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DIFFERENTIAL PULSE VOLTAMMETRY IN THE ANAESTHETIZED RAT: IDENTIFICATION OF ASCORBIC ACID, CATECHOL AND INDOLEAMINE OXIDATION PEAKS IN THE STRIATUM AND FRONTAL CORTEX

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1 Differential pulse voltammetric scans recorded using graphite paste working electrodes implanted in the striatum and frontal cortex of anaesthetized rats exhibited up to three distinct oxidation peaks at potentials between −0.1 and + 0.5 V.

2 The first peak at about + 0.12 V was selectively increased by the micro-infusion of ascorbic acid (5×10^{-6} M) close to the surface of the working electrode implanted in either the striatum or the frontal cortex.

3 The second peak at about + 0.22 V was selectively increased by micro-infusing either dopamine or 3,4-dihydroxyphenylacetic acid, (5×10^{-6} M).

4 The third peak at approximately + 0.35 V was selectively increased in a dose-related manner by the micro-infusion of either 5-hydroxytryptamine, (7.5×10^{-6} M to 7.5×10^{-5} M) or 5-hydroxyindole-3-acetic acid, (2.5×10^{-5} M to 6×10^{-4} M).

5 The results show that differential pulse voltammetry can be used to obtain qualitative and quantitative information about catechol and indoleamine neurones *in vivo*.

Introduction

Various techniques have been used to study neurotransmitter release *in vivo* such as cortical cups (Elliott, Swank & Henderson, 1950; Mitchell, 1963) and push-pull cannulae (Gaddum, 1961; Szerb, 1967). The application of electrochemical techniques *in vivo* offers an alternative approach to monitoring monoamine transmitter release and metabolism. The initial studies used chronoamperometric measurements; this involved the application of a predetermined potential to the working electrode for a fixed time (usually 1 s); however, this did not give qualitative data (Wightman, Strope, Plotsky & Adams, 1976; Conti, Strope, Adams & Marsden, 1978; Gonon, Cespuglio, Ponchon, Buda, Jouvet, Adams & Pujol, 1978; Marsden, Conti, Strope, Curzon & Adams, 1979; Huff, Adams & Rutledge, 1979).

Recently, differential pulse voltammetry has been used to provide qualitative and quantitative data. A ramp potential with superimposed regular square wave pulses of short duration and fixed amplitude is applied to the working electrode. Current measurement is made prior to the pulse and immediately before the end of the pulse, the current difference being recorded. Electroactive compounds, including many neurotransmitter substances, are oxidized at the surface of the working electrode as the applied potential attains their redox potential. In this way, distinct oxidation peaks are recorded corresponding to the compounds' redox potential. The peak height at a specific potential is directly proportional to the concentration of electroactive materials oxidized at that redox potential. The position and number of oxidation peaks observed depends on the type of working electrode employed and the nature of the electroactive compounds present at the surface of the electrode.

With graphite paste or carbon fibre electrodes up to three distinct peaks have been seen between −0.1 and + 0.5 V in various brain regions of the anaesthetized rat, (Lane, Hubbard & Blaha, 1978; Buda, Gonon, Cespuglio, Jouvet & Pujol, 1980; Cespuglio, Riou, Buda, Faradji, Gonon, Jouvet & Pujol, 1980; Gonon, Buda, Cespuglio, Jouvet & Pujol, 1980; Brazell & Marsden, 1981; Marsden, Bennett, Brazell, Sharp & Stolz, 1981). The compounds that give rise to these peaks can be identified by pharmacological manipulation, (e.g. Buda *et al.*, 1980; Cespuglio *et al.*, 1980; Gonon *et al.*, 1980). Alternatively micro-injections of the compounds suspected of contributing to the peaks can be made adjacent to the working electrode and changes in peak heights

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recorded (e.g. Lane *et al.*, 1978). In this study the second approach has been adopted to identify the three oxidation peaks seen in the striatum and frontal cortex of the anaesthetized rat.

Methods

Electrode preparation

Working electrodes were made from a length of Teflon coated silver wire (Clark, Pangbourne, England). The Teflon was drawn over the silver wire to leave a length of empty Teflon sheathing. This sheathing was packed with graphite paste to a depth of 3 mm. The graphite paste was made by slowly adding 2.1 g of carbon powder (Ultra Carbon UCP-1-1M), to a mixture of 0.9 g of liquid paraffin thoroughly dissolved in 15 ml of carbon tetrachloride followed by gentle continuous stirring until the carbon tetrachloride had evaporated; (Marsden, *et al.*, 1979). Electrodes were calibrated before use by trimming the tip with a razor-blade so that the packed Teflon sheath was 2.5 mm long, and recording their performance during differential pulse voltammetry in 0.1 M phosphate buffered saline, pH 7.4; then in the various standard solutions of either 5-hydroxytryptamine (5-HT) (5×10^{-6} M); 5-hydroxyindole-3-acetic acid (5-HIAA) (5×10^{-5} M); dopamine (5×10^{-4} M); 3,4-dihydroxyphenylacetic acid (DOPAC) (1×10^{-4} M) or ascorbic acid (1×10^{-4} M). Finally just before electrode implantation, the tip was trimmed to 1.8 mm.

