Measurement of transmitter release in vivo

At the 1980 Brighton meeting of the European Neuroscience Association (ENA) the organizers held a series of one-day workshops on special techniques. This proved the success of the 1981 congress in London. Three workshops were organized; one on neuroanatomical techniques, another on neuronal transplantation and experimentation, and a third (the subject of this article) on methods for studying transmitter release in vivo.

In recent years, a mass of information has been generated on anatomical, biochemical and physiological aspects of neurotransmission, but only circumstantial evidence has been produced linking the involvement of specific CNS neurotransmitters with particular physiological or behavioral responses. To establish such a connection one must obtain in vivo measurements of transmitter release, preferably in the freely moving animal.

Available in vivo release techniques

The workshop was divided into two parts and dealt with the push-pull technique in the morning, and in vivo electrophysiological detection after lunch with a 3-hour session in between. With the push-pull technique, perfusates are obtained in which either radioactively labelled or enzymatic transmitter concentrations can be measured. This important technique has mainly been applied to studies on anesthetized animals as described by A. Cheramy (Paris), though A. Phillips (Warburg) showed how the technique could be used to monitor catecholamine release in response to changes in blood pressure in the freely moving rat. Another adaptation to the perfusion approach is the use of cortical strips and E. Morest (Princeton) on the use of these in unanesthetized rats to monitor drug induced changes in acetylcholine and amino acid release. From the posters and general discussion, the aspect of the perfusion technique, which is rapidly improving, is the methods available to measure substances released into the perfusates. M. Weller-Bernhard (Zurich) described the use of gas chromatography and mass fragmentography to measure a wide range of amino acids and amines transmitters, while K. Le Quan-Bui (Paris) and E. Zentelstein (Stockholm) had both used HPLC with electrochemical detectors to measure endogenous amines released into perfusates which, in the case of the Stockholm group, had been collected by a novel brain dialysis technique.

In vivo electrophysiology

The afternoon session moved into the more controversial and unexplored field of in vivo electrophysical techniques. This approach to the measurement of transmitter release was developed by R. Adams (Cambridge) and, if sufficiently reliable, could be used to continuously monitor fluctuations in release (or metabolism) of electroactive transmitters.

Several studies have shown that various plant or carbon fibre electrodes can measure changes in oxidation current in specific brain regions in vivo in either anaesthetized or awake animals. The central question discussed at the workshop was the chemical identity of this oxidation current and, in particular, the separation of catechol and ascorbate oxidation. Notably among the studies was the development of the carbon fibre electrode (8 & mm diam) which after, and only after, special electrochemical pretreatment is able to distinguish between ascorbate and catechol oxidation using differential pulse voltammetry to measure oxidation (F. Gomar; Lyons). The pretreatment shifts the oxidation of ascorbate to a lower potential then that of either dopamine or DOPAC. Electrodes with rather similar characteristics have been developed by M. Wightman (Baltimore). The original in vivo electrochemical studies used fixed potential methods to monitor changes in current (chronopotentiometry) and demonstrated that aspartate incremented current while dopamine, in contrast, decreased it. It is now clear that this effect was not only related to release of dopamine as the new carbon fibre electrodes have demonstrated that ascorbate oxidation is also increased by amphetamine and kainic acid. The debate about which catechol is oxidized at the surface of these electrodes when they are in the striatum—dopamine or ascorbate—remains a lively topic and suggest that the technique is more suited to measurements rather than release.

Similar conclusions have been reached by R. Corelli (Lyons) and M. Bracci (Nottingham) regarding indolamine oxidation. Indolamines oxidize at a higher potential than catechols and pharmacological evidence points to 5HTRA rather than 5HT as the main contributor to indolamine oxidation peak, although the electrodes used are more sensitive to the parent amine than its metabolite. Does this indicate that the extraneuronal levels of 5HT
Multiple tubulins in a single neurone

The key to microtubule function?

One of the most prominent elements in the organization of axons and dendrites are the microtubules (or neurotubules). Their participation in axonal transport, neurosecretion, nerve cell growth and differentiation has long been recognized. It is only, however, with recent revelations as to the complexities of the microtubule at the molecular level that we begin to comprehend the many functions of which they are capable. A number of tubulin-binding proteins of neuronal cell types have been identified, and the evidence points conclusively to multiple genes encoding α- and β-tubulin.

How then do the neurones and brain contain similar tubulin subunits in different neuronal subpopulations? Presumably, the replicating cells of the neural tube at an early stage of neuronogenesis are capable of synthesizing the same or similar tubulins in different neuronal subpopulations. The wall of these microtubules may represent the homologous α- and β-tubulin or different ones from the same species, and one can visualize that neurones may contain a mixture of these two isoforms as indicated by the current models for microtubule assembly and disassembly.