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Anaesthesia abolishes the effect of valproate on extracellular 5-HIAA, DOPAC and ascorbate as measured in rat striatum by differential pulse voltammetry

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1 The effect of sodium valproate (VPA, 400 mg kg^{-1}, i.p.) on extracellular ascorbate, 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA) in the striatum was examined by differential pulse voltammetry in anaesthetized and freely-moving rats.

2 In rats anaesthetized with chloral hydrate (400 mg kg^{-1}, i.p.), pentobarbitone (50 mg kg^{-1}, i.p.) or phenobarbitone (60 mg kg^{-1}, i.p.), VPA produced no significant changes in peak 1 (extracellular ascorbate) or peak 2 (extracellular DOPAC), but produced a slight but statistically significant reduction in the height of peak 3 (extracellular 5-HIAA).

3 In contrast, in freely-moving rats the same dose of VPA greatly reduced extracellular ascorbate and DOPAC concentrations, and increased that of 5-HIAA.

4 These results suggest that VPA may reduce the release or turnover of dopamine, and increase that of 5-hydroxytryptamine in conscious rats. Our data also suggest that caution may be required in the interpretation of the effects of VPA in anaesthetized animals, as the results obtained may not always reflect the situation in the absence of anaesthesia.

Introduction

The antiepileptic drug sodium valproate (VPA) has been employed in clinical practice for a number of years (Pinder *et al.*, 1977; Chapman *et al.*, 1982). Although much emphasis has been placed on the increase in brain γ-aminobutyric acid (GABA) observed after VPA treatment (Godin *et al.*, 1969), the drug also modifies other neurochemical parameters, producing a reduction of aspartic acid (Schechter *et al.*, 1978), and an increase in the dopamine metabolite homovanillic acid (HVA), and the 5-hydroxytryptamine (5-HT) metabolite 5-hydroxyindoleacetic acid (5-HIAA) (Horton *et al.*, 1977; Hwang & Van Woert, 1979). It has been proposed that VPA raises brain HVA and 5-HIAA content by competition for the active transport mechanism for these acids (Horton *et al.*, 1977), but this conclusion has been disputed for 5-HIAA by subsequent workers, who have suggested that VPA increases the turnover of 5-HT (Hwang & Van Woert, 1979; Lazarova *et al.*, 1980; Whitton *et al.*, 1983). This effect of VPA on 5-HT turnover has been implicated in some pharmacological effects of the drug such as the production of wet dog shakes (Fletcher & Harding, 1981) and antimyoclonic activity (Whitton *et al.*, 1983).

Differential pulse voltammetry is a technique which has the advantage compared to classical biochemical methods of enabling a continuous analysis of extracellular 5-HIAA, ascorbic acid and the dopamine metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) *in vivo*. Recent developments of this technique enable the recording of these three compounds simultaneously by using a single, specifically pretreated carbon fibre electrode in discrete brain areas of the rat (Crespi *et al.*, 1984a,b). Furthermore, we have recently found that the same working electrode implanted chronically can be used to detect the three compounds simultaneously in freely-moving rats.

We have employed this improved methodology to determine the time course of the effect of VPA on extracellular 5-HIAA, DOPAC and ascorbate in

anaesthetized and freely-moving rats. A preliminary account of these results has been presented to the British Pharmacological Society (Crespi *et al.*, 1985).

Methods

Male Sprague-Dawley rats (CD, Charles River, France) weighing 250–300 g were anaesthetized with chloral hydrate (400 mg kg^{-1}, i.p.) and were prepared for differential pulse voltammetry analysis in the striatum (Crespi *et al.*, 1984a). The working electrodes were specifically pretreated as previously described in order to obtain three separate peaks in conjunction with the PRG5 polarograph (Tacussel, France) (Crespi *et al.*, 1984b). In one group of rats ($n = 5$), recording started immediately, while in a second group ($n = 5$), an 8 h period for recovery from anaesthesia was allowed before the start of the experiment. In this last condition, both mono (Gonon *et al.*, 1981a) or multi-fibre (Crespi & Jouvet, 1984) working electrodes were used, with identical results. To observe the influence of different anaesthetics on the effect of VPA, two other groups of rats were studied. Four rats received 5% pentobarbitone, and four others 6% phenobarbitone (both drugs at 1 ml kg^{-1}, i.p.), and the effects of VPA were studied immediately after the completion of surgery.

