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THE EFFECT OF SODIUM-*n*-DIPROPYL ACETATE ON γ-AMINOBUTYRIC ACID-DEPENDENT INHIBITION IN THE RAT CORTEX AND SUBSTANTIA NIGRA IN RELATION TO ITS ANTICONVULSANT ACTIVITY

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1 We have examined the time course of the anticonvulsant property of valproate sodium on electroshock-induced convulsions in rats and a comparison of this has been made with the action of the drug on single unit activity in the rat brain.

2 Intraperitoneal valproate sodium (100 to 400 mg/kg) protected rats from electroshock-induced convulsion. This effect was dose-dependent, the latency of the effect decreasing as a function of dose from 5 to 2 min.

3 The time course of this anticonvulsant property was paralleled by a pronounced inhibition of the spontaneous firing rate of cortical and nigral neurones, following intraperitoneal administration of valproate sodium (100 to 400 mg/kg).

4 The inhibitory action of microiontophoretically applied γ-aminobutyric acid (GABA) and muscimol on the firing rate of cortical neurones was potentiated within 1 to 3 min of microiontophoretic application of valproate sodium. In contrast, the inhibitory action of glycine on cortical neurones was unaffected during the microiontophoretic application of valproate sodium.

5 Microiontophoretically applied valproate sodium also potentiated inhibitory responses to GABA in rats which had received 100 mg/kg of a GABA-transaminase inhibitor, gabaculine, i.p. 16 h previously.

6 The duration of trans-synaptic inhibitory responses recorded in the substantia nigra and cortex following submaximal electrical stimulation of the striatum and cortex respectively was, in general, unaffected by either intraperitoneal or local application of valproate sodium.

7 These observations are discussed in terms of the mechanisms underlying the rapid onset of the anticonvulsant properties of valproate sodium.

Introduction

Sodium-*n*-dipropylacetate (valproate sodium; VPA) is an effective anti-convulsant agent both in animals (Simler, Ciesielski, Maitre, Randrianarisoa & Mandel, 1973; Horton, Anlezark, Sawaya & Meldrum, 1977) and in man (Simon & Penry, 1975). It was originally proposed that the drug's anticonvulsant action is related to its ability to prevent the catabolism of γ-aminobutyric acid (GABA) by inhibiting the enzymes GABA-transaminase (GABA-t; Maitre, Ciesielski, Cash & Mandel, 1978; Simler *et al.*, 1973) and succinic semialdehyde dehydrogenase (SSA-DH; Harvey, Bradford & Davison, 1975; Whittle & Turner 1978). However, there are several drawbacks to this hypothesis. A role for GABA in the mechanism of anticonvulsant action in general has never been firmly established (Vial, Claustre & Pacheco, 1974; Horton, Meldrum, Sawaya & Stephenson, 1976). VPA is an effective anticonvulsant at doses that do not elevate cerebral GABA levels in rats (Horton *et al.*, 1977) and a high dose of VPA is ineffective against cobalt-induced epilepsy, despite elevated cerebral GABA levels (Emson, 1976). Furthermore VPA is effective against electroshock-induced convulsion within 5 min (Schmutz, Olpe & Koella, 1979), whereas the first significant elevation of cerebral GABA is seen only after 30 min (Schechter, Tranier & Grove, 1978). In a preliminary communication it was shown that peripheral administration of an anticonvulsant dose of VPA produced a rapid depression in the firing rate of neocortical cells and that iontophoretically applied VPA potentiated the inhibitory responses to GABA on

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such cells (Schmutz *et al.*, 1979). We have now performed a systematic examination of both local and peripherally administered VPA on both spontaneous neuronal activity and inhibitory responses produced by microiontophoretically applied GABA, glycine and muscimol on single units in the rat cortex and substantia nigra. In addition, we have examined the effect of local and peripherally applied VPA on transsynaptic inhibitory responses in the cortex and substantia nigra following submaximal electrical stimulation of the cerebral cortex and striatum respectively. We have compared these responses to the time course of the anticonvulsant action of VPA on electroshock-induced convulsion.

Our results point to an ability of VPA to produce direct effects on postsynaptic inhibitory mechanisms which may be independent of its ability to inhibit enzymes in the catabolic pathway for GABA. These effects occur with a time course that may be more relevant to the anticonvulsant mode of action of VPA.

