REVIEW ARTICLE

Monitoring *in vivo* of transmitter metabolism by electrochemical methods

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Introduction

Conventional methods of studying the metabolism of central neurotransmitter amines (i.e. dopamine, noradrenaline and 5-hydroxytryptamine) in rats and other small laboratory animals have two major disadvantages. Firstly, animals under investigation must be killed before determinations can be made and secondly, intra- and extra-neuronal metabolism are not differentiated. Thus, determinations can only be made at one time per animal and information is not obtained on whether transmitters or metabolites measured reflect functional activity, i.e. transmitter molecules which had been available to receptors. Also, standard methods of measuring transmitter turnover cannot be applied to individual rats but require the use of groups of animals on which changes of amine, amine precursor or metabolite concentration are measured over a period after the administration of drugs which block transmitter synthesis or catabolism or egress of amine metabolites from the central nervous system. The various problems involved in these methods have been reviewed elsewhere (Pycock & Taberner, 1981).

Some of the above disadvantages can be avoided by local perfusion of brain tissue with artificial cerebrospinal fluid or physiological saline using the push–pull cannula (Glowinski, 1981), so that serial measurements of extraneuronal neurotransmitters (and their metabolites) can be made in the same animals. Thus, indices of transmitter release may be monitored following the administration of drugs (Nieoullon *et al.*, 1979) or sensory stimuli (Nieoullon *et al.*, 1977). However, this technique also has disadvantages. Thus, until recently (Loullis *et al.*, 1980; Nielsen & Moore, 1982), push–pull studies invariably involved anaesthetized animals so that parallel behavioural and biochemical measurements were impossible. Also the method is technically difficult; there are problems in balancing the push and pull perfusion pressures, otherwise excessive tissue damage can occur (Redgrave, 1977). Furthermore, metabolite concentrations in the perfusate are usually very low and thus difficult to assay. Therefore the technique has usually been applied to studies of the incorporation of labelled precursors

into transmitters and metabolites (Glowinski, 1981). Assay problems have been recently overcome by the use of high pressure liquid chromatography with electrochemical detection (Loullis *et al.*, 1980). However, another problem remains which is intrinsic to push–pull methods, that is they may disturb relationships between transmitter release and re-uptake. A technically more simple approach involves determination of endogenous metabolites (using high pressure liquid chromatography with electrochemical detection) in cerebrospinal fluid. This allows serial samples to be taken from conscious freely moving rats. Initial findings show comparable effects of partial inhibition of 5-hydroxytryptamine synthesis on its turnover, whether measured by brain or by cerebrospinal fluid determinations (Sarna *et al.*, 1983). The method has numerous potential applications but does not give data on specific brain regions.

Disadvantages of the above methods can be circumvented by voltammetry *in vivo*, which can monitor extraneuronal changes of electrooxidizable substances, including transmitter amines and their metabolites, at electrodes in brain regions of conscious freely moving animals. These changes may then be compared with concurrent behaviour. Electroanalytical techniques have been used *in vivo* for many years, e.g. to monitor oxygen tension (Bicher & Knisely, 1970) and cysteine concentration (Koryta *et al.*, 1971; Pradac *et al.*, 1971). When Kissinger *et al.* (1973) first used voltammetry to monitor electrochemical changes in the brains of anaesthetized rats the neurochemical complexity of the brain suggested that interpretation of results would be formidably difficult. However, McCreery *et al.* (1974a) were able to use voltammetry to study the oxidation in the brain of the neurotoxin 6-hydroxydopamine when injected close to the tip of the recording electrode. McCreery *et al.* (1974b) then showed that other oxidizable substances normally present in the brain, e.g. dopamine, noradrenaline and ascorbic acid, could also be monitored when similarly injected. The first use of voltammetry to detect central endogenously released oxidizable substances was by Wightman *et al.* (1976) who used anaesthetized rats and rabbits.

Fig. 1. Placement of Ag/AgCl reference electrode, stainless steel auxiliary electrode and carbon paste recording electrode implanted in the caudate nucleus
Electrical connection from these electrodes was made via gold pins in the pedestal to the electronic equipment.

Stimulating the substantia nigra (which contains the main group of cell bodies of dopaminergic neurones) to release dopamine from nerve terminals led to an increase in the cerebrospinal fluid of the dopamine metabolite homovanillic acid. Following this there have been numerous studies which have examined the effect of various stimuli on the release of electrooxidizable substances in the central nervous system of anaesthetized and conscious animals.

This review describes recent developments and problems associated with this potentially important method for monitoring transmitter amines and/or their metabolites *in vivo*.

Types of voltammetry

Voltammetry is the measurement of current-voltage relationships and involves applying a potential to a recording electrode at which susceptible compounds undergo electrochemical reaction so that currents are generated by which the compounds can be measured. The current depends on the rate of chemical reaction taking place at the electrode surface and is proportional to concentration of electroactive substances being oxidized (McCreery *et al.*, 1974b).

