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Correlation between catecholamine content and numbers of granulated vesicles in rabbit hypothalamus

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THE DISTRIBUTION of catecholamines in the brain has been investigated by several investigators and norepinephrine was present in the highest concentration in the hypothalamus.^{1,2,3,4} Within the hypothalamus itself we find conflicting reports about the content of norepinephrine; Vogt¹ showed somewhat larger content in the ventral and medial parts than in the dorsal and lateral ones respectively, while Kuntzman *et al.*⁵ demonstrated larger amounts in the anterior part than in the posterior. On the other hand the recent electron microscopic study by de Iraldi *et al.*⁷ has demonstrated the presence of large amounts of specific granulated vesicles especially in the anterior hypothalamus,

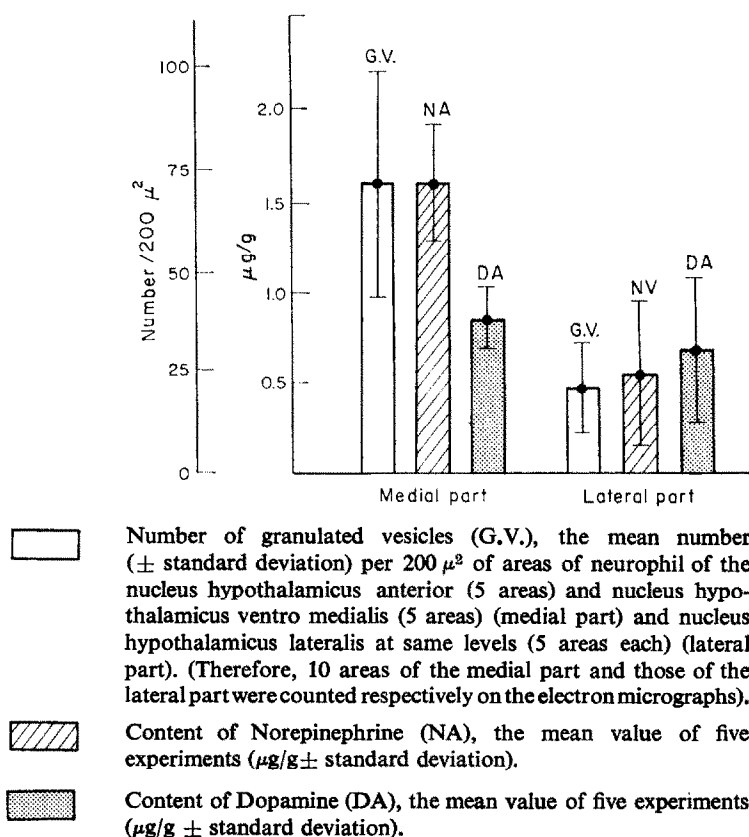


FIG. 1. Correlation between catecholamine content and number of granulated vesicles in rabbit hypothalamus.

which were located in the axons and synaptic terminals and supposed to be the site of storage of norepinephrine, but they did not study the lateral part of the hypothalamus. In the present investigation the content of norepinephrine and dopamine was estimated in the medial and lateral hypothalamic areas, which were divided by the dorso-ventral line passing through the fornix (and mamillothalamic tract). Then, their content were correlated with the number of the granulated vesicles,

which were observed by the electron microscope at the representative nuclei of the medial and lateral parts of the hypothalamus.

Male rabbits weighing about 2.0 to 2.5 kg were used. After killing by decapitation and excising the brain, the hypothalamus was divided macroscopically into the medial and lateral parts by the line stated above. The tissue pieces from ten animals were collected and analysed as one sample. Norepinephrine and dopamine were extracted from the brain tissues with 0.4 N perchloric acid. They were fractionated with a Duolite C-25 resin column,⁷ and norepinephrine was estimated by the trihydroxyindole method⁸ and dopamine by the ethylenediamine condensing method.⁹ In order to demonstrate the granulated vesicles,¹⁰ the brain of animals perfused with glutaraldehyde after the modified method of Palay *et al.*¹¹ was cut into frontal thin sections by the mechanical chopper and post-fixed in OsO₄. The necessary regions (nuclei hypothalamicus anterior, ventromedialis and hypothalamicus lateralis at the same levels) were cut out and embedded in Epon 812. The ultrathin sections stained with both uranyl acetate and lead hydroxide were observed under the JEM-6G electron microscope.

