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STUDY OF THE EFFECT OF SODIUM VALPROATE ON ENHANCEMENT OF MONOAMINE MEDIATED BEHAVIOURAL RESPONSES AFTER ELECTROCONVULSIVE SHOCK (ECS) ADMINISTRATION IN RATS

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ABSTRACT: Objectives: To obtain the data regarding the effects of sodium valproate on enhancement of monoamines mediated behavioural responses following administration of ECS for ten days to rats. **Methods:** Forty rats were selected for the study in which tonic-clonic seizures were developed fully after giving single maximal electroconvulsive shock (150V, 210mA). Four groups (n= ten in each) were received different treatment(duration= Ten days) 1) Control group- distilled water (2ml daily) 2) ECS pretreated group- single ECS daily 3) Valproate group- valproate suspension(5.4 mg/kg/day, p.o.) 4) Test group- Single ECS daily + valproate suspension one hour after ECS((5.4mg/kg/day, p.o.). Following behavioural studies were done on Day eleven - apomorphine induced stereotypy, lithium induced head twitches and clonidine induced sedation to study dopaminergic, serotonergic and noradrenergic functions respectively. **Results:** All Three scores i.e. apomorphine induced stereotypy; lithium induced head twitches and clonidine induced sedation were statistically significantly increased in ECS pretreated group as compared to control group. Apomorphine induced stereotypy score & lithium induced head twitches were statistically significantly decreased in test group as compared to ECS pretreated group. However there was no statistically significant difference in the clonidine induced sedation score between ECS pretreated and test group. **Conclusion:** ECS for ten days significantly enhanced the dopaminergic, serotonergic and noradrenergic mediated responses mimicking the role of ECT in treatment of depression. Sodium valproate decreases the activity of dopaminergic, serotonergic system and has no significant effect on noradrenergic system. This may play a role in therapeutic efficacy of valproate in various other CNS diseases.

INTRODUCTION: It is well accepted that there is a marked symptomatic improvement in severe depressive illness after electroconvulsive therapy (ECT). Although the antidepressant mechanism of ECT is not well established, the benefit may result from an enhancement of one or more of the monoamine mediated functions in the brain. The assumption is based on results obtained in rats and mice subjected to repeated administration of electroconvulsive shock (Hereinafter referred as ECS) mimicking ECT [1].

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The therapeutic efficacy of ECT is suggestive of a relationship between cortical seizure activity and symptomatic relief. Such a relationship is also suggested from the observation that abnormal psychic symptoms often arise in epileptic patients when their convulsions are controlled by the use of antiepileptic drugs [2], as if the convulsive episodes afford protection against abnormal psychic states. Under these circumstances, one possibility is that the drugs only control the convulsion and the abnormal psychic states results from the loss of protective effects of convulsions.

Another possibility is that the drugs have an inherent capacity to induce abnormal psychic states, which is unrelated to their anticonvulsant activity. While the adverse effect of anticonvulsant medication has been well documented, there is also

evidence that abnormal psychic state may supervene in epileptic patients who are not given drug therapy. In an attempt to define the role of anticonvulsant drugs in the genesis of abnormal mental states, a study was conducted by Bhavsar, Dhumal and Kelkar using phenytoin, carbamazepine and diazepam, with certain specific behavioural models to assess central dopamine (DA), 5-hydroxytryptamine (5-HT) and noradrenaline (NA) mediated functions in rats subjected to repeated administration of ECS. Since the electrically induced seizures mimic the epileptic convulsions, it was hoped that the influence of anticonvulsant drugs on any enhancement of monoamine mediated behavioural responses would be evident in these experiments [3].

sodium valproate is a unique antiepileptic drug with broad spectrum of activity and is widely prescribed in clinical practice. Hence, we selected this drug to study its interaction with monoamine mediated behavioural responses in rats. Such data is available for other antiepileptic medications like phenytoin, carbamazepine and diazepam but not for Sodium valproate.