Methods

Effect of valproate sodium on electroshock induced convulsion in the rat

Tonic convulsions of the hind extremities of male SIV-50 rats weighing 250 to 300 g were induced by passing alternating electrical currents of 50 Hz, 100 V for 0.63 s through corneal electrodes. Rats were pretreated with VPA (100 to 400 mg/kg, i.p.) at 1, 2, 3, 5, 10, 15 and 20 min before stimulation. Twenty animals per dose and time-point were used; one group served as control. The number of animals protected at each time interval from tonic hind limb extension seizure was determined in each dose group. The percentage of animals protected in each group was plotted against the pretreatment time for each dose.

Single cell recording and microiontophoresis

Experiments were performed on male rats (SIV-50) weighing 250 to 300 g. They were anaesthetized with chloral hydrate (400 mg/kg, i.p.). Additional injections of the anaesthetic were given as needed throughout the experiment. Body temperature was measured by a rectal probe and maintained between 37.0 and 38.5°C.

Glycine and chloral hydrate were purchased from Fluka (Buchs, Switzerland). Valproate sodium, muscimol and gabaculine were synthesized in the chemical laboratories of CIBA-GEIGY (Basel, Switzerland). GABA was obtained from Serva (Heidelberg, West Germany).

The conventional stereotactic approach and cell recording techniques were used to record from spontaneously active neurones in the parietal neocortex and the pars reticulata of the substantia nigra (Kelly, Simmonds & Straughan, 1975). Cortical neurones were sampled in an area 2 mm lateral from the midline and 3 to 5 mm anterior to lambda. Nigral neurones were approached stereotactically using the following co-ordinates: A, 2.5–3.0; L 1.5–2.0; V 7-8 mm from brain surface (De Groot, 1972). The action of VPA on trans-synaptic, GABA-mediated neuronal inhibition was investigated by activating inhibitory fibres, impinging upon nigral or cortical cells. Nigral neurones were selected which were inhibited by ipsilateral stimulation of the striatum and neocortical neurones were chosen which responded with inhibition following stimulation of the cortex locally, 2 mm anterior to the recording electrode. Stimulation consisted of single bipolar pulses applied through a modified Hess type electrode. The voltage varied between 2 to 20 V and the duration of the stimuli was 0.3 ms. Stimulation was always submaximal. Peristimulus-time histograms (PSTHs) were generated by a Nicolet 535 data system. PSTHs were obtained repeatedly from each neurone over periods of 15 to 75 min. Changes in inhibitory responses were determined by measuring the length of time the cell was quiescent following stimulation, determined from means of at least four reproducible responses.

Conventional microiontophoretic techniques were used to administer drugs near to cortical and nigral neurones. Three to four barrelled micropipettes were used. Ejection currents were balanced during all drug administrations. The following aqueous solutions were used: GABA (0.5 M, pH 3.5); glycine (0.5 M; pH 3.5); VPA (0.5 M, pH 8); bicuculline-methiodide (0.025 M in 165 mM NaCl, pH 3.5).

The interaction of VPA with GABA, muscimol or glycine was studied in microiontophoretic experiments on separate groups of cortical neurones. Submaximal inhibitory responses to either GABA, muscimol or glycine were obtained by passing ejecting currents of 10–90 nA during periods of 10 to 60 s. When at least two similar control responses were observed, VPA was administered continuously as an anion for periods of 5 to 30 min with currents from 40 to 80 nA. During this period GABA, muscimol or glycine were ejected at constant intervals from 30 s to 3 min. The interaction was evaluated by comparing the length of the inhibitory response and the maximal reduction of the firing rate achieved by the agents, before, during and after the application.

In one series of experiments, the effect of iontophoretically applied VPA was studied on inhibitory responses to GABA in a group of 10 rats which had been given gabaculine (100 mg/kg, i.p.) in 0.9% saline 16 h before the experiment. These animals were anaesthetized with chloral hydrate (100 mg/kg, i.p.).

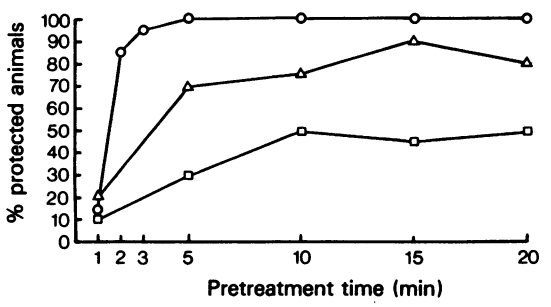

Figure 1 The time course of development of the anticonvulsive properties of valproate sodium (VPA) is shown for three different doses. Each point represents the percentage of rats protected from convulsion. Each point is derived from the number of animals protected in a single group of 20 rats: (□) 100 mg/kg i.p.; (△) 200 mg/kg i.p.; (○) 400 mg/kg i.p.