When the heights of the peaks 1 (ascorbate), 2 (DOPAC) and 3 (5-HIAA) recorded every 5 min had stabilised, a control period of 80 min was allowed before administration of VPA (400 mg kg^{-1}, i.p.) or its solvent (physiological saline) was injected. The mean height of each peak recorded after this treatment was compared to the mean height of the respective peak in the control recording period and results were expressed as percentage of the control values (± s.d.). The raw data were subjected to an analysis of variance, and comparisons between experimental points and their respective controls were performed using the Tukey test for multiple comparisons.

At the end of each experiment, the position of the electrode tip in the striatum was verified. An electrolytic lesion (5 mA, 5 s) was made using the working electrode, then the brain was rapidly removed for standard histological processing with cresyl violet dye.

Results

Figure 1a shows the response of a carbon fibre electrode to ascorbate (5 mM, peak 1 at − 50 mV), DOPAC (50 μM, peak 2 at + 100 mV) and 5-HIAA (25 μM, peak 3 at + 280 to 300 mV) *in vitro*. Figure 1b and c shows respectively typical *in vivo* recordings in the striatum of the anaesthetized and freely moving

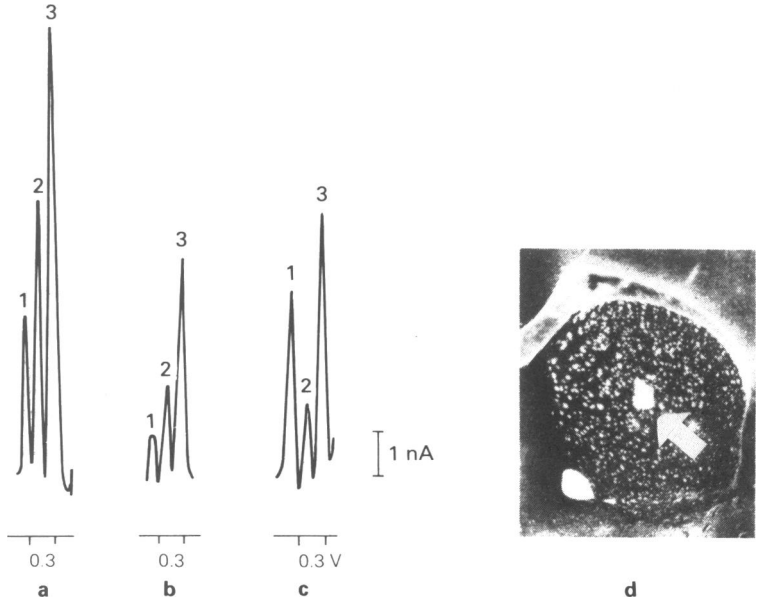

Figure 1 (a) Response of a carbon fibre electrode to ascorbic acid (5 μM, peak 1 at − 50 mV), 3,4-dihydroxyphenylacetic acid (50 μM, peak 2 at + 100 mV) and 5-hydroxyindoleacetic acid (25 μM, peak 3 at + 280–300 mV), *in vitro*; (b) typical *in vivo* recording in the striatum of an anaesthetized rat; (c) typical *in vivo* recording in the striatum of a freely moving rat; (d) typical electrode emplacement in the striatum of an anaesthetized rat.

rat. Figure 1d illustrates the typical position of the working electrode in the rat striatum. Peak 1 heights were always considerably higher in the conscious animal, suggesting that anaesthesia reduced extracellular concentrations of ascorbic acid in the striatum. Saline injections did not alter the size of any peak in our experimental conditions. The effects of VPA in rats anaesthetized with pentobarbitone or phenobarbitone were similar in all respects to those seen with chloral hydrate anaesthesia. Therefore only the results with the latter anaesthesia are illustrated in detail.