MATERIALS AND METHODS:

Experimental Animals:

The study was carried out in Pd. Dr. D. Y. Patil Medical College, Pimpri, Pune. Male Sprague-Dawley rats which were weighed from 140-160g were used in the present study. The animals were maintained on standard laboratory diet ('Amrut rat pellet feed' manufactured by Pranav Agro Food, Pune) and had free access to tap water (supplied by Pimpri Chinchwad Municipal Corporation). The rats were maintained under standard conditions of temperature (25ºC ± 5ºC) and relative humidity (55 ± 10%) and a 12/12 h light/dark cycle. The study was approved by the Institutional Animal Ethics Committee. (IAEC permission No. DYPMC/ IAEC/03/2006 dated 12 Dec 2006).

Chemical / Drugs:

Study drug : Valproate

Dose : 5.4 mg/kg body weight

Source : Sanofi-Synthelabo (India) Pvt Ltd. Bangalore

Chemicals used: (All the chemicals were procured from Sigma)

- apomorphine hydrochloride
- lithium chloride
- clonidine hydrochloride
- double distilled water (vehicle)

ECS administration:

The rats were selected at random and given a single maximal electroconvulsive shock (150 V, 50 Hz sinusoidal w1786ith intensity of 210 mA) for 0.5 sec through crocodile ear clip electrodes from a 'small for animal convulsiometer' without any anaesthesia. Only those rats in which tonic and clonic phases of seizure developed fully were taken up for the study.

Grouping of rat:

Rats were divided in four groups with ten rats in each group and treatment duration was kept for ten days in all the groups.

Group I- Control: Rats were given daily 2ml distilled water only.

Group II- ECS Pre-treated Group: Rats were administered single ECS daily for ten consecutive days to enhance the monoamine mediated behavioural responses as mentioned earlier.

Group III- Valproate Group: Rats were administered valproate suspension daily (5.4 mg/kg/day, p.o) only

Group IV- Test Group: Rats were given single ECS daily and valproate suspension (5.4 mg/kg/day, p.o) one hour after ECS administration .Since only fully developed tonic-clonic convulsions can lead to enhanced behavioural responses, the antiepileptic drugs were given not before, but one hour after administration of ECS every day.[2]

Behavioural Studies: The following models were used to study the effect of test drug i.e. valproate on central monoamine mediated functions:

- apomorphine- induced stereotypy.
- lithium-induced head twitches
- clonidine-induced sedation

These behavioural tests were performed on day 11, 24 hours after the administration of last dose of pretreatment according to their group respectively. All three behavioural tests were performed on day 11 with all groups and with same rats [3].

Apomorphine-induced stereotypy:
This model was used to study dopamine mediated behavioural changes.

Procedure:
The stereotypy following the injection of apomorphine hydrochloride (0.3 mg/kg, S.C.) was observed. Observation was started 15 min following the injection of apomorphine, was done for 2 min at 30 min interval for 2 hrs and then at 60 min interval for a total period of 4 h. The degree of stereotyped behaviour observed was assessed using the scoring system as described by Johnson et al. [4] as follows:

1= intermittent sniffing (bouts < 15 s)

2= persistent sniffing (bouts > 1 min)

3= licking or occasional biting

4 = intense or persistent biting

Lithium-induced head twitches:
This model was used to study serotonin (5-HT) induced behavioural changes.

Procedure
The numbers of head twitches induced by injecting lithium hydrochloride (200 mg/kg, i.p) were counted during 10 min interval, starting immediately from the time of injecting lithium hydrochloride up to a period of 90 min [5].

Clonidine-induced sedation:
This model was used to assess nor-adrenaline-induced behavioural changes.

Procedure:
The degree of sedation was assessed 30 min after injection of clonidine hydrochloride (100 µg/kg, i.p.) [6]. Sedation was assessed by removing rat from the cage and transferring them on to the laboratory shelf and observing for gross differences from the naïve rats. The following 6 indices were used and were scored on a 0-4 scale as follows:

- Lowered body posture (scored 0: if there was no change; scored 4: if the ventral surface of the abdomen touched the floor and the normal arched back was not distinctly visible);
- Slowness of gait (scored 0: if no change; increasing slowness increased the score; score 4: if the rat did not move at all);
- Depressed response of the rat to pressure by finger and thumb placed on either side of the body;
- Passivity (assessed by whether or not rat struggled when picked up gently by the dorsal fold of loose skin of neck);
- Impaired righting reflex (assessed by number of times the rat failed to land on all 4 feet when dropped 4 times from inverted position on to a tray of paddy husk);
- Ptosis (assessed by directly observing the eyes, scored 0: if the eyes were wide open; scored 4: if the eyes were shut);
- Scores for each index were summed for each dose group. The maximum possible score being 24.

