

## “Effect of p-chlorophenylalanine pretreatment on sodium valproate and dexfenfluramine induced wet dog shake behavior in rats.”

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### ABSTRACT:

**INTRODUCTION:** Sodium valproate, a broad spectrum antiepileptic drug elevates the brain gamma amino butyric acid (GABA) levels by various mechanisms. Behavioral studies in animals have provided additional evidence for interaction between the 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 (HTR) in mice is evoked by 5- hydroxytryptamine (5-HT, serotonin), the 5-HT precursor, the directly acting non-selective 5-HT receptor agonists and 5-HT releasers.

**METHOD:** In order to determine the GABAergic and 5-HTergic involvement in the induction of WDS behavior in rats the study was taken up to investigate the effect of clomipramine a 5HT depletor, pretreatment on sodium valproate, dexfenfluramine and 5-hydroxytryptamine (5-HTP) induced WDS behavior in rats.

**OBSERVATION:** It was observed that Valproate in the dose range of 200-500mg/kg induced dose dependent WDS in rats.

**RESULTS:** It was observed that pretreatment with PCPA significantly decreased the number of head and whole body shakes in rats caused by valproate and dexfenfluramine. This indicates that the induction of WDS behavior by valproate depends on the availability of brain 5HT and that valproate acts indirectly by releasing 5HT from the 5HTergic neurons.

**KEY WORDS:** Dexfenfluramine, p-chlorophenylalanine, Sodium valproate, Wet Dog Shake Behavior.

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**INTRODUCTION:** Sodium valproate, a broad spectrum antiepileptic elevates the brain gamma amino butyric acid (GABA) levels by increasing the GABA synthetic enzymes and by decreasing the GABA degradative enzymes <sup>[1]</sup>. Histological, electrophysiological and biochemical studies suggest a regulatory role of GABA on

dopaminergic (DAergic) neurons <sup>[2, 3]</sup>. Behavioral studies in animals have provided additional evidence for interaction between the GABAergic and DAergic systems<sup>[4]</sup>.

It was observed that valproate at 200-500mg/kg ip dosage induced head and whole body shake, i.e., the Wet Dog Shake (WDS) behavior in rats. The WDS behavior in rats and head twitch responses (HTR) in mice is evoked by 5-hydroxytryptamine (5-HT, 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 5-HT releasers p-

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chloramphetamine (PCA) and fenfluramine, through activation of the central 5HT<sub>2A</sub> receptors<sup>[5, 6]</sup>.

Hence in order to determine whether valproate induces WDS behavior by directly stimulating the central 5HT<sub>2A</sub> receptors or indirectly by releasing 5HT from 5HTergic neurons, the study was taken up to evaluate the effect of p-chlorophenylalanine(PCPA) pretreatment on sodium valproate and dexfenfluramine induced WDS behavior in rats.

### **MATERIALS AND METHODS:**

Albino rats of either sex, weighing between 100-180g, were used. They were allowed food and water ad libitum upon the time of experimentation. Each animal was used only once. All observations were made between 10-17hrs at 27-30°C in a noiseless, diffusely illuminated room. Each group consisted of 10 animals.

The drugs used were sodium valproate (Reckitt & Colman), dl-p-chlorophenylalanine methyl ester hydrochloride and dexfenfluramine. All the drugs were dissolved in distilled water. All drug solutions were prepared immediately before use and were injected intraperitoneally (ip). The volume of injection was 5ml/kg body weight for valproate, 10ml/kg body weight for dl-p-chlorophenylalanine and 2ml/kg body weight for dexfenfluramine. For observation of WDS behavior, the animals were placed individually in open topped perspex cages (30x20x 20cm) immediately after the injection of sodium valproate and dexfenfluramine, the number of head shakes and whole body shakes (WDS) were counted over the 30min period after administration of valproate and dexfenfluramine. The total count of WDS of each rat in the group was taken to compute the mean value of the group. P-chlorophenylalanine (PCPA, 100mg/kg/day) was

administered daily for 4 days, the last dose was given 18hrs before valproate & dexfenfluramine. Control groups received normal saline 10ml/kg body weight ip daily, for the same period before receiving valproate and dexfenfluramine.

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 IAEC (Institutional Animal Ethics Committee).

