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Immunocytochemistry of serotonin and galanin in the hypothalamus of the Japanese quail

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We investigated the interaction of serotonin and galanin (GA) by a double immunostaining method in the Japanese quail. Serotonin-immunoreactive (IR) cells were located in the paraventricular organ (PVO) and infundibular nucleus (IF). The number of the cells under short-day photoperiod (SD) was less in the dark phase than in the light phase. GA-IR cells were found in the PVO, IF and median eminence. The number of GA-IR cells in SD was significantly greater than that in long-day photoperiod (LD). Numerous GA- IR varicose fibers ran along serotonin- IR cell bodies and nerve fibers in the PVO and IF of the same sections. Very few serotonin-IR fibers ran along GA-IR cell bodies and GA-IR nerve fibers in the ventral part of the IF. The present results suggest that the possibility of functional interaction takes place between serotonin- and GA- IR neurons in the PVO and IF.

Key words: serotonin, galanin, immunocytochemistry, paraventricular organ, infundibular nucleus, quail , brain

INTRODUCTION

The photoperiodic responses of the testis and the cloacal gland were shown in blinded-pinealectomized quail [1]. Therefore, the photoreceptor for the photoperiodic time measurement seems to be located in somewhere other than the eye and the pineal. Since the rhodopsin-IR cells were located in the PVO of the Japanese quail, Yoshikawa and Oishi (1998) suggested that some cells of the PVO were deep brain photoreceptors in the Japanese quail [2].

Serotonin-IR cells were reported to be in the PVO of the Japanese quail [3-5]. These serotonin-IR neurons observed in the submammalian PVO had a feature of cerebrospinal fluid (CSF)-contacting neurons [6,7]. The function of these cells has not been known yet.

GA is a C- terminally amidated 29 residue peptide first discovered in extracts of the gastrointestinal tract of pigs [8]. In mammals, GA is widely distributed in the brain and has been implicated in the control of reproductive function [for reviews see 9,10]. However, only few studies have been reported in birds. GA-IR perikarya were located in the preoptic area, lateral septal nucleus, periventriclar nucleus, PVO and IF of the chicken [11] and Japanese quail [12] brain.

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Therefore, we investigated immuno-cytochemically the interaction of serotonin- and GA- IR neurons in the hypothalamus of the Japanese quail.

MATERIALS AND METHODS

Male Japanese quails (*Coturnix coturnix japonica*), 6-8 weeks old in age, were anesthetized with halothane fluothane, and perfused via the left ventricle of the heart with 0.9% NaCl and followed by Zamboni's fixative (4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) containing 0.2% picric acid at pH7.4). After perfusion, the brains were removed and post-fixed overnight in the same fixative at 4 °C.

Frozen sections (40-60 μ m) were prepared using a cryostat (Bright). Sections were mounted on gelatin-coated slides, air-dried and washed three times in 0.1 M phosphate-buffered saline containing 0.3 % Triton X-100 (PBS-T) for 10min.

The frozen sections were treated with the monoclonal rat antibodies against serotonin (diluted 1: 50, Chemicon) and galanin (diluted 1: 500, [12]). The antibodies were diluted in PBS-T containing 1 % bovine serum.

The sections were preincubated with 10 % normal goat serum in PBS-T for 30min at room temperature. In the first step, the sections were incubated with diluted antibodies

against serotonin overnight at room temprature. These were rinsed in PBS-T three times for 10 min, incubated with anti-mouse IgG-conjugated Alexa 488 (Molecular Probes ; diluted 1: 250 in PBS-T) for 5 h and rinsed with PBS-T three times for 10 min. In the second step, the same sections were incubated with the diluted antibodies against GA overnight at room temprature. These sections were rinsed in PBS-T three times for 10 min, incubated with anti-rabbit IgG-conjugated Alexa 568 (Molecular Probes ; diluted 1 : 250 in PBS-T) for 5 h, rinsed with PBS three times for 10 min and mounted using Gel/Mount (Biømeda corp).

The preparations were observed with a confocal laser scanning microscope (Leica,TCS-NT).

The numbers of serotonin- and GA-IR cell bodies were counted in each preparation under SD (8: 16 light-dark schedule with lights on at 0800 h) and LD (16: 8 light-dark schedule with lights on at 0400 h).

RESULTS

Serotonin-IR neurons were located in the PVO and IF (Fig.1; Fig.2-(a). upper panel). They were found along the third venrtricle. These cells extended knob-like structures to the third ventricle and therefore had characteristics of the CSF- contacting neurons, which we call the first layer cells (Fig.2-(a). upper panel). The axons of these serotonin-IR cells spreaded latelally and had many varicosities with the second layer cells (Fig.2-(a). upper panel). The number of the cells under SD was less in the dark phase than in the light phase ($p < 0.05$, Haida et al., in preparation).

Numerous GA-IR cells were observed in the ventral region of the IF (Fig.1; Fig.2-(b). middle panel). These cell bodies were round or spindle shape. Many GA-IR nerve fibers were located in the PVO and IF (Fig.2-(a). middle panel). The number of GA-IR cells in SD was significantly greater than that in LD ($p < 0.001$, Haida et al., in preparation).

Serotonin-IR and GA-IR neurons were observed in the same serial sections, and these neurons were closely associated in the PVO and IF (Fig.2. lower panel). Numerous GA-IR varicose fibers ran along serotonin-IR cell bodies and nerve fibers in the PVO and IF (Fig.2-(a). lower panel). Very few serotonin- IR fibers ran along GA-IR cell bodies and GA-IR nerve fibers in the ventral part of the IF (Fig.2-(b). lower panel). We could not observe co-localization of serotonin- and GA-immunoreactivities.