Effect of sodium valproate on peak 1 height in the rat striatum

Treatment with VPA (400 mg kg^{-1}, i.p.) in rats anaesthetized with chloral hydrate did not alter the height of peak 1 at any time during the recording period of 2.5 h. When the rats were allowed to recover from anaesthesia before the injection of VPA (400 mg kg^{-1}, i.p.), a highly significant reduction ($P < 0.01$) in peak 1 was noted starting approximately 30 min after injection, and reaching a maximum reduction of 80% after 90 min (Figure 2).

Effect of sodium valproate on peak 2 height in the rat striatum

In rats anaesthetized with chloral hydrate VPA (400 mg kg^{-1} i.p.) was ineffective in altering peak 2 height in the striatum (Figure 3), whereas in freely-moving rats the same drug treatment produced a marked fall in this parameter ($P < 0.01$). The reduction in extracellular DOPAC was already significant 30 min after injection, and gradually increased throughout the entire recording period (Figure 3).

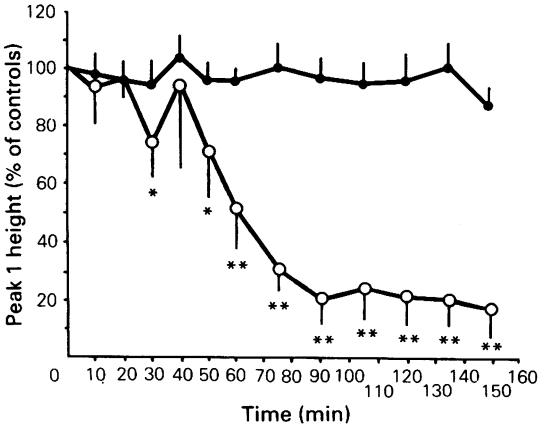

Figure 2 Effect of sodium valproate (400 mg kg^{-1}, i.p.) on peak 1 (extracellular ascorbic acid) in chloral hydrate anaesthetized (●) and freely-moving (○) rats ($n = 5$). Results are expressed as percentage of control peak height with s.d. shown by vertical lines. Statistical analysis was performed on raw data (nA) by analysis of variance followed by the Tukey test for multiple comparisons: * $P < 0.05$; ** $P < 0.01$.

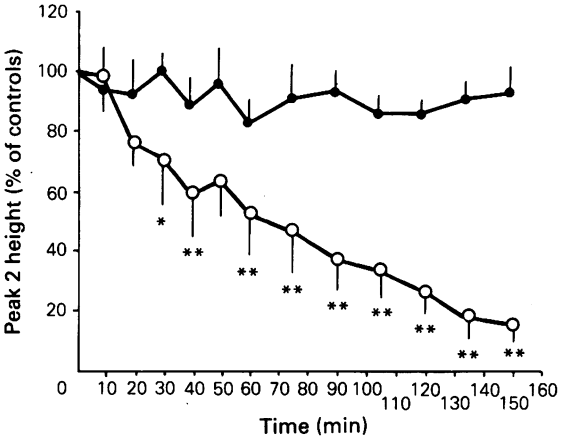

Figure 3 Effect of sodium valproate (400 mg kg^{-1}, i.p.) on peak 2 (extracellular 3,4-dihydroxyphenylacetic acid) in chloral hydrate anaesthetized (●) and freely-moving (○) rats ($n = 5$). Results are expressed as percentage of control peak height with s.d. shown by vertical lines. Statistical analysis was performed on raw data (nA) by analysis of variance followed by the Tukey test for multiple comparisons: * $P < 0.05$; ** $P < 0.01$.

Effect of sodium valproate on peak 3 height in the rat striatum

Injection of VPA into rats anaesthetized with chloral hydrate produced a slight fall in peak 3 height in the striatum, which was significant at 100, 130 and 150 min (Figure 4). The maximum reduction noted was 15% at 150 min. In contrast, in freely-moving rats, VPA produced the opposite effect. Ten minutes after injection, extracellular 5-HIAA was raised by 20% in the striatum, and a maximal increase of about 34% was noted 50 to 150 min after injection (Figure 4).