Voltammetry *in vivo* includes various electrochemical techniques which differ mainly in how the potential is applied to the recording electrode. They all make use of a three-electrode system: the recording electrode, a micro reference electrode (usually Ag/AgCl) and a silver or platinum auxiliary

electrode. The latter two electrodes are usually placed in contact with the surface of the brain, the exact position not being critical (Kissinger *et al.*, 1973). The auxiliary electrode controls the applied potential by providing sufficient current to maintain the recording electrode at a given potential relative to the reference electrode. The recording electrode is placed at the brain locus at which electrochemical changes are to be monitored, and is shown diagrammatically in Fig. 1. Its composition and construction crucially influence response and will be discussed in more detail later. Electrical connection between the electrodes and the electronic recording equipment is made via a suitable commutator (e.g. Andrews & Hutson, 1982) which allows the rat to move freely while maintaining electrical contact.

Chronoamperometry

This has been used for voltammetric recording *in vivo* by several workers (Conti *et al.*, 1978; Huff *et al.*, 1979; Lindsay *et al.*, 1980). It involves the repeated but brief application of a constant potential to the recording electrode and the measurement of currents generated as a function of time. Immediately following the application of the potential a charging current is manifest due to the electrode behaving as a capacitor. This current rapidly decays revealing the second component, the current due to the oxidation of electroactive species, which becomes apparent after about 1 s. The method has certain advantages for studies *in vivo* as (a) measurements can be repeated at frequent intervals,

Fig. 2. Series of linear sweep voltammograms recorded at 10 min intervals from a carbon paste electrode implanted in the caudate nucleus of a conscious freely moving rat

The voltage sweep was from 0 to +0.5 V at 20 mV/s. Two oxidation peaks are observed, following the initial current surge at approx. +0.12 and +0.26 V.

i.e. 120–180 s, and (b) electrode poisoning (due to the buildup of oxidation products) is minimized. An obvious disadvantage is that no information is provided on the nature of the oxidized material other than that it may be oxidized between zero volts and the potential applied. The following methods, which involve voltage scanning, can however provide some information of this type.

Linear sweep voltammetry

This has been used for voltammetry studies *in vivo* by, e.g., Curzon & Hutson (1981) and Kennett & Joseph (1982). A linearly increasing voltage ramp is applied between preset limits (e.g. 0–0.5 V) to the recording electrode. Rates between 10 and 40 mV/s are used, as faster rates produce very poorly defined voltammetric peaks. The resultant current is plotted as a function of the applied potential. Fig. 2 shows a series of typical linear sweep voltammograms recorded from a conscious freely moving rat using a carbon paste electrode implanted in the striatum. As the applied voltage increases the current rises to a maximum and then decays, producing a peak-shaped curve. The ascending side of the peak results from the rate of oxidative reaction increasing as voltage increases towards the oxidation potential of the material which is oxidized. The descending side reflects its depletion by oxidation exceeding its rate of accumulation. Under standard conditions the potentials at which the peaks occur are constant for specific oxidizable substances and their heights are proportional to concentration of the oxidizable substance. Thus, linear sweep voltammetry can

provide both qualitative and quantitative information on oxidizable substances present in the brain. However, a major disadvantage is that as the potential is applied for much longer periods (typically 25–30 s) than are used for chronoamperometry, it can deplete oxidizable substances for a considerable time. Thus, measurements have to be taken less frequently (e.g. every 10 min).

Cyclic voltammetry

Cyclic voltammetry has been used by Lane *et al.* (1978) for measurements of endogenous oxidizable substances *in vivo* and by Kruk *et al.* (1980) for monitoring of iontophoretically ejected oxidizable substances (catecholamines and 5-hydroxytryptamine). In principle it is similar to linear sweep voltammetry except that instead of a single voltage sweep the potential ramp is rapidly (100 V/s) and repeatedly applied first in a positive direction and then at the same rate in a negative direction so that both oxidation and reduction currents are generated. There are two advantages of this method. Firstly, there is relatively little poisoning at the electrode surface. Secondly, oxidized substances will tend to be reduced on reversing the direction of applied voltage so that a more constant milieu is maintained. A disadvantage of this method is that rapid sweep rates produce poor resolution between adjacent peaks.

Differential pulse voltammetry

This was originally applied to studies *in vivo* by Lane *et al.* (1976, 1978) and later by Gonon *et al.*

(1980) and Cespuglio *et al.* (1981*a,b*). Voltage pulses of very short duration (typically 10–50 ms) and fixed amplitude are superimposed at regular intervals on a linearly increasing voltage ramp. Current is measured before and after each pulse and the difference is recorded as a function of the applied voltage. The main advantage over other scanning methods is that the charging current is minimized so that electrode sensitivity is increased.

Semidifferential analysis

Semidifferential analysis is an inexpensive means of improving the poor separation between substances with rather similar oxidation potentials which is often obtained by linear sweep or cyclic voltammetry (Dalrymple-Alford *et al.*, 1977). The oxidation current is passed through a semidifferentiation circuit before recording it as a function of the applied voltage. Lane *et al.* (1979) using such a system in combination with cyclic voltammetry showed that peaks obtained from electrodes in the caudate nucleus were more clearly separated after semidifferentiation. Furthermore, the baseline was more stable, peaks more symmetrical and ratio of peak current to background current was increased.

