COGNITIVE DYSFUNCTION INDUCED BY PHENYTOIN AND VALPROATE IN RATS: EFFECT OF NITRIC OXIDE

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Abstract: Phenytoin (PHT) and Valproate (VPA) are known to induce cognitive dysfunction, in terms of long term memory loss. Nitric oxide (NO) on the other hand is said to help in long term potentiation and hence enhance memory. The effects of nitric oxide donor L-arginine (L-Arg) and nitric oxide synthase inhibitor N-W-L-Nitroarginine (L-NOARG) were studied on the cognitive dysfunction, induced by PHT and VPA in normal healthy rats, using the step-through passive avoidance test (PAT). It was observed that combining L-Arg with PHT significantly enhanced long term memory while, combining PHT with L-NOARG decreased it, as compared to PHT alone. When combined with VPA, L-Arg and L-NOARG increased the retention latency as compared to PVA alone but this was not statistically significant. We conclude that the No donor L-Arg is able to increase the difference in LTE in acquisition and retention trials with both PHT and VPA, but with VPA the increase is not statistically significant.

Key words: phenytoin valproate cognitive deficit passive avoidance test L-arginine L-NOARG nitric oxide

INTRODUCTION

Repetitive activation of N-methyl-D-aspartate (NMDA) synapses causes long term potentiation (LTP). LTP is a model form and proposed as a mechanism of memory (1, 2). Activation of receptors for excitatory aminoacids (notably NMDA receptors), in the central nervous system leads to production of nitric oxide (NO) postysynaptically from L-arginine (L-arg) (3, 4). The NO-mediated responses to NMDA receptor activation can be potently inhibited by the arginine analogue L-Nw-nitroarginine (L-NOARG), the inhibition being prevented by an excess of L-arginine (5).

Phenytoin (PHT) and valproate (VPA) are commonly used antiepileptic drugs. Epilepsy itself, as well as the use of antiepileptic drugs like PHT and VPA is often accompanied by severe cognitive deficits (6-8).

The aim of the present study was to investigate the effects of L-Arg and L-NOARG, alone and in combination with PHT and VPA on memory in rats, using
the step-through passive avoidance test paradigm.

METHODS

Animals: The experiments were conducted on Wistar strain albino rats (150-200 gm) of either sex, housed under standard laboratory conditions with standard diet and water ad libitum and maintained on a natural light-dark cycle. All experiments were carried out between 9 am to 12 noon, to avoid any circadian influences.

Drugs: PHT and VPA (gift from Torrent Pharmaceuticals, India) were dissolved in normal saline and injected intraperitoneally (i.p.), 120 and 30 minutes respectively, before the tests. The dose of PHT was 13 mg/kg and VPA 250 mg/kg. These doses were selected on the basis of their ED$_{50}$ as determined in our laboratory (9). L-Arg and L-NOARG (SIGMA Chemicals) were also dissolved in normal saline and injected i.p. at a dose of 750 mg/kg and 100 mg/kg respectively, one hour before the tests (10).

Grouping of Animals (n=10 in each group):

1. Control rats given normal saline i.p.
2. Rats given a single dose of either normal saline + L-Arg or normal saline + L-NOARG i.p.
3. Rats given a single dose of either PHT or VPA i.p.
4. Rats given a combination of PHT + L-Arg or PHT + L-NOARG or VPA + L-Arg or VPA + L-NOARG.
5. Rats given normal saline alone, PHT alone, PHT + L-Arg or PHT + L-NOARG for 21 days.

### Passive Avoidance Paradigm

A one trial step through passive avoidance test was carried out as previously described (11). The apparatus consisted of two compartments, an illuminated compartment (27 x 30 x 21 cm) and a dark compartment (10 x 30 x 21 cm) having a grid floor through which shock could be delivered. These compartments were separated by guillotine door. After drug treatment, each rat was placed in the illuminated compartment and 10 seconds later, the door was raised and the latency to enter (LTE) the dark compartment noted. Upon entry, the door was closed and a foot shock administered (0.5 mA for 2 sec). Twenty four hours after the acquisition trial, the rat was again placed in the illuminated chamber. The response (LTE) was noted up to a maximum of 300 second-retention trial. The difference between LTE in the acquisition and retention trial was noted. PAT was performed on day 1 for all the groups of animals and on day 1, 3, 7 and 21 for group 5.

