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Pharmacologic Analysis of Sodium Valproate-Induced Suppression of Secondary Components of Visual Evoked Potentials in Albino Rats[1]

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MYSLOBODSKY, M. S. AND M. MORAG. *Pharmacologic analysis of sodium valproate-induced suppression of secondary components of visual evoked potentials in albino rats.* PHARMAC. BIOCHEM. BEHAV. 15(5) 681–685, 1981.—Sodium Valproate (VPA) administered to rats in a dose of 10 or 200 mg/kg IP suppressed the slow negative wave (SNW) and photically-induced afterdischarge (SAD) of VEP (when they were present) within 15–30 min. The recovery of VEP amplitude began at 3 hr. This effect was antagonized by subconvulsive doses of convulsant benzodiazepine RO 5-3663 (2 mg/kg) and metrazol (15 mg/kg) but not by picrotoxin (2 mg/kg) and naloxone (10 mg/kg). The SNW suppression may be attributed to a disinhibitory action of a system located presynaptically on recurrent collaterals of the output neurons, or nerve terminals of inhibitory interneurons or both. Alternative conjecture suggests that VPA depolarizes the dendritic tree masking thereby somatic inhibiton produced by recurrent circuits.

GABA Sodium valproate Benzodiazepine Visual evoked potentials

ALTHOUGH the mechanism of action of the potent anticonvulsant, sodium valproate (VPA) is believed to be linked to the action of GABA [11, 12, 17, 28, 29, 34, 36, 37] contradictory evidence has been encountered. When administered to a rat, the drug has been shown to cause a suppression of photically-induced sensory afterdischarge (SAD) and its first component, a slow secondary negative wave (SNW) of the visual evoked potential [22,33]. The microelectrophysiological examination of these VEP components conducted by several groups [5, 7, 13, 20] has demonstrated that the slow negative wave of the VEP is most likely to be organised by recurrent strychnine-insensitive inhibition, mediated by a pathway through axon collaterals and interneurons feeding back synaptic inhibition on the output and/or adjacent cells. Given these findings it would seem puzzling that post-synaptic inhibition, supposedly a GABA-mediated process [9,15], is suppressed by a drug reportedly enhancing the concentration of GABA within nerve-endings.

Moreover, in a previous study we have shown that GABA-T inhibitor, γ-vinyl GABA produces a powerful and lasting facilitation of the SNW and SAD. VPA given on this background produced reversible suppression of hypersynchronized secondary potentials with a time-course similar to that after VPA administration alone [22]. These findings have been interpreted as suggesting that VPA acts via a mechanism (circuit) using a transmitter intimately connected with but not identical to GABA.

In view of this evidence the present study was designed (1) to replicate effects of VPA using a dose of the drug which caused SNW suppression in epileptic patients [21]; and (2) to examine VPA interaction with several antagonists of GABAergic synaptic transmission.

METHOD

Subjects and Surgery

VEPs were recorded in male and female rats (150–220 g) kept in a standard laboratory environment with food and water ad lib. Silver ball electrodes were implanted bilaterally extradurally over the visual cortex (4 mm laterally to lambda) as described elsewhere [32].

Procedure and Apparatus

Each rat was connected to a cable and placed into an electrostatically shielded cage filled with mirror panels on the sides and floor. A 10 μsec flash with intensity set at "4" (Grass PS22 model) was delivered randomly on artifact-free EEG background (monitored by Beckman type R Dynograph, band width 0.53 Hz to 30 Hz). Potentials were re-

