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EFFECTS OF VALPROIC ACID ON SPINAL REFLEXES AND [^{3}H]MUSCIMOL AND [^{3}H]DIAZEPAM BINDING IN BRAIN MEMBRANES IN RATS

MASAYOSHI GOTO, YASUSHI HASEBE, KOKI KATO, TSUGIO KANEKO AND HIDEOMI FUKUDA

Department of Toxicology and Pharmacology, Faculty of Pharmaceutical Sciences, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo, 113, Japan

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It was examined whether the anticonvulsant activity of valproic acid (di-*n*-propylacetic acid, DPA) was exerted *via* the inhibitory GABA system in the central nervous systems (CNS). Dorsal root reflexes (DR-DRR) were not augmented but intensely suppressed following the administration of DPA (50 and 200 mg/kg, *i.v.*). DPA did not depolarize the resting dorsal root potentials. These electrophysiological findings may indicate that DPA does not potentiate GABA system in the spinal cord of rats. DPA (1 and 10 mM) neither affected the binding of 2 or 40 nM of [^{3}H]muscimol nor the binding of [^{3}H]diazepam in the rat brain membranes; the same concentrations of DPA did not affect the enhancement of binding of [^{3}H]diazepam induced by the addition of GABA (100 μM). The result obtained from binding assays indicates that DPA does not interact with the GABA-benzodiazepine receptor complex. The findings in the present study do not support the proposal that the anticonvulsant action of DPA is exerted by potentiation of the GABA system in the CNS.

Keywords—valproic acid; anticonvulsant; spinal reflex; muscimol binding; diazepam binding

INTRODUCTION

Valproic acid (di-*n*-propylacetic acid, DPA), a short-chain branched fatty acid, is an effective anticonvulsant in both animals and man.[1,2] Despite extensive clinical experiences, little is known of the exact mechanism of action of DPA. There are some studies supporting a concept that the GABA system in the central nervous systems (CNS) is involved in the action of DPA. DPA inhibits convulsions produced by pentylenetetrazole, picrotoxin, bicuculline and strychnine, and among these agents picrotoxin-induced convulsion is most effectively prevented by DPA treatment.[2] This fact indicates that DPA might potentiate the GABA system in the CNS. In addition, increase in the brain GABA concentration has been demonstrated in rodents following the systemic administration of DPA[3] and this increase is attributable to the inhibition of succinic semialdehyde reductase, the enzyme responsible for the subsequent stage in GABA catabolism.[4–6]

Turner and Whittle pointed out that the structure of DPA was similar to that of GABA.[7] It was demonstrated in the cultured spinal neurons that DPA potentiated the electrophysiological response to GABA.[8] Further studies on single neurons of the supraspinal areas revealed the potentiation of the inhibitory action of GABA by DPA.[9–11] These facts suggest that the potentiation may be mediated by an action of DPA at the GABA receptor complex present in the postsynaptic membranes. On the other hand, Blume *et al.*[12] reported that DPA augmented the excitability of the neurons in the cerebral cortex and in the hippocampus of rats. It was also reported that DPA potentiated the seizure response of EEG induced by bicuculline or picrotoxin administration.[13]

Thus, the mechanisms of action of DPA remain uncertain. In the present study, we examined whether DPA exerts a pharmacological

effect by acting on GABA system as suggested. It is concerned with effect on spinal reflex, effect on [^{3}H]muscimol binding in brain membranes, and effect on [^{3}H]diazepam binding in brain membranes.