Statistical analysis:
The enhancement of monoamine mediated behavioural responses produced by ECS administration was assessed by comparing the result obtained in ECS pretreated group with those in control group. This was done irrespective of whether the animals were pretreated with a drug or not (naive rats). Result from the lithium test were

analysed by using student's t-test; result of apomorphine and clonidine tests were analysed using Mann-Whitney U test. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS:

Apomorphine-induced stereotypy:

Apomorphine-induced stereotypy scores were recorded at 0, ½, 1, 1½, 2, 3, 4 hr. The result of this study are shown in **Table 1** and **Fig.1**.

It was seen that in the control group maximum stereotypy score was 1.5 ± 0.15 (Mean ± SEM). In the ECS pretreated group the score increased to 3.16 ± 0.11 (Mean ± SEM) which was statistically significant as compared to control group (p <0.001 ECS pretreated Vs control group). In valproate group the score increased to 1.7 ± 0.15 (Mean ± SEM) which was not statistically significant as compared to control group (p >0.05 Valproate group Vs control group). In test group, the stereotypy score was decreased to 1.75 ± 0.13 and this decrease was statistically significant as compared to ECS pretreated group (p <0.001 Test group Vs ECS pretreated group).

TABLE 1: APOMORPHINE-INDUCED STEREOTYPY

Groups (n=10)	Stereotypy Score (at hours) (Mean ± S.E.M.)						
	0	½h	1h	1 ½h	2h	3h	4h
Control	0±0	1.5±0.15	0.33±0.14	0±0	0±0	0±0	0±0
ECS pretreated (p<0.001 Vs control group)	0±0	3.16 ±0.11	3.08 ±0.08	2.75±0.17	1.33±0.14	0.66±0.14	0±0
Sodium valproate pretreated (p>0.05 Vs control group)	0±0	1.6±0.16	1.6±0.16	1.7±0.15	1.3±0.15	1.5±0.16	1.5±0.16
Test group (p<0.001 Vs ECS pretreated group)	0±0	1.75±0.13	1.75±0.13	1.5± 0.15	1.33±0.14	1.08±0.83	0.58±0.14

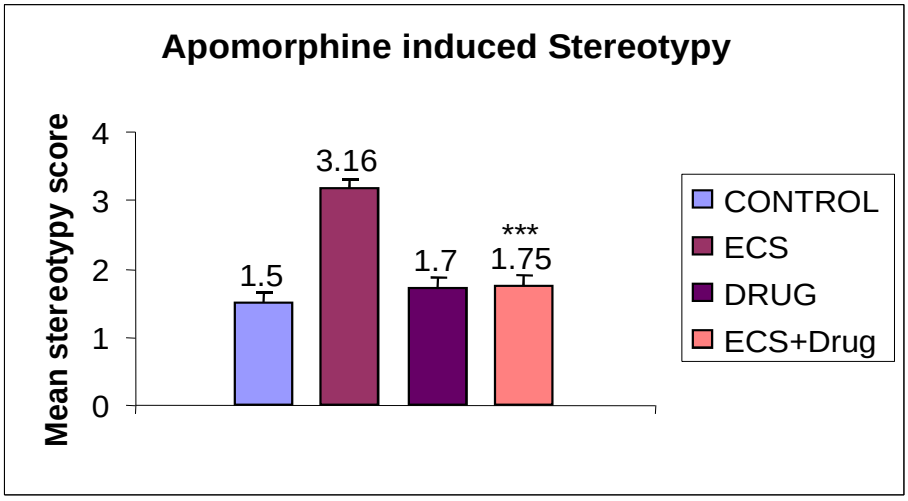

FIG.1: APOMORPHINE-INDUCED STEREOTYPY
(NS- not significant,*- p< 0.05, ** p < 0.01, *** p< 0.001)

Lithium-induced head twitches:

The numbers of head twitches induced by injecting lithium chloride (200 mg/kg, i.p.) were counted every 10 min, starting immediately from the time of injection up to a period of 90 min. The results of this study are shown in **Table 2** and **Fig.2**.

