

INVESTIGATION OF INFLUENCE OF DIAZEPAM, VALPROATE, CYPROHEPTADINE AND CORTISOL ON THE REWARDING VENTRAL TEGMENTAL SELF-STIMULATION BEHAVIOUR

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(Received on June 15, 1989)

Abstract : Experiments were carried on in the Wistar rats having self-stimulation (SS) electrodes implanted chronically in substantia nigra-ventral tegmental area (SN-VTA) to examine whether modulations of GABAergic, serotonergic, histaminergic, dopaminergic, and glucocorticoid neuronal receptor functions will affect or not the brain reward system and the SS behaviour. The modulators are the wellknown drugs : diazepam which is a facilitator of some of the GABA receptors, and used clinically for its tranquilising, anxiolytic, sedative-hypnotic and anti-convulsant properties; sodium valproate which is known to enhance the GABA synapse function, and used clinically for its anti-convulsant property; haloperidol which is a dopaminergic receptor (D2) blocker, and clinically used for its anti-psychotic property; cyproheptadine which is both anti-histaminic and anti-serotonergic (blocks 5-HT₂ receptor), used clinically for its antihistaminic and other beneficial properties; and hydrocortisone which is the stress-resisting glucocorticoid having direct effects on both brain and body cells, used clinically for the wide-ranging glucocorticoid therapeutic effects. The results revealed that systemic administration of these drugs, except haloperidol, caused no significant influence on the SS behaviour, thereby indicating that these nondopaminergic drugs have no effect on brain-reward system and also these categories of synaptic actions are not likely to be involved in the primary organization of the mechanisms of the brain-reward system.

Key words : brain-reward self-stimulation diazepam haloperidol
cyproheptadine cortisol drug effects

INTRODUCTION

Since a long time, it is known that subjects self-stimulate electrically certain regions of brain without getting satiated (1). Although the great deal of research of brain-reward system has not yet unravelled the rewarding neuronal mechanism, it has generated the hypothesis on hedonia, in drug addiction, psychosis, and of anhedonic actions of neuroleptic drugs (2). In order to understand the nature of neurons and synapses involved in the

self-stimulation (SS) reward system, and also to understand actions of drugs that have therapeutic advantages, studies using drugs have been going on since several years (3-5). As the neuroleptic drugs have been found to block primarily the dopaminergic synapse (receptors) and as they block self-stimulation behaviour, the hypothesis arose that dopaminergic synapse or neuron might form the basis of SS reward. However, it is also possible that the system may be very complex, and additional roles of cholinergic, opioid, serotonergic, steroid, GABA,

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and other important types of neuronal involvements have also to be investigated to delineate the neural substrate.

GABA neurons contribute the important inhibitory synapses (6). GABA synapses constitute the majority of synapses in substantia nigra compacta (7) which contains dopaminergic neurons and also is one of the best areas of self-stimulation. Benzodiazepines which are well-known anti-anxiety and tranquilising drugs have receptor sites complexed to some of the GABA receptors (8, 9) and act by facilitating the GABA synapses. The anti-convulsant drug sodium valproate has also been considered to exert facilitatory action in an unknown manner on the GABA synapses (10-15). Hence it was felt interesting to examine the effect of both diazepam (a benzodiazepine) and sodium valproate (an anti-convulsant) that have targets of influence on GABA synapses, on the brain-reward system of self-stimulation behaviour. Serotonergic system synapses are present in all regions of the brain (3, 16, 17) and the system is known to be involved also in the regulation of antidepressive mood states (18). Hence the effect of cyproheptadine which blocks serotonergic receptors (19) on the rewarding SS behaviour has been aimed to be examined. This drug is also an antagonist of histamine receptors, hence it is additionally interesting as histamine is a neuro-regulator (20, 21). Steroid hormones have been recognised in recent years to act on neurons of a number of limbic brain regions (22, 23). Hence, whether the glucocorticoid cortisol which has a major role in the relief of stress of the subject has any effect also on the positive brain-reward system has been examined in this study.

METHODS

Experiments were done on Wistar rats with self-stimulation bipolar stainless-steel electrodes implanted in substantia nigra-ventral tegmental area (SN-VTA) (24) at stereotaxic coordinates: -3.5 mm

from bregma: 1.2 mm lateral; and 8.5 depth from surface (25). After a few days of post-operative recovery, the rats were trained in the Skinner box to learn the operant behaviour to pedal press to obtain volitionally the electrical self-stimulation. With each pedal press, a train of sine waves of 50 Hz passed for 0.25 sec. The current was set in each rat at an optimal level that evoked the maximum possible pedal press rate, while causing no other incapacitating motor or aversive behavioural side effects. On an average the charge was $30 \mu\text{C}$ per train of stimulus.