Polarographic equipment

Voltammetric recordings *in vivo* can be readily made using commercial polarographs, e.g. those manufactured by Bioanalytical Systems (U.S.A.), Princeton Applied Research (U.S.A.) or Tacussel (France). We have used the Princeton Applied Research model 174A, which can apply different voltage wave forms for voltammetric scanning and detects the currents generated with a sensitivity of 2 nA/V. Choices of operating mode include linear sweep, differential pulse and normal pulse polarography. When a series of recordings are to be made, automatic operation can be performed using a microprocessor to switch on the recording electrode and chart recorder and then apply the potential sweep. The electrode and recorder are then switched off and the timing cycle started for the next recording. As an alternative to chart recording, data may be converted to digital form and stored on magnetic tape or floppy disc.

Recording electrodes for voltammetry

As already mentioned, the nature of the recording electrodes used in voltammetry is of crucial importance. Carbon electrodes of various types are commonly used as they are less susceptible to surface poisoning by the absorption of oxidation products than are platinum or gold electrodes. The use of carbon paste electrodes for studies *in vitro* was initially described by Adams (1969). The paste consists of graphite powder mixed with a water-

immiscible liquid, e.g. paraffin oil (Nujol). The oil reduces the porosity of the graphite so that the background or residual current is decreased. This is important for recordings *in vivo*, as with electrodes of convenient size oxidation currents are quite small (1–200 nA). Glassy or vitreous carbon (as used in electrochemical detectors for high pressure liquid chromatography) also gives low background current, but is less easy to form into the microelectrodes required for studies *in vivo*.

Carbon paste microelectrodes were the first type of recording electrode to be used for voltammetric studies *in vivo* (Kissinger *et al.*, 1973) and have since been extensively used for chronoamperometry (Conti *et al.*, 1978) and linear sweep (Curzon & Hutson, 1981), cyclic and differential pulse (Lane *et al.*, 1978) voltammetry. These electrodes are made by coating ultrafine graphite powder (1 μ m particle size) with Nujol and may be mixed with an epoxy resin. The resultant paste is then forced into glass capillaries (diameter approx. 100 μ m) and electrical contact made by inserting a silver wire into the paste which is then allowed to solidify. However, many such electrodes are defective due to the epoxy resin/carbon paste cracking inside the capillary so that electrical continuity is broken. This problem is overcome by using electrodes made of Teflon-coated wire. The Teflon sheath is pulled approx. 1 mm over the end of the wire forming a well which is then packed with carbon paste (Lane *et al.*, 1978; Curzon & Hutson, 1981).

Carbon fibres (approx. 8 μ m diameter) have also been used as recording electrodes for studies *in vivo*. These are threaded through borosilicate glass tubing (2 mm diameter) which is then drawn out in a microelectrode puller to give a micropipette with a length of fibre protruding from the tip. Electrical connection is made between the fibre and a silver wire by inserting the latter into an epoxy resin/graphite powder mixture previously pushed into the micropipette. The electrodes are left to solidify for 24–48 h at room temperature (Ponchon *et al.*, 1979; Wightman, 1981). Alternatively, electrical connection can be made by filling the micropipette with 0.9% NaCl solution into which is placed a silver wire (Armstrong-James *et al.*, 1980) or by using electrically conducting silver paint, providing the electrode tip is sealed afterwards (P. H. Hutson & G. Curzon, unpublished work). Wightman (1981) has used electrodes with the fibre cut flush with the tip of the micropipette, which give rather low oxidation currents of approx. 100 pA. They therefore require more sensitive recording equipment and may be more susceptible to noise but because of the small currents produced they are less destructive to the species being oxidized. Ponchon *et al.* (1979) used electrodes with 0.5 mm of exposed fibre giving low residual currents of approx. 1 nA.

Lane & Hubbard (1976) used platinum electrodes previously exposed to an aqueous solution of KI. This increases electrode sensitivity towards catecholamines, decreases background current and was claimed to result in a well defined oxidation peak for dopamine. Unfortunately, during use *in vivo* peak currents and resolution between adjacent peaks rapidly decline, probably due to the formation of an insulating film (Lane *et al.*, 1976).

Modified electrodes

Both carbon paste (Lane *et al.*, 1978) and fibre (Gonon *et al.*, 1980; Cespuglio *et al.*, 1981a,b) electrodes can differentiate various compounds of neurochemical interest *in vivo*. However, a serious problem is that ascorbic acid interferes with the detection of the monoamines and their metabolites (Kissinger *et al.*, 1973).