The reference electrode was prepared by removing 5 mm of the Teflon sheath from both ends of a length of Teflon coated silver wire. One end was anodized at +7 V, 25 mA for 1 min in 1 M HCl, and then immediately placed in a trimmed micropipette tip that was filled with 3 M NaCl containing 10% gelatine (weight by volume), the tip of which was sealed with a cotton wool plug. Gelatine was used to ensure that there was minimal leakage of NaCl out of the electrode. The other end of the micropipette was sealed with Araldite. These electrodes could be stored for up to 3 weeks in 3 M NaCl in a dark, sealed container at 4°C. Auxiliary electrodes were prepared as described previously (Marsden *et al.*, 1979).

Electrode implantation

Male Wistar rats (270–275 g), housed under a controlled 12 h light-dark cycle with food and water *ad libitum*, were anaesthetized with either pentobarbitone (60 mg/kg i.p.) or chloral hydrate (500 mg/kg i.p.) with supplementary doses when required. Electrode implantation followed standard stereotaxic techniques with the animals placed in a David Kopf frame with the upper incisor bar set 5.0 mm above the interaural line.

The cranium was exposed and cleaned. Holes 1.0 mm in diameter were drilled for the reference, auxiliary and working electrodes and also for an anchoring skull screw. The reference electrode was positioned so that it rested on the dura surface and the auxiliary electrode was screwed into the cranium till it also touched the dura. While the position of the reference and auxiliary electrodes was not critical, it was general policy to implant them as close to the working electrode as possible. Up to 4 working electrodes were implanted at a time or alternatively 1 working electrode/cannula combination, (see later). Co-ordinates from Pellegrino & Cushman, (1979); were from bregma as follows (a), striatum: rostral-caudal +1.6 mm; sagittal ±3.0 mm; vertical −4.5 mm from the dura surface, (b) frontal cortex: rostal-caudal +4.6 mm; sagittal ±2.0 mm; vertical −1.8 mm from the dura surface. Chronoplastic dental cement was used to secure the electrodes but not the working electrode/cannula combination.

The working electrode/cannula combination consisted of a modified 21G hypodermic needle tubing down the centre of which was secured a freshly trimmed working electrode, (Marsden *et al.*, 1981). This working electrode was secured in place by a wax cap and was positioned so that its tip projected 0.3 mm beyond the cannula tip. Teflon tubing connected the side arm of the cannula to a micro-litre infusion-system. Just before the implantation of the working electrode/cannula combination the 'system' was filled with the infusate. This consisted of either the test agent made up in 0.1 M phosphate buffered saline pH 7.4 at 37°C, or the vehicle. For the micro-infusions, 1 to 1.5 μl of the infusate was administered over 60 s. The following substances were infused: L-ascorbic acid, (5×10^{-6} M to 5×10^{-5} M) (Fisons); dopamine (5×10^{-6} M); DOPAC (5×10^{-6} M); 5-HT (7.5×10^{-6} M to 7.5×10^{-5} M); and 5-HIAA (2.5×10^{-5} M to 6×10^{-4} M); (all supplied by Sigma).

Electrochemical technique

All the results were obtained using differential pulse voltammetry (Princeton Applied Research polarograph, 174A) with the following settings. Scan rate 10 mV/s, voltage range −0.1 to approximately +0.5 V, modulation amplitude (step height) 50 mV peak to peak, modulation frequency 2/s, with the low pass filter set at 0.3 s. Differential pulse voltammetry was performed once every 8 min. This time period was used because it gave consistent peak heights while allowing frequent sweeps to be made. The first 5 sweeps taken between 0 and 32 min after electrode implantation were not used in the analysis of data as these values were considered to be contaminated by current produced by tissue damage following electrode implantation.