Diazepam pharmaceutical brand (Calmose, Ranbaxy) was administered intra-peritoneally in two doses: 1.25 mg/kg body weight, 2.5 mg/kg. In each experimental session, a 15 min control data of self-stimulation was obtained, a saline injection control data was again obtained for 15 min, before the diazepam injection. A post-injection time of 15 min for the diazepam to act was allowed and then the self-stimulation data for 15 min obtained.

Sodium valproate commercial product (Valparin-200, Torrent Labs) was administered orally in a dose of 90 mg/kg body weight. The post-drug self-stimulation was tested once at $\frac{1}{2}$ hr, and second time at 1 hr after the administration of drug. Before the drug administration the two types of control data were also obtained as mentioned above.

Cyproheptadine chloride commercial preparation (Periactin syrup, Merck, Sharpe and Dohme) was administered orally in a dose of $80 \mu\text{g}/\text{kg}$ body weight. The control data obtained as mentioned above, and the post-drug data was obtained at $\frac{1}{2}$ hr, 1 hr and 2 hrs after the administration of the drug.

Hydrocortisone sodium succinate commercial preparation (Efcorlin, Allen-burry's Pharmaceuticals) was dissolved in distilled water and administered intraperitoneally in the dose of 1 mg/kg body weight. The control data were obtained as mentioned above,

and post-drug self-stimulation data was obtained at 15 min, 45 min, 75 min, and 105 min after administration.

Haloperidol commercial injectable preparation (Serenace, Searle) was administered intra-peritoneally in a dose of 250 $\mu\text{g}/\text{kg}$ body weight. The control data were obtained as above, and the post-drug data obtained 15 min after administration of the drug.

At least 24 hours were allowed before testing the drug a second time on the same rat, to provide enough time to allow metabolism and lowering of the level of the previous dose.

The electrode sites of SS were confirmed post-mortem in hand-cut sections of the brains fixed in formalin.

The test period of SS, under each of the control and the drug conditions of the session, was of 15 min duration. At least 3 rats were used for each drug study, three experimental sessions repeated on each rat. The mean SS rate (per 15 min) of the control periods of the three experiments of each rat was tested against the mean rate of the three periods under the drug condition, to find significance of difference, using Student's t-test. In addition, group averages also were similarly assessed.

RESULTS

Diazepam: 13 experimental sessions were made on four rats with the low dose (1.25 mg/kg), and 16 experimental sessions were made on four rats with the high dose (2.5 mg/kg). The SS rates under the drug condition were compared with the rates under control condition. No statistically significant change in SS was caused by either the low dose or the high dose of the drug (Fig. 1). The lack of influence of diazepam was observed in relatively low SS rate as well as high SS rate rats. The study was also repeated in five experimental sessions on five more rats using

the high dose, and these results also confirmed lack of consistent effect of diazepam on the SS. In summary, SS rate data of the control and drug conditions in the 34 experimental sessions conducted for the diazepam study at two different doses, no significant effect on the brain reward system and self-stimulation behaviour was observed.

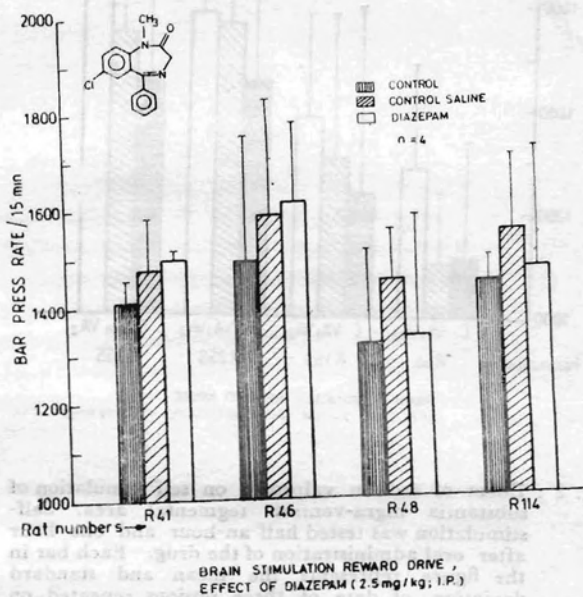


Fig. 1 : Effect of diazepam on self-stimulation of substantia nigra ventral tegmental area. Self-stimulation was tested 15 min after intraperitoneal administration of the drug. Each bar in the figure represents the mean and standard deviation of data of four sessions repeated on different days on each rat.

Sodium valproate: Experiments were conducted on four rats, 3 experimental sessions made on each rat. Each sessions had the control and the drug test data. The results showed that sodium valproate (90 mg/kg body weight) had no significant influence on the brain stimulation reward behaviour (Fig. 2.)