The content of norepinephrine and dopamine and the number of granulated vesicles in the medial and lateral parts of the hypothalamus were shown in Fig. 1. Figure 2a and Fig. 2b were the electron micrographs of the neuropil of the representative nuclei of both the medial and lateral parts of the hypothalamus, which exhibited the presence of granulated vesicles especially in large numbers in Fig. 2a. Figure 1 demonstrated that both norepinephrine content and the number of granulated vesicles in the medial part were about three times as large as the lateral part, while the content of dopamine was nearly the same in both parts. Therefore, the content of granulated vesicles corresponded fairly well with that of norepinephrine but not with that of dopamine in the hypothalamus. This result also indicated that granulated vesicles might have an intimate relation with the content of norepinephrine while far less relation with dopamine. Thus, our data for the first time show the clear correspondence of norepinephrine content with number of the granulated vesicles not only in the medial part but also in the lateral part of the hypothalamus, a finding which supports the opinion of the granulated vesicles being the site of storage of norepinephrine.

From the result of the experimental and histological studies, Kurotsu and his associates^{12,13} divided the hypothalamus into three zones; (1) the periventricular layer, 'A-zone', (2) the medial hypothalamic area, 'B-zone', (3) the lateral hypothalamic area, 'C-zone'. They demonstrated that electrical stimulation of 'B-zone' produced sympathetic effects while that of 'A and C-zones' parasympathetic effects. Accordingly, it is supposed that the sympathetic reaction produced by electrical stimulation of the 'B-zone' might be related in some way to the more amounts of norepinephrine and larger numbers of the granulated vesicles.

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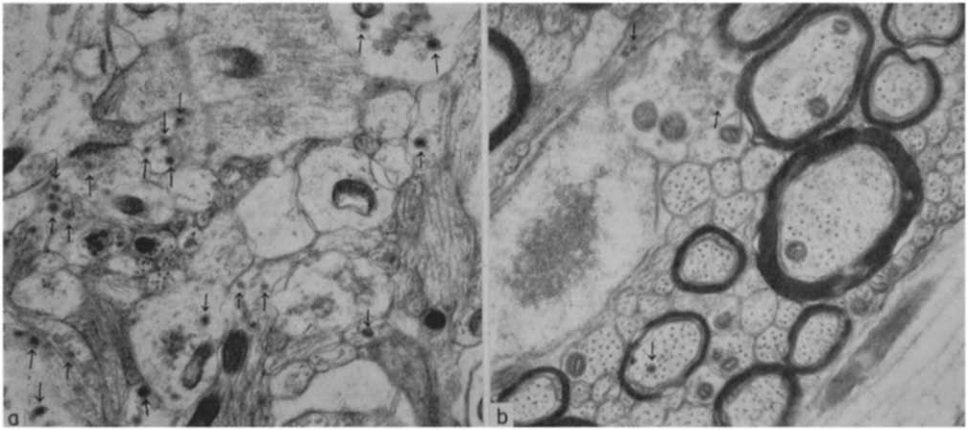


FIG. 2a. Electron micrograph of neuropil of the medial part of the hypothalamus (nucleus hypothalamicus ventromedialis) of rabbit. About twenty granulated vesicles (arrows) are seen within the axons and axon terminals. $\times 20,000$.

FIG. 2b. Electron micrograph of the lateral part of the hypothalamus (the nucleus hypothalamicus lateralis at the same level as Fig. 1a). About four granulated vesicles (arrows) are also found within the myelinated and non-myelinated nerve fibres of varying calibres, which constitute the medial forebrain bundle — 20,000.