HISTOCHEMICAL DEMONSTRATION OF MONOAMINE NEURON SYSTEMS IN THE HUMAN FETAL BRAIN

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CONSIDERING the great interest in the functional significance of the monoamine neurons in the CNS of man it is remarkable how so little is known about the cellular localisation of biogenic amines in the human brain. The major reason for this lack of knowledge is that this material offers special technical problems. Although the brain levels of catecholamines and serotonin decline rather slowly during the first hours after death (BERTLER and ROSENGREN, 1959; JOYCE, 1962; McGEER and McGEER, 1962) their optimal fluorescence histochemical visualisation in central nervous tissues is possible only when the tissue is processed within the first 30–60 min after death (BJÖRKLUND, FALCK and ROSENGREN, 1967; DE LA TORRE, 1972, BJÖRKLUND and NOBIN, unpublished observations). We have in our studies on human brain circumvented this obstacle by investigating fresh brains from 3–4 month-old human fetuses (NOBIN and BJÖRKLUND, 1973) and this paper is a brief account of these studies.

In chemical investigations on human fetal brain significant but rather low concentrations of dopamine, noradrenaline and serotonin have been reported (BERTLER, 1961; HYYPPÄ, 1972; NOBIN and BJÖRKLUND, 1973). Nevertheless abundant systems of fluorescent catecholamine-containing and indolamine-containing neurons have been demonstrated (NOBIN and BJÖRKLUND, 1973; OLSON, BOREUS and SEIGER, 1973). Thus, green-fluorescent (CA-containing) and yellow-fluorescent (IA-containing) cell bodies occurred in abundant systems in medulla oblongata, pons and mesencephalon and some green-fluorescent cell bodies occurred also in the hypothalamus (NOBIN and BJÖRKLUND, 1973). The cell body fluorescence was either rather diffuse, covering also the nucleus, or more distinct and confined to the cytoplasm. The fluorescent cell bodies could be referred to seven larger cell formations four CA-containing and three IA-containing: (1) A ventral and ventro-lateral system of CA-containing cells in the medulla oblongata; (2) CA-containing cells in the dorsal and dorsomedial regions of the medulla oblongata, partly within the area postrema; (3). A system of pontine CA-containing cells in the dorsal and lateral regions of the pons, mainly within the locus coeruleus and the subcoeruleus area (see Fig. 1); (4) An extensive system of CA cells in the ventral and medial regions of the mesencephalon (extending caudally into the rostral pons) mainly within the substantia nigra and the ventromedial tegmentum; (5 and 6). Two formations of IA-containing cells in the midline raphe regions, one in the rostral part of the medulla oblongata, and another more extensive system, extending in the raphe region of the pons and the caudal mesencephalon; (7) A less abundant group of IA-containing cells in the lateral reticular formation, in the caudal mesencephalon and the rostral pons. In our material the CA-containing cells were confined to the infundibular nucleus of the hypothalamus, whereas OLSON, BOREUS and SEIGER (1973) were able to demonstrate

FIG. 1.—Frontal section through the subcoeruleus area from a human fetus (CRL about 15 cm) showing green fluorescent cell bodies. Fluorescent processes and non-fluorescent dark nuclei are clearly seen. Fluorescent varicose fibres are distributed between the cell bodies (×210).

the existence of more extensive diencephalic CA neuron systems in the human fetal brain corresponding to groups A11, A12 and A13 in the rat brain (cf. FUXE *et al.*, 1969; BJÖRKLUND and NOBIN, 1973).

The monoamine axon bundles had a notably high fluorescence in the fetal brain (NOBIN and BJÖRKLUND, 1973; OLSON *et al.*, 1973) and could thus be traced for long distances through the lower brain stem and the hypothalamus up to the basal ganglia and the septal region. One major system of CA fibres could be followed from the region of the lower medulla oblongata through pons and mesencephalon; at the level of the red nucleus, it joined the medial forebrain bundle (MFB). Within the MFB, one system of CA fibres could be further traced up to the septal region. Along the course of this long, probably ascending fibre system it received fibres from the CA cell systems in medulla, pons and mesencephalon.