The concentration of ascorbic acid in brain is much greater than the concentrations of these substances (Milby *et al.*, 1982) and its oxidation peak potential is very similar to that of the catecholamines and related substances. While ascorbate can be distinguished from dopamine *in vivo* by means of KI-treated platinum electrodes in combination with differential pulse voltammetry, the electrodes quickly lose this ability (Lane *et al.*, 1976). Gonon *et al.* (1981a) report that the sensitivity of carbon fibre electrodes for dihydroxyphenylacetic acid, dopamine or dihydroxyphenylalanine is increased by pretreatment in phosphate-buffered saline (pH 7.4) with successive 20 s trains of alternating potentials (0–3–0 V, 0–2–0 V, 0–1–0 V; 70 Hz). When implanted in dopamine-rich brain regions and used with differential pulse voltammetry these electrodes give a peak which results from the oxidation of the dopamine metabolite dihydroxyphenylacetic acid and this is separated from ascorbic acid only after the above pretreatment. Cespuglio *et al.* (1981a,b) report that similar electrical pretreatment of carbon fibre electrodes also permits the 5-hydroxytryptamine metabolite 5-hydroxyindoleacetic acid to be distinguished from ascorbate and dihydroxyphenylacetic acid. However, with continued use *in vivo* the separation between ascorbate and dihydroxyphenylacetic acid is lost. We also find that electrical treatment of carbon fibres in phosphate-buffered saline (pH 7.4) enables ascorbate to be distinguished from dihydroxyphenylacetic acid and 5-hydroxyindoleacetic acid *in vitro* (Fig. 3) although the effects of pretreatment only lasted for approx. 3–4 h. Plotsky (1982) has used carbon fibre electrodes pretreated by immersing the fibre in concentrated 'dichromic acid' and then passing 250 μ A anodic current through the electrode for 30 s. This was reported to enhance sensitivity towards dopamine and moved the oxidation potential of ascorbate from +0.5 V to –0.05 V, thereby

Fig. 3. Differential pulse voltammogram recorded in 0.1 M-phosphate-buffered saline, pH 7.4, containing ascorbic acid (2×10^{-4} M), dihydroxyphenylacetic acid (2×10^{-3} M) and 5-hydroxyindoleacetic acid (5×10^{-6} M) using a carbon fibre electrode electrically pretreated in phosphate-buffered saline, pH 7.4

Peak 1 is due to oxidation of ascorbic acid and peaks 2 and 3 are due to oxidation of dihydroxyphenylacetic acid and 5-hydroxyindoleacetic acid respectively. The voltage sweep was from –0.2 to +0.4 V at 10 mV/s, modulation amplitude 25 mV.

permitting it to be distinguished from dopamine, the oxidation potential of which remained at +0.17 V. Beyond the suggestion by Plotsky (1982) that electrochemical pretreatment oxidizes the electrode surface and increases its surface area, thereby facilitating electron transfer from solution to the electrode surface, the mechanism by which pretreatment enables electrodes to differentiate between similar substances is unknown. Yamamoto *et al.* (1982) have recently reported that carbon paste electrodes containing a small amount of stearic acid are relatively insensitive to ascorbate and dihydroxyphenylacetic acid, which are electrostatically repelled by the stearic acid. Dopamine is not repelled and oxidizes at +0.2 V.

The above discussion indicates one of the major problems of voltammetry *in vivo*: the characterization of the oxidation peaks and the identification of the substances responsible for them. A large

fraction of the papers which have been published on the technique concern this problem. Relevant data can be obtained *in vitro*, while a useful procedure *in vivo* is to determine the voltammetric effects of substances of interest when injected or infused near the implanted recording electrode. The voltammetric effects of drugs with known actions on transmitter metabolism or disposition can also help to identify the substances response for voltammetric peaks *in vivo*, though it is important to check that the drugs or likely metabolites are not themselves oxidized to give interfering peaks, and it must be remembered that very few drugs have effects specific to one neuronal system.

Characterization *in vitro*

Table 1 summarizes data on oxidation potentials *in vitro* determined by different voltammetric

Table 1. Oxidation potentials *in vitro* determined by different voltammetric techniques and with different types of recording electrodes

The following details are given in order after each reference: type of carbon recording electrode, mode of voltammetry (DPV, differential pulse; LSV, linear sweep; NPP, normal pulse polarography), scan rate, voltage range. Any electrode pretreatments are also indicated. Abbreviation used: PBS, phosphate-buffered saline.

Reference/conditions	Oxidation potential (V)					
	Ascorbic acid	Dopamine	Dihydroxy-phenylacetic acid	Homo-vanillic acid	5-Hydroxy-tryptamine	5-Hydroxy-indoleacetic acid
McCreery <i>et al.</i> (1974b). Paste, LSV, 150 mV/s, 0–+0.6 V	—	+0.2	—	—	—	—
Ponchon <i>et al.</i> (1979). Fibre, NPP, 10 mV/s, –0.1–+1.0 V	—	+0.2*	—	+0.53*	+0.34*	+0.5*
Gonon <i>et al.</i> (1980). Fibre, DPV, 20 mV/s, –0.2–+0.2 V. Pretreatment 0–3 V, 70 Hz, 20 s in PBS, pH 7.4	–0.05†	+0.1†	+0.1†	—	—	—
Gonon <i>et al.</i> (1981a). Fibre, DPV, 10 mV/s, –0.2–+0.3 V. Pretreatment 0–3 V, 50 Hz, 20 s in PBS, pH 7.4	–0.08†	—	+0.07†	—	—	—
Plotsky (1982). Fibre, DPV, 5 mV/s, –0.2–+0.7 V. Pretreatment 250 μ A, 30 s in chromic acid	–0.05†	+0.12†	+0.42	—	—	—
Brazell & Marsden (1982). Paste, DPV, 10 mV/s, –0.1–+0.5 V	+0.3	+0.24–+0.3	+0.24–+0.3	—	+0.34	+0.34
Cespuglio <i>et al.</i> (1981a). Fibre, DPV, 20 mV/s, –0.05–+0.45 V. Pretreatment 0–3 V, 0–2.5 V, 0–1.5 V triangular voltage, 70 Hz, 20 s each in PBS, pH 7.4	–0.05†	—	+0.1†	—	+0.28–+0.3	+0.28–+0.3†
Kennett & Joseph (1982). Paste, LSV, 100 mV/s, 0–+1.0 V	—	—	—	—	+0.35–+0.4	+0.35–+0.4
P. H. Hutson & G. Curzon (unpublished work). Paste, LSV, 20 mV/s, 0–+0.5 V	+0.36	+0.15	+0.46	+0.46	+0.38	+0.42
P. H. Hutson & G. Curzon (unpublished work). Fibre, DPV, 10 mV/s, from –0.2 to +0.2 or +0.4 V. Pretreatment 0–1.5 V, 0–3 V, 0–4 V, 100 Hz, 10 s each (square wave pulses 0.1 ms duration in PBS, pH 7.4)	–0.05†	—	+0.12†	—	—	+0.22†