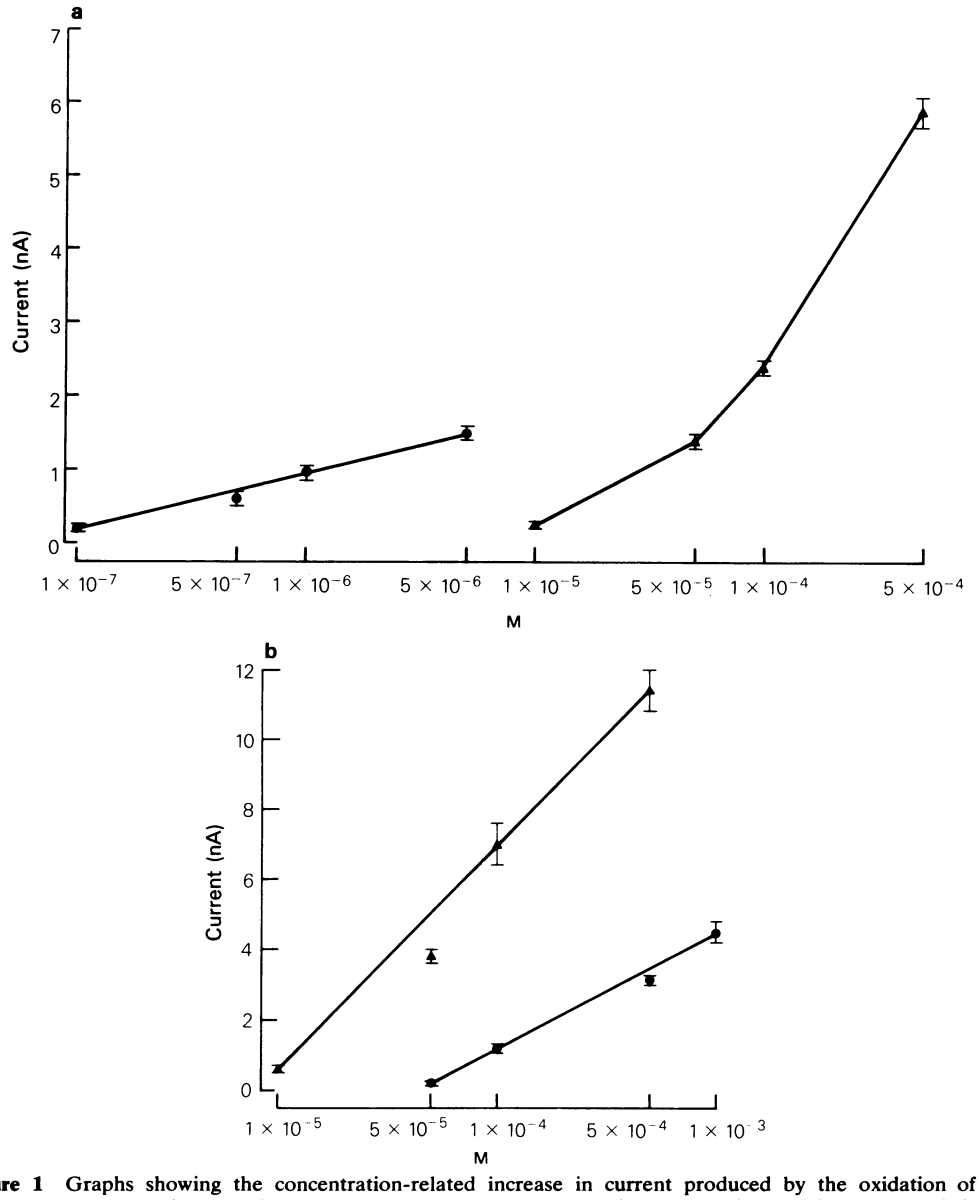

Figure 1 Graphs showing the concentration-related increase in current produced by the oxidation of (a) 5-hydroxytryptamine (5-HT, ●) and 5-hydroxyindole-3-acetic acid (5-HIAA, ▲) and (b) dopamine (●) and 3,4-dihydroxyphenylacetic acid (DOPAC ▲) at the surface of a micro (300 μm) graphite paste electrode, using differential pulse voltammetry *in vitro*. $n = 6$ in each case. The solutions were prepared in phosphate buffered saline, pH 7.4. Current was measured at the peak oxidation potentials which for 5-HT and 5-HIAA was + 0.35 V while for dopamine and DOPAC it was + 0.24 V. Scan parameters were; scan rate 10 mV/s; voltage range − 0.1 to + 0.5 V; modulation amplitude 50 mV (peak to peak); filter 0.3 s.

Differential pulse voltammetry was performed for at least 90 min before any infusion to allow the baseline to stabilize. The micro-infusions were timed so that a voltammogram could be obtained during the last 30 s of the infusion.