A second large system of ascending CA fibres was observed to originate in the fluorescent cell bodies in the substantia nigra and via the tegmental fields of Forel, the lateral hypothalamus, and the zona incerta, it ran into the internal capsule, towards the basal ganglia. This ascending pathway is probably identical with the so-called nigrostriatal dopaminergic pathway. Although there is much indirect evidence for such a system also in man, e.g., from observations in parkinsonian patients (see HORNYKIEWICZ, 1966), its actual demonstration has been lacking. The course of the nigrostriatal fibres revealed in the human fetal brain has clear similarities to that described by CARPENTER and PETER (1972) in the monkey, and by MOORE *et al.*,

(1971) in the cat. Fluorescent varicose, probably terminal, axon parts were, in the fetal material studied, detected to a more limited extent and only in certain regions. The most abundant CA terminal systems had developed in the basal ganglia (Fig. 2) and the olfactory and septal regions. In the basal ganglia not only the caudate nucleus and putamen (Fig. 2) but also the developing globus pallidus exhibited a dense CA innervation. This conforms well to the biochemical findings of moderate to high concentrations of dopamine in these regions in adults (BERTLER, 1961; BERTLER and ROSENGREN, 1959, EHRINGER and HORNYKIEWICZ, 1960; FAHN *et al.*, 1971; HORNYKIEWICZ, 1966; SANO *et al.*, 1959) and in a 8-months-old human fetus published by BERTLER (1961). As the globus pallidus appears to be devoid of CA-containing terminals in the rat (FUXE, 1965, FUXE *et al.*, 1969) it has been questioned whether the dopamine present in this structure in man was confined to non-terminal fibres passing through the nucleus (HORNYKIEWICZ, 1966). The present findings provide evidence for a direct CA (probably mainly dopamine-containing) fibre supply to the globus pallidus, suggesting that in man the mesencephalic dopamine containing neurons might project also to globus pallidus. Nigropallidal fibre connexions have been presumed to exist in the human brain (HORNYKIEWICZ, 1966) but neither in cat (MOORE *et al.*, 1971) nor in monkey (CARPENTER and PETER, 1972) a substantial termination of nigral fibres has been possible to demonstrate in the globus pallidus with silver staining of degenerating terminals. Thus, until direct evidence for a nigro-pallidal projection has been obtained in man, the possibility has to be considered that the CA-fibres in the globus pallidus originate in other areas, e.g. in mesencephalic CA cell bodies outside the substantia nigra.

Green-fluorescent varicose, probably terminal, fibres occurred also in several hypothalamic and hypophyseal areas whereas in the thalamus, CA fluorescence was observed only in some scattered varicose fibres in ventricle-near regions. A variable density of fluorescent varicose fibres was detected in the subthalamic nucleus. The pineal was supplied with CA fibres organised into thick bundles at the surface or within interlobular septa. OLSON *et al.* (1973) reported in fetuses with a CRL about 15 cm scattered parenchymal cells exhibiting a yellow fluorescence.

Significant CA fibre supplies were found in the posterior hypothalamic area, the dorsomedial nucleus, the perifornical area, the mamillary body, the tuberal nucleus, the periventricular nucleus, the infundibular nucleus, the paraventricular nucleus, the suprachiasmatic nucleus, and in the median eminence-pituitary region. Some scattered fibres were also observed in the dorsal, lateral and preoptic hypothalmic areas, and in the ventromedial nucleus, where they appeared as parallely arranged fibres traversing the nucleus in ventral and ventrolateral directions. In the rostral infundibulum two systems of fibres extended between the infundibulum and the region of the MFB in a manner suggestive of a commisural arrangement.