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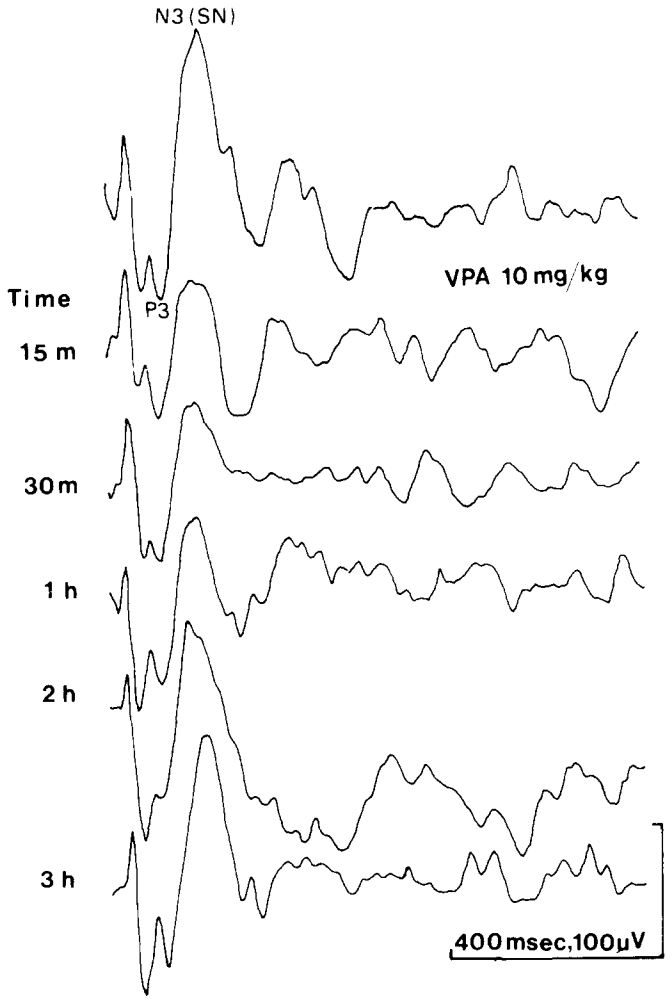

FIG. 1. Suppression of the SNW component of VEP by VPA (10 mg/kg, IP). The top trace depicts the control response. Numbers on the left denote time after VPA administration. Each trace is an average of 20 individual responses. Negativity of the active electrode is upwards. The presentation of the stimulus coincides with the beginning of the trace.

corded monopolarly against a reference electrode positioned over the frontal lobe. Blocks of 20 VEPs were averaged online with PDP-8 minicomputer and plotted by X-Y plotter (Hewlett-Packard 7035B).

Rats were taken to the experiment after at least five days had elapsed after surgery. The VPA effect was studied in three different samples. VPA (200 mg/kg) (n=3); VPA (10 mg/kg) and control Saline group (n=3). VEPs were taken at 0.25, 0.5, 1, 2 and 3 hr after injection of either the drug of saline.

In a separate series of experiments an attempt was made to antagonize VPA effects with picrotoxin (2 mg/kg) (n=3), metrazol (15 mg/kg) (n=3), convulsant benzodiazepine Ro 5-3663 (2 mg/kg) (n=3), and naloxone (10 mg/kg) (n=3). The results were compared to Saline-injected rats (n=3) and rats treated with VPA (200 mg/kg) alone (n=3). All drugs were dissolved in Saline solution and administered IP 15 min after

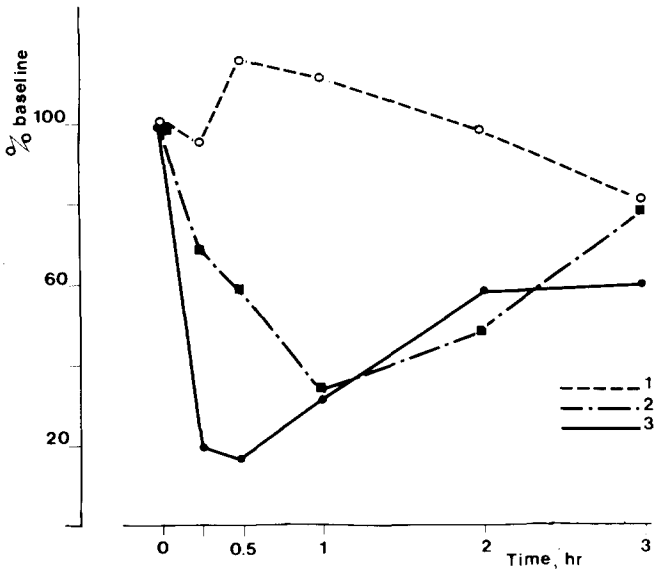

FIG. 2. The IP injection of VPA caused a decrease in the area covered by SNW-SAD during 3 hr of 4 hr of observation period. 1=Saline control; 2=VPA (10 mg/kg) and 3=VPA (200 mg/kg). Each point represents the average value of 3 experiments obtained from three rats.