Analysis of data

A peak current value (nA) was determined by constructing a tangent to the shoulders of the peak and measuring the perpendicular height between the

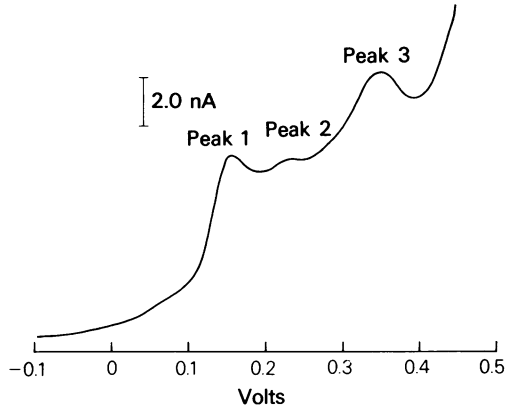

Figure 2 Differential pulse voltammogram obtained *in vivo* from the striatum of a rat anaesthetized with chloral hydrate; note the three oxidation peaks at +0.13, +0.22 and +0.34 V. Scan parameters and electrode as in Figure 1.

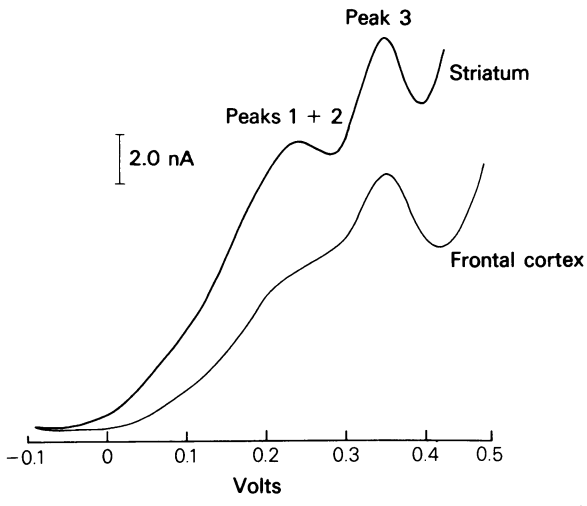

Figure 3 Comparison of the differential pulse voltammograms obtained *in vivo* in the rat anaesthetized with chloral hydrate from the striatum and frontal cortex. Scan parameters and electrode as in Figure 1. Note the relatively large peak 1 + 2 in the striatum.

tangent and the centre of the peak. Changes in peak height following a micro-infusion were determined by calculating the mean height of the peak using the four voltammetric sweeps immediately prior to the infusion and comparing the value with the height of the peak during maximal response.

The peak heights have been corrected to take into account the internal amplification factor of ×10 present in the Princeton Applied Research polarograph, 174A. The peak heights thus differ from our previously reported values by a factor of ×10, (Brazell *et al.*, 1981; Marsden *et al.*, 1981).

Results

Differential pulse voltammetry of 5-hydroxytryptamine, 5-hydroxyindole-3-acetic acid, dopamine, 3,4-dihydroxyphenylacetic acid and ascorbic acid in vitro

The 5-hydroxyindoles, 5-HT and 5-HIAA produced an oxidation peak in phosphate buffered saline pH 7.4: at between +0.33 and +0.35 V while dopamine and DOPAC oxidized at between +0.24 and +0.30 V. Ascorbic acid gave an oxidation peak at approximately +0.30 V. For all these compounds there was a dose-related increase in current over the concentration ranges tested: 5-HT 1×10^{-7} M to 5×10^{-6} M; 5-HIAA 1×10^{-5} M to 5×10^{-4} M; dopamine 5×10^{-5} M to 1×10^{-3} M; DOPAC 1×10^{-5} M to 5×10^{-4} M (Figure 1a and b); and ascorbic acid 5×10^{-4} M to 1×10^{-3} M. Phosphate buffered saline, 0.1 M, pH 7.4 was used as the solvent for the *in vitro* study, and on its own it gave no distinct oxidation peaks between −0.1 and +0.5 V.

Differential pulse voltammetry in the striatum and frontal cortex in vivo

Voltammograms recorded in the striatum and frontal cortex exhibited up to 3 distinct oxidation peaks, referred to as peaks 1, 2 and 3, (Figure 2). Oxidation peak 1 was seen between +0.12 and +0.15 V. Peak 2 was between +0.22 and +0.24 V and so overlapped the *in vitro* oxidation potential range of dopamine and DOPAC. Peak 3 occurred between +0.33 and +0.35 V which corresponded to the *in vitro* oxidation potential range of 5-HT and 5-HIAA. Peak 1 was not consistently seen in all animals and when it did occur it was most apparent shortly after electrode implantation and was short lived. It was sometimes observed briefly following manipulation of the animal, (i.e. after i.p. drug or anaesthetic administration). Usually peak 1 was 'hidden' in the shoulder of peak 2; for this reason the first peak seen was a combination of peaks 1 and 2, and will thus be referred to as peak 1 + 2.

A regional variation in the height of peak 1 + 2 was observed between the striatum and the frontal cortex. After allowing the readings to stabilize the peak was smaller in the frontal cortex than in the striatum, (Figure 3). Striatal peak 1 + 2 had a mean value of 1.3 nA ($n=6$) while in the frontal cortex it was 0.5 nA ($n=6$). The regional difference was less marked with peak 3; in the striatum it had a mean value of 3.1 nA ($n=6$), while in the frontal cortex it was 2.8 nA ($n=6$).

