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Chronic administration of valproic acid induces a decrease in rat striatal glutamate and taurine levels

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Summary. The effect of acute and chronic (10 days) administration of 200 mg/kg (i.p.) of valproic acid (VPA) on endogenous levels of aspartate, glutamate, alanine, glycine and taurine in the cerebral frontal cortex and corpus striatum of rats was studied. Quantification of the amino acid levels was performed by HPLC.

Valproic acid (VPA) did not either induce changes on these neurotransmitters contents in corpus striatum after acute treatment. After chronic administration we found a decrease on the endogenous levels of glutamic acid (24%, $p < 0.05$) which was related to an increase (250%, $p < 0.02$) of the in vitro KCl evoked release of glutamate. We found decrements in taurine endogenous levels (22%, $p < 0.05$) which was not associated with an increase of its release.

In cerebral frontal cortex there was not found any change neither under the acute nor under the chronic condition.

Thus, it may be conclude that chronic treatment with VPA produces decreases on the endogenous levels of glutamate and taurine. However the relevance of this effect concerning it therapeutic action remains unclear.

Keywords: Amino acids – Glutamate – Taurine – Valproic acid – Cerebral frontal cortex – Corpus striatum

Introduction

During the last years the study of pathophysiology of epilepsy have focused on amino acid neurotransmission in the central nervous system.

Recent studies have demonstrated changes in the excitatory amino acid mediated neurotransmission in epileptogenic brain areas. There is growing body of data that implicate NMDA receptors in the development of some forms of epilepsy (Vezzani et al., 1988; McNamara et al., 1988). Glutamate, acting at its site of binding at the NMDA receptor, has also shown an

epileptogenic effect. Kamphius et al. (1991) reported interactions between GABA and glutamate neurotransmission in pyramidal neurons after kindling epileptogenesis. They showed that kindling induced epileptogenesis diminishes the inhibitory effect of GABA on glutamate responses. Concerning the antiepileptic action of amino acid blockers, Hwa and Avoli (1992) demonstrated that both NMDA and non-NMDA receptor antagonists may reduce the peak amplitude of the excitatory postsynaptic potential of epileptogenic human neocortex maintained in vitro. It was also demonstrated that antagonists of NMDA receptors block the burst discharge in dentate gyrus of epileptic patients during epileptiform responses (Masukawa et al., 1991). Furthermore, felbamate, a relatively new antiepileptic drug, exerts its action blocking glycine binding to the strichnine insensitive glycine site at NMDA receptor, thus inhibiting glutamate action (McCabe et al., 1992).

Valproic acid (VPA) is an antiepileptic drug used to treat absences, myoclonic and tonic-clonic seizures. VPA increases endogenous levels of GABA and this is thought to be its principal mechanism of action. This effect may be partially explained by enhancement of the synthesis enzyme activity, the glutamic acid decarboxylase (Löscher, 1981) and by inhibition of GABA aminotransferase and succinic semialdehyde dehydrogenase (Goldin et al., 1969; Fowler et al., 1975; Harvey et al., 1975; Löscher, 1980; Van der Laan, 1979). However, intimate mechanism of action of VPA remains unclear.

Recent advances about alterations of cerebral amino acids in epilepsy stimulated the study of the modifications produced by VPA on amino acid function. Some reports (Farrant and Webster, 1989; Biggs et al., 1992) indicate that acute treatment with this drug may have an effect on amino acid levels in different brain areas of the central nervous system. Most studies were performed under acute conditions. With respect to the effects of the drug after chronic administration, which is actually its clinical schedule of usage, there are fewer reports. Patsalos and Lascelles (1981) reported an increase in taurine, GABA, aspartate and glutamate concentration in different brain areas after chronic administration of two injections of 150 mg/kg/day and Simila et al. (1979) found an increase in glycine concentration in human plasma, urine and cerebral spinal fluid after chronic oral treatment with 14–21 mg/kg but it may be noted that the peripherical inhibitory and muscle relaxant function of glycine is dramatically different to the glutamate co-agonist role that this amino acid plays in the central nervous system.

The main goal of our study was to investigate the effect of acute and chronic treatment with VPA on endogenous levels of aspartate, glutamate, glycine, taurine and alanine in corpus striatum and cerebral frontal cortex of the rat.

Material and methods

We used male Wistar rats (150–200 g) maintained in a 12–12 light dark cycle (lights on 8:00 am) with free access to food and water.

Acute treatment with VPA

Rats were injected either with VPA, 200 mg/kg i.p. (dissolved in Tween 20%) or vehicle. Thirty minutes after the animals were killed by decapitation, the brain was removed and cerebral frontal cortex and corpus striatum were dissected out on a Petri dish at 0°C according to Glowinski and Iversen (1966). Samples were frozen until amino acid quantification was performed.