Discussion

The present study shows that VPA reduces extracellular ascorbate and DOPAC concentrations, and increases extracellular 5-HIAA, in the striatum of freely-moving rats. However, in rats anaesthetized with chloral hydrate, pentobarbitone or phenobarbitone, the only consequence of VPA administration was a slight but significant fall in extracellular 5-

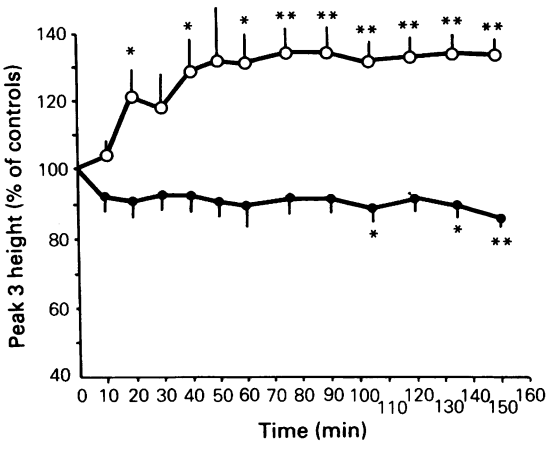

Figure 4 Effect of sodium valproate (400 mg kg^{-1}, i.p.) on peak 3 (extracellular 5-hydroxyindoleacetic acid) in chloral hydrate anaesthetised (●) and freely-moving (○) rats. ($n = 5$). Results are expressed as percentage of control peak height with s.d. shown by vertical lines. Statistical analysis was performed on raw date (nA) by analysis of variance followed by the Tukey test for multiple comparisons: * $P < 0.05$; ** $P < 0.01$.

HIAA. This reduction by VPA of peak 3 in the striatum of anaesthetized rats is in agreement with a previous voltammetric study in which the amide analogue of VPA was used (Scatton *et al.*, 1984).

Thus anaesthesia appears to abolish the effects of VPA on ascorbate and DOPAC, and reverse its effect on 5-HIAA. Trulson & Trulson (1983) have found that the presence or absence of anaesthesia can profoundly influence the response of cells in the 5-HT containing raphe nuclei to a wide variety of pharmacological agents. Although the effects of most drugs they studied were increased by anaesthesia, the action of diazepam was greatly attenuated. Our present results suggest that this conclusion may also be valid for VPA. In this context, it is interesting to note that VPA shares many of the pharmacological properties of the benzodiazepines (Simiand *et al.*, 1983).

The reduction of extracellular DOPAC in the striatum of freely-moving rats may suggest that VPA can reduce the release or turnover of dopamine in this brain region. This is supported by classical biochemical studies indicating that VPA reduces the rate of synthesis of striatal dopamine (Walter *et al.*, 1979). Although Horton *et al.* (1977) found that VPA increased the whole-brain content of HVA in mice, a number of studies have indicated that GABAergic drugs reduce the levels of dopamine metabolites in the brain of naive and neuroleptic-treated rats (Keller *et al.*, 1976; Scatton *et al.*, 1982).

The increase in extracellular 5-HIAA in the striatum of freely-moving rats may suggest that VPA increases striatal 5-HT turnover. Alternatively, a probenecid-like inhibition of the transport of this metabolite from brain tissue by VPA has been proposed (Horton *et al.*, 1977). Although the present results are consistent with either hypothesis, the former possibility is supported by experiments indicating some 5-HT-mimetic effects of VPA (Hwang & Van Woert, 1979; Fletcher & Harding, 1981). Furthermore, the VPA-induced reduction of striatal DOPAC, another acid whose transport is inhibited by probenecid (Spano & Neff, 1972), also supports this conclusion.