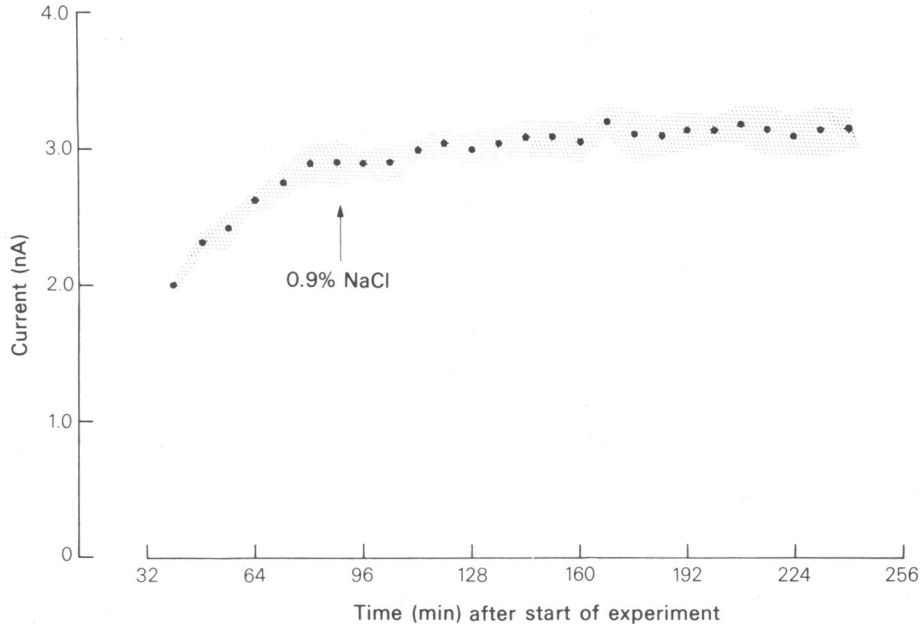

Figure 4 Graph showing the stability of peak 3 in the striatum of a rat anaesthetized with chloral hydrate. The current values were taken at the potential which gave maximum current readings, i.e. at about + 0.35 V. After electrode implantation, the current was recorded once every 8 min for 240 min. Current values are the mean of 6 (40 to 184 min) and 4 (192 to 240 min) experiments; stippled area indicates s.e.mean. Scan parameters and working electrodes as in Figure 1.

Stability studies over 240 min of peaks 1 + 2 and 3 in the striatum showed that peak 3 stabilized approximately 90 min after the start of the recording (Figure 4). In contrast peak 1 + 2 was not very stable exhibiting an overall increase in height during the initial 160 min and then a general decrease, (Figure 5). The control injection of saline (0.9%, 2.0 ml/kg i.p.), had no appreciable effect on the height of peaks 1 + 2 and 3, (Figures 4 and 5).

Chloral hydrate was used as the anaesthetic because studies showed that under pentobarbitone anaesthesia the peaks were 30 to 50% smaller than under chloral hydrate anaesthesia (Table 1).

Effect of 5-hydroxytryptamine and 5-hydroxyindole-3-acetic acid infusion on the height of peak 3

Peak 3 could be selectively increased by the micro-

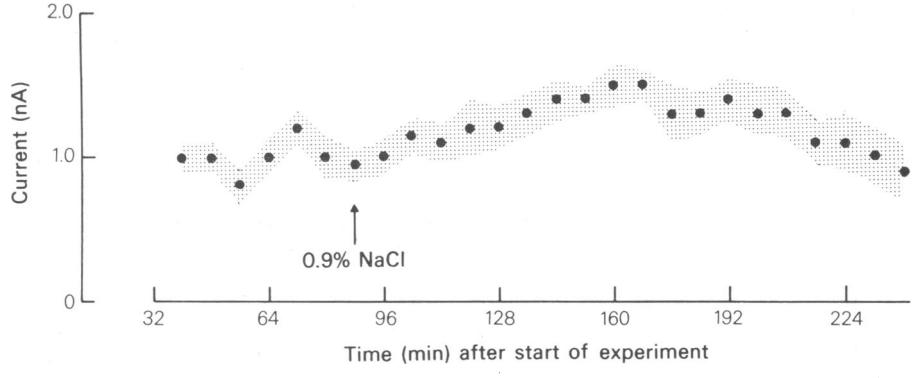

Figure 5 Graph showing the stability of peak 1 + 2 in the striatum of a rat anaesthetized with chloral hydrate. The current values were taken at the potential which gave maximum current readings, i.e. at + 0.22 V; recorded every 8 min from 40 to 240 min after the start of the experiment. Current values are the mean of 6 (40 to 184 min) and 4 (192 to 240 min) experiments; stippled area indicates s.e.mean. Scan parameters and electrodes as in Figure 1.