The maximum numbers of head twitches in different groups were compared. In the control group a score of 0.4 ± 0.16 (Mean ± SEM) was seen. In the ECS pretreated group the score increased to 40.6 ± 14.56 and this increase was statistically significant as compared to control group (p <0.01 ECS Pretreated Vs Control group). The valproate group showed increase in head twitches to 6.1±1.7 which was statistically significant in comparison with control (p <0.01 Valproate group Vs Control group). In the test group score was 1.7 ± 3.02 which was statistically significant decrease as compared to ECS pretreated group (p <0.001Test group Vs ECS pretreated group).

TABLE 2: LITHIUM-INDUCED HEAD TWITCHES

Time in mins (n=10)	Control (n=10)	ECS pretreated (n=10) (p<0.01 Vs control group)	Sodium valproate pretreated (n=10) (p<0.01 Vs control group)	Test group (n=10) (p<0.001 Vs ECS pretreated group)
10	0±0	10.8±4.9	0±0	0.3±0.67
20	0±0	8.2±2.9	0.1±0.1	1.13±0.16
30	0±0	24.2±10.08	0±0	1.7±3.02
40	0.4±0.16	40.6±14.56	0.2±0.133	0.6±1.26
50	0±0	31.6±8.0	1.1±0.60	1.1±1.91
60	0±0	20.5±6.8	2.2±0.89	1.1±1.44
70	0±0	0	6.1±1.7	0.2±0.42
80	0±0	0±0	0±0	0±0
90	0±0	0±0	0±0	0±0

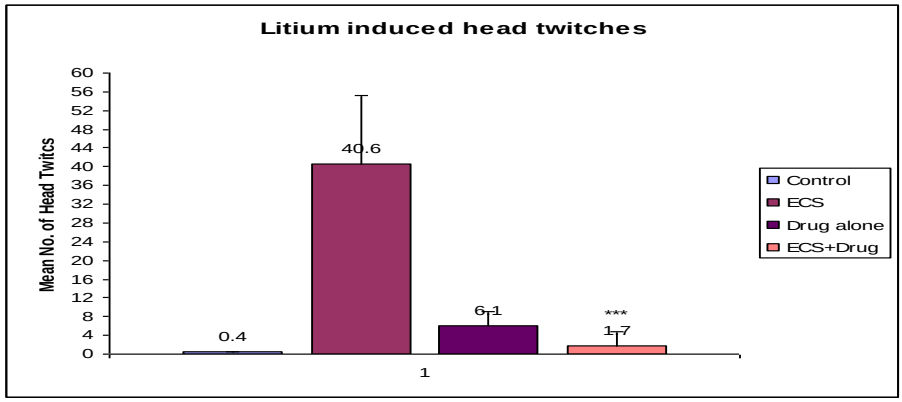

FIG.2: LITHIUM-INDUCED HEAD TWITCHES (MAXIMUM MEANS)
(NS- not significant, *- p< 0.05, ** p < 0.01, *** p< 0.001)

Clonidine-induced sedation:

After injecting clonidine (100µg/kg, i.p.), total sedation score was calculated as described in methodology. The result has been shown in **Table 3** and **Fig.3.** The control group had a total sedation score of 4.66± 0.30 (Mean ± SEM). In the ECS pretreated rats the score increased to 12 ± 1.35 which was statistically significant as compared to control group(p <0.001 ECS pretreated Vs control group). In valproate group the score was 5 ±0.81 which was not statistically significant as compared to control (p > 0.5, valproate Vs control group). In test group the score was 8.91 ± 1.03 which does not show statistically significant difference when compared with ECS pretreated group (p >0.05Test group Vs ECS Pretreated group)

