EXISTENCE OF DOPAMINERGIC NERVE TERMINALS IN THE RAT CORTEX

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CATECHOLAMINERGIC nerve terminals in the cerebral cortex of mammals have been assumed to be mainly represented by arborisations of the dorsal noradrenergic (NE) pathway (FUXE, 1965; OLSON and FUXE, 1972; UNGERSTEDT, 1971). However, the relative amounts of dopamine (DA) and norepinephrine (NE) found in the cortex of different strains of rats (VALZELLI and GARATTINI, 1968; THIERRY *et al.*,1973) or in various cortical area of the cat (MCGEER *et al.*,1963) are much higher than those estimated in the cerebellum (KOSLOW *et al.*, 1972). This latter structure is, as the cortex, innervated by NE neurons originating from the locus coeruleus. The high DA levels found in the cerebral cortex cannot be only explained by the role of precursor played by DA in NE terminals. We thus postulated the occurrence of DA neurons or nerve endings in the cerebral cortex. To test this hypothesis, the effect of various lesions of ascending catecholaminergic pathways on the endogeneous cortical DA and NE levels were estimated in rats of the Charles River strain.

Two types of lesions were made. Electrolytic bilateral lesions were performed with high frequency current (100 kHz, 2 mA, 10 sec) in the locus coeruleus (group A_6) or in the area ventralis tegmenti (ATV; group A_{10}). Such lesions destroy respectively the cell bodies of the dorsal NE pathway and of the DA mesolimbic system. Chemical lesions of the ventral and dorsal ascending NE pathways were performed by local microinjections of 6-OH-DA (2 μg in 1 μl injected in 5 min) made laterally to the pedunculus cerebellaris superior (L-PCS) or in the medial forebrain bundle (MFB) in the lateral hypothalamus. In all cases catecholamine levels were estimated three to five weeks later with the help of biochemical techniques (THIERRY *et al.*, 1971).

Cortical NE levels were markedly reduced (75–98 per cent) after lesions of the locus coeruleus (5 weeks), the L-PCS (4 weeks) and the MFB (3,5 weeks) (Fig. 1). These effects were expected since the dorsal NE pathway originates or passes through all the lesion sites selected. The selective degeneration of the ventral NE pathway induced by electrolytic lesion of the ATV did not affect NE content in the cortex. On the other hand, surprisingly, DA cortical levels were not significantly reduced either after lesion of the locus coeruleus or after degeneration of the ascending NE pathway induced by an injection of 6-OH-DA into the L-PCS (Fig. 1). Cortical DA levels were also slightly but not significantly reduced after chemical lesion of the MFB although this lesion affected not only NE systems but also the nigrostriatal pathway. Indeed DA levels in the neostriatum were markedly reduced ($12{\cdot}8 \pm 1{\cdot}9$ μg/g in sham operated; $1{\cdot}3 \pm 0{\cdot}04$ μg/g in lesioned rats). Finally as revealed by the electrolytical lesion of the ATV the DA mesolimbic system does not innervate the cerebral cortex since DA cortical levels were not affected.

In vivo and *in vitro* formation of DA from tyrosine should persist in the cortex, after degeneration of the dorsal NE pathway if DA is synthesised in sites independent