MATERIALS AND METHODS

Spinal Reflexes in Rats — Male Wistar rats weighing 250—400 g were anesthetized with urethane (1 g/kg, *i.p.*) and chloralose (25 mg/kg, *i.p.*). Laminectomy was performed in the lumbosacral region. Ventral and dorsal roots below L4 were cut bilaterally, and dorsal and ventral roots of the segments L4 and L5 were isolated. A skin pouch was formed at the site of the dissection to cover the exposed tissues with liquid paraffin kept at 33—37°C. The dorsal root of L5 was placed on a bipolar silver electrode for stimulation (frequency 0.1 Hz, pulse duration 0.05 ms, supramaximal). The ventral root of L5 and the dorsal root of L4 were placed on bipolar silver electrodes for recordings. Monosynaptic (MSR) and polysynaptic (PSR) reflex potentials, and dorsal root-dorsal root reflex potentials (DR-DRR) were evoked in the ipsilateral ventral root and in the ipsilateral dorsal root, respectively. MSR, PSR and DR-DRR were amplified with an a.c.-amplifier and displayed on an oscilloscope (Nihonkohden VC-9). Five responses were photographed and averaged at each time. To record dorsal root potentials (DR-DRP) and resting dorsal root potentials (resting DRP), a pair of silver-silver chloride electrodes were placed on the dorsal root L4. The interelectrode distance was approximately 20 mm as described previously.[14] Potentials were amplified and recorded using a d.c.-recorder (Toa Electronics ERP-2T).

Valproic acid was dissolved in physiological saline and injected through a cannula inserted into the femoral vein after adjusting pH to around 7.1 with NaOH solution.

Assay of [^{3}H]Muscimol and [^{3}H]Diazepam Binding to Rat Brain Membranes — Male Wistar rats weighing 200—250 g were exsanguinated from the carotid arteries and brains except cerebellum, pons and medulla were removed. Crude synaptic membranes were obtained according to the method of Zukin *et al.*[15] Membrane fraction was suspended in buffer (50 mM Tris-citrate, pH 7.1, for [^{3}H]muscimol and 50mM Tris-HCl, pH 7.4, for [^{3}H]diazepam) and was centrifuged at 48000 × ***g*** for 20 min and the pellet was stored at −80°C until the measurement of binding.

To determine [^{3}H]muscimol binding, at the day of assay the frozen membranes were thawed, suspended in 50 mM Tris-citrate buffer containing 0.05% Triton X-100, incubated at 37°C for 30 min and centrifuged at 48000 × ***g*** for 20 min. The pellet was washed twice with the buffer without Triton X-100 by resuspension and recentrifugation. For [^{3}H]diazepam binding, the frozen pellet was thawed and washed three times with 50 mM Tris-HCl buffer by resuspension and recentrifugation at 48000 × ***g***.

For the standard binding assay for [^{3}H]muscimol, aliquots of final pellet were incubated in duplicate in 1 ml of 50 mM Tris-citrate

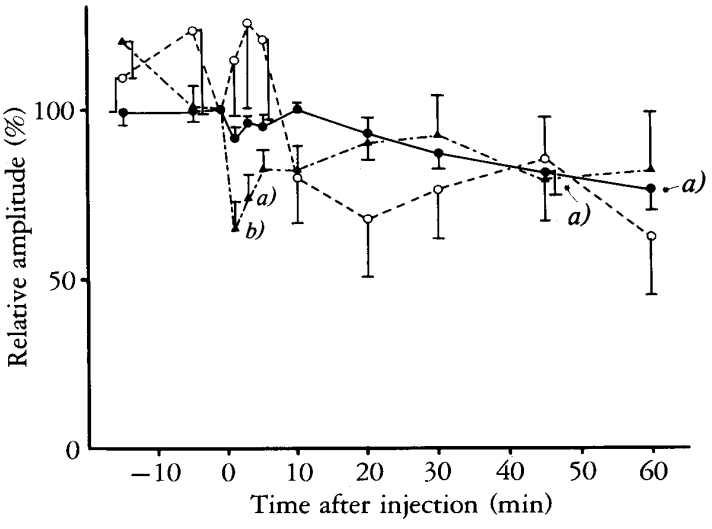

FIG. 1. *Effect of Valproic Acid (50 mg/kg, i.v.) on the Amplitudes of the Spinal Reflexes in the Anesthetized Rats*

Each point represents the mean of response amplitude in per cent of control, with the SEM indicated. (●) monosynaptic reflexes (n=5), (○) polysynaptic reflexes (n=4) and (▲) dorsal root reflexes (n=5).
a) $p<0.05$, b) $p<0.01$, calculated according to Student's t-test.

