BRAIN INDOLE METABOLISM ASSESSED USING IN VIVO DIALYSIS

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INTRODUCTION

The relationships between tryptophan (Trp) and its major CNS metabolites serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) may be studied by analysis of post-mortem tissue or by analyzing concentrations in the extracellular fluid. Major advances in our understanding of the physiological importance of Trp supply to the brain and subsequent 5-HT synthesis and metabolism was derived, in part, from regional brain tissue analysis following e.g. Trp loading, stress, fasting (Fernstrom and Wurtman, 1971; Knott and Curzon, 1974; Curzon and Marsden, 1975). However, tissue analysis does not differentiate between functionally active substrate concentrations available to receptors in the extracellular compartment and intracellular concentrations. Furthermore, as only a single value is obtained from each animal, large numbers of animals are required to obtain temporal profiles of drug action. The primary advantage of in vivo monitoring of extracellular substrate concentrations is that repeated measurements may be made in the same animal over time. When applied to the conscious, freely moving animal, this allows associations between neurochemical changes and their roles in behaviors to be determined.

A variety of in vivo methods had been described (Sarna et al., 1983; Westerink et al., 1987; Knott, 1988). These include microdialysis, sampling of cerebrospinal fluid (CSF), push-pull, cortical-cup, ventricular-cisternal perfusion and voltammetry. The present chapter will focus specifically on our studies with cerebral microdialysis in investigations of the roles of 5-HT following physiological, pharmacological and neurological interventions.

PRINCIPLES OF MICRODIALYSIS

The use of cerebral dialysis was first described by Bito et al. (1966). A small dialysis bag containing artificial CSF was implanted into the brain and the contents allowed to equilibrate with the extracellular fluid compartment (Fig. 1A). After 10 weeks the bag was removed and the dialysate analysed for different amino acids. Delgado et al. (1972) and Ungerstedt and Pycock (1974) subsequently reported a perfusion-dialysis system whereby a microdialysis bag is continuously perfused with artificial CSF, the dialysate collected over timed periods (e.g. 20 min) and analysed (Fig. 1B). As well as sampled extracellular substrates, drugs may be delivered to the surrounding fluid. The recent widespread use of microdialysis may be attributed to
the major improvements in analytical methods, especially high-performance chromatography (HPLC) with electrochemical and fluorometric detection (Hutson et al., 1985; Church and Justice, 1986). It is now possible to separate all biogenic amines and their metabolites within a single chromatographic run in less than 15 min from injected sample volumes of only a few microlitres (Fig. 2).

Dialysis probe

There now many different designs of dialysis probes (Knott, 1988) and we currently use the concentric probe shown in Fig. 3. It is similar in construction to a push-pull cannula but the presence of the dialysis membrane reduces potential tissue damage due to flowing perfusion fluid being in direct contact with brain tissue.

Factors influencing recovery

A number of factors influence the passage of substrates across the dialysis membrane including membrane composition, chemical interactions with membrane, molecular weight, molecular shape, perfusion rate, surface area,