Few pharmacological manipulations have been shown to alter extracellular ascorbate levels in the brain. Dopamine receptor agonists can increase peak 1 in the rat striatum (Gonon *et al.*, 1981b; Clemens & Phebus, 1984). This suggests that the reduction in dopamine turnover produced by VPA is a potential mechanism for the observed effect on peak 1. A second hypothesis concerns the excitatory neurotransmitter aspartate. Grunewald & Fillenz (1984) found that glutamate and aspartate produced an efflux of ascorbate from brain synaptosomes *in vitro*, and O'Neill *et al.* (1984) showed that parenteral or local administration of excitatory amino acids provoked an increase in the height of peak 1 in the rat striatum. Numerous studies have shown that VPA administration reduces cerebral aspartate concentrations (Godin *et al.*, 1969; Schechter *et al.*, 1978; Chapman *et al.*, 1983). If as proposed by Grunewald & Fillenz (1984), changes in extracellular ascorbate concentration reflect the activity of neurones which secrete excitatory amino acids, the reduction in aspartate produced by VPA could explain the drug's effect on peak 1.

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References

CHAPMAN, A., KEANE, P.E., MELDRUM, B.S., SIMIAND, J. & VERNIÈRES, J.C. (1982). Mechanism of anticonvulsant action of valproate. *Prog. Neurobiol.*, **19**, 315–359.

CHAPMAN, A.G., MELDRUM, B.S. & MENDES, E. (1983). Acute anticonvulsant activity of structural analogues of valproic acid and changes in brain GABA and aspartate content. *Life Sci.*, **32**, 2023–2031.

CLEMENS, J.A. & PHEBUS, L.A. (1984). Brain dialysis in conscious rats confirms in vivo electrochemical evidence that dopaminergic stimulation releases ascorbate. *Life Sci.*, **35**, 671–677.

CRESPI, F. & JOUVET, M. (1984). Differential pulse voltammetric determination of 5-Hydroxyindoles in four raphe nuclei of chronic freely moving rats simultaneously

recorded by polygraphic technique: physiological changes with vigilance states. *Brain Res.*, **299,** 113–119.

CRESPI, F., SHARP, T., MAIDMENT, N.T. & MARSDEN, C.A. (1984a). Differential pulse voltammetry: simultaneous in vivo measurement of ascorbic acid, catechols and 5-hydroxyndoles in the rat striatum. *Brain Res.*, **322,** 135–138.

CRESPI, F., PARET, J., KEANE, P.E. & MORRE, M. (1984b). An improved differential pulse voltammetry technique allows the simultaneous analysis of dopaminergic and serotonergic activities in vivo with a single carbon-fibre electrode. *Neurosci. Lett.*, **52,** 159–164.

CRESPI, F., KEANE, P.E. & MORRE, M. (1985). Effect of valproate on striatal ascorbic acid, DOPAC and 5-HIAA in rats as measured by voltammetry, *Br. J. Pharmac.*, **85,** 372P.

FLETCHER, A. & HARDING, V. (1981). An examination of the "wet dog" shake behaviour in rats produced by acute administration of sodium n-dipropylacetate. *J. Pharm. Pharmac.*, **33,** 811–813.

GODIN, Y., HEINER, L., MARK, J. & MANDEL, P. (1969). Effect of di-n-propylacetate, an anticonvulsant compound, on GABA metabolism. *J. Neurochem.*, **16,** 869–873.

GONON, F., BUDA, M., CESPUGLIO, R., JOUVET, M. & PUJOL, J.F. (1981a). In vivo electrochemical detection of catechols in the neostriatum of anaesthetised rats: dopamine on DOPAC? *Nature (Lond.)*, **286,** 902–904.

GONON, F., BUDA, M., CESPUGLIO, R., JOUVET, M. & PUJOL, J.F. (1981b). Voltammetry in the striatum of chronic freely moving rats: detection of catechols and ascorbic acid. *Brain Res.*, **223,** 69–80.

GRUNEWALD, R.A. & FILLENZ, M. (1984). Release of ascorbate from a synaptosomal fraction of rat brain. *Neurochem. Int.*, **4,** 491–500.