Statistical analysis:

The results of PAT were expressed as mean ± sem, significance of the latencies were assessed using the Wilcoxon rank sum test. P value < 0.05 was taken as significant.

RESULTS

a) Single dose studies:

In control group given only normal saline, the difference in LTE in acquisition and retention trial was 147.4 ± 43.4. With L-Arg 750 mg/kg the difference increased to
217.3 ± 29.9 (p<0.05 compared to control). With L-NOARG the difference decreased compared to control, but was not statistically significant.

When L-Arg was combined with PHT there was a significant increase in the difference in LTE in acquisition and retention trials as compared to PHT alone (p<0.01 vs PHT alone), whereas the combination of L-NOARG and PHT produced no significant changes in the difference in LTE as compared to PHT alone.

When VPA was combined with L-Arg it was observed that the difference in LTE increased when compared to VPA alone, the same was the case when VPA was combined with L-NOARG. However, the increase was insignificant when compared to control groups (Table I).

b) Chronic studies:

The increase in the difference in LTE in acquisition and retention trials which was observed when L-Arg was combined with PHT, persisted on days 3, 7 and 21 (P<0.01 on days 3 and 7, p<0.001 on day 21). With combination of PHT and L-NOARG there was a further decrease in the difference in LTE on days 3, 7 and 21, which was not significant as compared to PHT alone (Table II).

<table>
<thead>
<tr>
<th>TABLE I: Differences in latency to enter in acquisition and retention trials (in sec) in passive avoidance task following single dose administration (n = 10 in each Group)</th>
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</thead>
<tbody>
<tr>
<td><strong>Drugs</strong></td>
</tr>
<tr>
<td>1) CONTROL</td>
</tr>
<tr>
<td>2) PHT</td>
</tr>
<tr>
<td>3) VPA</td>
</tr>
<tr>
<td>4) L-ARG</td>
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<tr>
<td>5) L-NOARG</td>
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<tr>
<td>6) VPA + L-ARG</td>
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<td>7) VPA + L-NOARG</td>
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<td>8) PHT + L-ARG</td>
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<td>9) PHT + L-NOARG</td>
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*P<0.05 vs. Control
**P<0.01 vs. PHT alone.

<table>
<thead>
<tr>
<th>TABLE II : Differences in Latency to enter in acquisition and retention trials (in sec) in passive avoidance task following chronic administration (n = 10 in each Group)</th>
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<tr>
<td><strong>Drugs</strong></td>
</tr>
<tr>
<td>1) CONTROL</td>
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<tr>
<td>2) PHT</td>
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<td>3) PHT+L-ARG</td>
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<td>4) PHT+L-NOARG</td>
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*P<0.01 and
**P<0.001 as compared to PHT alone.
DISCUSSION

The results of the study show that L-Arg, a NO donor increases memory as assessed by difference in LTE in acquisition and retention trials in PAT in saline treated rats, whereas the NO synthase inhibitor L-NOARG decreases memory in saline treated rats. This is in concordance with the view that NO has a role in synaptic plasticity and influences some of the neurophysiological phenomena underlying memory, such as long term potentiation (12, 13).

When L-Arg was combined with PHT, (which induces memory loss), it was able to reverse the memory loss and bring it above the control values, this effect persisted on repeated administration upto 21 days. With the combination of L-NOARG and PHT the memory loss was same, as compared to PHT alone.

Also in this study it was seen that in rats given a single dose of VPA, both L-Arg and L-NOARG improved the memory loss induced by VPA, but this improvement was not statistically significant.

NMDA preferring glutamate receptors have been implicated in memory processing. Psychometric tests suggest that anticonvulsant drugs including PHT and VPA, adversely affect memory in normal people and epileptics (14). PHT has been shown to block excitation mediated by sustained fast activity along NMDA/glutamate pathway which may have some effect on memory (15), whereas VPA has not been shown to influence NMDA responses. NO acts on the NMDA receptors as a retrograde messenger (12) to influence learning and memory. Hence it may be postulated that this action of NO on the NMDA receptors may be responsible for the reversal of PHT induced memory loss.

In conclusion, this study has demonstrated that the NO donor L-Arg is able to increase the difference in LTE in acquisition and retention trials with both PHT and VPA, but with VPA the increase is not statistically significant.

REFERENCES


