

# Is the Site of Action of Ethosuximide in the Hindbrain ?

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## Summary

The action of ethosuximide, valproate and clonazepam against pentylenetetrazol-induced epileptic EEG phenomena was studied in acute experiments in rats with intercollicular brainstem transection. Ethosuximide lost its action against both rhythmic metrazol activity (model of human absences) and EEG seizures. On the contrary, the action of valproate and clonazepam in *cerveau isolé* rats was the same as in intact animals. The site of anticonvulsant action of ethosuximide may be localized in hindbrain structures, whereas the actions of both valproate and clonazepam may be demonstrated even if hindbrain structures had been eliminated.

## Key words

Pentylenetetrazol – Ethosuximide – Valproate – Clonazepam – Epileptic EEG activity – Brainstem transection

## Introduction

There are many data on the action of individual antiepileptic drugs in various seizure models (for review see Woodbury *et al.* 1972, 1982, Vida 1977, Glaser *et al.* 1980, Levy *et al.* 1989, Rogawski and Porter 1990) and there is an increasing amount of findings on the action of antiepileptics at the cellular and molecular level (for review see Rogawski and Porter 1990, de Lorenzo 1991, Macdonald 1991). On the contrary, the studies of structures necessary for the action of different antiepileptics are only exceptional (Gale 1989). These studies may be useful for molecular biology to avoid analyses performed on irrelevant models.

One of the classical antiepileptic drugs with unknown mechanisms of action is ethosuximide (ESI), (for review see Ferrendelli and Klunk 1982, Ferrendelli and Holland 1989). Recently, the studies of Prince's group demonstrated one possible mechanism of action of this drug – blockade of low threshold calcium channels in thalamic neurones (Coulter *et al.* 1989a,b). On the other hand, there are some older data of Kästner *et al.* (1968) hypothesizing that the brainstem is of primary importance for the anticonvulsant ESI action. The brainstem reticular formation plays a role in the generation of absence seizures (Gloor 1984,

Gloor *et al.* 1990), which represent the only type of human seizures sensitive to ESI (Sherwin 1982). We therefore decided to test the possible brainstem site of action of ESI in rats with mesencephalic transection of the brainstem at the intercollicular level. Pentylenetetrazol-induced electrocorticographic phenomena (primarily rhythmic metrazol activity elicited by low doses of pentylenetetrazol as a possible model of human absences – Schickerová *et al.* 1989, Snead 1992) were used in this study. Two other antiepileptic drugs active against human absence seizures – valproate and clonazepam – were included for comparison.

## Material and Methods

Experiments were performed in adult male albino rats of the Wistar strain. Surgical preparation (trepanation of the skull, tracheostomy and insertion of the tracheal cannula) as well as stereotaxic intercollicular transection of the brainstem at the level 6.5 mm posterior to the bregma (the classical "*cerveau isolé*" preparation of Bremer (1936), prepared according to the method of Burešová *et al.* 1962) was made under ether anaesthesia. In this preparation all

sensitive nerves (including the trigeminal nerve) enter the central nervous system below the level of the transection and thus somatosensory information could not reach higher brain levels and pain perception is eliminated. The animals were then immobilized by curare (0.2 mg/kg i.p.), connected to a positive pressure respirator with a frequency of 60/min and ether anaesthesia was disrupted. The extent of the transection was controlled macrophotographically on sagittal sections of the brainstem after the end of the experiments and only animals with complete transection (approximately 70 % of operated rats) were included in this study.

