into the hypothalamus (B). Note that the injection resulted in heavy labeling of fibers which was extremely confined to the PP. The injected WGA-HRP spread to the hypothalamus including the paraventricular and arcuate nuclei, and thalamic nuclei (black area in B). IP, intermediate pituitary; PP, posterior pituitary, PVH, paraventricular nucleus of the hypothalamus; T, thalamic nulei; VMH, Ventromedial nucleus of the hypothalamus; ARH, arcuate nucleus of hypothalamus. Calibration bar = 50 µm.*

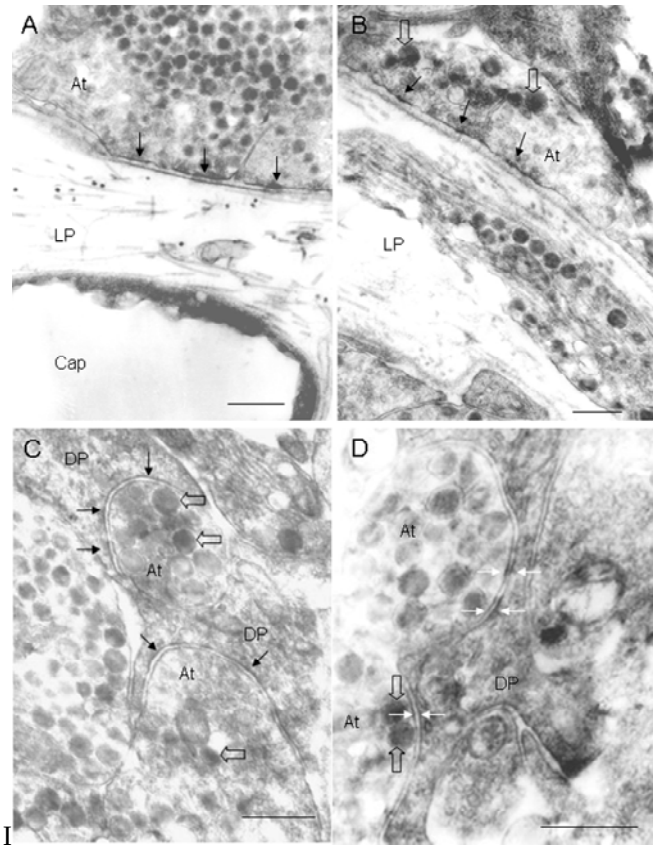

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Figure 2: *Electron micrographs of neuronal contacts*

with pituicytes. Terminals containing large dense core and small clear vesicles (A) and additional HRP-RP (B) made synaptoid contacts with light pituicytes. Note that a few of these terminals containing HRP-RP apparently made asymmetrical (C) and symmetrical synaptic contacts (D) with dark pituicytes. Large arrows indicate HRP-RP in hypothalamic terminals, while small arrows indicate the sites of neuronal contacts with pituicytes. At, axon terminal; DP, dark pituicytes; LP, light pituicytes; Cap, capillary. Calibration bars = 0.5 µm in A-D.

Discussion

The PP has been well known to contain nerve terminals originating the hypothalamus and pituicytes playing a role in the regulation of neurohypophysial hormone output [9,11]. With respect to the relationship between the hypothalamic terminals and pituicytes, formation of synaptic contacts has been reported in mammals [2,4]. In these studies, the structure of synaptic contacts was indicated to be characterized by presynaptic thickening which was much thicker than that on the postsynaptic side as shown in Figs. 2A and B. These synaptic contacts are considered to have neuronal functions because of accumulation of small clear synaptic vesicles in vicinity to the presynaptic thickening. However, the findings of asymmetrical and symmetrical synapses in the present study appearently seem to mean the existence of hypothalamic excitatory or inhibitory effects on pituicytes in the PP as well as transmission in the central nervous system. Particularly, these suggest the neuronal regulation on dark pituicytes which have functions of controlling secretion [15].

The release of oxytocin and vasopressin mainly depends on the pattern of firing of their synthesizing hypothalamic neurons located in the paraventricular, supraoptic and other nuclei [16,17]. In the present study the injected WGA-HRP seems to stimulate the hypothalamic neurons whose axons deliver the hormones to the PP, and take part in making the asymmetrical and symmetrical synapses which have not yet been reported. It was pointed out that terminals containing oxytocin and cholecystokinin form synaptoid contacts with pituicytes in the PP as well as terminals containing enkephalin [18], gamma-aminobutyric acid [19] and neuropeptide FF [20]. In contrast to these studies of terminals forming synaptoid contacts, there is no biological evidence about terminals forming Gray's type I and II synaptic contacts in the PP. Further studies about functional significance of such contacts should be needed.

The present findings provided the morphological evidence for tight structural relationship between neurons and pituicytes in the PP, as well as neuro-neuronal connections in the central nervous system, and additionally for pituicyte modulation of neurohormone output.

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Correspondence to:

Yoshiki Takeuchi
Department of anatomy and neurobiology
Faculty of Medicine, Kagawa university
1750-1 Ikenobe, Miki-cho
Kagawa 761-0793
Japan