VPA. The latency of these drug effects was considered in order to obtain the maximal change at about 30 min after VPA administration.

RESULTS

The averaged VEPs were similar in pattern to those described by this and other groups [7, 20, 22, 32, 33]. In brief, the VEP consisted of a primary negative (N_1) or positive-negative (P_1-N_1) complex with a peak latency at about 18 msec. This was followed by a positive-negative complex (P_2-N_2) and a high amplitude slow negative wave (SNW or N_3) with peak latency at about 170 msec. In 14 of 24 cases the SNW was the first component of the sensory after-discharge (SAD), composed of 2–7 waves.

VPA Effect

VPA adminstration in a dose of 200 mg/kg (IP) caused a distinct reduction of the SNW and SAD in *all* animals studied (Fig. 1). Reduction varied from 40 to 60% below the baseline. Group values of this effect for the SNW and SAD areas are shown in Fig. 2. The figure demonstrates that the effect was time-dependent, with the SNW decrement at 15–30 min and slow recovery between 2 and 3 hr after the drug. ANOVA (treatment group × recording session) showed a significant interaction between the groups and recording sessions, $F(1,5)=4.51$, $p<0.01$, and $F(1,5)=10.15$, $p<0.01$, for 200 and 10 mg/kg, respectively. This interaction effect results mainly from the difference between groups in sessions at 15 min, $t(4)=6.05$; $p<0.01$ and $t(4)=2.42$; $p<0.05$) and at 30 min, $t(4)=4.87$; $p<0.01$, and $t(4)=2.60$; $p<0.05$ for 200 and for 10 mg/kg, respectively.