Results

Time course of the anticonvulsive effect of valproate sodium

The anticonvulsive action of VPA as a function of dose and time is shown in Figure 1. There is a dose-dependent increase in the percentage of animals protected from electroshock induced convulsions. With increasing dosage a reduction of the latency of the anticonvulsive effect can be observed. At a dose of 400 mg/kg (i.p.) 85% of all animals are protected within 2 min after application of the drug.

The effect of intraperitoneally applied valproate sodium on spontaneous neuronal activity

The neocortical cells under investigation were unidentified and located in various cell layers. The nigral neurones showed characteristics of GABA-sensitive neurones found in the pars reticulata. Cells were firing irregularly at a frequency greater than 5 action potentials per second. In addition these cells were inhibited in a bicuculline-sensitive manner by stimulation of the striatum. The predominant effect of systemically administered VPA on spontaneous cell firing in both brain areas was an inhibitory one (Table 1). At a dose of 200 mg/kg (i.p.) the majority of cortical and nigral neurones were potently inhibited. At a dose of 400 mg/kg, all cortical cells were depressed. Mean latencies to the depressant effect varied between 4.0 and 5.7 min (Table 1).

The effect of microiontophoretically applied valproate sodium on the cell depressant action of GABA, muscimol and glycine

VPA alone often had a slight excitatory effect which developed within 2 to 5 min of the start of application. A highly significant potentiation ($P < 0.001$) of the cell-depressant action of GABA was observed on 58 unidentified neocortical cells (Figure 2). Significance was achieved for both the length of the inhibitory responses and the maximal reduction of the firing rate. The latency of this effect varied from 1 to 3 min. The cell depressant action of muscimol on cortical neurones was also significantly potentiated ($n = 15$, $P < 0.05$) by microiontophoretically applied VPA (Table 2). In contrast, the inhibitory effects

Table 1 Summary of the effect of intraperitoneal valproate sodium (VPA) on the activity of 28 neocortical and 10 nigral cells

	Dose of VPA								
Brain area	100 mg/kg i.p.			200 mg/kg i.p.			400 mg/kg i.p.		
	n	Effect	Mean latency (min)	*n*	Effect	Mean latency (min)	*n*	Effect	Mean latency (min)
Cortex	2	↑	4.4						
	1	↓	5.3	16	↓	4.5	4	↓	4
	2	ϕ		3	ϕ				
Substantia nigra	2	↓	5	3	↓	5.7	1	↓	5
	2	ϕ		2	ϕ				

The effects of VPA on the spontaneous activity of neurones are represented as: ϕ, no effect; ↓, depression of firing rate; ↑, increase in firing rate. Mean latencies for the onset of the observed effects are indicated. n = number of neurones.

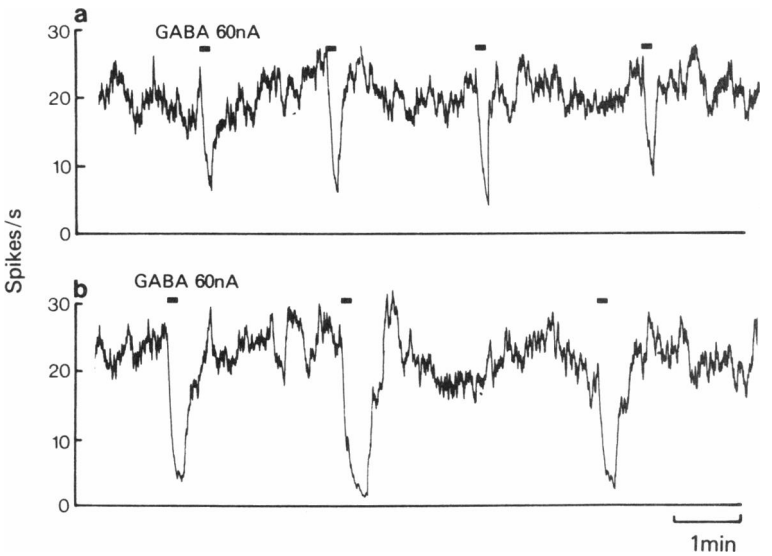

Figure 2 Potentiation of submaximal inhibitory responses to γ-aminobutyric acid (GABA) of the spontaneous firing rate of a rat cortical cell by microiontophoretically applied valproate sodium (VPA) (40 nA): (a) before VPA; (b) during VPA.

observed with glycine on such cells were unaffected by VPA ($n = 11$) (Table 2).

