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Research Article

EFFECT OF CLOMIPRAMINE PRETREATMENT ON SODIUM VALPROATE, DEXFENFLURAMINE AND 5-HYDROXYTRYPTAMINE INDUCED WET DOG SHAKE BEHAVIOR IN RATS

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ABSTRACT

Sodium valproate, a broad spectrum antiepileptic drug elevates the brain gamma amino butyric acid (GABA) levels by various mechanisms. 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.

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 pretreatment on sodium valproate, dexfenfluramine and 5-hydroxytryptamine (5-HTP) induced WDS behavior in rats.

Keywords: Clomipramine, Dexfenfluramine, 5-Hydroxytryptamine, Sodium valproate, Wet Dog Shake Behavior.

INTRODUCTION

Sodium valproate, a broad spectrum antiepileptic drug elevates the brain GABA levels by various mechanisms [1, 2]. Histological, electrophysiological and biochemical studies suggest a regulatory role of GABA on dopaminergic (DAergic) neurons [3,4,5].Additionally the evidence for the interaction between the GABAergic and DAergic system is obtained by the behavioral studies conducted in animals [6].

We observed that valproate at 200-500mg/kg ip dosage induced head and whole body shakes, 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-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 $5HT_{2A}$ receptors [7, 8].

In order to determine the involvement of GABAergic and 5-HTergic systems in the induction of WDS behavior in rats the study was undertaken to investigate the effect of clomipramine pretreatment on sodium valproate, dexfenfluramine and 5-HTP 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 up to the 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.

The drugs used were sodium valproate (Reckitt& Colman), clomipramine hydrochloride, dexfenfluramine hydrochloride, l-5-hydroxytryptophan and carbidopa.

Carbidopa and 5-hydroxytryptophan were dissolved in distilled water acidified with a minimum amount of hydrochloric acid. All drug solutions were prepared immediately before use and were injected intraperitoneally. The volume of injection was 3 ml/kg body weight for 5-hydroxytryptophan, 5 ml/kg body weight for valproate while for remaining drugs it was 2 ml/kg body weight.

For observations of Wet Dog Shake behavior the animals were placed individually in open topped perspex cages (30x20x20) immediately after the injection of sodium valproate, dexfenfluramine and 5-hydroxytryptophan (5-HTP). The number of head shakes and whole body shakes were counted over the 30 min period after administration of valproate, dexfenfluramine and 5-HTP. The total count of WDS of each rat in a group was taken to compute the mean value of the group.

Clomipramine was injected 1 hr before valproate, dexfenfluramine and 5-HTP. Control groups received normal saline (2ml/kg ip) 1hr before receiving valproate, dexfenfluramine or 5-HTP. Further animals receiving 5-HTP were pretreated with carbidopa 30 min prior to the injection of 5-HTP.Clomipramine was tested in the doses of 5 and 10 mg/kg.

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).

Statistics

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

RESULTS

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

Table 1: Dose dependency of WDS response induced by sodium valproate (VAL)

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

The WDS behavior manifested within 5-7 min of valproate administration, with maximum frequency between 10-15 min time interval after valproate injection and depending on dose used lasted for about 30-45 min 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.

Clomipramine 5 and 10 mg/kg did not induce WDS behavior in rats. Pretreatment with 5 and 10 mg/kg clomipramine significantly increased the number of head and whole body shakes induced by 200 and 300 mg/kg sodium valproate and by 75 mg/kg 5-hydroxytryptophan in carbidopa 25 mg/kg pretreated rats.

Pretreatment with 5 and 10 mg/kg clomipramine however, significantly decrease the number of head and whole body shakes induced by 10 mg/kg dexfenfluramine (Table-2).

Table 2: Effect of clomipramine (CIMI) pretreatment on sodium valproate (VAL), dexfenfluramine (DEX) and 5-hydroxytryptophan (5-HTP) induced WDS behavior in rats.

Study	No	Treatment in mg/kg	Number of head and whole body shakes. Mean ± S.E.M
I	1	NS + VAL 200	16.6±1.4
	2	CIMI 5 + VAL 200	29.8±1.7
	3	CIMI 10 + VAL 200	37.5±2.1
II	1	NS + VAL 300	38.9±1.9
	2	CIMI 5 + VAL 300	51.8±2.1*
	3	CIMI 10 + VAL 300	58.1±2.3**
III	1	NS + DEX 10	69.8±3.5
	2	CIMI 5 + DEX 10	48.9±3.2*
	3	CIMI 10 + DEX 10	29.8±2.7**
IV	1	NS +CAR 25 + 5-HTP 75	67.2±3.4
	2	CIMI 5 + CAR 25 + 5-HTP 75	89.6±3.9*
	3	CIMI 10+ CAR 25 +5-HTP 75	111.4±4.3**

*P<0.05, **P<0.01, NS= normal saline.

DISCUSSION

Intraperitoneal administration of high doses (300 to 600 mg/kg) of sodium valproate induced WDS behavior in rats. However it was observed by the authors that pretreatment with lysergic acid diethylamide (LSD), which acts as a partial or full agonist at $5HT_{2A}$ receptors, antagonized the valproate induced WDS behavior, and suggested that central 5HTergic systems are involved in the induction of WDS behavior by valproate in rats [9]. It has also been reported based on the studies that 15 min after the injection of valproate (400 mg/kg) whole brain 5HT levels were unchanged whereas 5HIAA levels were significantly increased suggesting that valproate increases brain 5HT turnover [10].

Histological studies have demonstrated an anatomical connection between central ascending serotonergic pathway and nigrostriatal dopaminergic pathway.Hyperfunctioning of the central serotonergic systems is responsible for the occurrence of WDS behavior in rats [11].

In our study it is observed that valproate, in the dose range of 200-500 mg/kg had induced dose dependent degree of WDS behavior in the rats as shown in table-1. Dexfenfluramine, after its 5HT uptake carrier mediated entry into the 5HTergic neurons, causes rapid release of 5HT from the 5HTergic neurons with resultant induction of WDS behavior in rats. The 5HT uptake blockers e.g., Fluoxetine, clomipramine, antagonize dexfenfluramine induced WDS behavior by blocking its 5HT uptake carrier mediated entry into the 5HTergic neurons and preventing the subsequent release of 5HT [12].

5-HTP, the precursor of 5HT, enters the 5HTergic neurons via the amino acid transport system which is different from the 5HT uptake carrier system responsible for the neuronal uptake of 5HT and drugs like dexfenfluramine. In the neuron 5-HTP is decarboxylated in the cytoplasm to 5HT by the 5-HTP decarboxylase enzyme. Thus 5-HTP treatment increases the intraneuronal stores of 5HT with consequent excessive release of 5HT and occurrence of the WDS behavior [13]. Clomipramine, by blocking the neuronal uptake of 5HT, increases the concentration of 5HT in the synaptic cleft and there by potentiates 5-HTP induced WDS behavior [14].

With the pretreatment of clomipramine we found that it potentiates valproate and 5-HTP induced WDS behavior and significantly antagonized dexfenfluramine induced WDS behavior in rats. Since clomipramine pretreatment potentiated valproate induced WDS behavior it indicates that valproate enters the 5HTergic neurons independently of the 5HT uptake carrier system and subsequently releases 5HT from the 5HTergic neurons which demonstrates that elevated GABA levels and increased 5HT levels are responsible for the induction of WDS behavior in rats.

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