Table 1 Comparison of the effects of chloral hydrate and pentobarbitone anaesthesia on the size of peak 3 pre-infusion and following the infusion of 5-hydroxytryptamine (5-HT, 7.5×10^{-6} M) or 5-hydroxyindole-3-acetic acid (5-HIAA, 2.5×10^{-5} M)

	Current (nA)		
	Pre-infusion	*Post-infusion*	
		5-HT (7.5×10^{-6} M)	*5-HIAA* (2.5×10^{-5} M)
Chloral hydrate ($n=4$)	30.2 ± 0.02	38.7 ± 0.06 [+28%]	–
(500 mg/kg) ($n=3$)	29.7 ± 0.11	–	38.3 ± 0.19 [+29%]
Pentobarbitone ($n=3$)	18.0 ± 0.09	22.6 ± 0.07 [+25%]	–
(60 mg/kg) ($n=3$)	14.7 ± 0.07	–	19.0 ± 0.09 [+29%]

Current values were obtained at the peak oxidation potential for peak 3 (i.e. about + 0.35 V). 5-HT and 5-HIAA (1 μl) were slowly infused 90 min after the start of the recording. The pre-infusion current value is the mean of the peak height recorded during the last four differential pulse voltammetric scans performed before infusion. The post-infusion value is the peak height attained during the first scan immediately after the infusion (i.e. 8 min post-infusion). Scan parameters and electrode were as in Figure 1. Note the smaller size of peak 3 under pentobarbitone anaesthesia while the % increase post-infusion is the same with both anaesthetics. Results are given as mean ± s.e.mean.

infusion of either 5-HT or 5-HIAA into the striatum or frontal cortex. The micro-infusion of 1 to 1.5 μl of 7.5×10^{-6} M 5-HT over 60 s increased striatal peak 3 by about 28%, (Table 1). This response was dose-dependent over the range tested, (7.5×10^{-6} M to 7.5×10^{-5} M), (Figure 6).

Infusion of 5-HIAA (2.5×10^{-5} M), into the striatum, 1 to 1.5 μl over 60 s also increased the height of peak 3 by about 29%, (Table 1). Again this change was dose-dependent with increases of 82 and 164% being obtained when 8×10^{-4} M and 6×10^{-4} M were infused respectively. Similar results were obtained following the micro-infusion of 5-HT and 5-HIAA into the frontal cortex.

The selective increase in the height of peak 3 produced by the micro-infusion of either 5-HT or 5-HIAA was short lived, normally disappearing within the next 2 sweeps (i.e. 16 min). Sometimes the height of peak 1 + 2 as well as the overall base line was reduced following the infusion, this effect lasting for not more than 16 min.

Phosphate buffered saline 0.1 M, pH 7.4 at 37°C was used as the vehicle, and when given on its own (1.5 μl) it either had no effect or slightly reduced all the peaks and the base line.

Effect of dopamine, 3,4-dihydroxyphenylacetic acid and ascorbic acid on peak 1 + 2

Infusion of dopamine (5×10^{-6} M) (Figure 7) or DOPAC (5×10^{-6} M) increased the height of peak 1 + 2 in the striatum and frontal cortex of rats anaesthetized with chloral hydrate. The micro-infusion of dopamine or DOPAC did not alter the height of peak 3.

Ascorbic acid ($5 + 10^{-6}$ M) infused into the striatum or frontal cortex also produced an increase in the height of peak 1 + 2, with a 'new' peak occurring at about + 0.12 V; this 'new' peak had a height of 6.8 nA. Increasing the concentration of the infused ascorbic acid to 5×10^{-5} M resulted in a peak at + 0.12 V of 17.0 nA.

Similar effects were observed following the micro-infusion of either dopamine, DOPAC or ascorbic acid into the frontal cortex.

Discussion

The present study confirms that differential pulse voltammetry can be used to record catechol and indoleamine oxidation peaks in the anaesthetized rat brain, (Lane *et al.*, 1978; Buda *et al.*, 1980; Cespuglio *et al.*, 1980; Gonon *et al.*, 1980; Marsden *et al.*, 1981). With the micro-infusion technique we have been able to show that the three peaks seen *in vivo* in the striatum and frontal cortex relate, in part at least, to the oxidation of ascorbic acid (peak 1), dopamine and/or DOPAC (peak 2) and 5-HT and /or 5-HIAA (peak 3). The most reliable results were obtained following micro-infusion of either 5-HT or 5-HIAA into the striatum and frontal cortex as both of these produced consistent increases in the size of the oxidation peak at about + 0.35 V (i.e. peak 3), irrespective of the anaesthetic used. The results support recent pharmacological studies using electrically treated carbon-fibre, rather than carbon paste working electrodes, which indicated that the peak observed at about + 0.35 V was caused by indoleamine oxidation, (Cespuglio *et al.*, 1980). Cespuglio and co-workers further suggested that the major contribution to the peak was from the oxidation of 5-HIAA

