MORPHOLOGICAL STUDY OF SOME HYPOTHALAMIC CENTERS IN RATS AFTER DEAFFERENTATION OF THE MEDIOBASAL HYPOTHALAMUS

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The volume of the nuclei and nucleoli of certain hypothalamic centers (SON, PVN, SCN, AN, VMN) was determined in control rats and in rats after deafferentation of the mediobasal hypothalamus. Sex differences were found in the parvocellular formations of the control animals: The volumes of nuclei and nucleoli of neurons of AN and VMN, and also of the nucleolus of SCN neurons were larger in females than in males. After deafferentation of the mediobasal hypothalamus the volume of the cell nuclei was increased, especially in hypothalamic formations located outside the isolated zone. This increase was more clearly defined in rats constantly in a state of estrus after the operation. Statistically significant differences between volumes of both nuclei and nucleoli of the cells in subgroups of rats with permanent estrus and with permanent diestrus were found only in the case of SCN. No such differences were found for AN, despite the considerable difference in the constant of luteinizing hormone in the pituitary of the same rats. It is suggested that gonadotropin releasing factors are not produced by the cells of AN and that control over the succession of phases of the sex cycle may be exerted by SCN.

KEY WORDS: mediobasal hypothalamus; deafferentation; karyometry.

After deafferentation of the mediobasal hypothalamus (MBH) in rats the succession of phases of the sex cycle is disturbed. Predominance of diestrus, permanent diestrus, or permanent estrus is observed. Differences in the disturbances produced are due to variations in the course of the knife during the operation, especially in the frontal part of the isolated region. In the modern view hypothalamic regulation of the functions of the gonads is effected by two centers: a tonic center, located in the tuberoinfundibular region, producing gonadotropin-releasing factor [14], and a cyclic center, located in the anterior hypothalamus or in the preoptic region. Separation of the cyclic center from the tonic center after deafferentation of MBH is accompanied not only by disturbance of the succession of phases of the sex cycle, but also by a disturbance of the concentrations of luteinizing hormone (LH) in the blood and pituitary gland and of LH-releasing factor in the hypothalamus [1, 8, 15].

One indicator of the activity of neurons and neurosecretory cells is the size of their nuclei and, in particular, of their nucleoli. However, during the normal succession of phases of the sex cycle in female rats no significant changes have been found in the size of the nuclei and nucleoli of the cells in various hypothalamic centers. The reason for this is that the state of estrus in female rats is of very short duration, and during this time no appreciable increase in size of the nuclei or nucleoli of the cells takes place [2, 4, 7, 11]. Under experimental conditions, by changing the length of the period of daylight, a significant increase in the size of the cell nuclei was obtained in several hypothalamic centers during prolonged estrus and a decrease in their size during prolonged diestrus [11].

The object of this investigation was to measure the volume of the cell nuclei and nucleoli in certain hypothalamic centers in rats in a state of permanent estrus or permanent diestrus after deafferentation of MBH. It was expected that the morphometric data would show differences in the functional state of the various hypothalamic centers in the rats of

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TABLE 1. Volume (in μ^3) of Cell Nuclei and Nucleoli of Certain Hypothalamic Centers in Control Rats and in Rats with Deafferented MBH (M±m)

Hypothalamic centers	Groups of rats			
	control animals		deafferented rats in a state of permanent	
	males	females	diestrus	estrus
Nuclei				
SON	553±6,5	501±13,6	671±17,0	734±20,0
PVN	496±5,3	510±6,8	611±13,7	687±20,0
SCN	231±4,3	224±5,8	307±6,5	362±10,0
VMN	438±5,5	486±6,6	599±11,5	569±14,8
AN	345±5,0	396±6,9	422±7,4	442±10,7
Nucleoli				
SON	22,8±0,4	22,0±0,4	28,7±0,7	27,8±1,0
PVN	20,2±0,3	18,1±0,4	22,0±0,6	21,8±0,4
SCN	5,5±0,1	6,2±0,2	7,1±0,1	8,2±0,3
VMN	11,8±0,2	13,8±0,4	14,5±0,4	13,6±0,6
AN	8,6±0,1	10,1±0,2	10,1±0,2	10,2±0,3

Legend. Lines connect data differences between which are not significant ($P > 0.05$).

these two subgroups, kept for a long time in polar phases of the sex cycle. It was assumed that structures responsible for releasing factor production would show the greatest differences.

EXPERIMENTAL METHOD

Albino rats weighing about 200 g underwent an operation by Halasz' method [10]. During 3 weeks after the operation, phases of the sex cycle of the rats were determined from vaginal smears. From the large number of rats seven with the clearest changes were chosen for morphometric analysis: two rats with permanent estrus and five with permanent diestrus. Intact rats — four females and four males — served as the control. The hypothalamic region of the brain was fixed in Bouin's mixture. Serial paraffin sections 5-7 μ thick were stained with paraldehyde-fuchsin by the Gomori–Gabe method and counterstained with azan by Heidenhain's method. The diameters of the nuclei and nucleoli were measured with the MOV 15× screw ocular micrometer with an overall magnification of 3000. Measurements were made in the following hypothalamic nuclei: supraoptic (SON), paraventricular (PVN), suprachiasmatic (SCN), ventromedial (VMN), and arcuate (AN). Altogether 30 cells were counted from each hypothalamic nucleus, as a rule on each side. The volume of the nuclei was calculated by the equation for an ellipsoid of rotation, the volume of the nucleoli by the equation for a sphere. The data were processed on the "Nairi" computer.*