In the median eminence-pituitary region varicose CA fibres were demonstrated both in the internal and external layers of the developing median eminence, in the stalk, and in the neural lobe. In the external layer of the median eminence, some of the fibres were oriented perpendicular to the surface (for details see NOBIN and BJÖRKLUND, 1973).

The demonstration of CA-containing cell bodies and axons in the tuberohypophyseal region of the human brain (NOBIN and BJÖRKLUND, 1973) is of particular interest in view of the important role these neurons play in the release of

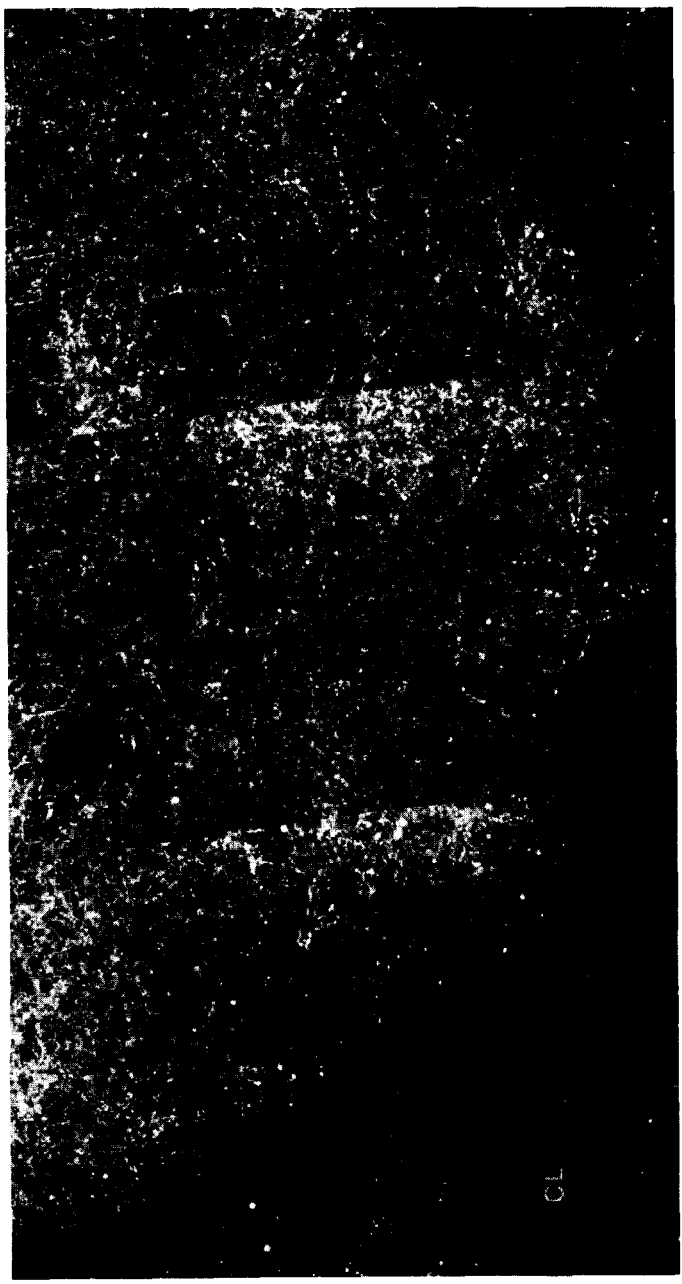

FIG. 2.—Frontal section at the level of caudal neostriatum of a human fetal brain (CRL about 12 cm). Photomontage of the ventral part of the putamen showing the very dense CA-innervation of this nucleus. Some CA fibres are also present in the claustrum (CL) (×130).

hypophysiotrophic hormones from the median eminence in the rat (for reviews see HÖKFELT and FUXE, 1972; MCCANN *et al.*, 1972). The morphological basis for such a function thus seems to exist also in man.

It can be concluded that although there are many differences with respect to details the principles of organisation of monoamine neuron systems are notably similar in rat and man.

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