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### Original article

# Effect of picrotoxin and cyproheptadine pretreatment on sodium valproate induced wet dog shake behavior in rats

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#### ABSTRACT

Sodium valproate, a broad spectrum antiepileptic elevates the brain GABA levels by various mechanisms. Histological, electrophysiological and the biochemical studies suggest a regulatory role of GABA on dopaminergic neurons. Behavioral studies in animals provide an additional evidence for interaction between GABAergic and DAergic systems. Valproate at 200-500mg/kg induces Wet Dog Shake (WDS) behavior in rats. The WDS behavior in rats and head twitch responses in mice is evoked by 5- hydroxy-tryptophan (5-HT), the 5-HT precursor, the directly acting non-selective 5-HT receptor agonists and 5-HT releasers. In order to determine the involvement of GABAergic and 5-HTergic mechanism in the induction of WDS behavior by valproate in rats, the study was taken upto investigate the effect picrotoxin and cyproheptadine pretreatment on valproate induced WDS in rats. It was observed that subconvulsant doses of picrotoxin failed to antagonize valproate induced WDS while cyproheptadine effectively antagonized valproate induced WDS behavior in rats. Based on our study we propose that GABAergic and 5-HTergic systems are involved in production and controlling WDS behavior evoked by valproate in rats.

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### 1. Introduction

Sodium valproate, a broad spectrum antiepileptic drug elevates the brain GABA levels by stimulating the synthetic enzyme, and by inhibiting GABA degradative enzymes [1,2]

Histological, electrophysiological and the biochemical studies suggest a regulatory role of GABA on dopaminergic neurons [3,4]. Behavioral studies in animals have provided additional evidence for interaction between GABAergic and DAergic systems [5].

It was observed in our study that sodium valproate at 200-500 mg/kg ip dosage induced wet dog shake (WDS) behavior, in rats. The WDS behavior in rats and head twitch responses (HTR) in mice is evoked by 5-hydroxy -tryptamine (5HT, serotonin) or the 5-HT precursor 5-hydroxytryptophan (5 HTP), the directly acting non selective 5HT receptor agonists ergometrine and 5 methoxy-N, N-dimethyl tryptamine (5-MeODMT) and the 5HT releasers p-chloramphetamine (PCA) and fenfluramine, through activation of the central 5HT2A receptors [6,7,8].

In order to determine the involvement of GABAergic and 5HTergic mechanism in the induction of WDS behavior by valproate in rats, the study was taken up to investigate the effect of picrotoxin and cyproheptadine pretreatment on sodium valproate induced WDS behavior in rats.

### 2. Materials and Methods:

Albino rats of either sex, weighing between 100-180g, were used. They were allowed food and water ad libitum upto time of experimentation. Each animal was used only once. All observations were made between 10-17hrs at 27°C in a noiseless, diffusely illuminated room. Each group consisted of 10 animals.

Drugs used were sodium valproate (Reckitt & Colman), picrotoxin and cyproheptadine. All drugs were dissolved in distilled water only. All drug solutions were prepared immediately before use and were injected intraperitoneally. Volume of injection was 2ml/kg body weight for picrotoxin while 5ml/kg body weight for valproate and cyproheptadine.

For observation of WDS behavior the animals were placed individually in open topped perspex cages (30X20X20 cm) immediately after injection of sodium valproate. The number of head shakes and whole body shakes were counted over 30 min periods after administration of valproate.

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Picrotoxin was injected 30 min before valproate while the control group received normal saline 2ml/kg, 30 min before receiving valproate. Cyproheptadine was injected 45min before valproate while the control groups received normal saline 5ml/kg ip, 45min before receiving valproate.

Picrotoxin was tested in the dose of 1, 2, and 3mg/kg, while cyproheptadine was tested in the dose of 5 and 10mg/kg.

The total count of WDS of each rat was taken to compute the mean value of the group.

The study was undertaken at Krishna Institute of Medical Sciences, Karad. Maharashtra. All the procedures were performed in accordance with CPCSEA guidelines & the study was carried on following the approval of the approval of IAEC (Institutional Animal Ethics Committee).

### 2.1. Statistics

The results were statistically analyzed by the students compared t-test with differences considered significant at  $P < 0.05$ .

### 3. Results

In preliminary experiments 50, 100, and 150mg/kg of sodium valproate did not produce WDS behavior in rats. However in the dose range 200 to 500mg/kg sodium valproate induced dose dependent degree of head and whole body shakes in rats (Table-1).

**Table-1 shows: Dose dependency of WDS response induced by sodium valproate (VAL) in rats. Values are Mean  $\pm$  S.E.M (n=10) of the number of head and whole body shakes occurring in 30min period following VAL administration.**

Drug and dose	Number of WDS, Mean $\pm$ S.E.M.
VAL 50	0.0
VAL 100	0.0
VAL 150	0.0
VAL 200	16.2 $\pm$ 1.8
VAL 300	38.7 $\pm$ 2.2
VAL 400	58.4 $\pm$ 2.4
VAL 500	67.7 $\pm$ 2.7

The WDS behavior manifested within 5-7 min of valproate administration, with maximum frequency between 10-15min time interval after valproate injection and depending on dose used lasted for about 30-45min after which animals became sedated, exhibited ptosis, piloerection and hunched back posture. Though the animals were sedated and exhibited ptosis they however, gave a negative response when tested for catalepsy.

