

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 successful and the idea was extended at the recent 1981 congress in Liège. Three workshops were organized; one on neuroanatomical techniques, another on neural transplantation and explantation, 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 pharmacological aspects of neurotransmission, but only circumstantial evidence has been produced linking the involvement of specific CNS neurotransmitters with particular physiological or behavioural 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* electrochemical detection after lunch with a 3 h poster session in between. With the push-pull technique, perfusates are obtained in which either radioactively labelled or endogenous transmitter concentrations can be measured. This important technique has mainly been applied to studies on anaesthetized animals as described by **A. Cheramy** (Paris) though **A. Phillipu** (Wurzburg) 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 perfusate approach is the use of cortical cups and **F. Moroni** (Florence) discussed the use of these in unanaesthetized rats to monitor drug induced changes in acetylcholine and amino acid release. From the posters and general discussion, the aspect of the perfusate technique, which is rapidly improving, is the methods available to measure substances released into the perfusates. **M. Wolfensberger** (Zurich) described the use of gas chromatography and mass fragmentography to measure a wide range of amino acid and amine transmitters, while **K. Le Quan-Biu** (Paris) and **T. Zetterström** (Stockholm) had both used HPLC with electrochemical detection 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 electrochemistry

The afternoon session moved into the more controversial and unexplored field of *in vivo* electrochemical techniques. This approach to the measurement of transmitter release was developed by **R. Adams** (Kan-

sas) and, if sufficiently reliable, could be used to continuously monitor fluctuations in release (or metabolism) of electroactive transmitters.

Several studies have shown that various graphite 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. Notable among the studies was the development of the carbon fibre electrode (8 μm tip diameter) 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. Gonon**, Lyon). The pretreatment shifts the oxidation of ascorbate to a lower potential than that of either dopamine or DOPAC. Electrodes with rather similar characteristics have been developed by **M. Wightman** (Indiana). The original *in vivo* electrochemical studies used fixed potential methods to monitor changes in current (chronoamperometry) and demonstrated that amphetamine increased this current when the electrodes were in the striatum. 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. Furthermore, there is also debate about which catechol is oxidized at the surface of these electrodes when they are in the striatum - dopamine or its metabolite DOPAC - as both are oxidized at the same potential. Extensive pharmacological studies by the group in Lyon indicate DOPAC as the most likely candidate and suggest that the technique monitors metabolism rather than release.

Similar conclusions have been reached by **R. Cespuglio** (Lyon) and **M. Brazell** (Nottingham) regarding indoleamine oxidation. Indoleamines oxidize at a higher potential than catechols and pharmacological evidence points to 5HIAA rather than 5HT as the main component of the indoleamine oxidation peak, although the electrodes used are more sensitive to the parent amine than its metabolite. Does this indicate that the extraneuronal levels of

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either dopamine or 5HT are too low to be detected by existing electrodes under normal conditions? There is obviously need for further studies in this area.

It is clear that the major improvements to the technique will come from developing electrodes with increased selectivity and sensitivity. While there are obviously technical and interpretative problems with *in vivo* electrochemistry, they are worth overcoming as it can be readily used in the freely moving animal and not only in the rat as illustrated by **J. Justice** (Atlanta) who showed that signal amplitude from electrodes in the neostriatum of the rhesus monkey was increased during emotional excitation and feeding. Other groups described changes during sleep (**R. Cespuglio**, Lyon), stress (**G. Kennett**, London), and circadian rhythms (**P. Hutson**, London). Further applications are combined electrophysiological and electrochemical recordings as discussed by **R. Adams** (Kansas) or the use of a single *in vivo* carbon-fibre electrode for combined

unit recordings and quantitative iontophoresis. As was pointed out, electrochemistry is not just about amines; many drugs and neuropeptides are electroactive, so providing the right electrodes can be found the future looks interesting and certainly not dull. At present, *in vivo* electrochemistry can produce important information about amine neurone function *in vivo* and along the way should improve our understanding of the role of ascorbic acid in the CNS.

The workshop showed the perfusate techniques to be reliable and well established, but somewhat limited due to the size of the cannulae while electrochemistry is new, promising but still enigmatic. Hopefully, one outcome of the workshop will be more work including a detailed side by side comparison in the same animal of the well established with the enigmatic.

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