Article

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Reference

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Vasoactive intestinal peptide-containing neurons in the paraventricular nucleus may participate in regulating prolactin secretion

(neuroendocrine regulation/adrenalectomy/lactation)

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ABSTRACT **Vasoactive intestinal polypeptide (VIP) immunoreactivity is present in varicosities and fibers in the hypothalamic paraventricular nucleus (PVN) in normal control animals. Adrenalectomy and lactation combined with colchicine treatment results in the appearance of a large population of VIP-immunopositive cell bodies in the parvocellular part of the PVN. Adrenalectomy, as well as lactation, significantly increases the number of VIP-positive fibers in the external zone of the median eminence. These observations suggest that the VIP-immunopositive neurons in the PVN may participate in regulating prolactin and corticotropin secretion.**

Vasoactive intestinal polypeptide (VIP) was isolated from lung extracts (1, 2), and shortly after its discovery it was shown to be present in a wide variety of peripheral and central neuronal elements (3, 4). Its presence in hypothalamic nerve endings (5) as well as in the portal blood (6) indicates that VIP might be involved in the regulation of anterior pituitary hormone secretion. Intracerebroventricularly or intravenously administered VIP causes a dose-related increase in plasma prolactin levels (7); in ovariectomized rats it also stimulates the release of growth hormone and luteinizing hormone (8). The effect of VIP on corticotropin (ACTH) secretion is moot (9, 10).

At present, then, it is generally agreed that VIP affects pituitary function, but the anatomical substrate of this function is unknown. Work on this problem has been impeded by the absence of data indicating that VIP-positive nerve terminals are present in the external zone of the median eminence. The internal zone, on the other hand, does contain VIP-positive neuronal elements (cf. ref. 4). Below we describe a significant population of VIP-positive cell bodies in the parvocellular region of the hypothalamic paraventricular nucleus (PVN) that project to the external zone of the median eminence. This cell population represents the missing link between the "pharmacological" effects of VIP and studies of its neuroendocrine function.

METHODS AND MATERIALS

Male and female Sprague–Dawley rats (200 g) were used in all of our studies. Three groups of rats were studied: control animals, adrenalectomized animals (1 week after surgery), and lactating animals (2 days after delivery). In all groups, 3 or 4 animals received colchicine treatment 48 hr prior to perfusion to enhance immunoreactivity in cell bodies. The colchicine (90 μg per rat) was injected into the lateral ventricle (coordinates: 1 mm lateral to the midline, 1 mm anterior to the bregma; 4.5 mm ventral to the dura, 3.3 mm nose down, in 15-μl vol at a flow rate of 1 μl/min).

The rats were anesthetized with sodium pentobarbital (40 mg/kg) and were perfused through the ascending aorta with 4% paraformaldehyde/0.2% saturated picric acid in 0.16 M sodium phosphate buffer (pH, 7.2). The brains were removed and postfixed in the same fixative for 90 min, then rinsed overnight in phosphate-buffered saline. Fifty-micrometer-thick Vibratome sections were cut and processed using the avidin–biotin technique (11, 12). The sections were incubated overnight at 4°C with the primary antisera and then processed as described (13). Diaminobenzidine (Polysciences, Warrington, PA) was used as a chromogen (40 mg/100 ml) in 0.05 M Tris·HCl (pH, 7.6). For double staining with VIP and corticotropin-releasing factor (CRF), after the incubation with the second primary antibody, $NiCl_2$ was added to the diaminobenzidine solution (final concentration, 40 mg/100 ml) to give a grey reaction product. The sections were dehydrated, mounted with permount, and photographed.

Antisera and controls used were as follows: antiserum against VIP was purchased from Immuno Nuclear (Stillwater, MN) and was used in a 1:1500 dilution. This working dilution was preincubated with 100 μg of VIP per ml (Sigma), 100 μg of PHI (a peptide with NH_2-terminal histidine and COOH-terminal isoleucine amide) per ml (Bachem Fine Chemicals, Torrance, CA), and 100 μg of secretin per ml (gift from Tom O'Donohue) for control studies. The CRF antiserum was a gift from Robert Eskay, and its specificity has been described (13).

