CATECHOLAMINES OF THE ORAL REGION OF THE BRAIN STEM UNDER NORMAL CONDITIONS AND DURING RAPIDLY DEVELOPING STRESS (HISTOCHEMICAL INVESTIGATION)

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Differences are found in the distribution of catecholamines in structures of the oral brain stem of albino mice under normal conditions and in asphyxia.

Functional systems formed in states of stress are characterized by a pacemaker physiological architecture, with the formation of what P. K. Anokhin (1970) describes as energetically nodal mechanisms in the region of the limbic system.

The object of the present investigation was to study the "catecholamine architecture" of the functional system of stress simulated by asphyxia. The role of catecholamines in the organization of stress states has been demonstrated [4-6, 10].

EXPERIMENTAL METHOD

Adult albino mice were used for the experiments. A rubber finger stall was placed over the animal's snout. The state of asphyxia was judged from the development of motor excitation. The animals were decapitated after the first (25-30 sec) or second motor excitation (50 sec-2 min 30 sec). The brain was quickly removed from the skull, the required part was excised and mounted together with the same part of the brain of a normal animal, and placed on a freezing microtome. Sections were cut to a thickness of 10 μ and subsequently treated (lyophilic drying and heating in formaldehyde vapor) by the method of Falck et al. [7] in the modification adopted in V. A. Govyrin's laboratory at the Institute of Evolutionary Biochemistry and Physiology. The intensity of fluorescence was estimated visually.

EXPERIMENTAL RESULTS

Under normal conditions the greatest intensity of fluorescence was found in the following structures of the oral division of the brain stem: locus coeruleus; substantia nigra; grisea centralis, and adjacent ventrolateral portions of the mesencephalic reticular formation; the region of the raphe; ganglion interpendunculare, together with regions lying laterally to it (Fig. 1). Fluorescence of the locus coeruleus was particularly strong (Figs. 2-3), and that of the substantia nigra slightly weaker (Fig. 3b); the other three regions mentioned above gave about equal fluorescence, somewhat weaker than the first two. In the substantia grisea centralis of the oral division of the pons, the fluorescent region was small and occupied a small area in the midline in the periventricular gray matter (Fig. 1a). At the level of the inferior colliculi, the region of intensive fluorescence occupied the ventral part of the substantia grisea centralis, much of the adjacent reticular formation, and also an area of the raphe in direct contact with the grisea centralis (Fig. 1b). At the level of the superior colliculi, the substantia nigra and an area of the raphe located between the right and left halves of the substantia nigra were strongly fluorescent (Fig. 1c).

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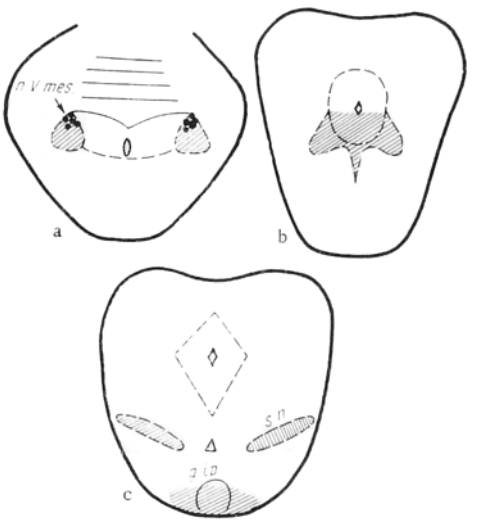

Fig. 1

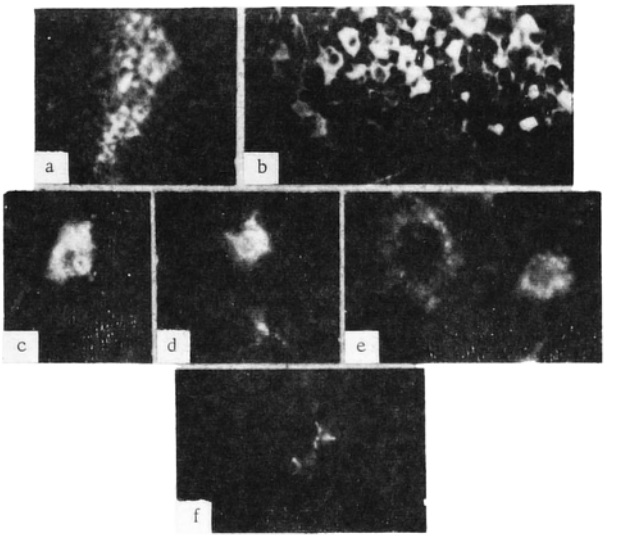

Fig. 2

Fig. 1. Scheme of fluorescent regions of oral division of the brain stem of an albino mouse: a) oral division of pons; b) level of inferior colliculi; c) level of superior colliculi. S.n.) substantia nigra; g.ip.) ganglion interpedunculare; n.V mes.) mesencephalic nucleus of trigeminal nerve.

Fig. 2. Catecholamines in region of locus coeruleus: a) locus coeruleus, normal (objective 10, ocular Homal 3); b) the same (objective 40, ocular Homal 3); c–f) fluorescent cells of locus coeruleus on soma of which noradrenalin–dopamine terminals can be seen. In e on the left, cells of mesencephalic nucleus; on the right, cells of locus coeruleus (objective 90, imm., ocular Homal 3).