* Versus standard calomel electrode. All other potentials are versus Ag/AgCl reference electrode.

† Indicates substances that are differentiated from each other following electrode pretreatment.

methods and with different types of recording electrodes.

Sensitivity and linearity

Electrodes must be able to detect the very low (10^{-5} – 10^{-6} M) concentrations of amines or metabolites present in the extracellular space of the brain.

Carbon fibre electrodes are reported to respond linearly *in vitro* to dopamine over the range 2×10^{-6} – 10^{-3} M with a detection limit of 10^{-6} M (Ponchon *et al.*, 1979). After various pretreatments they responded linearly to dopamine (up to 2×10^{-6} M), dihydroxyphenylacetic acid (up to 6×10^{-5} M) and ascorbic acid (up to 10^{-3} M) (Gonon *et al.*, 1980) and to 5-hydroxytryptophan and 5-hydroxytryptamine (10^{-7} – 10^{-6} M) and 5-hydroxyindoleacetic acid (10^{-7} – 2×10^{-5} M) (Cespuglio *et al.*, 1981a). Carbon paste electrodes also produce linear increases of oxidation current *in vitro* to solutions of 4-methylcatechol (electrochemically identical but more stable than dopamine) (10^{-5} – 10^{-3} M) (Lindsay *et al.*, 1980). We find that with carbon paste electrodes dopamine, dihydroxyphenylacetic acid, ascorbic acid, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid give linear responses over the range 10^{-6} – 10^{-3} M.

Selectivity and stability

As many transmitter amines and related substances have rather similar oxidation potentials, electrode selectivity is crucially important. This may be improved by chemical or electrical pretreatment as described previously. It is also important that electrodes regain sensitivity and selectivity for as long as possible after implantation, not least because a period is required for recovery from the effects of operative procedures. With untreated carbon paste electrodes, stable recordings have been made over several hours commencing 24–72 h after implantation (Andrews & Curzon, 1981). Curzon & Hutson (1981) found that such electrodes were viable over 3 days when recording circadian changes of oxidation current. However, pretreated electrodes appear to be less stable. Thus Lane *et al.* (1976), Gonon *et al.* (1981b) and Cespuglio *et al.* (1981b) found that pretreated electrodes only gave stable recordings *in vivo* for a few hours, and therefore it was only possible to use them with anaesthetized rats. Buda *et al.* (1981), however, was able to use these electrodes in conscious rats that had been implanted under light anaesthesia and had recovered within minutes after surgery.

Characterization in vivo

Central dopamine and 5-hydroxytryptamine terminals are shown schematically in Fig. 4, which gives the pathways of metabolism of the above

transmitters and mechanisms of action of the drugs referred to below.

Catecholamines

Most of these studies have been made with electrodes implanted in the striatum. This region contains little noradrenaline but a particularly high concentration of dopamine, being innervated by terminals of dopamine neurones with their cell bodies in the substantia nigra.

McCreery *et al.* studied the fates of intracerebrally injected 6-hydroxydopamine (1974a), ascorbate, dopamine and noradrenaline (1974b). Subsequently, Lane *et al.* (1976), using a surface-modified platinum electrode and differential pulse voltammetry, recorded two peaks *in vivo* from the striatum at -0.05 and $+0.2$ V and showed that injecting dopamine or noradrenaline solution in the vicinity of the electrode selectively increased the peak at $+0.2$ V and that injection of ascorbic acid selectively increased the peak at -0.05 mV. Using carbon paste electrodes, both dopamine and ascorbate increased the oxidation peak at $+0.14$ V, whilst 5-hydroxyindoleacetic acid and homovanillic acid increased the peaks at $+0.35$ and $+0.48$ V respectively (Lane *et al.*, 1978).