GABA Antagonists-VPA Interaction

To test the hypothesis that VPA effects are mediated via

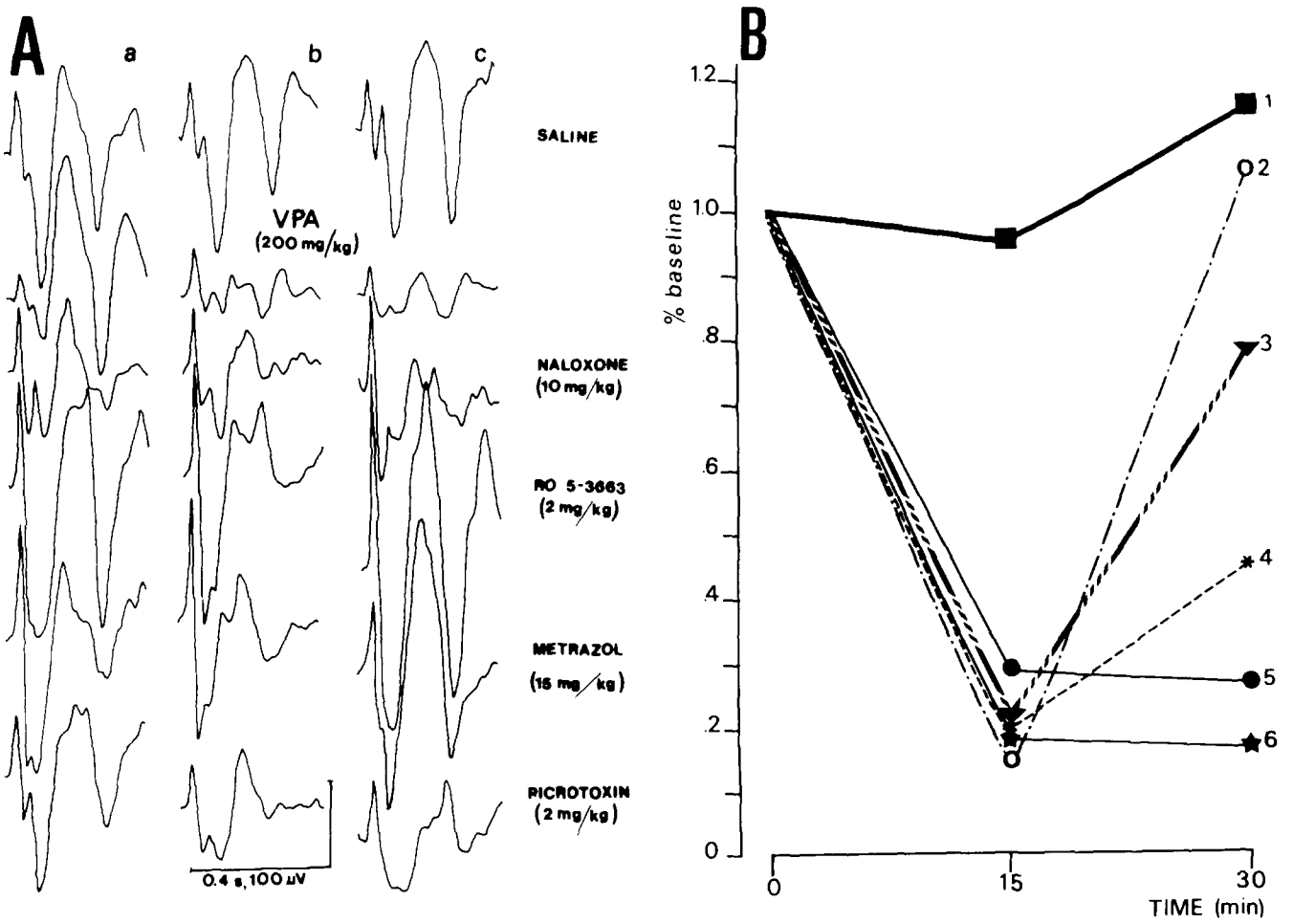

FIG. 3. Effects of GABA antagonists on VPA-induced suppression of the SNW. (A) Traces from selected experiments taken prior to drugs administration (a) and (b) 15 min after the injection of VPA (200 mg/kg). (c) Effects of saline, naloxone, Ro 5-3663, metrazol and picrotoxin. VEPs were recorded 30 min after VPA. The compounds were administered 5–10 min before the records were obtained. (B) Time dependent effect of VPA (6) and the effects of Ro 5-3663 (2), metrazol (3), picrotoxin (4) and naloxone (5). Only Ro 5-3663 and metrazol reliably antagonized VPA effects. The area covered by SNW-SAD was not different from saline alone (1) and different from VPA alone at $p<0.05$ (t-test, two-tailed comparison based on ANOVA). Each point represents the average value of experiments with three drug-naive rats.

the GABA system, several compounds with GABA antagonistic properties were administered after SNW suppression was achieved. These are picrotoxin, metrazol, and Ro 5-3663. In addition, the opiate antagonist naloxone was employed which is known also to act as a GABA antagonist [10].

The effects of metrazol and picrotoxin alone have been described in detail elsewhere [20,24]. The antagonism of GABA-mediated activity in the spinal cord by Ro 5-3663 has been demonstrated recently by Schlosser and Franco [31]. Our preliminary exploration of this compound showed that at 2 mg/kg (IP) it activated within 2–5 min, a hypersynchronisation of EEG in the theta-frequency band and caused a powerful augmentation of the photically induced SAD. The effect was similar to that caused by about 10–15 mg/kg of metrazol. Initially infrequent myoclonic jerks became more evident and regular at 3 mg/kg. A dose of 3–4 mg/kg evoked an interesting form of generalized seizure with a brief tonic phase followed by an extended period of clonus executed in a reared ("kangaroo") position [23].

All drugs were administered to drug naive rats after the clear signs of VPA suppression (observed 15 min after VPA administration) were documented. The latent period of the drugs was considered in order to obtain the maximal effect at about 30 min after VPA. The results of these experiments conducted in six groups of animals are summarized in Fig. 3A and B. It demonstrates that Ro 5-3663 completely antagonized and metrazol partially antagonized the VPA-induced SNW suppression. Picrotoxin was least effective while naloxone was not effective at all. An interesting aspect of the difference between picrotoxin and metrazol effects was that metrazol facilitated the SNW suppressed by VPA but not the SAD; Ro 5-3663 enhanced both (data not shown).