In rats pretreated with gabaculine, VPA potentiated GABA-elicited cortical cell depression ($n = 22$; $P < 0.005$) as in untreated animals. The effect of VPA on inhibitory responses to muscimol and GABA was reversible within 1 min, whereas the excitatory effect of VPA was often maintained.

Absence of effect of microiontophoretically and systemically applied valproate sodium on trans-synaptic effects mediated by GABA

In most instances, during systemic application of VPA, interpretation of the results was often hampered by the fact that VPA had a pronounced cell depressant action of short latency (see above). Under such circumstances, a potentiation of trans-synaptic inhibition was seen, particularly with higher doses of VPA (Figure 3). In the substantia nigra, 19 cells were sampled following systemically administered VPA. The inhibition was shown to be mediated by GABA since it was antagonized by microiontophoretically applied bicuculline in 4 cells tested randomly. VPA potentiated inhibition in 2 out of these 19 cells. In the neocortex such treatment prolonged trans-synaptic inhibitory effects in only 1 out of 21 cells. Upon microiontophoretic application of VPA, trans-synaptic

Table 2 The effect of iontophoretic application of sodium valproate (40 to 80 na) on the reduction in firing rate of spontaneously active neocortical cells produced by submaximal ejections of muscimol or glycine

	Number of cells	*% increase in inhibition response*	*Current range*	*P*
Muscimol	15	48 ± 6%	0–60 nA	<0.05
Glycine	11	6.2 ± 3.9%	20–80 nA	NS

Muscimol or glycine were ejected for periods of 10 to 30 s at 1 min intervals. Continuous ejections of sodium valproate were superimposed for 10 to 30 min. The percentage increase in response is calculated from the mean reduction in firing rate during three ejections in the absence of sodium valproate, compared to the mean reduction in firing rate of three ejections during sodium valproate. Untransformed data from individual pairs was subjected to a 2 factor analysis of variance (Paired t test). The standard errors presented were calculated from the individual percentage values.

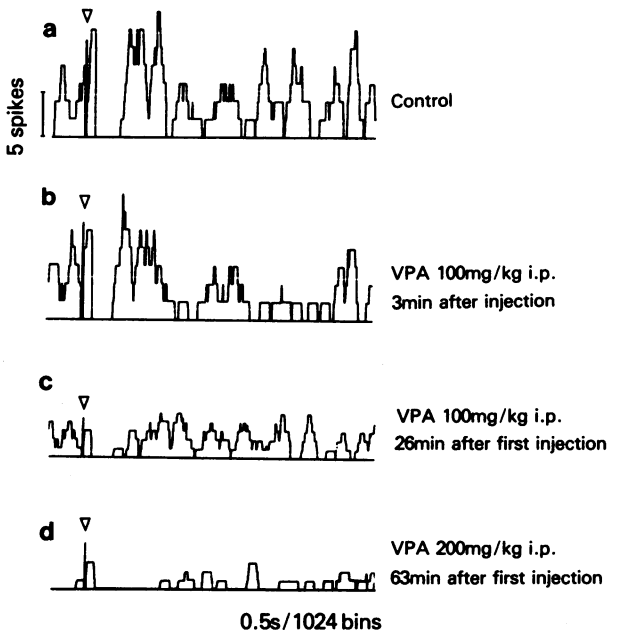

Figure 3 Peri-stimulus time histograms showing the effect of systemically applied valproate sodium (VPA) on trans-synaptically evoked inhibition of a nigral cell elicited by stimulation of the ipsilateral striatum: (▽) denotes onset of stimulation (single bipolar pulse, 6 V, 0.3 ms). After two control recordings VPA 100 mg/kg i.p. was administered (b); 30 min later an additional 100 mg/kg i.p. of VPA was given (c); a further 200 mg/kg i.p. was administered 30 min later (d).

inhibitory effects elicited on nigral cells by striatal stimulation could be potentiated on 4 out of 19 neurones.

