Changes in Monoamine-Related Enzymes in Cobalt-Induced Epilepsy

PAUL R. CLAYTON and PIERS C. EMSON

MRC Brain Metabolism Unit, University Department of Pharmacology, 1 George Square, Edinburgh EH8 9JZ, U.K.

The evidence that points to the involvements of aminergic neurons in epilepsy can be divided into two sets. First, the work that indicates that drugs acting on amine metabolism can modify seizures, including data on the use of reserpine to lower brain amine concentrations in animals (Schlessinger et al., 1968) and man (Pallister, 1959), and p-chlorophenylalanine to decrease brain 5-hydroxytryptamine in rats (De La Torre & Mullan, 1970). These studies all demonstrated that a general decrease of amines tended to increase susceptibility to epileptic seizures. This has been the subject of a review by Carlsson (1974).

Conversely, drugs that potentiate the activities of brain amines, including monoamine oxidase inhibitors (Prockop et al., 1959) or tricyclic anti-depressants (Lehmann, 1967), have at least moderate protective effects. Recently this series of data has been refined into an analysis of the roles of the different amines; dopamine (3,4-dihydroxyphenethylamine) and noradrenaline are now thought by some workers to exert opposing influences on an epileptic focus, inasmuch as dopamine and its agonists can be shown to decrease ‘spiking’ in the cobalt model, whereas noradrenaline agonists have the reverse effect (Ashcroft et al., 1974).

The second set of information consists of work that indicates that an alteration in the concentrations of brain amines, presumably parameters of a functional change in specific cortical circuits, occurs in the development of audiogenic seizures (Schlessinger et al., 1967) and in cobalt epilepsy (Emson & Joseph, 1975). However, other workers have found no changes in brain amines related to epilepsy (Hansen et al., 1973), so the case cannot be said to be by any means complete.

In an attempt to clarify the problem of amine involvement in epilepsy, a study was undertaken of the following amine-related enzymes in the cobalt model (Dow et al., 1972): tyrosine hydroxylase, catecholamine O-methyltransferase and monoamine oxidase. γ-Aminobutyrate aminotransferase was also investigated. The purpose was to use these enzymes as cell markers to study the integrity of aminergic circuitry in the cortex around a cobalt focus, and to ascertain whether these specific neurons could be demonstrated to react differentially.

Tyrosine hydroxylase was assayed by the method of Hendry & Iversen (1971), it being assumed that this would be the most specific marker for noradrenaline neurons, and the catechol O-methyltransferase (Axelrod & Tomchick, 1958) and monoamine oxidase (McCaman et al., 1965) assays were mainly corroborative. γ-Aminobutyrate aminotransferase was examined (Hall & Kravitz, 1967) in the hope that it would provide data on the integrity of local inhibitory gabaminergic fibres and their postulated role in the eventual suppression of a focus, and also as an index of the glial reaction that is associated with the cobalt implant.

Histological studies of the cobalt model done in our laboratory (Emson & Joseph, 1975) by using the Fink–Heimer (Fink & Heimer, 1967) technique have demonstrated degenerating terminals, axons and cells around the primary lesions after 4 days, extending to the 29th day after implant, at which time spikes are still present in the electrocorticogram. Other enzymes so far studied, including glutamate decarboxylase, choline acetyltransferase and aromatic amino acid decarboxylase, tend to follow a similar pattern, reaching their lowest activities in the primary (1") focus at around day 8 and re-
covering at 24 days. It is probable that these changes, which are reflected in the secondary (2") focus to a lesser extent, represent a specific neuronal involvement; lactate dehydrogenase, a cytoplasmic enzyme, is only decreased in the lesion area, and is not a part of the pattern of 2" focus development at all. The rate of spiking is inversely correlated with these findings, reaching a peak at days 8–12 and decreasing towards day 24, and probably reflects the enzyme picture (Emson & Joseph, 1975).

Of the enzymes studied, only γ-aminobutyrate aminotransferase returned to normal activity at around day 30 (Fig. 1a); the peak activity of γ-aminobutyrate aminotransferase occurring in the 1" focus, and to a lesser extent the 2" focus, at around day 9, probably indicates the intense glial reaction that is developing at this stage.