TABLE 3: CLONIDINE-INDUCED SEDATION SCORE

Groups(n=10)	Total sedation score (Mean ± SEM)
Control	4.66 ± 0.30
ECS pretreated (p<0.001 Vs control group)	12 ± 1.35
Sodium valproate pretreated (p>0.5 Vs control group)	5 ± 0.81
Test group (p>0.05 Vs ECS pretreated)	8.91 ± 1.03

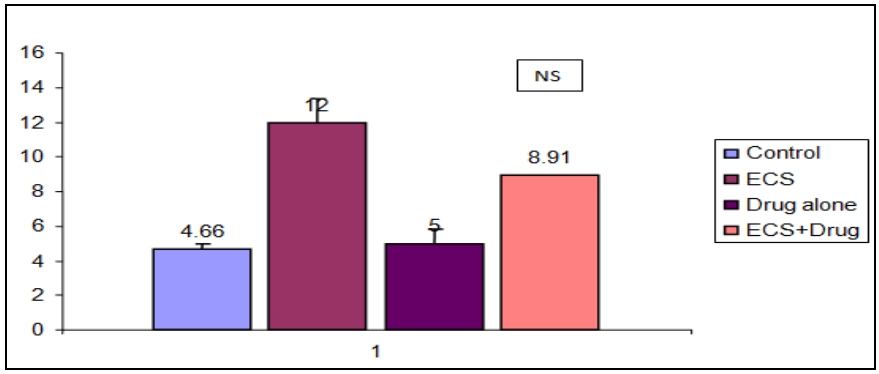

FIG.3: CLONIDINE-INDUCED SEDATION SCORE
(NS- not significant, *- p< 0.05, ** p < 0.01, *** p< 0.001)

DISCUSSION: Previous studies conducted have given the possibility that in case of ECT, the freedom from occurrence of abnormal mental states enjoyed by epilepsy cases during the active phase of the disease is due to enhanced central monoamine- mediated functions and the antiepileptic drugs retard such enhancement.[3] From this standpoint, experimental models involving the use of repeated ECS should be useful to indicate the role of antiepileptic drugs.

The present study was planned to find out the data regarding the effect of sodium valproate on enhancement of monoamine mediated behavioural responses following administration of ECS for ten days to rats. Such data is available for drugs like phenytoin, carbamazepine and diazepam; but not for valproate.

In the present work, we have examined the central monoamine mediated functions using behavioural animal models. It is reported that behavioural changes produced in the rats and mice by increasing brain 5-hydroxytryptaminergic (serotonergic), dopaminergic or noradrenergic functions can be enhanced if animals are pretreated with a single daily ECS for 8-10 days closely resembling the administration of electroconvulsive therapy (ECT) [3, 7, 8]. Therefore ECS has been used as a pretreatment in the present study to enhance the monoamine mediated functions using respective agonists for these monoamines i.e. apomorphine-induced stereotypy for dopamine, lithium-induced head twitches for serotonin and clonidine-induced sedation for noradrenaline.

Evidences for Behavioural responses and monoaminergic systems:

It is well established that administration of apomorphine to rats lead to the production of stereotyped sniffing, licking, and biting behaviour, probably due to stimulation of dopaminergic mechanisms in the striatum [4, 9]. Head twitches, induced by lithium chloride is a useful model for quantifying 5-HT activity in the brain and screening of potential antagonists of 5-HT receptors [5].

It is well known that central α_2-adrenergic receptor agonists like clonidine produces sedation [6], which is its major side effect. Also this is established fact that wakefulness is maintained by the activities of the cell bodies of locus coeruleus which form the major noradrenergic pathway in the brain. Cedarbaum and Aghajanian[10] have shown that microiontophoretic application of α-agonists into this region inhibits the spontaneous firing of locus coeruleus neurons by stimulating the α_2 adrenergic receptors located on or near the cell bodies of these neurons, thereby producing sedation.