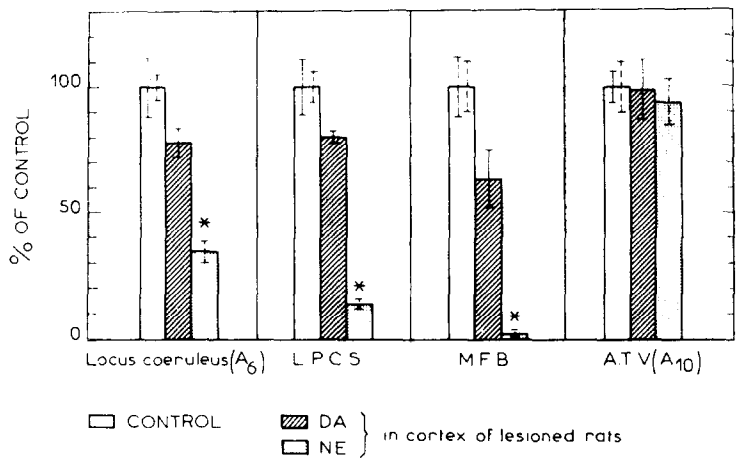

FIG. 1.—Effect of bilateral lesions of the NE ascending pathways on DA and NE content of the rat cerebral cortex. Lesions were made as described in the text. Degeneration of the dorsal NE bundle was induced by electrolytic lesion of the locus coeruleus; degeneration of both ventral and dorsal NE bundle was induced by microinjection of 6-OH-DA either laterally to the PCS (L-PCS) or into the MFB; degeneration of the ventral NE bundle was induced by electrolytic lesion of the ATV. In all cases controls were sham operated animals. Control values for each group of operated rats were respectively for DA: 0·200 ± 0·027; 0·133 ± 0·015; 0·145 ± 0·099; 0·164 ± 0·010 μg/g and for NE: 0·244 ± 0·15; 0·154 ± 0·008; 0·204 ± 0·018 and 0·157 ± 0·017 μg/g. Results are the means of groups of eight rats and are expressed as percentage of respective control values ± SEM; $\times P < 0{\cdot}001$ when compared with control values.

of NE terminals. Therefore, sham operated rats and animals injected with 6-OH-DA (2 μg in 1 μl) into the L-PCS received 4 weeks later an intracisternal injection of L-3·5-^{3}H-tyrosine (42 Ci/mM, 80 μCi) and were sacrificed 20 min after the ^{3}H-amino-acid administration. ^{3}H-NE formation was markedly reduced (57 per cent) in 6-OH-DA lesioned rats (control: 4·05 ± 0·25 nCi/g; lesioned: 1·77 ± 0·38 nCi/g) whereas ^{3}H-DA levels were slightly but not significantly affected (control: 15·9 ± 1·5 nCi/g; lesioned 13·0 ± 1·4 nCi/g).

In a second group of experiments, purified synaptosomes were prepared from cortex of sham operated and 6-OH-DA pretreated rats according to the technique of GRAY and WHITTAKER (1963). As previously 6-OH-DA was injected bilaterally in the L-PCS 4 weeks before the experiment. Synaptosomes were incubated for 15 min with L-3·5-^{3}H-tyrosine (40 Ci/mM; 53 μCi) and total ^{3}H-DA and ^{3}H-NE content of the tissues and incubating medium were analysed. As observed *in vivo* the synthesis of ^{3}H-DA (24·8 ± 0·8 nCi) from ^{3}H-tyrosine exceeded that of ^{3}H-NE (14·7 ± 0·5 nCi) in sham operated animals. ^{3}H-NE formation was completely abolished in cortical synaptosomes prepared from lesioned rats since it represented only 11 per cent (1·58 ± 0·01 nCi) of control values (14·7 ± 0·5 nCi). DA synthesis was only slightly affected and ^{3}H-DA formation (16·8 ± 0·7 nCi) from ^{3}H-tyrosine in lesioned animals still represented 67 per cent of the control levels (24·8 ± 0·8 nCi). In complementary experiments the persistence of ^{3}H-DA formation could also be demonstrated in cortical synaptosomes 14 days after bilateral injection of 6-OH-DA

into the L-PCS. Finally, the ^{3}H-catecholamines from ^{3}H-tyrosine in cortical synaptosomes was also examined 4 weeks after bilateral lesions of the locus coeruleus. ^{3}H-NE synthesis in cortical synaptosomes of lesioned animals (1·64 ± 0·19 nCi) was markedly reduced (74 per cent) when compared to sham operated rats (6·25 ± 0·13 nCi), but ^{3}H-DA synthesis was not modified (control: 12·3 ± 0·2 nCi; lesioned: 11·4 ± 0·3 nCi).

In conclusion degeneration of the dorsal NE pathway was associated with a disappearance of cortical NE synthesis both *in vivo* and *in vitro*. The selective persistence, in lesioned animals, of cortical DA content and of ^{3}H-DA synthesis from ^{3}H-tyrosine *in vivo* as well as *in vitro* in synaptosomal preparations, demonstrate the existence of dopaminergic nerve endings independent of noradrenergic terminals in the cerebral cortex. Innervation of the cortex by collateral sprouts of dopaminergic neurons from neighbouring structures can be excluded since ^{3}H-DA synthesis occurred in cortical synaptosomes at short (14 days) or at long (4 weeks) time after degeneration of the dorsal NE pathway. Moreover the endogeneous levels of DA and the synthesis of the transmitter are comparable in sham operated and lesioned animals. The DA mesolimbic system does not appear to be involved in the DA cortical innervation. The localisation of the cell bodies of the dopaminergic neurons which innervate the cerebral cortex must still be determined. The occurrence of DA interneurons in the cortex can be postulated since some cortical cell bodies have been shown to take up exogenous catecholamines (ARBUTHNOTT, 1969; DESCARRIES and LAPEIRRE, 1973).

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