Unilateral lesions of dopamine neurones by injecting 6-hydroxydopamine into the substantia nigra caused a peak at electrodes in the ipsilateral but not the contralateral striatum to disappear (Gonon *et al.*, 1980; Buda *et al.*, 1981). The electrodes distinguished between ascorbate and the dopamine metabolite dihydroxyphenylacetic acid. The tyrosine hydroxylase inhibitor α -methyl-*p*-tyrosine, which decreases brain catecholamine synthesis, also decreased this peak (Gonon *et al.*, 1980). Conversely, it increased following electrical stimulation of the dopamine cell bodies in the substantia nigra, which causes increased release and metabolism of striatal dopamine. These experiments all suggest that oxidation current recorded from the striatum reflects oxidation of catecholamines. However, destroying the dopamine neurones with 6-hydroxydopamine did not diminish the striatal oxidation peak measured by Lane *et al.* (1978) or by Dayton *et al.* (1981). However, these workers used electrodes that may well have not been able to distinguish between ascorbate and dihydroxyphenylacetic acid. Also, a serious controversy has emerged concerning the nature of the major contributor to the above striatal oxidation peak. Originally, it was assumed to result from the oxidation of dopamine, but *in vitro* dopamine, dihydroxyphenylalanine and dihydroxyphenylacetic acid all oxidize at similar potentials to that of the striatal peak *in vivo* (Gonon *et al.*, 1980). Dihydroxyphenylalanine is unlikely to be a major contributor *in vivo*, as not only is its concentration

Fig. 4. *Neurochemistry and pharmacology of dopamine and 5-hydroxytryptamine*

Dopamine metabolism: tyrosine is converted by tyrosine hydroxylase (TH) to dihydroxyphenylalanine which is decarboxylated by L-aromatic amino acid decarboxylase (AAAD) to give dopamine. This is released and acts on post-synaptic receptors (T). Dopamine is removed from the synaptic cleft either through metabolism by catechol *O*-methyltransferase (COMT) to 3-methoxytyramine or by neurone reuptake. Dopamine and 3-methoxytyramine are metabolized by monoamine oxidase (MAO) to dihydroxyphenylacetic acid and homovanillic acid respectively. 5-Hydroxytryptamine metabolism: tryptophan is converted by tryptophan hydroxylase (TPH) to 5-hydroxytryptophan which is decarboxylated by aromatic amino acid decarboxylase to 5-hydroxytryptamine. This is released and acts on post-synaptic receptors (Y). 5-Hydroxytryptamine is removed from the synaptic cleft by neuronal reuptake and metabolized by monoamine oxidase to 5-hydroxyindoleacetic acid. Pharmacology: drugs acting on the metabolism or disposition of the above substances are indicated in square brackets. Mechanisms involving inhibition or blockade are shown by broken lines, mechanisms involving enhancement of transmitter release are shown by solid lines.

in the brain very low but also the peak was decreased after its decarboxylation to dopamine was prevented by the dopa decarboxylase inhibitor NSD 1015 (Gonon *et al.*, 1980). However, dihydroxyphenylacetic acid, the product of action of monoamine oxidase on dopamine, could not be excluded since inhibiting the enzyme with pargyline, which would increase dopamine but decrease dihydroxyphenylacetic acid, also decreased the peak *in vivo*. Dopamine agonists, i.e. haloperidol (Lane *et al.*, 1978; Gonon *et al.*, 1980), pimozide and spiroperidol (Lane *et al.*, 1978), increased the striatal oxidation peak but these compounds increase not only dopamine release (Bartholini *et al.*, 1976) but also its turnover, and hence the concentration of dihydroxyphenylacetic acid (Roth *et al.*, 1976). Other investigations of the striatal oxidation peak have focused on the effects on it of systemic injection of amphetamine. As amphetamine increases dopamine release (e.g. Lloyd & Bartholini, 1975; Perlow *et al.*, 1980) but decreases striatal dihydroxyphenylacetic acid concentration (Roth *et al.*, 1976; Fadda *et al.*, 1977), and as neither

amphetamine nor its metabolites are electroactive (Lane *et al.*, 1976), it was assumed that the increase of striatal oxidation current after systemic amphetamine injection reported by many workers was due to an increase of extracellular dopamine (Lane *et al.*, 1976, 1978; Conti *et al.*, 1978; Huff *et al.*, 1979; Lindsay *et al.*, 1980; Dayton *et al.*, 1981). However, Gonon *et al.* (1980, 1981b), using electrodes which differentiated ascorbate from catecholamines, found that amphetamine decreased the striatal catecholamine oxidation peak and therefore deduced that it largely reflected extracellular dihydroxyphenylacetic acid. Gonon *et al.* (1980) suggest that the presumed dopamine oxidation peak obtained with less selective electrodes was due largely to extracellular ascorbic acid. Ascorbic acid is present in brain at 1–2 mM (Subramanian, 1977) and recently Milby *et al.* (1982) have shown it to occur at particularly high concentrations in nerve terminal regions such as the nucleus accumbens, hippocampus, amygdala and neocortex. It is released with dopamine from synaptosomes *in vitro* following depolarization with K^+ , and from the

cortex on electrical stimulation of the medial lemniscus (Milby *et al.*, 1981). The finding by Dayton *et al.* (1981) (using less selective electrodes) that amphetamine increased oxidation currents at striatal electrodes comparably in control rats and in animals with 6-hydroxydopamine lesion also indicates that much of the net voltammetric response to amphetamine is independent of dopamine. On the other hand, Lane *et al.* (1976), using KI-treated platinum electrodes which also were claimed to differentiate ascorbic acid from catechols, found that amphetamine did not increase the ascorbic acid peak. Also Conti *et al.* (1978) found that amphetamine did not increase the efflux of ascorbic acid from striatal slices *in vitro*, and Milby *et al.* (1981) found that amphetamine did not induce efflux of ascorbate from a cortex synaptosome preparation.