Chronic treatment with VPA

Rats were injected with VPA, same dose as specified for acute treatment, or vehicle during 10 days. The last day, thirty minutes after injection, the rats were decapitated and the same procedure for acute treated animals was followed.

Endogenous amino acid quantification

Amino acid levels, expressed in μg/g of tissue were quantified by precolumn derivatization with o-phtalaldehyde in the presence of 2-mercaptoethanol followed by chromatographic separation and detection of the derivatives by HPLC-EC at +0.6 V (Durkin et al., 1988).

Potassium evoked release of amino acids

In a separated group of chronically treated animals, the K^+ induced release of amino acids was studied. Immediately after decapitation rat corpus striatum was dissected out and cut into 100 μm thick slices using a tissue sectioner (Smith and Farquhar Sorval TC-2). Slices were incubated in 5 ml Krebs solution gassed with 95% O_2- 5% CO_2 at 37°C, Krebs buffer was composed as follows (mM): NaCl 118, KCl 5, $CaCl_2$ 2.5; $MgCl_2$ 1.2, NaH_2PO_4 1.0; glucose 11.1, EDTA 0.004, ascorbic acid 0.11. The media was replaced every 5 min and after 30–40 min basal amino acid release was achieved. Amino acid release measured in the 5 min sample obtained immediately before stimulation with KCl was considered as basal (pre-stimulation) release. At that time amino acid release was evoked by addition of KCl 50 mM. Five minutes later fresh media, with no KCl added were replaced for a subsequent 5 min period (post-stimulation). The media corresponding to the stimulation, post-stimulation and pre-stimulation periods were collected and amino acids were quantified by high pressure liquid chromatography and electrochemical detection (HPLC-EC) as described for quantification of endogenous levels of the compounds.

The results were analysed using paired Student's test.

The following drugs were used: O-phtalaldehyde (Sigma), 2-mercaptoethanol (Sigma). Valproic acid was provided by Armstrong Laboratories from Argentina.

Results

VPA did not produce any change on endogenous levels of excitatory (aspartate, glutamate) or inhibitory (glycine, taurine or alanine) amino acids in cerebral frontal cortex neither after acute nor after chronic treatment (Table 1).

The drug did not either induce changes on these neurotransmitters levels in corpus striatum after acute treatment but after chronic administration we found a decrease on the endogenous levels of glutamate (24%, $p < 0.05$) and

Table 1. Effect of acute and chronic treatment with 200 mg/kg (i.p.) of valproic acid on endogenous levels of aspartate, glutamate, taurine, glycine and alanine in cerebral frontal cortex of the rat

	Acute treatment		Chronic treatment	
	Control μg/g tissue	Valproic acid μg/g tissue	Control μg/g tissue	Valproic acid μg/g tissue
Aspartate	782 ± 83	780 ± 76	911 ± 35	1,004 ± 92
Glutamate	2,041 ± 205	1,985 ± 152	1,999 ± 94	1,764 ± 234
Taurine	872 ± 131	808 ± 47	477 ± 26	400 ± 51
Glycine	134 ± 39	152 ± 17	40 ± 5	37 ± 7
Alanine	82 ± 13	106 ± 27	82 ± 10	64 ± 7

Results are expressed as mean ± SEM of 5 or 6 experiments per group.

taurine (22%, $p < 0.05$). Aspartate, glycine and alanine were not modified by chronic administration (Table 2). It may be noted that the control values of endogenous levels of taurine and glycine in cerebral frontal cortex and of aspartate, glutamate, glycine and taurine in the corpus striatum were not the same in the acute compared with the chronic treated groups. Levels of amino acids were lower for taurine and glycine in cerebral frontal cortex. In the corpus striatum it was found an increase on the endogenous levels of aspartate and glutamate and, as seen in cerebral frontal cortex, a decrease on the contents of glycine.

As changes induced by VPA were observed only in corpus striatum of chronically treated animals, we investigated the effect of in vitro K^+ stimulation on the release of taurine and glutamate in the above mentioned area after 10 days of injection of 200 mg/kg of VPA. Basal values of treated tissues were increased by 328% ($p < 0.05$). Incubation during 5 min with KCl 50 mM increased the release of glutamate both in the control and in the VPA treated animals but the increment observed in the latter was greater (250%, $p < 0.02$) than the increase found in the control group (Fig. 1A). During the post-

Table 2. Effect of acute and chronic treatment with 200 mg/kg (i.p.) of valproic acid on endogenous levels of aspartate, glutamate, taurine, glycine and alanine in corpus striatum of the rat

	Acute treatment		Chronic treatment	
	Control μg/g tissue	Valproic acid μg/g tissue	Control μg/g tissue	Valproic acid μg/g tissue
Aspartate	772 ± 109	561 ± 82	1,395 ± 58	1,418 ± 98
Glutamate	1,200 ± 109	1,017 ± 60	1,965 ± 136	1,494 ± 123*
Taurine	610 ± 62	458 ± 122	653 ± 34	510 ± 43*
Glycine	211 ± 50	136 ± 46	80 ± 7	79 ± 11
Alanine	85 ± 12	105 ± 26	83 ± 5	68 ± 8

Results are expressed as mean ± SEM of 4 to 9 experiments per group.
*$p < 0.02$ compared with the respective control.