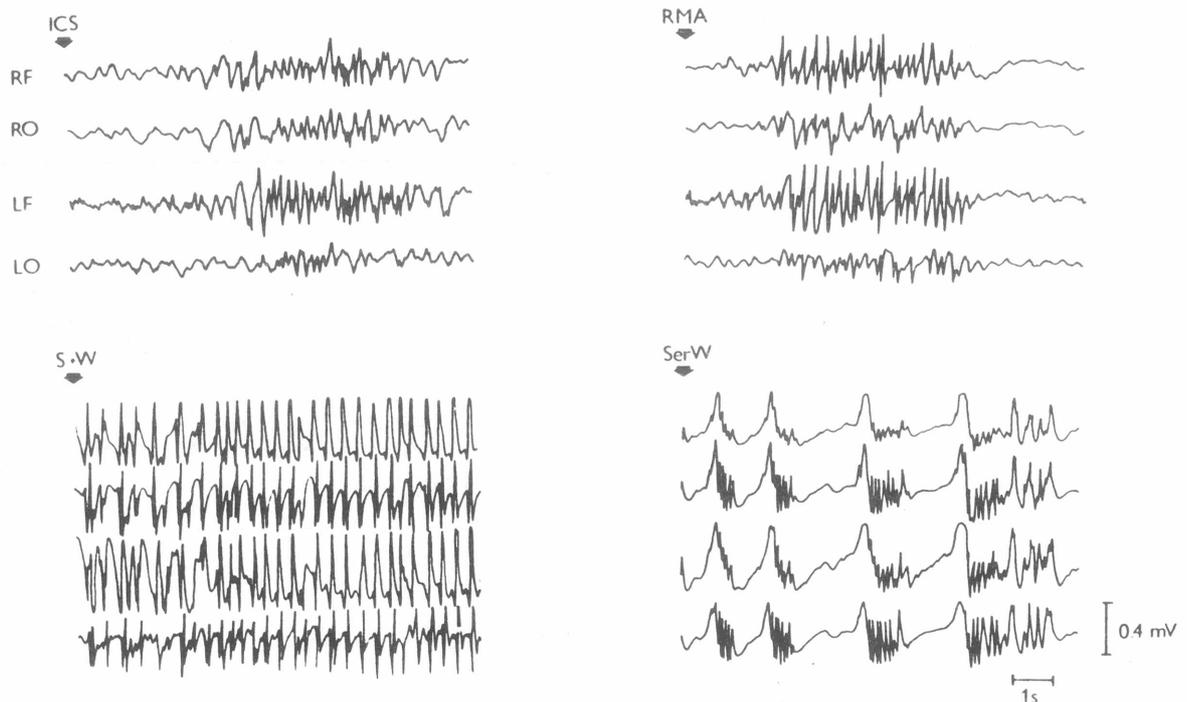
Silver ball recording electrodes were placed on the surface of the frontal (sensorimotor) and occipital (visual) cortical regions of both hemispheres, an indifferent electrode was on the nasal bone. EEG activity was recorded in both reference and bipolar connections, ECG recording served as a control of the animal's state.

All animals received an injection of pentylenetetrazol (PTZ, metrazol, freshly prepared 10 % solution). Doses of 20 mg/kg were repeatedly administered every 5 min by means of an intraperitoneal cannula up to the elicitation of the generalized electrocorticographic seizure activity. The maximum number of PTZ doses was restricted to 15 (i.e. the last dose was given 70 minutes after the first one) to avoid the elimination of PTZ from the brain (Brink *et al.* 1970). Rats not exhibiting EEG seizures even after 15 doses of PTZ, were considered as fully protected. Four experimental groups were studied:

1. Ten rats given only PTZ
2. Eleven rats pretreated with ethosuximide (ESI, Arzneimittelwerke, Dresden, 125 mg/kg i.p.)
3. Twelve rats pretreated with valproate (VPA, Gerot Pharmazeutica, 300 mg/kg)
4. Eight rats pretreated with clonazepam (CZP, Rivotril<sup>R</sup> Roche, 0.1 mg/kg)

All antiepileptic drugs were administered intraperitoneally 10 min before the first dose of PTZ.