RESULTS

In hypothalami of control animals, a few VIP immunoreactive cell bodies were present in the suprachiasmatic nucleus, and stained fibers and varicosities were seen in the peri- and paraventricular nuclei, as described by others (cf. ref. 4; Fig. 1*A*). After colchicine treatment, there was a marked increase in the number of immunopositive cell bodies in the suprachiasmatic nucleus, and a few faint cells could be seen in the parvocellular subdivisions of the paraventricular nucleus. The brains of adrenalectomized animals were similar to those of controls, except the surgery resulted in the appearance of numerous VIP-immunopositive fibers in the external zone of the median eminence (Fig. 1 *C* and *D*). Adrenalectomy plus colchicine treatment revealed a large population of VIP-positive neurons in the medial parvocellular subdivision of the paraventricular nucleus (Fig. 1*B*). Double staining with CRF and VIP antibodies suggests that although these two peptides are present in the same subdivision of the PVN, they do not coexist in the same cells.

Brains from lactating rats were quite similar to brains from adrenalectomized animals. Without colchicine treatment a

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Abbreviations: ACTH, corticotropin; CRF, corticotropin-releasing factor; PVN, hypothalamic paraventricular nucleus; VIP, vasoactive intestinal peptide; PHI, peptide with NH_2-terminal histidine and COOH-terminal isoleucine amide.

