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Review

In vivo neurochemical monitoring and the study of behaviour

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Abstract

In vivo neurochemical monitoring techniques measure changes in the extracellular compartment of selected brain regions. These changes reflect the release of chemical messengers and intermediates of brain energy metabolism resulting from the activity of neuronal assemblies. The two principal techniques used in neurochemical monitoring are microdialysis and voltammetry.

The presence of glutamate in the extracellular compartment and its pharmacological characteristics suggest that it is released from astrocytes and acts as neuromodulator rather than a neurotransmitter. The changes in extracellular noradrenaline and dopamine reflect their role in the control of behaviour. Changes in glucose and oxygen, the latter a measure of local cerebral blood flow, reflect synaptic processing in the underlying neuronal networks rather than a measure of efferent output from the brain region.

In vivo neurochemical monitoring provides information about the intermediate processing that intervenes between the application of the stimulus and the resulting behaviour but does not reflect the final efferent output that leads to behaviour.

Keywords: Microdialysis; Glutamate; Noradrenaline; Dopamine; Glucose; Oxygen; Brain metabolism

Contents

Animal behaviour includes complex motor responses to external and internal stimuli. The aim of the experimental study of behaviour is to establish the neuronal activity that underlies the behaviour. Approaches to this problem include electrophysiological recording of neuronal discharge during the behaviour and the effect on the behaviour of lesions of specific brain regions. Electrophysiological recording of single unit activity, that has been very successful in the study of sensory processing, is difficult to use in freely moving animals. The effect of brain lesions identifies the regions necessary for a certain behavioural function, but studies the dysfunctional brain, rather than determining the role of brain regions under physiological conditions. What is needed is a technique that can monitor changes in brain function as the behaviour is taking place.

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1. Synaptic transmitters

The activity of the brain consists of the transmission of nerve impulses, initiated by the activation of receptors, through neuronal networks that ultimately results in the activation of muscles. The transmission of action potentials through the neuronal networks is mediated by the interaction of excitatory and inhibitory chemical transmitters. The main excitatory transmitter is glutamate and the main inhibitory transmitter is γ-amino-butyric acid (GABA); they give rise to excitatory and inhibitory synaptic potentials. The release of neurotransmitters is triggered by the entry of Ca^{2+} on arrival of the action potential at the presynaptic nerve terminal. Transmitter release is abolished by drugs that block the Na^{+} channel responsible for the propagation of the action potential as well as drugs that block the Ca^{2+} channel. The effect of such drugs is the test for neuronal release of a transmitter.

2. In vivo monitoring techniques

Early attempts at measuring transmitter release consisted of various techniques for monitoring chemical changes in the extracellular compartment of the brain. This involved a number of sampling and assay techniques used in freely moving behaving animals. The earliest was the push pull technique developed by Gaddum (Gaddum, 1961) and Myers (Myers, 1972), which was followed by the microdialysis technique of Ungerstedt (Myers et al., 1998). With microdialysis, a concentric probe, originally modelled on a capillary, is implanted into a selected brain area. The microdialysis probe consists of a semipermeable membrane that surrounds two fine cannulae, through which fluid flows into and out of the portion containing the semipermeable membrane. The perfusion rate varies from 0.5 to 3 μl/min and collection time ranges from 1 to 20 min. Compounds reach the perfusion fluid by diffusion; the size of the solutes penetrating the probe is limited by the properties of the dialysis membrane. The concentration of the solute in the dialysate is not the same as the extracellular concentration but depends on probe recovery. Recovery in vivo is a measure of the rate at which a substance is delivered to the perfusate. This in turn depends on diffusion barriers within the brain, properties of the dialysis membrane and the rate of perfusion. The dialysis probe, in addition to collecting samples of extracellular fluid, can also be used for the local application of drugs by adding them to the perfusion fluid.

Dialysate samples are applied to a chromatography column that separates the various compounds according to their size, charge and lipophilicity. They are assayed electrochemically at a downstream electrode where the sequentially eluted compounds are oxidised and give rise to a series of currents. The identity of the compounds is established by injecting standards that serve both to identify the various compounds by their elution time and to calibrate the current, so that it can be translated into a concentration.

An alternative to microdialysis is in vivo voltammetry. In this technique, rather than measuring monoamines ex vivo in a sample of extracellular fluid, the electro active compound is measured at the surface of an implanted carbon electrode. Voltammetry involves the oxidation or reduction of analyte molecules at the surface of an electrode, as the result of an applied potential. Each compound has a characteristic oxidation potential; the oxidation results in a current, whose magnitude is a measure of the concentration of the compound. One of the problems of in vivo voltammetry arises from the fact that the differences between oxidation potentials are so small, that specificity is a serious challenge. A number of techniques have been developed to deal with this problem. One such technique is fast cyclic voltammetry (FCV). FCV provides a good temporal and chemical resolution. A 75–250 μm length of a 3 μm diameter carbon fibre, sealed in a glass capillary, is implanted in a brain area. The potential of the electrode is linearly ramped to a value sufficient to oxidise the analyte, and then returned to its original value in 10 ms. The potential change gives rise to a large background current, on which are superimposed the characteristic oxidation and reduction currents, which can be isolated by subtraction of the background current. In voltammetry using enzyme-based sensors, the specificity of the enzyme establishes the identity of the compound and the product of the enzyme reaction is oxidised at the surface of the implanted electrode.

3. Glutamate release

Glutamate is the main excitatory transmitter and there are now a number of techniques for monitoring extracellular glutamate. These include new sampling techniques combined with capillary electrophoresis and fluorescence assay (Kennedy et al., 2002) as well as a number of enzyme-based biosensors (Ryan et al., 1997; Kulagina et al., 1999; Burmeister et al., 2002; Pomerleau et al., 2003). The use of these techniques has revealed basal extracellular concentrations of glutamate that vary between 3 μM (Miele et al., 1996b) and 2 μM (Baker et al., 2002). In order to determine whether this basal glutamate was of neuronal origin, TTX was added to the perfusion medium. The effect was variable; many reports found that neither the addition of TTX, nor lowering of extracellular Ca^{2+} had any effect on basal glutamate (Biggs et al., 1995; Miele et al., 1996a; Timmerman and Westerink, 1997). One study suggested that the non-synaptic basal extracellular glutamate resulted from the activity of the cystine-glutamate antiporter. This conclusion was based on the reduction of glutamate by inhibitors of the antiporter (Baker et al., 2002).

Although K^{+}-induced and drug-induced increases in glutamate have been reported, there are only two studies of