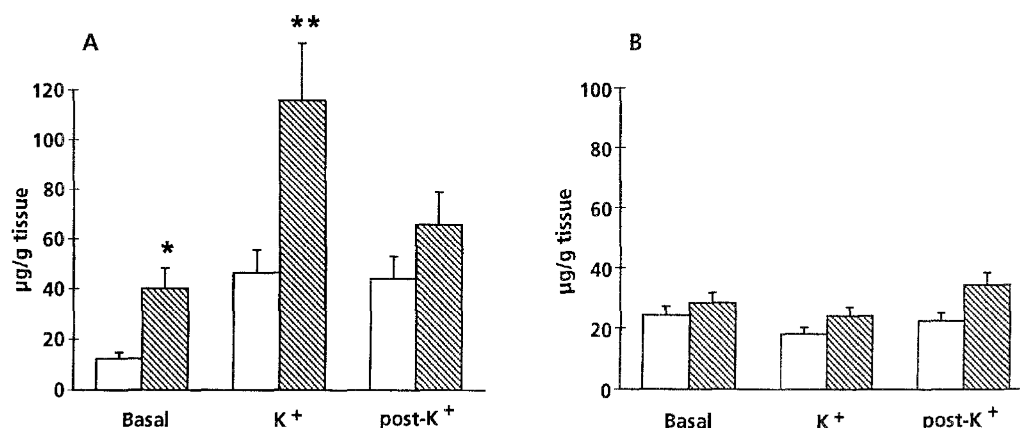

Fig. 1. Effect of chronic treatment with valproic acid on the evoked release by KCl 50 mM of glutamate (**A**) and taurine (**B**) on corpus striatum. Amino acid release is expressed in μg/g of tissue. Open columns represent the release of tissues control and hatched columns the release of tissues of animals chronically (10 days) treated with VPA (200 mg/kg). *Basal* release during a 5 min period without KCl. K^+ release during a 5 min period of stimulation with ClK 50 mM. *post-*K^+ release during a 5 min period after the stimulation period, without ClK. The results are expressed as the mean ± SEM of 5 experiments. * $p < 0.05$ compared with the respective control; ** $p < 0.02$ compared with the respective control

stimulation period no changes were observed among the control and experimental groups.

On the other hand, KCl 50 mM did not induce increments on the release of taurine during the 5 min period of stimulation, but it was found a tendency toward an increment in the post-stimulation period. In fact, as showed in Fig. 1B, during the stimulation period it was observed non statiscally significative decrements in the release of taurine both in the control and in the experimental group. When the experimental group was compared with the control one, we found an increment of the taurine release during the post-stimulation period which, however, did not reach statistical significance.

Discussion

The present study shows that chronic, but not acute, treatment with VPA induces a decrease on glutamate and taurine levels in corpus striatum. On the other hand we demonstrate the absence of effect of VPA on amino acid levels in cerebral frontal cortex after both acute and chronic treatment. We also observed that even in control animals there was a change in the contents of some of the amino acids measured. This unexpected finding might be evaluated in the context of the changes induced by stressful manipulations, in our case repetitive handling and injection, on amino acid contents and release. Although the response of GABA system to stress was widely studied there is little information about the effect of acute or chronic stress on other amino acid neurotransmitters. Palkovits et al. (1986) found an increase in aspartate and glutamate levels in cortical areas after pain stress and Bartanusz et al.

(1995) described that acute immobilization stress induces an increase in the cellular level of NR1 subunit messenger RNA in hyppocampus and hypothalamus with a concomitant decrease in the levels of AMPA subunit A glutamate receptor RNA messenger in hyppocampus. No reports were found on the effect of chronic handling on the endogenous neurotransmitters levels. So, as some authors demostrate a participation of the amino acid neurotransmission in the stress response, it would be possible to hypothesized that the differences on control values among acute and chronic treated groups may be due to the stressful experimental situation.