The pattern of changing γ-aminobutyrate aminotransferase activity agrees well with those studies of enzymes already carried out, and with the general time-course of cellular degeneration (Emson & Joseph, 1975). However, tyrosine hydroxylase activity (Fig. 1b) is still low at 30 days, at only 30% of control values. Monoamine oxidase and catecholamine O-methyltransferase activities are also low, at 20% and 40% respectively of control value. All these enzymes appear to have returned to normal by days 75 and 100, and the fact that they all share a similar time-course, and at 30 days are proportionately very similarly affected (20%, 30% and 40%), could indicate that noradrenaline neurons do not regrow into the area of an epileptic focus at the same rate as, for example, cholinergic neurons or cell regeneration in general (Emson & Joseph, 1975).

These results could be due to either an increased susceptibility to Co2+ or a genuinely slower rate of repair; the fact that this pattern is also found in the 2° focus, where Co2+ is probably not involved, tends to support the latter.

The rate of spiking in a 1° or 2° cobalt focus, followed over a period of some months, tends to form a distinct pattern. Firing rates reach a peak at 8–12 days and then decrease over the next 20 days, until at day 30 they are at 10–15% of the peak values. After day 30, spiking continues to decrease, though less rapidly, over a period of several months. It could be that this apparently biphasic response represents an initial intracortical phenomenon during the first month, involving cholinergic and gabaminergic fibres in the suppression or resolution of the focus, followed by a slower regrowth of aminergic fibres.

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Fig. 1. γ-Aminobutyrate transaminase (a) and tyrosine hydroxylase (b) activities in the frontal cortex of cobalt-treated rats

▼, Primary focus; ●, secondary focus.
fibres from outside the cortex. The time-course for central-nervous-system regrowth has been found by many workers to be of the order of 50–100 days, for example, the re-innervation of the tongue after hypoglossal axotomy (Sumner & Sutherland, 1973). This correlates reasonably well with the rates of return to normal of the aminegic enzymes.

To extend these results, data on cortical and striatal concentrations of noradrenaline, dopamine and 5-hydroxytryptamine are being collected.

P. R. C. is a Medical Research Council research student, and P. C. E. is a Beit Memorial Research Fellow.


Bottomline: The Effect of Neurotransmitter Release upon Phospholipid Composition and Fatty Acid Turnover in Synaptic Vesicles of Torpedo marmorata Electric Organ and Guinea-Pig Cerebral Cortex

R. ROY BAKER, MICHAEL J. DOWDALL and VICTOR P. WHITTAKER

Abteilung für Neurochemie, Max-Planck-Institut für Biophysikalische Chemie, D-3400 Göttingen-Nikolausberg, Am Fassberg, Postfach 968, West Germany

It has been reported that lysophosphatidylcholine can promote the fusion of erythrocytes and fibroblasts in vitro (Poole et al., 1970) and, further, that high concentrations of this lysophosphoglyceride exist in chromaffin granules isolated from adrenal medulla (Blaschko et al., 1967). Thus it has been proposed that this lysolipid may be involved in exocytosis, the mechanism whereby compounds stored in intracellular vesicles or granules are released to the extracellular medium (Poole et al., 1970; Winkler et al., 1974). Some support has been given to this proposal by the demonstration of phospholipase A activities in synaptic-vesicle fractions isolated from brain (Heilbronn, 1972; Gullis & Rowe, 1973). Yet a number of other types of vesicle have only low concentrations of lysophosphatidylcholine [as cited by Winkler et al. (1974)], and an enhanced turnover of [32P]orthophosphate and [1-14C]glycerol has not been demonstrated in lysophosphatidylcholine of adrenal-medulla slices stimulated by acetylcholine (Trifaró, 1969).

The present communication is based on a more detailed investigation of the possible involvement of lysophosphatidylcholine or other lysophosphoglycerides in synaptic vesicles during the release of neurotransmitters. For this purpose we have analysed synaptic vesicles from Torpedo marmorata electric organ and guinea-pig cerebral cortex.