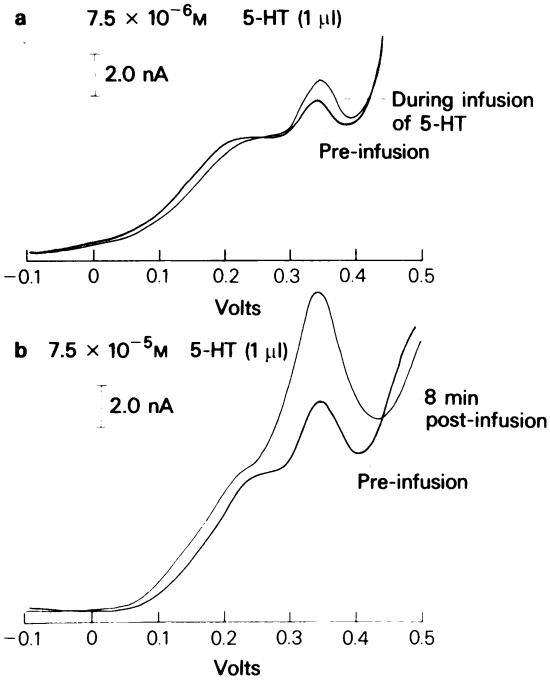

Figure 6 Voltammograms showing the effect of the micro-infusion of 5-hydroxytryptamine (5-HT, 7.5×10^{-6} M in (a) and 7.5×10^{-5} M in (b)), on the oxidation peaks observed during differential pulse voltammetry in the striatum of the rat anaesthetized with chloral hydrate. The top trace shows the scan immediately prior to the micro-infusion of 7.5×10^{-6} M of 5-HT (1 μl) and the first scan after the infusion, (i.e. 8 min post-infusion). The lower trace also shows the voltammograms immediately prior to and 8 min after a micro-infusion of 5-HT, but this time the concentration of 5-HT was 7.5×10^{-5} M. Note the selective dose-related increase in the peak at + 0.35 V in the post-infusion voltammogram. Scan parameters and electrodes as in Figure 1.

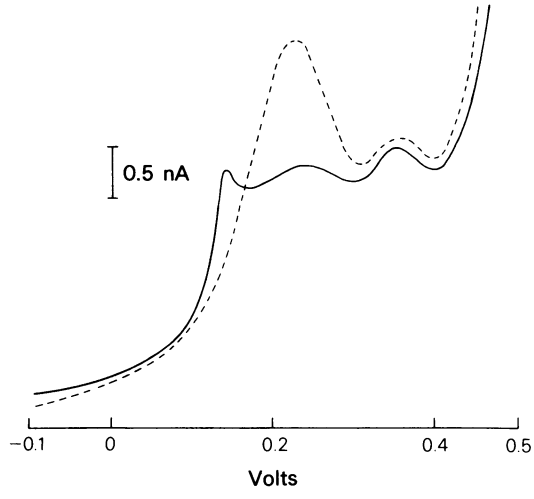

Figure 7 Effect of micro-infusion (1 μl) of dopamine (5×10^{-6} M) into the striatum of a rat anaesthetized with chloral hydrate on the oxidation peaks observed during differential pulse voltammetry. The trace shows the voltammogram immediately before infusion (solid line) and the first voltammogram after the infusion (i.e. 8 min post-infusion, dashed line). Note the selective increase in the oxidation peak at + 0.22 V. Scan parameters and electrodes as in Figure 1.

rather than 5-HT. Recent studies in this laboratory would support this view, (Brazell & Marsden, unpublished observations). This is despite the fact that the present study has shown that the carbon paste electrode is almost 100 times more sensitive to 5-HT than to 5-HIAA *in vitro*. Thus the *in vivo* electrochemical technique may monitor changes in amine metabolism (Gonon *et al.*, 1980), rather than release (Conti *et al.*, 1978; Marsden *et al.*, 1979). The responses originally recorded using fixed potential methods (i.e. chronoamperometry) will need revaluation since although the current change observed may relate to the activity of particular aminergic neurones it may not represent release of the amine alone as the current recorded may have some other electroactive component, i.e. ascorbic acid, (Gonon *et al.*, 1980).