Discussion

The ability of VPA rapidly to depress cortical cell firing and to potentiate inhibitory responses to GABA extend and confirm our preliminary findings (Schmutz *et al.*, 1979) and those of others (Blume, Lamour, Arnauld, Layton & Renaud, 1979; MacDonald & Bergey, 1979). These effects were rapid in onset with a time course similar to VPA's ability to prevent electroshock-induced convulsion in the rat. This effect is rapid compared to VPA's ability to elevate cerebral GABA (Maitre *et al.*, 1978). Furthermore we have attempted to rule out enzymatic effects as a possible mechanism for the presently observed electrophysiological effects. Thus, we examined the effects of locally applied VPA on responses to muscimol, a directly acting GABA agonist (Johnston, Curtis, De Groat & Duggan, 1968) which is not a substrate for GABA-t or SSA-DH and which has only low affinity for the GABA uptake and release carriers (Johnston & Kennedy, 1978). Similarly we attempted to pre-empt VPA's ability to elevate synaptic GABA levels by pretreating a group of rats with the GABA-t inhibitor, gabaculine (Rando & Bangarter, 1976). The dose and pretreatment time used ensures a maximal elevation of brain GABA concentrations (R. Bernasconi, personal communication). In the presence of such high GABA levels it is unlikely that VPA will exert any appreciable effect on synaptic GABA levels through catabolic inhibition. Thus it was found that VPA applied locally by iontophoresis still potentiated inhibitory responses to GABA in gabaculine-treated rats. Similarly local iontophoresis of VPA potentiated inhibitory responses to muscimol.

In our experiments it was not possible to demonstrate potentiation of synaptic inhibition produced by submaximal stimulation of presumed GABA pathways. However, this does not necessarily argue against the ability of VPA to potentiate GABA-mediated inhibition, since the drug itself produced profound changes in firing rate. Thus, while peripherally applied VPA clearly potentiated the phase of inhibition in some cells, this was always associated with a decrease in cell firing, thus hampering analysis. Similarly the phase of inhibition was apparently unchanged in cells when applied locally. However, in these cases the drug was often excitatory.

The rapid decline in firing rate of cells following peripheral administration and the potentiation of muscimol and GABA responses upon local administration, still present following pretreatment with gabaculine point to a direct postsynaptic site of action.

The mechanism of action must remain a matter of speculation. The drug produces no perceptible changes in [^{3}H]-muscimol binding (Löscher, 1979). Similarly in our radioreceptor assay, VPA displaced [^{3}H]-muscimol from cortical membranes only at concentrations greater than 500 μM. No enhancement of binding was seen at concentrations of VPA from 1 to 100 μM (H. Bittiger & R.W. Kerwin, unpublished). It seems, therefore, unlikely that VPA interacts directly with the GABA receptor. In *Aplysia* neurones, VPA can increase membrane conductance to potassium (Slater & Johnston, 1978), leading to hyperpolarization. This seems an unlikely explanation for the effects described here. Notwithstanding the excitatory effects of local VPA, such changes in ionic conductance would also be expected to lead to a potentiation of responses to glycine, since GABA and glycine utilize similar ionic mechanisms (Barker, McDonald & Ransom, 1978). No potentiation of glycine responses was observed in this study.

It is possible that VPA may act presynaptically to release GABA or prevent reuptake. Indeed such an effect has been postulated by Harvey (1976). It is unlikely that this is an important factor in these experiments, because of the excitation seen after local application. The potentiation of muscimol-induced inhibition also argues somewhat against this theory, although it should be noted that muscimol can be taken up with low affinity and this may be important in terminating its action in iontophoretic experiments (Johnston & Kennedy, 1978; Lodge, Curtis & Johnston, 1978) but only potent GABA uptake blockers can potentiate muscimol-induced inhibition (Lodge *et al.*, 1978).

The contrast between the inhibitory effects of intraperitoneal VPA and the excitatory effects of local VPA is of interest. At present we have no explanation, although the involvement of a metabolite seems likely following peripheral administration. The major metabolite is 3-ketovalproic acid, formed by β-oxidation (Schäfer & Lührs, 1978). These authors suggest that this or a derivative may be responsible for some of the drug's action *in vivo*.

While the mechanism of action must remain unsolved, it is suggested that the apparent ability of VPA to augment GABA receptor-mediated postsynaptic inhibition seen by ourselves and by others (McDonald & Bergey, 1979) and the rapid depression of spontaneous activity after peripheral VPA, paralleling the drug's anticonvulsant activity should receive consideration as a possible mechanism of action in the therapeutic effects of the drug.

RWK is an MRC scholar. Reprint requests to Ciba-Geigy, please.

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