With respect to the effect of VPA on other than GABA amino acid levels there is little information. Schechter et al. (1978) reported a decrease in brain concentration of aspartate after acute treatment with 400 mg/kg in whole brain of mice. Similarly, Chapman et al. (1982) reported a reduction in aspartate concentration in whole brain both in rats and in mice after an acute treatment with 200 and 400 mg/kg, i.p. We could not replicate these results. As mentioned above, aspartate levels were not modified in our experimental conditions. Differences among those results and ours may be due to differences in the experimental approaches, as we dissected out and studied specific areas of the central nervous system, while the above mentioned authors worked with the whole brain. In fact, Biggs et al. (1992), who also studied the effect of VPA intraperitoneally administered (100, 200 and 400 mg/kg) but performed their quantifications in a discrete area of the rat brain (i.e. ventral hippocampus) reported absence of effect on aspartate together with glutamine and taurine contents. With respect to glycine concentration, 30 min after the injection we found no changes on its endogenous levels. Some authors demostrated time-course modifications on this parameter. In fact Martin-Gallardo et al. (1985) reported increments in the levels of such amino acid in whole brain after the acute administration of VPA (700 mg/kg, i.p.), but while this increment was detectable 1 hour after the intraperitoneal injection, higher values were achieved 2 hours after it.

Chronic effect of VPA on amino acid levels has not been almost subject to investigation. Patsalos and Lascelles (1981) reported that chronic treatment with two daily doses of 150 mg/kg i.p. of VPA increase aspartate and glutamate concentration in hippocampus and cerebral cortex. They also showed elevated brain taurine levels, specifically in cerebellum, pons/medulla, midbrain and cerebral cortex. They did not report changes in the content of glutamate or taurine in corpus striatum as we did. Differences among these and our results may be due to different dosage regime (two daily doses of 150 mg/kg versus one daily dose of 200 mg/kg). Furthermore these authors reported a plasma mean concentration of 139 μg/ml of the drug 1 hour after the last injection of 150 mg/kg, while we found a mean plasma concentration of 356 μg/ml of VPA at the time of decapitation 30 min after the last dose. Differences in regime dosage may account for pharmacokinetic diverse parameters. Dickinson et al. (1979) described that the decline of VPA in the blood of rats given different doses is not a single first order process. On the other hand Hönnack et al. (1992) signaled that the active metabolite of the drug, the trans-2-en VPA shows a concentration dependent decline, being the

half life of a dose of 100 mg/kg of 2–6 h and of a dose of 200 mg/kg of 4–11 h. Different regime dosage and different doses may produce different pharmacokinetic profiles. Our regime of administration results in a non accumulative kinetic neither of the drug nor of its metabolite, while regime dose used by Patsalos and Lascelles may produce a slight accumulation of the metabolite, whose effect on glutamate endogenous levels is not specifically investigated.

The decrease of striatal endogenous glutamate levels after chronic administration may be due either to an increment of its release or to a diminution of its synthesis. When we examined the release of glutamate evoked by KCl we observed that the tissue of chronically treated animals showed a greater release both in basal conditions and during the period of stimulation. These results may explain the decrease of endogenous levels seem above. Nevertheless, although GABA effects on glutamate levels or activity on glutamatergic neurons has not been described, we can not discard that the changes we found on glutamate levels and its release would be an epiphenomenon of the increments in GABA contents induced by VPA.

On the other hand we found that the level of the inhibitory amino acid taurine is diminished. Nevertheless, decrements in taurine levels were not accompanied by increments in its KCl evoked release in the in vitro experiments. Evoked release of taurine differs from that of other neurotransmitter amino acids in being of lesser magnitud (Oja and Kontro, 1987; Lehmann et al., 1986). Some other differences were described among taurine and other amino acids release. A singular aspect of K^+ stimulated taurine release is a delay phenomenon which has been seen with no other neurotransmitter. Philibert et al. (1988) reported that potassium produces an efflux of taurine from astrocytes with a lag time of 3–5 min. This was called an "off" effect of K^+ on taurine release (see Rev. Huxtable, 1989). In fact, in our post-stimulus sample (5 min after stimulation) we began to detect an increment of the release of taurine, which, however, did not reach statistical significative values.

As explained for glutamate the decrements on taurine levels either may be the result of a direct mechanism of VPA on taurine synthesis, storage or release (which we could not demostrate) or of an indirect action of GABA well documented modifications. Interactions between GABA and taurine systems were studied by various authors. Kontro and Oja (1987) demonstrated that GABA provoked an enhancement of the efflux of taurine in slices of cerebral cortex in mouse. Similary, and although GABA effects on glutamate levels or activity on glutamatergic neurons has not been described, we can not discard that the changes we found on glutamate levels and its release would be an epiphenomenon of the increments in GABA contents induced by VPA.

In summary our results demonstrate that chronic administration of VPA decreases the endogenous levels of glutamate and taurine in corpus striatum. The diminution of glutamate contents may be at least partially explained by an increment of its release, while changes in the content of taurine may be due to a different mechanism not elucidated. Further studies are required in order

to clarify the relationship between our finding and the still controversial mechanism of action of this antiepileptic drug.

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