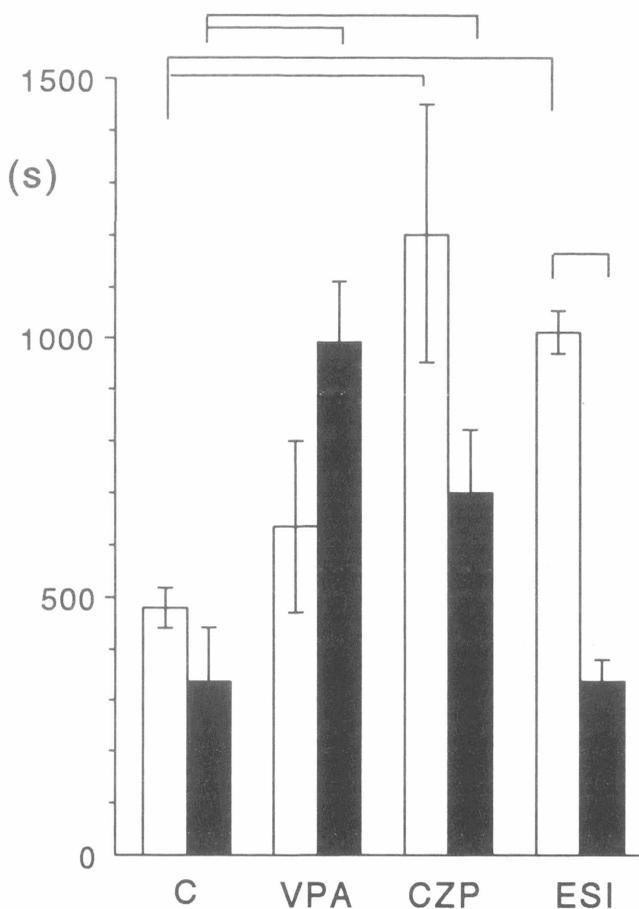
#### Metrazol Activities after ICS



**Fig. 1**

Examples of spindles appearing spontaneously in cerebellar slice preparations (upper left, ICS) and three types of EEG epileptic activity: rhythmic metrazol activity (upper right), EEG seizures formed by spike-and-wave rhythm (lower left) and EEG seizures formed by "serrated waves", i.e. delta waves with superimposed fast activity (lower right). In all sections the leads from top to bottom: right frontal (RF) and occipital (RO), left frontal (LF) and occipital (LO) cortical regions in reference connection. Time mark is 1 s, amplitude calibration 0.4 mV.

The latencies of the first electrocorticographic spike-and-wave rhythm (rhythmic metrazol activity), of the first ictal activity and of the first generalized ictal activity were measured and the values were compared by means of two-way ANOVA with grouping factors drugs (four levels) and surgery (two levels). The four groups of rats with an intact brainstem were taken from the previous experiments performed by the same technique in rats of the same strain, sex and weight (Mareš and Velišek 1986, Velišek and Mareš 1987). These four groups without brainstem section consisted of seventeen (controls), nine (ESI-pretreated), eight (VPA-pretreated) and eleven (CZP-pretreated) rats, respectively. Statistical significance was set at the 5% level.



**Fig. 2**  
Latencies (mean  $\pm$  S.E.M.) of rhythmic metrazol activity in control untreated rats (C), animals pretreated with valproate (VPA, 300 mg/kg i.p.), clonazepam (CZP, 0.1 mg/kg i.p.) and ethosuximide (ESI, 125 k/kg i.p.). Abscissa - individual drugs, open columns denote results from animals with an intact brainstem, black columns indicate rats with a transection of the brainstem. Ordinate - latency in hundreds of seconds. Statistically significant differences are marked with bars over columns.

## Results

### *Electrocorticogram of "cerveau isolé" rats (Fig. 1)*

Spontaneous ECoG of animals with brainstem transection was characterized by the appearance of rhythmic activity ("spindles"). This activity was always formed by waves, spikes were never recorded. This feature served for differentiation of this phenomenon from rhythmic metrazol activity.

### *Rhythmic metrazol activity (Figs 1 and 2)*

Intercollicular transection of the brainstem changed neither the shape nor latency of the spike-and-wave activity. Significant differences were found among the drugs. ESI, which exhibited a specific effect against this activity in rats with an intact brainstem, completely lost its efficacy. VPA, which exhibited only a tendency to prolongation of the latency in intact rats, demonstrated a significant effect in animals with intercollicular transection. CZP was effective under both conditions, in intact as well as *cerveau isolé* rats but the action was less expressed after the brainstem transection.