Haloperidol: Nine experimental sessions done on 3 rats, under the haloperidol (250 $\mu\text{g}/\text{kg}$), and control conditions showed that haloperidol caused a highly significant effect of reducing self-stimulation behaviour by about 96% of control. Under the dose

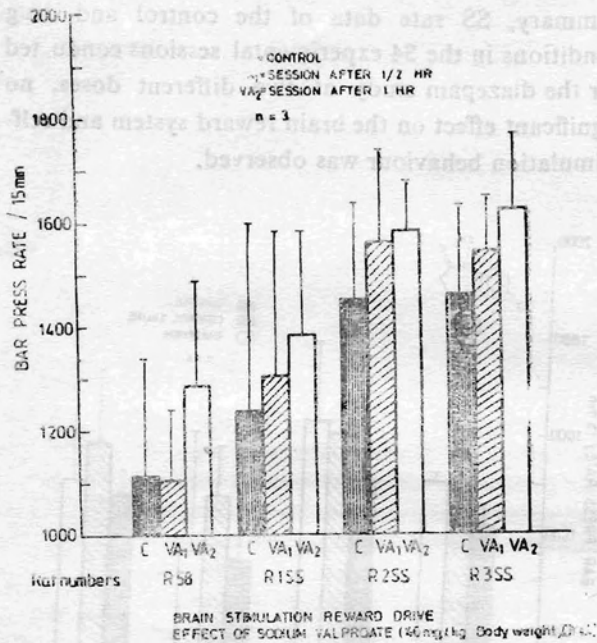


Fig. 2 : Effect of sodium valproate on self stimulation of substantia nigra-ventral tegmental area. Self-stimulation was tested half an-hour and one hour after oral administration of the drug. Each bar in the figure represents the mean and standard deviation of data of three sessions repeated on different days on each rat.

of haloperidol used, the rat would initially try to self-stimulate but soon would give up in a few seconds, like in the non-rewarded extinction type behaviour.

Cyproheptadine : 9 experimental sessions on 3 rats, including the control and drug conditions were conducted. The data revealed that cyproheptadine (80 μ g/kg) caused no significant alteration of the self-stimulation behaviour (Fig. 3).

Hydrocortisone : The data of 9 experimental sessions, including the control and drug conditions were conducted on three rats. Cortisol (1 mg/kg, IP), caused no significant change in the self-stimulation behaviour (Fig. 4).

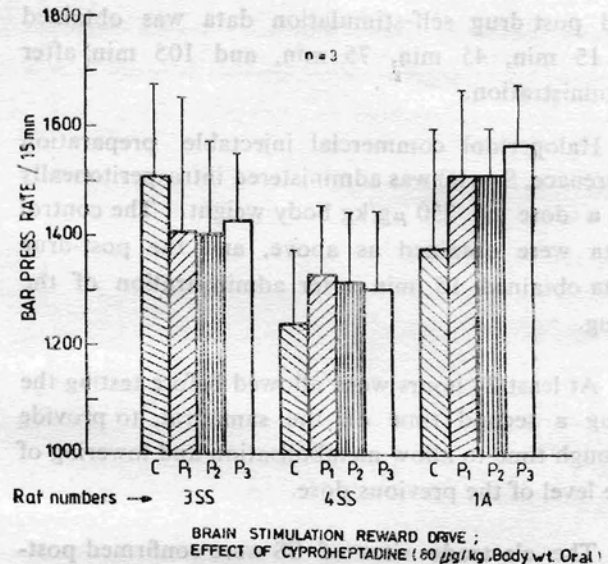


Fig. 3 : Effect of cyproheptadine on self-stimulation of substantia nigra ventral tegmental area. Self-stimulation data was obtained after 30 min (P₁), 60 min (P₂), and 120 min (P₃) of oral administration of the drug. Each bar represents the mean and standard deviation of data of three sessions repeated on different days on each rat.

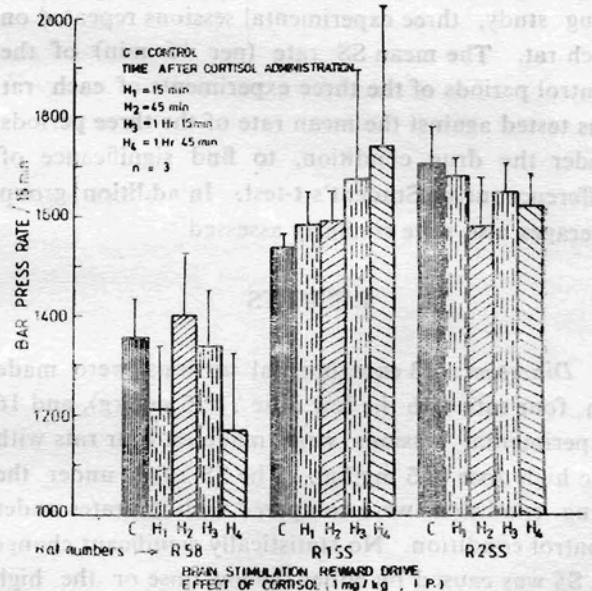


Fig. 4 : Effect of cortisol on self stimulation of substantia nigra-ventral tegmental area. Self-stimulation data was obtained after 15 min, 45 min, 75 min, and 105 min of intraperitoneal administration of the drug. Control data was obtained with saline injection. Each bar represents mean and standard deviation of data of three sessions repeated on different days on each rat.