Micro-infusion of dopamine and DOPAC into the striatum and frontal cortex selectively increased the oxidation peak observed at about + 0.22 V. This peak corresponds to the DOPAC oxidation peak identified pharmacologically by Gonon *et al.* (1980). While it is likely that striatal peak 2 is caused by dopamine and/or DOPAC, as dopamine is by far the most dominant catecholamine in this region (Broch & Marsden, 1972); the fact that noradrenaline and its initial metabolite 3,4-dihydroxyphenylglycol (DOPEG) also oxidize *in vitro* at about + 0.22 V means that in other brain regions it will be important to determine the relative contribution to peak 2 by these latter two compounds. However, as most of the DOPEG found *in vivo* is in the sulphated form (Nielsen & Braestrup, 1976) which oxidizes at a higher potential than DOPEG, then the peak observed at approximately + 0.22 V in other brain regions may only have a small DOPEG component.

Ascorbic acid (5×10^{-6} M) produced a peak at about + 0.12 V *in vivo* when micro-infused into the striatum or frontal cortex, however, a clear oxidation peak at this potential was only seen in the pre-infusion state during a manipulation such as drug injection. Gonon *et al.* (1980), have reported a peak at − 0.05 V corresponding to the *in vitro* oxidation of ascorbic acid at treated carbon-fibre electrodes. The position of this oxidation peak *in vitro* and *in vivo* is very dependent on the electrochemical pretreatment

of the carbon-fibre electrodes used by Gonon and his co-workers. The failure of the present study to observe a consistent endogenous ascorbic acid peak, either in the striatum or frontal cortex, probably reflects differences between the electrodes used in this study and those used by other groups.

The carbon paste electrodes of this study show several interesting properties. *In vitro* they are more sensitive to 5-HT than 5-HIAA, DOPAC or dopamine while showing equal sensitivity to 5-HIAA and DOPAC (Figures 1a and b). However, there are problems when using *in vitro* calibration with standard solutions to establish the sensitivity of carbon paste electrodes *in vivo*, as the present study shows that these electrodes are less sensitive *in vitro* than *in vivo*. While there is no clear explanation for this effect it may be due to *in vivo* 'priming' of the electrode surface with free-NH_3 groups from the CSF or brain tissue, (R.N. Adams, personal communication). Another unexplained observation with the existing carbon paste electrodes is the marked difference in oxidation potential of ascorbic acid *in vitro* (about +0.35 V) from that observed following its infusion *in vivo* (about +0.12 V).

The oxidation profiles of the frontal cortex and striatum show an inter-regional variation over the potential range −0.1 to +0.5 V. Thus peak 3, the indoleamine peak, has approximately the same height in the two regions while peak 1+2, the ascorbic acid and catecholamine peak, is larger in the striatum than in the frontal cortex. More recent work (Bennett, Brazell, Marsden & Sharp, 1981) has shown that the nucleus accumbens also has a distinctive oxidation profile, with peaks 1+2 and 3 having similar heights within this region. In general, the oxidation profile recorded from these various brain regions follow the reported endogenous levels of catecholamines and 5-hydroxyindoles. Thus high levels of dopamine and DOPAC in the striatum and nucleus accumbens (Lindvall & Bjorklund, 1974), are associated with a relatively large endogenous peak 1+2 while in the frontal cortex a large peak 3 but small peak 1+2 corresponds with a relatively dense 5-HT innervation (Steinbusch, 1981), and more restricted dopaminergic innervation (Lindvall, Bjorklund & Divac, 1978).

Two anaesthetics were used in these studies, pentobarbitone and chloral hydrate. With the former, the endogenous oxidation peaks were always smaller than those recorded under chloral hydrate anaesthesia. Previous studies have failed to observe drug-induced changes in electrochemical signals in rats anaesthetized with pentobarbitone (Marsden *et al.*, 1979). While the absence of drug responses may be due to effects of barbiturates on drug uptake by neurones (Aldridge & Marsden, 1981), the present results indicate that endogenous *in vivo* metabolism of dopamine and 5-HT may also be reduced by the barbiturates and that this group of anaesthetics is best avoided during *in vivo* recording experiments.

In conclusion, the results show that two, and sometimes three, oxidation peaks can be observed by differential pulse voltammetry in the striatum and frontal cortex of anaesthetized rats using untreated carbon paste electrodes. The peaks relate to the oxidation of ascorbic acid, (approximately +0.12 V) dopamine and/or DOPAC, (approximately +0.22 V) and 5-HT and/or 5-HIAA, (about +0.35 V). The position and size of these oxidation peaks is very dependent on the type of working electrode used and its pretreatment. In view of this, the peaks need to be re-identified whenever the electrode is modified. However, within these limitations *in vivo* voltammetry using differential pulse techniques allows qualitative and quantitative information to be obtained about amine metabolism and possibly release as well as offering an opportunity for investigating the undefined role of ascorbic acid in the brain, (Subramanian, 1977).

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