buffer (pH 7.1) containing 2 or 40 nM [^{3}H]muscimol (9.5 Ci/mmol) in the presence or absence of 1 mM unlabelled GABA for 30 min in an ice-cold bath. The binding assay for [^{3}H]diazepam (76.8 Ci/mmol) was the same as for [^{3}H]muscimol binding except that 50 mM Tris-HCl buffer (pH 7.4) containing 1 nM [^{3}H]diazepam in the presence or absence of 3 μM unlabelled diazepam was used. The incubation was terminated by adding 4 ml of ice-cold buffer and filtration under vacuum through Whatman GF/B, which was washed with 4 ml of ice-cold buffer. Radioactivity was measured by liquid scintillation spectrometry (Packard 3255). Specific binding was obtained by subtracting "non-specific" binding (the radioactivity bound in the presence of unlabelled ligand) from "total" binding (the radioactivity bound in the absence of unlabelled ligand). Protein was measured by the method of Lowry *et al.*[16] Drugs used were valproic acid (Tokyo Kasei), [^{3}H]muscimol (RCC Amersham) and [^{3}H]diazepam (New England Nuclear).

RESULTS

Effects of DPA on Spinal Reflexes and Dorsal Root Potentials

When the MSR, PSR and DR-DRR were measured following intravenous injection of 1 ml/kg of saline, these amplitudes did not vary significantly during at least 60 min. Although MSR and PSR remained unaffected by 50 mg/kg of DPA, the dose of 200 mg/kg suppressed MSR slightly (Figs. 1 and 2). DPA at 50 and 200 mg/kg significantly suppressed DR-DRR by 40 and 60%, respectively (Figs. 1 and 2). The recovery of DR-DRR to the control level was observed within a few minutes in the case of DPA at 50 mg/kg, but it was not observed within 10 min at the dose of 200 mg/kg.

DPA did not depolarize the resting DRP and delayed the repolarization of DR-DRP without

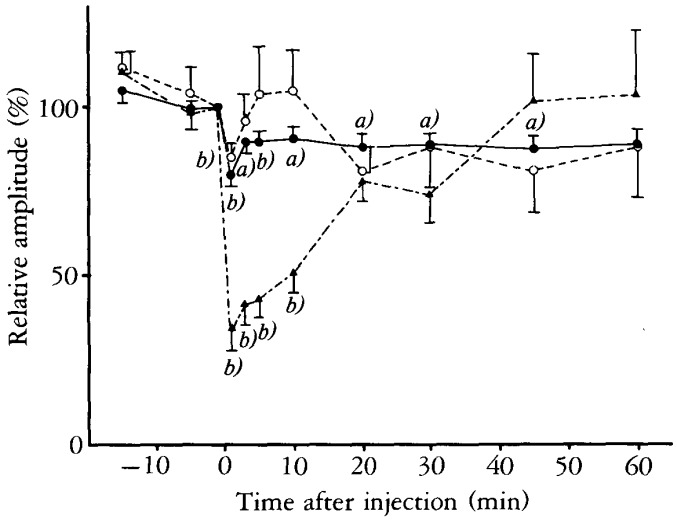

FIG. 2. *Effect of Valproic Acid (200 mg/kg, i.v.) on the Amplitudes of the Spinal Reflexes in the Anesthetized Rats*

(●) monosynaptic reflexes (n=10), (○) polysynaptic reflexes (n=4) and (▲) dorsal root reflexes (n=7).

a) $p<0.05$, b) $p<0.01$, calculated according to Student's t-test

Others are the same as in Fig. 1.

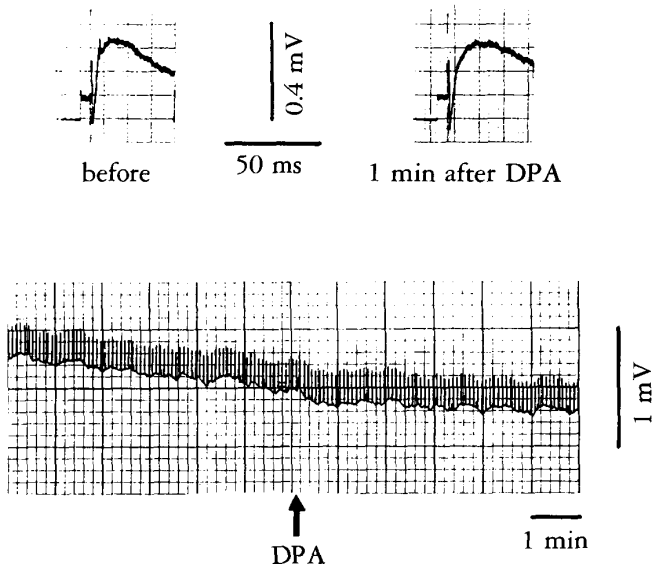

FIG. 3. *Effect of Valproic Acid (200 mg/kg, i.v.) on the Resting Dorsal Root Potential in the Anesthetized Rats*