5-Hydroxyindoles

Voltammetric studies *in vivo* on the detection of 5-hydroxytryptamine and its metabolite 5-hydroxyindoleacetic acid have been less extensive than those on the catecholamines. Initial evidence suggesting that 5-hydroxytryptamine release could be monitored voltammetrically was reported by Conti *et al.* (1978). Using an electrode in the globus pallidus and chronoamperometry they showed that oxidation current at +0.8 V was transiently increased by the 5-hydroxytryptamine-releaser *p*-chloroamphetamine. This was confirmed by Marsden *et al.* (1979), who, using chronoamperometry and cyclic voltammetry demonstrated the appearance of a previously undetectable striatal oxidation peak between +0.4 and 0.5 V on treatment with *p*-chloroamphetamine. This was prevented by the 5-hydroxytryptamine synthesis inhibitor *p*-chlorophenylalanine or by fluoxetine, which prevents uptake of *p*-chloroamphetamine into 5-hydroxytryptamine neurones. Conversely, electrical stimulation of the medial raphe nuclei (which contain 5-hydroxytryptamine cell bodies projecting rostrally to the forebrain), so that 5-hydroxytryptamine was presumably released from terminals, markedly increased oxidation current in the hippocampus and this response no longer occurred in rats pretreated with *p*-chlorophenylalanine. Cespuglio *et al.* (1981a) showed a 5-hydroxyindole peak at +0.3 V at striatal electrodes using differential pulse voltammetry. This peak was selectively decreased after treatment with *p*-chlorophenylalanine or by lesioning of ascending 5-hydroxytryptamine neuronal tracts from the raphe nuclei with the neurotoxin 5,7-dihydroxytryptamine. Similarly, Rivot *et al.* (1982), using pretreated carbon fibre electrodes and differential pulse voltammetry, reported a 5-hydroxyindole peak in the dorsal horn of the spinal cord which was attenuated by *p*-chlorophenylalanine.

Cespuglio *et al.* (1981b) found that the decarboxylase inhibitors NSD1015 and R044602, which inhibit synthesis of 5-hydroxytryptamine (and catecholamines) and also inhibit 5-hydroxytryptamine degradation to 5-hydroxyindoleacetic acid, decreased the peak at +0.3 V as well as brain 5-hydroxyindoleacetic acid concentration but had little effect on brain 5-hydroxytryptamine. Also the monoamine oxidase inhibitor clorgyline decreased both this peak and brain 5-hydroxyindoleacetic acid by about 50%, but comparably increased brain 5-hydroxytryptamine. These results show that the voltammetric peak reflected changes of 5-hydroxyindoleacetic acid, rather than 5-hydroxytryptamine concentration. Similarly, probenecid, which blocks the transport of 5-hydroxyindoleacetic acid from the central nervous system, increased both the peak at +0.3 V and brain 5-hydroxyindoleacetic acid, but not 5-hydroxytryptamine. Although these studies using differential pulse voltammetry and electrochemically treated electrodes did not indicate that ascorbate interfered with the detection of the 5-hydroxyindole peak, this may not be the case in studies using unmodified carbon paste electrodes and chronoamperometry or linear sweep voltammetry.

A further complication when monitoring brain 5-hydroxyindoles is that penetration of the dura or damage to the brain tissue during electrode implantation may cause blood contamination around the electrode tip and this can give a voltammetric peak at the same potential as 5-hydroxyindoleacetic acid. Cespuglio *et al.* (1981a) have overcome this problem by doing the surgery necessary for electrode implantation several days before this is performed.

Application of voltammetry *in vivo*

Voltammetry has not yet been applied extensively *in vivo*, attention having largely been concentrated on the characterization of the oxidation peaks. However, a few applications have been reported which indicate some possible ways in which the technique might be used. For example, Marsden (1980) reported that when α -methyltryptamine was given to rats, behaviours indicating stimulation of 5-hydroxytryptamine receptors (forepaw treading, head weaving, hind limb abduction and Straub tail) occurred and voltammetric current increased. The latter was obtained using chronoamperometry and carbon paste electrodes implanted in the striatum and hippocampus. It rose to a maximum by 30 min after injection of the drug, somewhat preceding the maximum value of the behavioural score. Pretreatment with the 5-hydroxytryptamine synthesis inhibitor *p*-chlorophenylalanine considerably reduced both behavioural score and current. α -Methyltryptamine itself was found to be oxidized

at +0.8 V and was thus clearly distinguished from 5-hydroxytryptamine oxidation at +0.4 V *in vitro*.