The color of the fluorescence, and, consequently, the composition of catecholamines, differed in the structures listed above. The locus coeruleus and substantia nigra gave a distinctive bright green fluorescence, indicating a high concentration of noradrenalin and dopamine in these structures. Yellow terminals, evidence of the presence of serotonin in the endings, were observed in the region of the locus coeruleus only around cells of the mesencephalic nucleus of the trigeminal nerve, imbedded in the locus coeruleus. Predominantly serotonin terminals were characteristic of the region of the raphe and substantia grisea centralis. In the ventrolateral horns of the substantia grisea centralis, the ganglion interpedunculare, and adjacent areas lateral to it the content of serotonin and of catecholamines was evidently equal.

Fluorescence of the locus coeruleus (and, to a lesser degree, of the substantia nigra) is associated with the cytoplasm (Fig. 2c–f). Against the background of the bright green fluorescence it was difficult to detect the greenish-yellow terminals. This feature distinguishes these regions from the others in which fluorescence was found mainly as the higher concentration and more intensive fluorescence of the terminals (Fig. 2e, cell on left, and Fig. 3c–f); cells with a fluorescent cytoplasm were very rare.

Apparently in the locus coeruleus and substantia nigra catecholamines are synthesized not only in the terminals, but also in the some of the cells. So far as the substantia nigra is concerned, this has been demonstrated electron-microscopically also [3].

During stress, no new fluorescent structure appeared in the region investigated. After the first motor excitation the intensity of fluorescence of all the structures fell appreciably, and after stronger stress it usually was slightly more marked than normally. This applies to all structures, but most of all to the locus coeruleus, the substantia grisea centralis (Fig. 3a) and its horns. However, the great lability of the catecholamine levels in these structures and the precise dependence of the intensity of fluorescence on the experimental conditions must be pointed out.

A high concentration of catecholamines and monoamine oxidase in the locus coeruleus has been observed by all workers who have investigatigated the region of the isthmus. The monoamine oxidase content in the locus coeruleus is very high even in the embryonic period [12, 13]. The suggestion has been made [1] that the locus coeruleus liberates adrenalin during the stress reaction to secure additional activation of the whole sympathico-adrenal system.

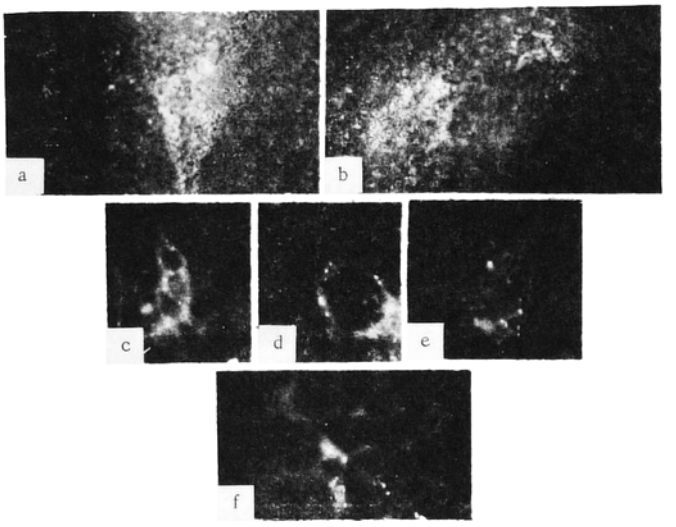

Fig. 3. Catecholamines in oral division of brain stem: a) region of raphe, experiment (objective 10, ocular Homal 3); b) substantia nigra, normal (magnification as in a); c) serotonin endings on cell of oral reticular nucleus of the pons, experiment (objective 90 imm., ocular Homal 3); d, e) serotonin endings on cells of mesencephalic nucleus of trigeminal nerve, experiment and normal respectively (magnification as in c); f) region of raphe, noradrenalin–dopamine terminals, normal (magnification as in c).

Some cells of the mesencephalic nucleus, especially those in the region of the isthmus, possess the highest sensitivity (compared with all other structures of the brain stem) to changes in the composition of the atmosphere.

This involvement of the locus coeruleus in asphyxia of short duration suggests that this structure plays a leading role in the organization of stress states connected with disturbance of respiration. Information regarding changes in the composition of the atmosphere may reach cells of the locus coeruleus through the mesencephalic root. The presence of sensory endings capable of detecting changes in the constance of the air has been demonstrated in the respiratory part of the nasal mucous membrane [2]. The possibility is not ruled out that these sensory bodies are formed by peripheral branches of cells in the mesencephalic nucleus. It can also be postulated that cells of the mesencephalic nucleus react to changes in the gas composition of the blood directly because of their close contact with the abundant capillary network [8].

The role of the locus coeruleus in the organization of the protective response in stress may be twofold. Stimulation of the locus coeruleus leads to the liberation of catecholamines into the blood stream, from which they act on several brain structures, notably the hypothalamus. In addition, there is reason to suppose that extensive caudal and oral nervous projections of this structure exist. These connections may bring about activation of the general motor and respiratory responses.

These experimental results suggest the existence of a corticopetal system of catecholamine fibers running from the brain stem to the neocortex. Evidence in favor of this has recently been obtained by Swedish workers using a histochemical method to detect amines [9].

The localization of the fluorescent regions indicates that start of this projection ascends in the bundle of Schutz (which is concerned in the maintenance of homeostasis [11]) to the basal regions of the forebrain.

The localization of fluorescence in the region of the ganglion interpedunculare, the regions lateral to it, and portions of the reticular formation lying ventrolaterally to the substantia grisea centralis indicates that in stress ascending projections also reach the hippocampus through the tract of Meinert and the flocculus and also, evidently, through ascending projections of the mesencephalic reticular formation.

All these ascending systems contribute to the organization of the means of escape from the terminal state, with the participation of the neo-, archi-, and paleocortex.

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