ECS effect on monoamine mediated responses:

In the present study, the results of apomorphine-induced stereotypy shows that as compared to the control group there is a significant increase in the stereotypy score in ECS pretreated group ($p < 0.001$ ECS pretreated Vs control group). In various animal models it is reported that there is an acute elevation in the brain content of dopamine and with each ECS [11, 12]; there is also an increase in D_1-receptor density in the region of rat brain [13]. This explains the above observations in our present study.

In the present study, the result of lithium-induced head twitches showed a statistically significant enhancement in the number of head twitches in the ECS pretreated group as compared with the control group ($p < 0.01$ ECS Pretreated Vs Control group). Animal studies suggest enhanced responsivity of serotonin mediated behaviours and increased density of ($5\text{-}HT_2$) receptors after ECS [14]. This explains the enhancement in the Lithium-induced head twitches following ECS.

In the Clonidine-induced sedation model there was statistically significant increase in the sedation score following ECS as compared to the control group ($p < 0.001$ ECS pretreated Vs control group). This shows that central α_2 agonist like clonidine produces sedation by activating noradrenergic pathways.

Modulation of dopaminergic system by valproate:

Apomorphine-induced stereotypy is described in the literature as a model to study the dopaminergic system. But valproate prevented the ECS induced enhancement in the stereotypy score in the test group ($p < 0.01$ Test group Vs ECS pretreated

group). This can be explained by GABA mimetic effect of valproate. GABAergic agents are known to potentiate the action of neuroleptics and exert some intrinsic antidopaminergic activity as inhibition of stereotypy or hyperactivity induced by dopamenergic drugs. This action is being attributed to GABA ergic inhibition of dopaminergic neurons[15]

However, this alteration of dopaminergic functions by valproate might play some role in its antipschycotic activity. This inhibitory effect of valproate is seen after ECS pretreatment, but not when given alone.

Modulation of Serotonergic system by valproate:

In the present study, head twitches were significantly reduced in test group as compared to ECS pretreated group ($p<0.001$)Test group Vs ECS pretreated group). Animal studies have suggested enhanced responsivity of serotonin-mediated behaviour after repeated ECS and increased density of 5-hydroxytryptamine receptors [15]. This attenuation of serotonergic action by valproate might explain its therapeutic efficacy in migraine.

Modulation of noradrenergic system by valproate:

As seen from the results there was no statistically significant difference in the sedation score between ECS pretreated and test group ($p<0.001$) Test group Vs ECS Pretreated group). Valproate is not known to have statistically significant interaction or modulation at the level of noradrenergic system, which explains our results.

Bhavsar and Dhumal have reported that administration of phenytoin to experimental animals neither altered the monoamine mediated behavioural responses by itself, nor influenced the development of enhanced behavioural responses after ECS[3]. However administration of carbamazepine has been reported to retard the enhancement of dopamine mediated and 5HT–mediated behavioural responses in ECS pretreated rats. It did not have any significant effect on the enhancement of NA-mediated functions caused by ECS administration. The same results are seen with valproate. Lack of efficacy of phenytoin in other CNS disorders may be because it has no influence on enhancement of monoamine mediated behavioural responses, whereas carbamazepine have some role in treatment of bipolar disorder, trigeminal neuralgia and mania which might be due to the retardation in the monoamine enhancement, caused by this drug. This might be true with valproate as well and our results are in conjunction with this hypothesis.

CONCLUSION: It can therefore be concluded that valproate decreases the activity of dopaminergic, serotonergic system and has no significant effect on noradrenergic system. It is possible that restoration of enhanced monoamine level in the brain, to nearby normal level may play a role in the therapeutic efficacy of valproate in other CNS disorders. Further it might be concluded that attenuation in dopaminergic mediated responses could be due to GABA like action of valproate which may substantiate its known action in the treatment of epilepsy patients.

LIMITATIONS OF STUDY: Our study has certain shortcomings. Small sample size of animals was selected for the study. Secondly, the effect of valproate on monoamine mediated responses in animal models of other CNS disorders could have been studied to elucidate the action of valproate in other CNS disorders.

CONFLICT OF INTEREST: The authors do not have any conflict of interest.

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