Identification of Corticotropin-Releasing Factor in Neurosecretory Cells of Rat Hypothalamus "Gomori-Positive" Accessory Centers

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Apart from the supraoptic, paraventricular, and postoptic nuclei there are some small groups of large nonapeptidergic neurosecretory cells (NSC) invariably located in the rat hypothalamus which are called accessory groups (AG) or centers. Their microanatomy is well known [9], but their functional significance is still to be discovered. Polenov *et al.* [1] put forward a hypothesis about functional differences between these groups and speculated that their functions are not the same as those of the chief neurosecretory centers. According to Palkovits [7], the NSC of each AG synthesize their own neurohormone, an assumption which is consistent with Polenov's hypothesis, although they stained uniformly with paraldehyde-fuchsin. Immunocytochemically AG NSC have been shown to be vasopressin (VP)- and oxytocin (OT)-ergic, as well as the cells in the supraoptic and paraventricular nuclei. It must be stressed that no quantitative analysis of the share of NSC of each kind has ever been performed [10] and that we have no reliable data about AG NSC other than VP- or OT-ergic.

The synergic effect of nonapeptide neurohormones and corticotropin-releasing factor (CRF) on the pituitary ACTH cells is also well known [6,13,14]. This research was aimed at detecting CRF-immunoreactive (CRFir) cells in rat hypothalamus AG.

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MATERIALS AND METHODS

Two groups of male Wistar rats weighing 200-250 g were used, 5 animals in each group. In group 1 rats deafferentation of the mediobasal hypothalamus was carried out one month before decapitation. To group 2 rats colchicine (75 μg/10μl saline) was injected into the third ventricle 48 h before sacrifice. The experiments were directed at blocking neurohormonal transport along with NSC processes to reveal more immunoreactive cells. Five intact male Wistar rats were controls.

Hypothalami were fixed in Bouin's fluid. Serial paraffin sections were stained immunohistochemically with the PAP method, using antisera to CRF, arginine-VP, and OT. Then OT and VP NSC in each of the AG (circular complex, periforniceal (PFAG), dorso- and ventrolateral AG, and the extrahypothalamic group near the capsula interna) were estimated, taking into account only the sections of cells containing nucleoli.

RESULTS

The location of the AG studied is presented in Fig. 1.

More than 600 NSC were found in AG of intact rats, mostly OT-ergic (58%). No CRFir cells

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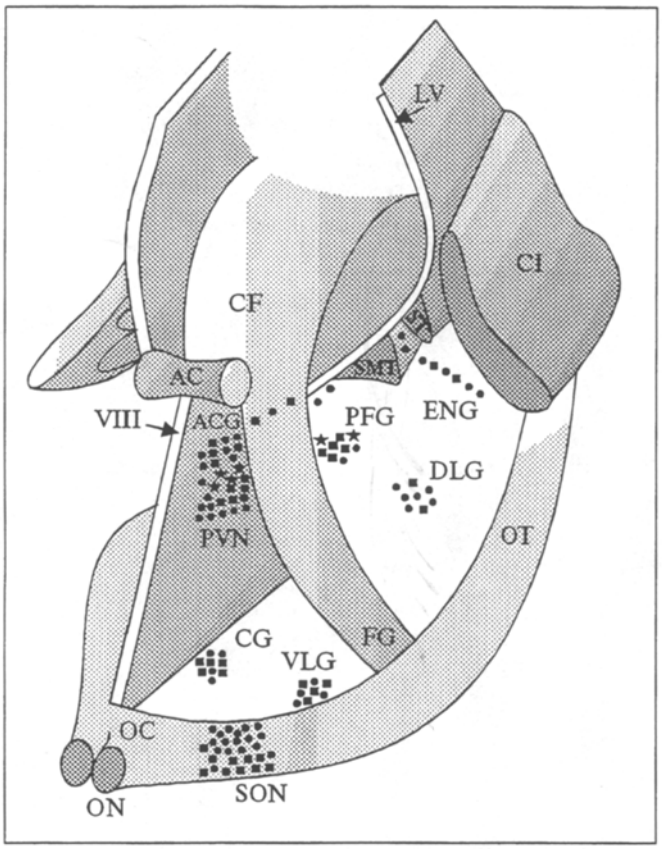

Fig. 1. Localization of oxytocin– (circles), vasopressin– (rectangle), and corticotropin–releasing factor– (stars) immunoreactive cells in the rat hypothalamus and adjacent regions. VIII: third ventricle; LV: lateral ventricle; CI: capsula interna; VLG: venrolateral group; DLG: dorsolateral group; ST: stria terminalis; FG: forniceal column; SMT: stria medullaris thalami; ON: optic nerve; OT: optic tract; OC: optic chiasma; PVN: paraventricular nucleus; AC: anterior commissure; ACG: anterior commissural group; PFG: periforniceal group; SON: supraoptic nucleus; CF: corpus fornicis; CG: circular group; EHG: extrahypothalamic group.