DISCUSSION

In the present study, we have investigated the morphological distribution of serotonin and GA neurons in the PVO and IF of the Japanese quail using a double immunostaining technique. With regard to the general

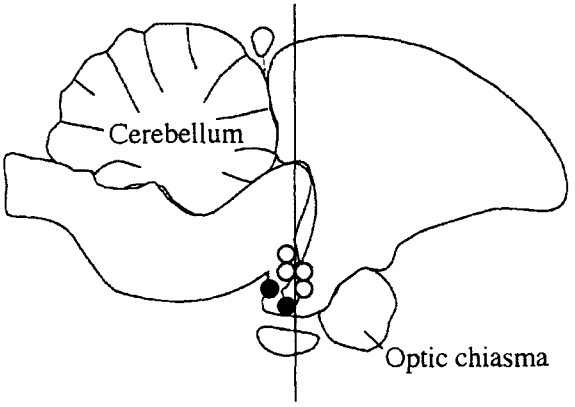

Fig.1. Schematic drawing of the Japanese quail brain.
A drawing of the sagittal section. Coronal sections were made at the position of the vertical line. White circles (○) indicate serotonin-IR cell bodies. Dots (●) indicate GA-IR cell bodies.

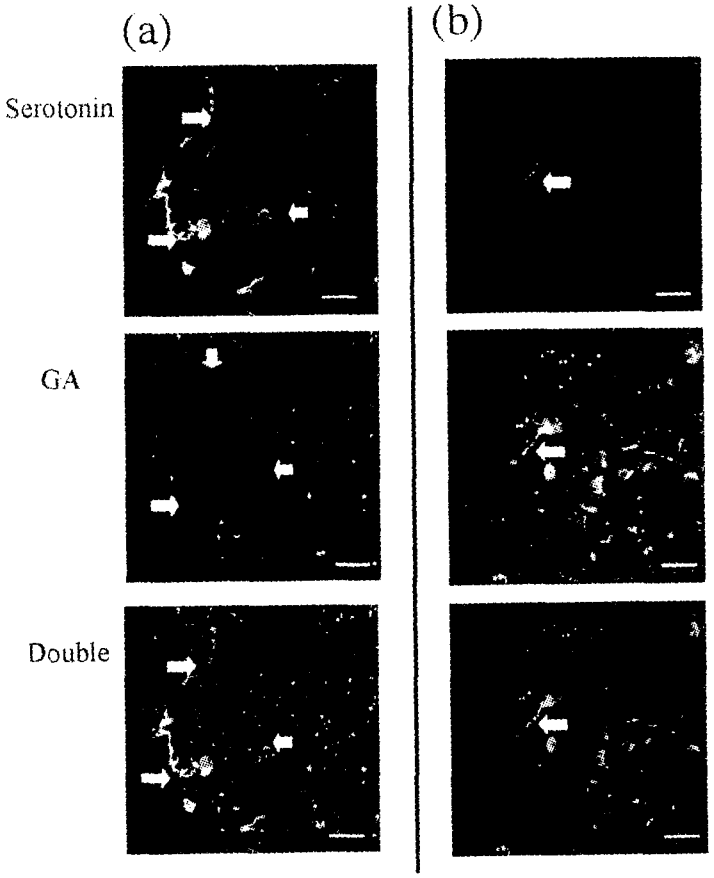

Fig.2. Confocal images of the PVO and IF (a) and the ventral region of the IF (b) double-immunostained with antibodies against serotonin and GA. Serotonin- and GA-immunoreactivities are separately shown in the upper and the middle panel, respectively. Lower panels indicate double immunostaining images. Arrows show serotonin-IR neurons and GA-IR neurons in close appositions. Bars : 20μm

distribution of the serotonin- and GA-IR neurons in the PVO and IF of the Japanese quail, our present study is in agreement with previous reports (serotonin: [3-6], GA: [11,12]).

In the brain of some animal species, the pineal and parapineal photoreceptive molecules have been sequenced, and they were named pinopsin [13] and parapinopsin [14], respectivity. Furthermore the rhodopsin gene expression was detected in the pigeon lateral septum [15]. The rhodopsin-IR cells were shown in the PVO of the Japanese quail [2]. In the lateral septal area and the IF of the pigeon, rhodopsin-IR [16,17] and $Gt_1\alpha$ - like IR cells were reported [17], although rhodopsin immunoreactivity was very week in the IF [16]. These results suggest the possibility that deep brain phtoreceptors were in the PVO, IF and lateral septum in birds. Serotonin-IR CSF-contacting neurons were found in the same regions. Since serotonin is one of the neurotransmitters in the central nervous system, they may have important roles to modulate photoreception in the deep brain. In the brain of mammals, GA coexpresses in a subset of gonadotoropin-releasing hormone [18], suggesting that GA may have an important role in reproduction. In the present study using Japanese quail, the GA-IR fibers were seen in close apposition to serotonin-IR cell bodies and fibers in the PVO and IF. These results suggest that there is a possibility of interaction between GA-IR and serotonin-IR neurons and these IR neurons may participate in modulating reproduction in birds as well as mammals.

The number of vasoactive intestinal peptide (VIP)-IR cells in the IF of SD males was significantly lower than that of LD males [19]. The numbers of serotonin- and GA-IR cells were changed by the photoperiod. These results suggest that the possibility of interaction among VIP, serotonin and GA in regulation of photoperiodic-gonadal response in birds.

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