HORTON, R.W., ANLEZARK, G.M., SAWAYA, M.C.B. & MELDRUM, B.S. (1977). Monoamine and GABA metabolism and the anticonvulsant action of di-n-propylacetate and ethanolamine-O-sulphate. *Eur. J. Pharmac.*, **41,** 387–397.

HWANG, E.C. & VAN WOERT, M.H. (1979). Effect of valproic acid on serotonin metabolism. *Neuropharmacology*, **18,** 391–397.

KELLER, H.H., SCHAFFNER, R. & HAEFELY, W. (1976). Interaction of benzodiazepines with neuroleptics at central dopamine neurons. *Naunun-Schmiedebergs Arch. Pharmac.*, **294,** 1–7.

LAZAROVA, M. ROUSSINOV, K., KLEINROK, Z. & RAJTAR, G. (1980). On some relationships between GABA-ergic and 5-HT-ergic mechanisms in pentylenetetrazol convulsive-seizure reactions. *Agressologie*, **21,** 253–257.

O'NEILL, R.D., FILLENZ, M., SUNDSTROM, L. & RAWLINS, J.N.P. (1984). Voltammetrically monitored brain ascorbate as an index of excitatory amino acid release in the unrestrained rat. *Neurosci. Lett.*, **52,** 227–233.

PINDER, R.M., BROGDEN, R.N., SPEIGHT, T.M. & AVERY, G.S. (1977). Sodium valproate: a review of its pharmacological properties and therapeutic efficacy in epilepsy. *Drugs*, **13,** 81–123.

SCATTON, B., ZIVKOVIC, B., DEDEK, J., LLOYD, K.G., CONSTANTINIDIS, J., TISSOT, R. & BARTHOLINI, G. (1982). γ-Aminobutyric acid (GABA) receptor stimulation, III, Effect of progabide (SL76002) on norepinephrine, dopamine and 5-hydroxytryptamine turnover in rat brain areas. *J. Pharmac. exp. Ther.*, **220,** 678–688.

SCATTON, B., SERRANO, A., RIVOT, J.P. & T. NISHIKAWA. (1984). Inhibitory GABAergic influence on striatal serotonergic transmission exerted in the dorsal raphe as revealed by in vivo voltammetry. *Brain Res.*, **305,** 343–352.

SCHECHTER, P.J., TRANIER, Y. & GROVE, J. (1978). Effects of n-dipropylacetate on amino acid concentrations in mouse brain: correlation with anti-convulsant activity. *J. Neurochem.*, **31,** 1325–1327.

SIMIAND, J., KEANE, P.E., EYMARD, P., SANTUCCI, V., MORRE, M. & RONCUCCI, R. (1983). Antiaggressive, anxiolytic and antianoxic effects of valproate in animals. *Br. J. clin. Pract.*, suppl. **27,** 155–160.

SPANO, P.F. & NEFF, N.H. (1972). Metabolic fate of caudate nucleus dopamine. *Brain Res.*, **42,** 139–145.

TRULSON, M.E. & TRULSON, V.M. (1983). Chloral hydrate anaesthesia alters the responsiveness of dorsal raphe neurons to psychoactive drugs. *Life Sci.*, **32,** 949–956.

WALTER, J.R., LAKOSKI, J.M., ENG., N. & WASZCZAK, B.L. (1979). Effect of muscimol, AOAA, and Na valproate on the activity of dopamine neurones and dopamine synthesis. In *GABA-Neurotransmitters: Pharmacochemical, Biochemical and Pharmacological Aspects*, ed. Krogsgaard-Larsen, P., Scheel-Kruger, J. & Kofod, H. pp. 118–134. New York: Academic Press.

WHITTON, P.S., ORESKOVIC, D., MARKOVIC, Z., SKARPA, D. & BULAT, M. (1983). Effect of the antiepileptic di-n-propylacetamide on 5-hydroxytryptamine turnover in the brain and 5-hydroxyindoleacetic acid level in the cerebrospinal fluid. *Eur. J. Pharmac.*, **91,** 57–62.

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