ANOVA conducted for a period of 15 and 30 min gave a significant interaction (treatment group × session) for Ro 5-3663, metrazol and picrotoxin, $F(1,4)=8.80$, $p<0.05$;

$F(1,4)=15.50$, $p<0.05$; $F(1,4)=11.83$, $p<0.05$, respectively. However, only Ro 5-3663, $t(4)=2.80$, $p<0.05$, and metrazol, $t(4)=3.55$, $p<0.05$, were significantly different from VPA alone at 30 min. Neither Ro 5-3663 nor metrazol were significantly different from saline at 30 min.

DISCUSSION

In the present as well as the preceding studies, the VPA-induced SNW-SAD suppression was robust and maximal within 15–30 min after the drug's administration with a slow decline of the effect at 3 hr. An interesting feature of this time course is that it seemed to correspond perfectly to changes in brain GABA content, reported by several groups [29, 34, 37]. While the doses of VPA employed in the present study are known *not* to cause detectable changes of whole brain GABA levels [4], it may be argued that electrophysiological changes after VPA are mediated through a synaptosomal GABA increase [11,28]. However, it remains to be proven that a dose as low as 10 mg/kg may affect presynaptic GABA content.

In view of this uncertainty the findings with GABA antagonists may be suggestive of a possible site of VPA action.

Of four compounds tested only Ro 5-3663 and metrazol reliably antagonized VPA-induced SNW suppression. O'Brien and Spirt [25] believe that Ro 5-3663 may interact at both the picrotoxin-sensitive chloride ionophore and allosteric GABA-benzodiazepine complex. However, picrotoxin which has convulsant potency near that of Ro 5-3663 was least effective in reversing the VPA effect. Also, glycine reponses which are mediated by an increase in chlorine conductance are not affected by VPA [19]. Given this evidence it is likely that Ro 5-3663-VPA interaction occurs at GABA-benzodiazepine receptor sites. Whether this speculation should be extended to metrazol is yet to be explored. Macdonald and Barker [18] have reported that in mouse spinal cord neurons (tissue culture preparation) metrazol antagonized reversibly and in a dose-dependent manner the conductance changes and voltage response produced by the GABA inontophoresis. Only further analysis can determine to what extent this mechanism would apply to GABA-benzodiazepine-mediated inhibition in the sensory system of the mammalian brain.

The nature of SAD generation has been interpreted [1, 13, 20] on the basis of inhibitory phasing theory [2]. In this theory recurrent IPSPs play a dominant role in phasing the activities of thalamocortical relay cells while the post-anodal excitation ("rebound") is believed to reset a new inhibitory event. According to Shearer *et al.* [33] VPA presumably suppresses "rebound excitation." However, an inhibition of the SNW produced by VPA in the present study clearly indicates that postanodal excitation may be inhibited along with preceding long lasting IPSP, which is recorded by a cortical electrode as the SNW.

A possible site for disinhibitory effects of the type obtained with VPA may be the presynaptic terminals impinging on terminals of inhibitory neurons and/or recurrent axon collaterals of the output neurons. Benzodiazepines are known to enhance presynaptic inhibition on the spinal cord primary afferent [27,30] and in the cuneate nucleus [26]. The presence of a similar arrangement in the visual cortex and the lateral geniculate, or both, is likely. While benzodiazepine receptors are found throughout the CNS, the density of binding sites is highest in cortical areas [35]. The likelihood of VPA action via the benzodiazepine receptor localized on GABA terminals is strengthened by the fact that diazepam has been reported to suppress the SNW and SAD in the rat [6,8]. Alternatively, given that a depolarizing response to GABA [1] and benzodiazepines [14] may be a property of axonal terminals as well as dendrites, the possibility that VPA may cause the depolarizing response of the dendritic tree should be considered. This mechanism has been demonstrated to promote somatic hyperpolarization in the hippocampal pyramidal cells [1, 3, 14]. It is conceivable that this "tonic" hyperpolarization masks an actual *increment* of inhibitory action produced by "classical" recurrent chains, registered as the SNW depression. This hypothesis is in agreement with findings that VPA potentiates GABA-mediated postsynaptic inhibitory responses [19].

In summary, the present findings add to the conjecture [19,29] that VPA acts via a mechanism independent of elevation of CNS GABA. The decision as to whether the therapeutic effects of VPA are related to the benzodiazepine or other neurotransmitter systems must await further *in vitro* and *in vivo* biochemical, electrophysiological and behavioral studies.

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