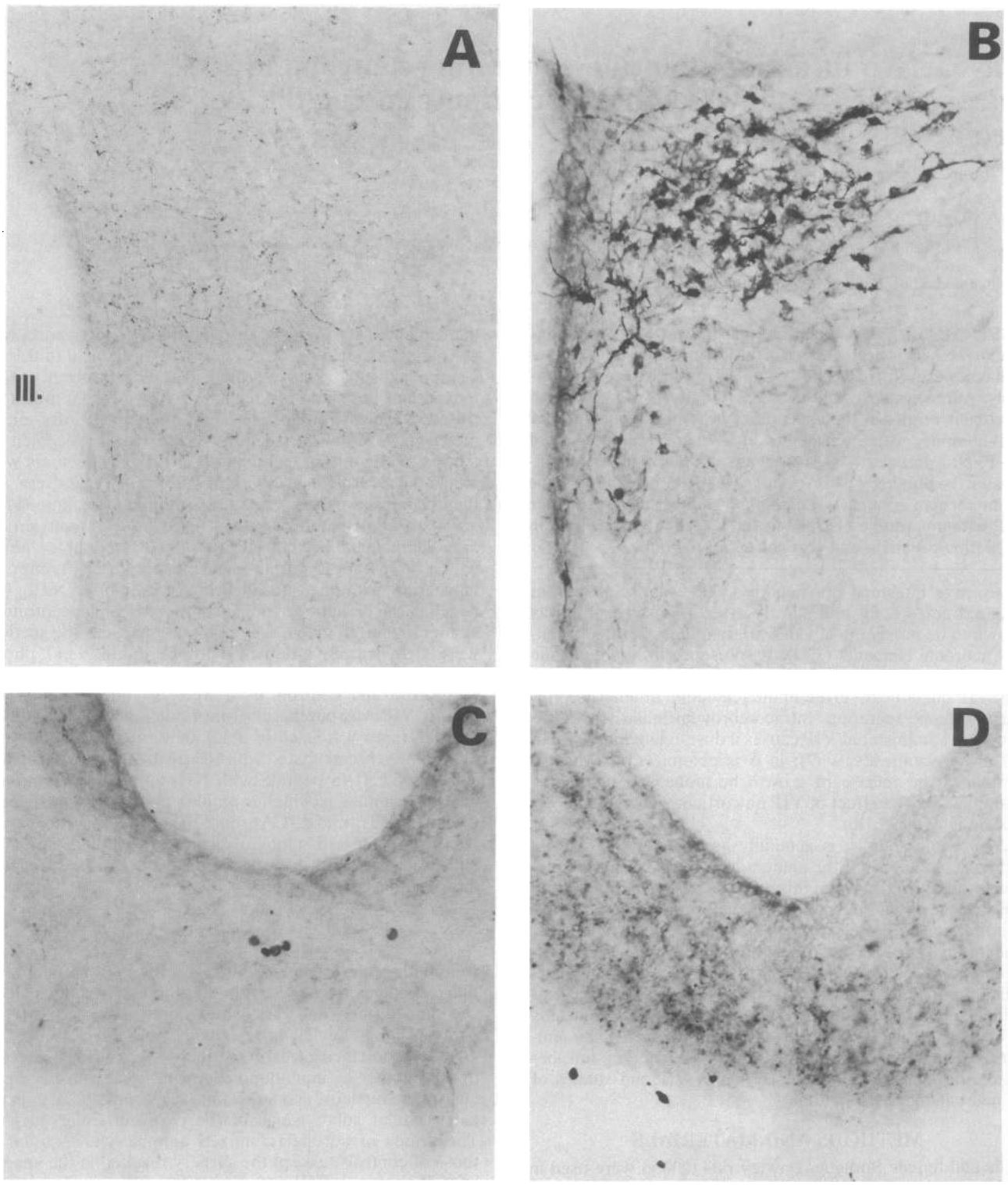

FIG. 1. Immunostaining for VIP in control (*A*) and adrenalectomized (plus colchicine-treated) (*B*) paraventricular nucleus. The median eminence of a control animal (*C*) contains only a few VIP-immunopositive terminals, while adrenalectomy results in a strong, dense network of VIP-immunopositive fibers and terminals in the external zone (*D*). III, third ventricle. (*A* and *B*, ×130; *C* and *D*, ×330.)

few VIP-positive cell bodies could be observed in the PVN of lactating rats; colchicine treatment resulted in the appearance of numerous VIP cell bodies (somewhat less, however, than those observed after adrenalectomy and treatment with colchicine).

DISCUSSION

Our results suggest that there is a population of neurons in the PVN that produce VIP. These neurons are found in the medial parvocellular subdivision of the nucleus, which was shown to project to the neurohemal "external" zone of the median eminence; they are thus intimately involved in neuroendocrine regulation (14). The parallel changes in VIP immunoreactivity in the median eminence and PVN indicate that the VIP-containing PVN neurons are the source of this peptide in the external layer of the median eminence (i.e., in the portal blood). The fact that adrenalectomy and lactation dramatically increase VIP immunostaining in these neurons suggests that VIP may contribute to the regulation of ACTH

and prolactin secretion from the anterior lobe of the pituitary. Previous observations suggested that VIP might be a releasing factor for prolactin, ACTH, and growth hormone (7, 8, 10, 15, 16). Adrenalectomy results in high plasma levels of ACTH and prolactin (17). A prolactin-releasing hormone was suspected to act during lactation. We chose those two conditions to see whether the altered plasma levels of the two peptides are accompanied by altered VIP immunostaining in the central nervous system. The effect of adrenalectomy on VIP immunostaining seems to support the hypothesis that VIP is involved in ACTH release. Moreover, this hypothesis is further supported by earlier findings that corticosteroids affect hypothalamic VIP synthesis (18) and that VIP stimulates ACTH release from human and mouse pituitary tumor cells (16).

The most consistent effect of VIP is on prolactin release (cf. ref. 19). The fact that in lactating animals many neurons in the PVN become VIP immunopositive supports the hypothesis that VIP might act as a prolactin-releasing factor. These neurons must have a very low level of VIP synthesis and/or rate of release under "normal" conditions, because colchicine treatment alone does not allow them to be stained. Manipulations that presumably increase VIP synthesis in these cells (such as adrenalectomy or lactation) together with blockade of axonal transport result in a high enough concentration of the peptide for detection with immunocytochemistry.

Hökfelt *et al.* (20) have reported that CRF and PHI, a peptide closely related to VIP (21), coexist in PVN neurons. Our absorption control studies, however, indicate that the immunoreactivity we see is not attributable to PHI; absorption with PHI does not affect the staining, while VIP absorption eliminates it. Absorption with secretin also does not effect staining.

A number of hypophyseotrophic neurohormones, such as CRF (22), vasopressin (13, 23, 24), and thyrotropin-releasing hormone (25) have been found in PVN parvocellular neurons. It is clear now that CRF and vasopressin, which have a synergistic effect on ACTH secretion (26), are produced by one and the same neuron (13, 23, 24). The close topographical relationship of VIP and CRF immunoreactive neurons suggests that these cells may contribute to producing parallel changes in plasma ACTH and prolactin observed in response to various stressors.

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