were found in the hypothalamic AG of intact rats. They appeared in the rats of both experimental groups but only among PFAG NSC, and therefore the AG deserves more attention. PFAG is situated at the level of the paraventricular nucleus, above the forniceal column. CFRir cells are situated between large multipolar nonapeptidergic NSC which encircle an arteriole running through this AG. About 102±3 nonapeptidergic NSC are found in this AG in an intact rat, VP-NSC (68%) being more numerous than OT-NSC. Fibers filled with neurohormone leave the perikarya in various directions. Most of them follow the arteriole, proceeding to the base of the hypothalamus; a lesser number of fibers are directed mainly to the paraventricular nucleus and laterodorsally to extrahypothalamic AG.

Two types of CFRir NSC are seen in PFAG of experimental rats. The first are single cells with perikarya 15-20 μ in diameter with immunoreactive processes and eccentrically located nuclei (Fig. 2). In addition, there are single large (40-50 μ in diameter) multipolar cells with a light nucleus and large nucleolus. Their morphology is close to nonapeptidergic NSC. The cytoplasm of these NSC stains weakly after reaction to CRF antibody.

Modern RIA [3] and immunohistochemical [2,12] data as well as hybridization *in situ* [15] show that CRF is synthesized in the cells of the paraventricular nucleus, mainly in its dorsomedial part. No other location is described, except by Kawata *et al.* [5], who found CFRir cells not only in the paraventricular nucleus, but also in the supraoptic and postoptic nuclei, the circular complex of AG, and in the lateral hypothalamus. We suspect that such a wide distribution of CFRir NSC may be the result of using an insufficiently specific antiserum. Paraventricular nucleus destruction in rats led six weeks later to the appearance of CFRir NSC in the supraoptic nucleus and in the periforniceal area, but not in PFAG. It is thought to be a compensatory reaction [8]. In our study CFRir NSC became visible in PFAG after section of nerve fibers by deafferentation of the mediobasal hypothalamus and after the blocking of neurohormone transport by colchicine injection. Both interventions impeded CRF secretion and led to its accumulation in the cell body. It seems possible that both PFAG and the paraventricular nucleus originate from the same source. Their localization is close and the type of CFRir NSC similar. It seems that CFRir NSC are present even in intact rat hypothalamus, but the amount of hormone in their perikarya is low. This is why it could not

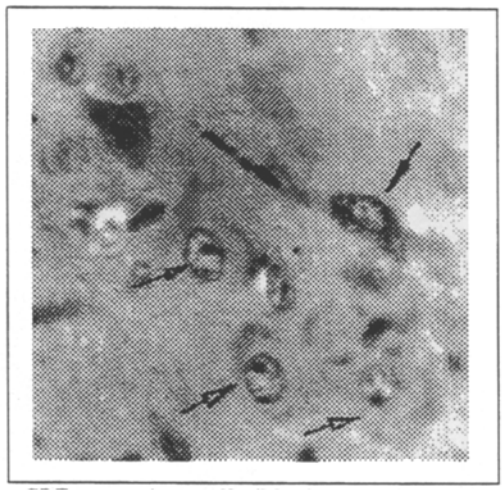

Fig. 2. CRF–reactive cell (black arrow) among immunoreactive nonapeptidergic cells (light arrows) of PFG of rat hypothalamus intraventricularly injected with colchicine. Reaction with antiserum to CRF; staining with hematoxylin after Ehrlich. ×252.

be found in intact animals and became visible only under experimental conditions. This also points to a high velocity of CRF transport from NSC under normal conditions.

The second type of CFRir NSC seen under experimental conditions is thought to be nonapeptidergic cells with small amounts of CRF in the cytoplasm. As PFAG is the only AG where VP NSC prevail, this is highly probable. Moreover, a similar phenomenon has been described in the paraventricular nucleus after adrenalectomy [11]. Colocalization of OT and CRF was also shown in the paraventricular nucleus NSC [4]. For a final conclusion as to which of the nonapeptides is colocalized with CRF, experiments with double immunoreactive staining are needed, with CRF and VP or OT antibodies simultaneously.

Thus, only PFAG of all the AGs contains CFRir NSC. This may be evidence of PFAG NSC involvement in the stress reaction, confirming Polenov's supposition [1] about AG functional variety. The possible ways and mechanisms of PFAG participation in the stress reaction need further investigation.

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