EXPERIMENTAL RESULTS

It will be clear from Table 1 that statistically significant differences were found in the size of the nuclei and nucleoli in rats of different sexes and they were particularly clear in cells of the parvocellular formations. The volume of the nuclei and nucleoli as a

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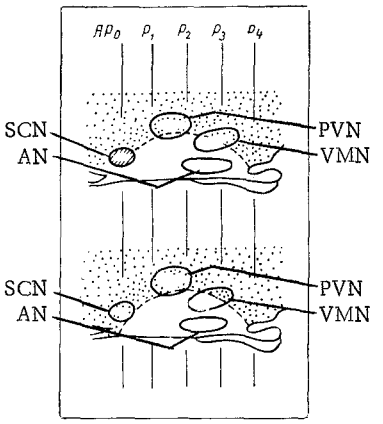

Fig. 1. Scheme showing course of knife during deafferentation of MBH in rats kept after operation in state of permanent estrus (E) or diestrus (D). PVN) Paraventricular, SCN) suprachiasmatic, VMN) ventromedial, and AN) arcuate nucleus of hypothalamus. AP_0-P_4) Frontal planes taken from atlas of Szentagothai et al. [14].

rule was less in cells from males. Nuclei of SCN cells, which were larger, but not significantly, than the corresponding cells in females, were the exception. In rats with considerable injuries to the suprachiasmatic region, permanent estrus was observed, although in the frontal part of the insular, in planes AP_0 according to the atlas of Szentagothai et al. [14] the knife did not reach as far as the basal surface of the brain (Fig. 1). In the deafferented rats a clear difference was observed between the size of the nuclei and nucleoli in cells of SON, PVN, and SCN, i.e., in centers lying mainly outside the isolated region. In cells of VMN and AN in the deafferented rats the volume of the nuclei was increased, but there was no statistically significant change in the volume of the nucleoli (Table 1). Comparison of the volumes of the nuclei and nucleoli of cells from the hypothalamic centers in the two subgroups of deafferented rats showed that in rats with permanent estrus the measured structures were in most cases larger than in rats with permanent diestrus. However, statistically significant differences between the volumes of both nuclei and nucleoli were observed only in the case of cells in SCN.

Differences in the size of cell nuclei of the hypothalamic centers in rats of different sexes were observed previously also [12, 13]. These data coincide to some extent with our own. It is difficult to say whether the presence of larger cell nuclei in females reflects differences in the hormonal balance, but their presence also in females in a state of permanent estrus suggests a different explanation. The larger size of the nuclei and nucleoli in cells of SON, PVN and, possibly, SCN in the deafferented rats may also be connected with regeneration or compensatory hypertrophy of the cells of these centers [3]. It should be noted that significant and very considerable differences in the volumes of the cell nuclei and nucleoli in rats in a state of estrus or diestrus were seen most clearly in SCN. It can therefore be postulated that this formation (compared with the other centers investigated) may be most directly related to the maintenance of one or other phase of the sex cycle. Attention is drawn particularly to data on AN obtained by Baranov et al. [1], regarding the blood estrogen levels and content of luteinizing hormone in the pituitary of these same rats. Rats in a state of estrus and diestrus after deafferentation of MBH differed sharply in their content of LH and, consequently, also in the content of LH-releasing factor entering the portal blood stream from the hypothalamus; meanwhile differences in the volumes of the cell nuclei and nucleoli of AN between these subgroups of rats were not statistically significant, and the volume of the nucleoli in the two subgroups was indistinguishable from the control.

This fact confirms the view that AN in rats does not participate in the synthesis of LH-releasing factor, although it is involved in the regulation of the gonadotropic function of the anterior lobe of the pituitary [5, 9].

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INTERNEURONAL CORTICAL CONNECTIONS STUDIED BY KAINIC ACID INJECTION

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Changes taking place in the cat cerebral cortex 1, 3, 7, and 14 days after injection of 0.2% kainic acid solution were studied. Kainic acid was found to cause local injury to bodies of neurons and their processes, by leaving intact afferent fibers entering this region. This was demonstrated both by the use of Golgi and Golgi–Kopsch methods and also by injecting horseradish peroxidase after kainic acid into the cerebral cortex. Survival for 3 days after injection of kainic acid provides the optimal time for studying interneuronal connections. Pericellular plexuses formed by afferent fibers were discovered.

KEY WORDS: kainic acid; cerebral cortex; interneuronal connections.

The use of microinjections of kainic acid (KA) — a cyclic analog of glutamate — is justified by the fact that KA causes degeneration of neurons which selectively accumulate glutamate, but does not injure axons passing through or terminating in the given region [1, 2, 6]. It has been shown by the use of KA-^{3}H that it possesses high affinity for glutamate receptors and has a powerful excitatory-toxic action on them [9]. After injection of 2 μg KA into the striatum activity of certain enzymes — glutamate decarboxylase, choline-acetyltransferase — is sharply reduced, and ultimately this leads to neuropathological changes similar to those observed in Huntington's chorea [5], the lateral hypothalamic syndrome [10], etc.; for that reason, KA is nowadays used to create models of these diseases. On the basis of biochemical, histological, and electron-microscopic investigations it has been concluded that KA has spe-

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