### **STATISTICS:**

The results were statistically analyzed by the student's unpaired t-test with differences considered significant at p< 0.05.

### **OBSERVATIONS AND RESULTS:**

It was observed in our preliminary studies that 50-150mg/kg sodium valproate produced neither gross behavioral change nor induced WDS behavior in rats. However, in the dose range 200-500mg/kg valproate induced dose dependent degree of head and whole body shakes in the rats (WDS) (Table-1).

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.

It was observed that pretreatment with PCPA (100mg/kg /day x 4days) significantly decreased the

number of head and whole body shakes induced by 300 & 400 mg/kg sodium valproate and 10mg/kg dexfenfluramine (Table-2).

#### **DISCUSSION AND CONCLUSION:**

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<sup>[7]</sup>.

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 we found that it did not induce WDS behavior which signifies that in these doses it did not release 5HT. However pretreatment with 200,300and 400mg/kg doses of valproate did induce WDS behavior which indicates the release of 5HT.

It is evident with the previous studies that pretreatment with cyproheptadine a 5HT receptor antagonist effectively antagonized valproate induced WDS behavior in rats<sup>[8]</sup>. This indicates that hyperfunctioning of the central serotonergic system is responsible for occurrence of WDS behavior in rats. The directly acting non-selective 5HT receptor agonists, ergometrine and 5-MeODMT, induce the WDS behavior by directly stimulating the central 5HT<sub>2A</sub> receptor whereas the indirectly acting 5HT agonists viz 5HTP, precursor of 5HT releasers p-chloramphetamine (PCA) and dexfenfluramine

through the released 5HT,activate the central 5HT<sub>2A</sub> receptors with resultant induction of WDS behavior<sup>[9]</sup>.

In present study valproate in the dose range of 200-500mg/kg had induced dose dependent degree of WDS behavior in the rats. Hence to determine whether valproate induces WDS behavior by directly stimulating the central 5HT<sub>2A</sub> receptors or by indirectly releasing the 5HT from the 5HTergic neurons we investigated the effect of pretreatment of PCPA, a drug which depletes brain 5HT by inhibiting tryptophan hydroxylase on valproate and dexfenfluramine induced WDS behavior in rats<sup>[10]</sup>.

Pretreatment with PCPA significantly antagonized valproate and dexfenfluramine induced WDS behavior in rats. This indicates that the induction of WDS behavior by valproate depends on the availability of brain 5HT and that valproate acts indirectly by releasing 5HT from the 5HTergic neurons. Our observations support the earlier studies which show that pretreatment with PCPA antagonized where as pretreatment with 5HT precursor L-tryptophan potentiated valproate induced WDS behavior<sup>[11]</sup>. On the basis of these findings it is proposed that both GABAergic and 5HTergic systems are involved in controlling the WDS behavior evoked by valproate in rats and suggest the possibility of an interaction between GABAergic and 5HTergic systems in the production of WDS behavior by valproate in rats.

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**Table-1:** 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 50mg/kg	0.0
VAL 100mg/kg	0.0
VAL 150mg/kg	0.0
VAL 200mg/kg	16.2 $\pm$ 1.8
VAL 300mg/kg	38.7 $\pm$ 2.2
VAL 400mg/kg	58.4 $\pm$ 2.4
VAL 500mg/kg	65.7 $\pm$ 2.7

**Table-2 :** Effect of p-chlorophenylalanine (PCPA), pretreatment on sodium valproate (VAL) and dexfenfluramine (DEX) induced WDS behavior in rats.

Study	No	Treatment mg/kg ip	Number of Head and Whole body shakes. Mean $\pm$ S.E.M.
<b>I</b>	1	NS + VAL 300	38.5 $\pm$ 1.8
	2	PCPA 400 + VAL 300	11.2 $\pm$ 1.3*
<b>II</b>	1	NS + VAL 400	58.9 $\pm$ 2.6
	2	PCPA 400 + VAL 400	32.3 $\pm$ 2.2*
<b>III</b>	1	NS + DEX 10	69.2 $\pm$ 3.3
	2	PCPA 400 + DEX 10	41.1 $\pm$ 2.4*

\*P < 0.01, NS=normal saline.

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