Interpretation of regional changes in brain dopamine after stimulation

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With the development of sensitive assays for dopamine and noradrenaline there has been an increase in experimental investigation of the regional response of brain catecholamines to stimulation. An example is a report by St Laurent, Roizen & others (1975) on the effect of self-stimulation on catecholamine content in several discrete nuclei of rat brain. In this experiment the authors placed a stimulating electrode in the ventral tegmental area (VTA). After one week of self-stimulation rats were killed and dopamine and noradrenaline were assayed. Along with changes in concentrations of the two amines, there were alterations in their *ratios* within each area. The ratio changes appeared to differ according to the relative amounts of the two amines. Thus, in areas such as nucleus accumbens, where the ratio of dopamine to noradrenaline exceeds 3·0, the ratio decreased with stimulation. In noradrenergic areas like the median forebrain bundle and nucleus paraventricularis where the ratio is less than 0·5, there was an increase, sometimes as much as 2-fold.

In a similar experiment by Miliaressis, Thoa & others (1975), rats with stimulating electrodes in VTA were pretreated with α-methyltyrosine and self-stimulation produced essentially no change in the ratios. This suggests that the change in ratio is related to events occurring during normal catecholamine synthesis and is not due to different rates of release of dopamine and noradrenaline.

The changes in noradrenergic brain regions in the experiment of St Laurent & others (1975) resemble those which have been reported in the peripheral nervous system. Stimulation of rat adrenal medulla by systemic 6-hydroxydopamine causes a rapid rise in the ratio of dopamine to the β-hydroxylated catecholamine (normally about 0·01) which lasts several days (Snider, 1974). This increase is apparently a consequence of the precursor/product relation of peripheral dopamine and noradrenaline which approximates first-order reaction kinetics, i.e. the concentration of precursor dopamine varies with the synthesis rate of the product, noradrenaline (Levitt, Spector & others, 1965).

To test the possibility that a similar relationship between dopamine and noradrenaline might exist in the central nervous system, we decided to investigate the cerebellar cortical dopamine and noradrenaline ratio after locus coeruleus (LC) stimulation. LC is known to project ipsilaterally to the cerebellar cortex and its neurotransmitter which is thought to be noradrenaline (Chu & Bloom, 1974) can be released by stimulation (Olson & Fuxe, 1971).

Five cats were anaesthetized with sodium methohexitone and a 25-gauge bipolar stainless steel insulated stimulating electrode tapered at the tip was placed in the left LC according to the coordinates of Snider & Niemer (1961) and Chu & Bloom (1974). Electrode placement was confirmed in each case by histologic examination. The LC was stimulated at 5 Hz (5 ms pulses, 6 V) 4 min out of 5 for 45 min. After stimulation, the cerebellum was immediately removed and dissected on ice into right and left halves. Standard slices of anterior cerebellar cortex weighing about 2 g were taken from each half and homogenized in iced 0·4 N perchloric acid to extract the two amines. They were separated on a strong cation exchange column (Fahn, Snider & others, 1975) and fluorimetric assays were performed on the eluates Laverty & Taylor, 1968; Atack, 1973).

Mean unstimulated side values for dopamine and noradrenaline (Table 1) were similar to those reported for normal cat cerebellum by Bertler & Rosengren (1959) (20 and 130 ng g^{-1}, respectively). Considerable variation in amine concentrations occurred within groups, perhaps because of minor differences in electrode placement, depth of anaesthesia or proportion of white matter. However, the ratio was relatively constant and on the stimulated side was consistently higher than on the unstimulated side (Table 1). The comparatively small (20%) difference in noradrenaline between sides may reflect replacement by new synthesis although some contralateral effect of the stimulation, e.g. via partial contralateral innervation, cannot be ruled out.

These data indicate that a stimulation-induced increase in noradrenaline turnover in LC terminals is accompanied by an increase in the ratio of dopamine to noradrenaline. Although the increase could conceivably be the result of activation of dopaminergic fibres, there is no evidence that dopamine is released as a

Table 1. *Effect of unilateral stimulation of locus coeruleus on dopamine and noradrenaline concentrations in cat cerebellar cortex.* Locus coeruleus was stimulated 4 min out of 5 for 45 min. The cerebellum was immediately removed and standard slices of anterior cortex from each half of the cerebellum were taken for analysis. Shown are the means ± s.e.

	Unstimulated side (n)	Stimulated side (n)
Dopamine, ng g^{-1}	31 ± 14 (5)	69 ± 21 (5)
Noradrenaline, ng g^{-1}	107 ± 34 (5)	84 ± 31 (5)
DA/NA ratio	0·29 ± 0·05 (5)	0·82 ± 0·09 (5)*

* = $P < 0.05$ *vs* unstimulated side (Mann-Whitney U-test). Other differences not significant (Student's *t*-test.

primary neurotransmitter in the cerebellar cortex (Chu & Bloom, 1974). A more likely explanation is that it serves mainly as a precursor to noradrenaline and its elevation is caused by increased noradrenergic activity and noradrenaline synthesis. An analagous relationship might exist between dopa concentration and dopamine synthesis in brain (Lindqvist, Kehr & Carlsson, 1975) and adrenal (Snider, 1974) although unlike dopamine, dopa does not appear to have both precursor and neurotransmitter functions in mammals.

The possibility that measured changes in dopamine in predominantly noradrenergic brain regions reflect precursor dopamine turnover and not neurotransmitter dopamine should be kept in mind when its concentrations are related to experimental physiologic or pharmacologic stimulation.

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