DISCUSSION

Diazepam: The site of action of diazepam in the brain is the benzodiazepine site of GABA receptor complex (8). GABA synapses are widely distributed in the brain, one of the richest regions being substantia nigra (7). Substantia nigra is also the dopaminergic neuronal region merging with the ventral tegmental area (3). This dopaminergic region is known to be among the highest SS rate giving areas (24). Benzodiazepine binding sites of GABA receptors cause facilitation of GABA mediated post-synaptic inhibition (5, 8, 9). Hence, the dopaminergic neurons having the GABAergic synapses are expected to be inhibited by diazepam administration, and thereby reduce the self-stimulation. However, this type of an effect was not observed in the present experiments, hence a different explanation has to be thought of. As the self-stimulation currents excite neurons of the SN-VTA region the inhibitory effects of the GABA synapses might be of little impediment. Or some other type of synapse on the dopaminergic neurons may be more relevant than the GABA-benzodiazepine for self-stimulation.

In previous studies also, either marginal facilitation or no consistent effects of other benzodiazepine compounds were reported on SS (4, 2, -28). Our study being on SN-VTA region, differed from the previous studies made on lateral hypothalamus. Further more, another study (29) reported depression effect of diazepines (high doses) on the SS behaviour. The present results with diazepam on SS of SN-VTA region showed no significant effect on SS, hence suggest that the benzodiazepines produce their effects of tranquillity and well-being not through their action on the brain-reward or hedonic system, but perhaps through action (inhibition) on anxiogenic neuronal systems of the brain. Chronic usage of benzodiazepines bring drug dependence behaviour (30), and in such a situation the influence of the GABA synapses on neurons of brain-reward system could have developed into a different and effective kind.

Sodium valproate: Sodium valproate is known to be a broad-spectrum anti-convulsant, Its bio-availability on oral administration is 100% and peak time is about 2 hrs (31, 32). Its mode of therapeutic action is not fully known, but is generally considered to be due to a facilitatory effect on the GABAergic synapse (11-14), but at higher doses (15). It has been found to increase the potassium conductance (10). Since dopaminergic neurons are known to be important in self-stimulation reward, and as these neurons have GABAergic synapses (as cited above), the self-stimulation behaviour could be affected by sodium valproate facilitating the GABA synapses. But the present results with valproate also did not confirm such a hypothesis in the role of the GABA synapses on the dopaminergic neurons of SN-VTA. Sodium valproate does not seem to significantly affect the brain reward system in the dose level (90 mg/kg) that is normally used in the clinical situation. However, with higher doses (200 mg/kg and higher) of valproate, SS is depressed (29).

Haloperidol: Haloperidol is widely used in the treatment of major psychosis. Its higher affinity is to block the D-2 receptors of dopaminergic synapses. The results with haloperidol are comparable to the results previously reported on neuroleptic drugs in the literature (2), hence it is not discussed further here.

Cyproheptadine: Cyproheptadine blocks the 5-HT₂ receptors (16, 17). It also antagonises the action of histamine. 5-HT axons are present throughout the brain including the cerebral cortex. However, little is known about the physiological and synaptic functions of the 5-HT synapses. Among functions known, 5-HT neurons have been implied to influence also the antidepressive state (18). However, the present results revealed that there is no effect of cyproheptadine on the brain-reward or SS behaviour. The preliminary studies of the past reported contradicting results that serotonergic system is both facilitatory and inhibitory to SS (33),

and only inhibitory to SS (34). The present work with cyproheptadine showed clearly that there is no significant effect of 5-HT₂ receptor mediated action on SS. The results also revealed that histaminergic synaptic role is negligible in the brain-reward mechanisms and SS behaviour, because cyproheptadine is also a major antihistaminic (19).

Hydrocortisone : Steroids have a fast access to all the parts of the nervous system and exert both genomic and non-genomic actions of both short latency as well as long latency (23). Glucocorticoid

receptors have been found in high concentrations in septum, amygdala, and hippocampus (23). Since these structures were known to play in mnemonic and affective processes, and also in SS behaviour (septum), it was anticipated that cortisol might alter the brain stimulation reward behaviour. In the old reports on adrenal steroid effects, one (35) indicated an increase in the SS, and the other (36) a decrease. The present results with cortisol clearly show that the glucocorticoids do not modify significantly the brain-reward system and self-stimulation behaviour.

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