### *Electrocorticographic seizure activity (Figs 1 and 3)*

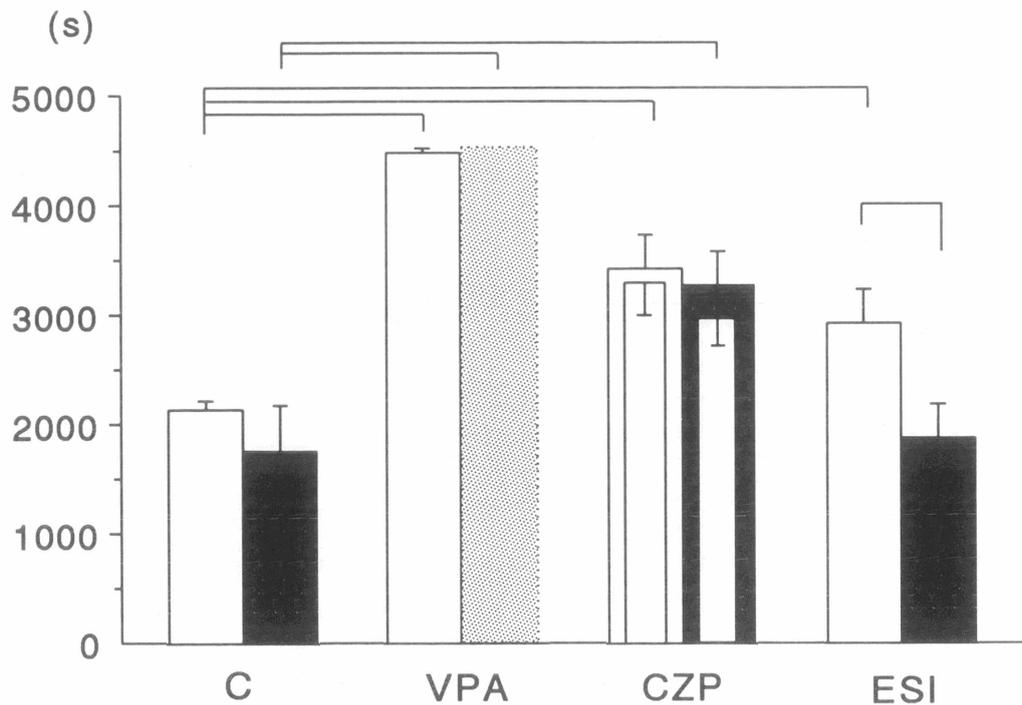
There was again no change in the latency of ECoG seizures in *cerveau isolé* rats compared to intact animals, but the pattern of seizures changed. The EEG seizure pattern formed by delta waves with superimposed fast activity represented in *cerveau isolé* rats an alternative to the spike-and-wave rhythm recorded in animals with an intact brainstem. The effects of ESI were exactly on the border of statistical significance in intact animals. The latencies in control and ESI-pretreated *cerveau isolé* rats were practically the same while those of the first seizure after ESI pretreatment were significantly shorter in *cerveau isolé* rats than in intact animals. The efficacy of both VPA and CZP was preserved after brainstem transection; in pretreated intact and sectioned rats the latencies of seizures were identical and always significantly longer than in the appropriate controls. In addition, VPA completely blocked the appearance of seizures in *cerveau isolé* preparations. The specific ability of CZP to change the generalized onset of EEG seizures to a partial one was also demonstrated in *cerveau isolé* animals.

## Discussion

Pentylentetrazol was found to elicit the same epileptic EEG phenomena in both intact and brainstem-transected rats. These results disagree with those of Velasco *et al.* (1975) since they speak in favour of the brainstem as a site of generation of metrazol-induced epileptic seizures. The disagreement might be explained by the differences in experimental

paradigms: fractionated low doses of pentylenetetrazol and EEG phenomena taken as an endpoint in our experiment in comparison with a single dose of PTZ and multiple unit activity in Velasco's experiments. Our schedule might be more convenient for activation of the thalamocortical system (generator of rhythmic

metrazol activity) which is highly sensitive to pentylenetetrazol according to the EEG symptoms (Zouhar and Mareš 1972). The role of the brainstem is probably in the regulation of the excitability more or less favourable for the generation of the spike-and-wave rhythm (Gloor 1984, Jinnai *et al.* 1969).



**Fig. 3**

Latencies (mean ± S.E.M.) of EEG seizures. Value for cerveau isolé animals pretreated with VPA is drawn with dotted column because of complete suppression of seizures in this group; the duration of the experiment (4500 s) is therefore represented. Inset columns in the two clonazepam-pretreated groups denote latencies of the very first, nongeneralized EEG seizures.

Our results demonstrated the dependence of ESI action in the PTZ model on the intact brainstem. Not only the high efficacy against rhythmic metrazol activity but also the less expressed action against ECoG seizures was lost in rats with the transected brainstem. Our results are thus in agreement with the data of Kästner *et al.* (1968) localizing the site of action of ESI into hindbrain structures. It is not probable that ESI exerts its action through the cerebellum; a more plausible mechanism could act through some of the systems originating in the brainstem which send their projections diffusely into diencephalic and telencephalic structures. Noradrenergic, dopaminergic, serotonergic and possibly also cholinergic systems have to be taken into account – especially in connection with the hypothetical involvement of the dopaminergic system in the action of ESI (Klunk and Ferrendelli 1980). Our unpublished data demonstrating a

possibility to block ESI action against pentylenetetrazol-induced motor seizures by means of yohimbine, but not ritanserin, haloperidol or atropine, speak in favour of the role of the noradrenergic  $\alpha_2$  system in the anticonvulsant ESI action.

The unchanged action of VPA and CZP in animals with the brainstem transection was not surprising. Both these drugs exhibit their anticonvulsant action by increasing the efficacy of the inhibitory GABAergic system albeit at different sites (for review see Johnston and Slater 1982, Johnston 1984, Haefely 1989). The GABAergic system is widely distributed in the central nervous system so that these drugs may probably influence both supra- as well as infracollicular structures.

The variance with results of Coulter *et al.* (1989a,b) might signify that ESI like other anticonvulsant drugs acts by more than one

mechanism. The different mechanisms of action may play a role in various seizure models and their individual contributions still have to be analyzed.

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