Andrews & Curzon (1981) have made a detailed comparison of the behavioural and voltammetric effects of D-amphetamine. At high dosage (25 mg/kg intraperitoneally) the drug causes a complex spectrum of behaviours, each with its own time course. Numerous previous non-voltammetric experiments with drugs and brain lesions (Lees *et al.* 1979; Andrews *et al.*, 1982) indicate different roles of dopamine and 5-hydroxytryptamine release in these various behaviours. Voltammetric study using a linear sweep and graphite/silicone oil electrodes showed that amphetamine treatment caused striatal oxidation peaks at +0.12 and 0.26 V to increase. Other experiments showed that drug treatments thought to release selectively dopamine and 5-hydroxytryptamine increased the heights of the first and second peaks respectively. While this by no means proves that the two peaks obtained on amphetamine injection specifically reflect changes of these transmitters, there were nevertheless striking relationships between the time courses of the voltammetric and behavioural changes. Thus, during the first hour after giving the drug, the peak at +0.26 V rose much more sharply than that of +0.12 V, and 5-hydroxytryptamine-dependent behaviours were particularly marked. Backward walking, which requires release of both transmitters (Curzon *et al.*, 1979), was also present. During the next hour there was little change of the +0.26 V signal or of two of the 5-hydroxytryptamine behaviours (head weaving and forepaw treading), while backward walking showed somewhat equivocal changes and the other two 5-hydroxytryptamine behaviours (hind limb abduction and body shakes) declined. This can be explained in terms of the marked concurrent rise of the signal at +0.12 V (if it reflects dopamine release), as lesion experiments indicate that the latter behaviours require 5-hydroxytryptamine but are inhibited by dopamine (Andrews *et al.*, 1982). Using a lower dose of amphetamine (5 mg/kg), only the signal at +0.12 V increased, 5-hydroxytryptamine behaviours were absent and behaviours specifically associated with dopamine release (i.e. head bobbing, sniffing, rearing) were manifest.

While these experiments show a very suggestive relationship between the behavioural and voltammetric changes, the time courses of the former changes (as in the above work of Marsden using α -methyltryptamine) tended to precede those of the latter. This could possibly reflect a lag between neurochemical changes at synapses mediating behaviour and resultant changes in the more diffuse extraneuronal pools presumably monitored by the relatively large electrodes used in voltammetric techniques (Cheng *et al.*, 1979).

Other pharmacological applications have been reported. Thus Broxterman & Mos (1980) showed that HA-966, which depresses central nervous system activity and more specifically decreases dopamine cell activity, also markedly decreased striatal oxidation current, suggesting a decrease of dopamine release. Similar results were found by Curzon & Hutson (1981), who showed that γ -butyrolactone, a compound with similar actions on dopamine cell activity, decreased the voltammetric peak in the striatum at +0.12 V, but not that at +0.26 V, in association with a marked decrease of motor activity.

Some physiological studies have also been performed using voltammetry *in vivo*. Thus Joseph & Kennett (1983) found that immobilization stress provoked an increase of oxidation current at +0.4 V which was thought to reflect 5-hydroxyindole oxidation (Kennett & Joseph, 1982) and was consistent with the increase of brain 5-hydroxytryptamine turnover also shown as indicated by the raised concentrations of brain tryptophan and 5-hydroxyindoleacetic acid. These stress-induced changes were all attenuated by treatment with valine, which competes with tryptophan for uptake into the brain.

Cespuglio (1981) showed using electrically pretreated carbon fibres implanted in the rat striatum that the current at +0.3 V (resulting predominantly from oxidation of 5-hydroxyindoleacetic acid) decreased during slow wave and paradoxical sleep and increased when the rat was awake.

The oxidation peak at +0.12 V recorded in the striatum using graphite/silicone oil electrodes and linear sweep voltammetry was also shown to fluctuate with changes of the light-dark cycle, being maximal during the dark phase (when motor activity was greatest) and minimal during the light phase (when it was lowest) (Curzon & Hutson, 1981).

Lindsay *et al.* (1981) found, using chronoamperometry in the conscious rhesus monkey, that oxidation current in the putamen was increased following the presentation of food or a glove previously used during handling and persisted for 40–50 min after removal of the stimulus.

Future developments

It is not easy to predict the status which voltammetry will eventually attain in the armoury of neuroscience. Much will depend on the development of improved electrodes, a field of research which has a somewhat empirical aspect. Voltammetry *in vivo* will possibly prove of most use in the study of 5-hydroxytryptamine metabolism, which is relatively simple, rather than in the more complex catecholamine field where the greater number of metabolites per amine and the considerable extent to which they are conjugated *in vivo* (Elchisak *et al.*,

1977) results in relatively severe problems of specificity and sensitivity. It seems likely that voltammetry will be most applicable in the monitoring of amine metabolites rather than (as originally thought) of their parent amines, as the latter appear to occur extraneuronally at relatively low concentrations (Ungerstedt *et al.*, 1982). It may well be that *ex vivo* voltammetric procedures in which small extraneuronal samples are taken either from the cerebrospinal fluid space (Sarna *et al.*, 1983) or from brain perfusates (Ungerstedt *et al.*, 1982) and their constituents separated by high pressure liquid chromatography before voltammetric assay will prove more widely useful than the fully *in vivo* method.

This review has specifically described the application of voltammetry to the monitoring *in vivo* of transmitter amine metabolism in the brain. However, there is no essential reason why the method should not also be used to monitor other oxidizable substances of interest, e.g. ascorbic acid. As discussed above, this substance may well have a hitherto unsuspected role in the brain. It can be readily monitored, being present in tissues at much higher concentrations than the transmitter amines and their metabolites. Voltammetry might also be used in the field of drug metabolism to monitor oxidizable drugs or their metabolites *in vivo*.

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