In the group receiving 500mg/kg dose of sodium valproate there was 20% (n=10) mortality. Hence for subsequent studies this dose of sodium valproate was not used.

Picrotoxin 1, 2 and 3mg/kg and cyproheptadine 5 and 10mg/kg did not induce WDS behavior in rats. Picrotoxin, at 1 & 2mg/kg did

not induce convulsions while at 3mg/kg it did induce mild intensity, brief episodic convulsions without mortality. Pretreatment with 1 & 2mg/kg (subconvulsant doses) and 3mg/kg (convulsant dose) of picrotoxin had no significant effect on the WDS behavior, induced by 200mg/kg sodium valproate (Table-2).

**Table-2 shows: Effect of picrotoxin (PIC) and cyproheptadine (CYP) pretreatment on sodium valproate (VAL) induced WDS behavior in rats.**

Treatment mg/kg ip	Number of head and whole body shakes, Mean $\pm$ S.E.M.
NS + VAL 200	16.8 $\pm$ 1.6
PIC 1 + VAL 200	17.1 $\pm$ 1.9
PIC 2 + VAL 200	16.5 $\pm$ 1.7
PIC 3 + VAL 200	17.3 $\pm$ 1.5
NS + VAL 300	16.4 $\pm$ 1.3
CYP 5 + VAL 200	0.0
NS + VAL 400	39.2 $\pm$ 2.1
CYP 5 + VAL 300	8.4 $\pm$ 0.9*
CYP 10 + VAL 300	0.0
NS + VAL 400	59.2 $\pm$ 2.8
CYP 5 + VAL 400	28.3 $\pm$ 2.4*
CYP 10 + VAL 400	6.2 $\pm$ 1.1**

\* $P < 0.01$ , \*\* $P < 0.001$ , NS=Normal saline

Pretreatment with 5mg/kg cyproheptadine abolished the WDS behavior induced by 200mg/kg sodium valproate and significantly decreased the head and whole body shakes induced by 300 & 400mg/kg of sodium valproate. However pretreatment with 10mg/kg cyproheptadine abolished WDS behavior induced by 300mg/kg sodium valproate and significantly decreased the number of head and whole body shakes induced by 400mg/kg sodium valproate (Table-2).

The effect of picrotoxin pretreatment on WDS behavior induced by 300 and 400mg/kg sodium valproate was not studied as picrotoxin had failed to antagonize the WDS behavior induced by 200mg/kg dose of sodium valproate.

### 4. Discussion

Behavioral studies in animals have demonstrated that the drugs influencing central GABAergic system also modulate the intensity of behaviors dependent on the functional status of nigrostriatal and mesolimbic DAergic systems. Our study demonstrates that at higher doses (200mg/kg and above) valproate enhances the central 5HTergic neurotransmission by releasing 5HT [9].

Histological studies have demonstrated an anatomical connection between the central ascending serotonergic pathway and the nigrostriatal dopaminergic pathway. The biochemical and electrophysiological studies suggest that 5HT inhibits DAergic neurotransmission in the nigrostriatal DAergic pathway.

In our study pretreatment with valproate 100 & 150mg/kg did not induce WDS behavior which signifies that in these doses it did not release 5HT. However pretreatment with 200,300 and 400mg/kg doses of valproate did induce WDS behavior which indicates the release of 5HT.

Hyperfunctioning of the central serotonergic system is responsible for the occurrence of WDS behavior in rats [8,9]. In earlier studies by De Boer et al it was observed that pretreatment with GABAA receptor antagonists bicuculline and picrotoxin had suppressed valproate induced WDS behavior in rats, which suggested GABA involvement in the induction of WDS behavior by valproate in rats.

To confirm GABA involvement we studied the effect of pretreatment with picrotoxin on valproate induced WDS behavior in rats. It was observed that subconvulsant doses of picrotoxin failed to antagonize valproate induced WDS behavior which is explained as follows. The valproate induced WDS reaches peak after 5-6 min of valproate administration and disappears after 15min, while the maximum increase in brain GABA levels occurs 45min after valproate administration.

However, in our study pretreatment with cyproheptadine a 5HT<sub>2A</sub> receptor antagonist effectively antagonized valproate induced WDS behavior. This indicates that 5HT<sub>2</sub> mechanisms are involved and that valproate induces WDS behavior either by directly stimulating 5HT<sub>2A</sub> receptors or indirectly by releasing 5HT from 5HT<sub>2</sub> neurons with resultant activation of central 5HT<sub>2A</sub> receptors by the released 5HT [10,11]. The authors based on their results proposed that GABAergic and 5HT<sub>2</sub> systems are involved in controlling the WDS behaviors evoked by valproate in rats and suggested possibility of interaction between the GABAergic and 5HT<sub>2</sub> systems in production of WDS behavior by valproate in rats [12].

## 5. Conclusion

Observations made by the authors that valproate increases 5HT turnover (i.e. increases the synthesis and release of 5HT) in rat brains supports our contention that valproate induces WDS behavior by releasing 5HT from the central 5HT<sub>2</sub> neurons [13].

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