The amplitudes of the dorsal root potentials were recorded as an upward pen deflection. The above traces are representative records.

affecting the amplitudes of DR-DRP (Fig. 3).

Effects of DPA on [^{3}H]Muscimol and [^{3}H]Diazepam Binding in Rat Brain Membranes

To examine the effect of DPA on [^{3}H]muscimol binding, two concentrations (2 and 40 nM) of [^{3}H]muscimol were used, because it was demonstrated that there were two components of the binding, high and low affinities, in the brain membranes.[17] Addition of DPA (1 and 10 mM) did not affect the binding of 2 and 40 nM of [^{3}H]-muscimol (Table I). These results may indicate that DPA does not act on GABA receptors directly.

Addition of DPA (1 and 10 mM) did not affect the [^{3}H]diazepam binding in the brain membranes (Table I); DPA did not affect the enhancement of [^{3}H]diazepam binding induced by 100 μM GABA (Table I).

DISCUSSION

The present study suggests that DPA does not potentiate the GABA system in the CNS, although several papers indicate pharmacological activation of GABA system by DPA.

It was examined whether DPA acted as a GABA receptor agonist or potentiated the GABA system like benzodiazepines in the CNS. Electrophysiological studies on the several neurons revealed that the action of DPA was ascribed to the potentiation of GABA system.[8-11] If DPA is a GABA mimetic agent, the followings will be observed as a result of DPA treatment: inhibition of [^{3}H]muscimol binding, potentiation of [^{3}H]diazepam binding[17,18] and depolarization of resting DRP.[19,20] If DPA potentiates the GABA system like benzodiazepines, DR-DRR will be increased.[21-23]

The results supported the finding that DPA did not affect the binding of GABA in the brain membranes,[24] although kinetics of the binding were not determined in this study. The idea that DPA is not a GABA mimetic agent comes also from our observations; binding of [^{3}H]diazepam in the brain membranes was not potentiated by the addition of DPA; DPA could not displace the binding of [^{3}H]muscimol, and DPA did not depolarize resting DRP.

DR-DRR reflects the presynaptic inhibition mediated by GABAergic interneurons and benzodiazepines increase DR-DRR by activation of the GABA system[25]; thus, benzodiazepines potentiate the presynaptic inhibition.[24] It was demonstrated in this study that DPA inhibited DR-DRR without depolarizing the resting DRP. This observation indicates that DPA does not potentiate the presynaptic inhibition. Another evidence is the fact that DPA did not affect the [^{3}H]diazepam binding.

It is known that DPA increases GABA content in the nervous tissue by inhibiting the metabolic enzymes of GABA[4-6] but it can not explain the anticonvulsant effect of DPA because the increase of GABA does not begin within 30 min.[3,25] It is also indicated that the anticonvulsant activity of DPA develops before the increase of GABA content in the nervous tissue.[10]

Thus, the findings in the present study do not offer the evidence that the anticonvulsant activity

TABLE I. *Effect of Valproic Acid on [^{3}H]Muscimol and [^{3}H]Diazepam Binding in Rat Brain Membranes*

Valproic acid (mM)	[^{3}H]Muscimol binding (fmol/mg protein)		[^{3}H]Diazepam (1 nM) binding (fmol/mg protein)	
	2 nM	40 nM	− GABA	+ GABA (100 μM)
0	705 ± 64	4644 ± 74	313.4 ± 12.2	623.2 ± 17.4
1	679 ± 81	4657 ± 64	315.6 ± 10.3	651.7 ± 2.9
10	683 ± 69	4535 ± 111	325.8 ± 9.9	663.4 ± 21.2

Each Value represents mean of 4 separate experiments with the SEM indicated.

of DPA is exerted by the potentiation of GABA system.

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