Effect of combined treatment of thioperamide with some antiepileptic drugs on methionine-sulfoximine induced convulsions in mice

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Methionine-sulfoximine (MSO), a convulsant is known to increase the activity of histamine N-methyl transferase. The effect of a selective H3 receptor agonist R- (α) methylhistamine (RAMH) and antagonist (thioperamide, THP) and some antiepileptic drugs (gabapentin and sodium valproate) have been evaluated on MSO-induced convulsions in mice. The effect of THP was also evaluated in combination with these antiepileptic drugs. Sodium valproate (300 mg/kg, po) and gabapentin (400 mg/kg, po) offered protection against MSO-induced convulsions as evidenced by a significant prolongation of latency to abnormal dorsoflexion and complete protection against mortality within 6 h of administration. THP (15 mg/kg, ip) alone and in combination with sub-effective doses of gabapentin (75 mg/kg, po) and sodium valproate (75 mg/kg, po) revealed no significant differences from the control group or either drug alone. Hence, the convulsant action of MSO does not appear to be mediated via histaminergic mechanism.

Keywords: Gabapentin, Methionine-sulfoximine, Sodium valproate, Thioperamide

Histamine is considered to be an anticonvulsant inhibitory neurotransmitter. Drugs that deplete brain histamine (for example, histidine decarboxylase inhibitors etc) are considered to have proconvulsant effects in experimental animals. On the other hand, agents that enhance brain histamine levels (histamine itself or histamine precursor l-histidine etc) show potent anticonvulsant effects in experimental models. Presynaptic control via histamine H3 receptors (autoreceptors) is an important mechanism of histamine-mediated neurotransmission. Thioperamide (THP), a selective H3 receptor antagonist, is reported to have antiepileptic effects against a wide range of experimental models including the maximal electroshock (MES) test, pentylenetetrazole (PTZ)-induced seizures, amygdaloid kindling etc. Such effects of THP are known to be mediated via enhancement of neuronal histamine release through an action on H3 receptors. Methionine-sulfoximine (MSO), a convulsant, increases the activity of histamine N-methyl transferase, the enzyme primarily responsible for the catabolism of histamine. There are various reports of agents inhibiting histamine N-methyl transferase to have anticonvulsant effects by increasing the brain histamine levels. An example of such an agent is metoprine. Thus, it is possible that MSO, by increasing the activity of histamine N-methyl transferase and hence decreasing brain histamine, is responsible for its convulsant effects. To test this hypothesis, the effects of an H3 receptor antagonist, THP, which acts as an anticonvulsant via histaminergic mechanism, have been evaluated on MSO-induced convulsions. In addition, the effect of R- (α) methylhistamine (RAMH), a selective H3 receptor agonist, was also investigated in the MSO model.

Several studies indicate that GABA plays a role in the convulsive mechanisms of MSO. Hence, the present study also evaluated the effects of combination of THP with antiepileptic drugs (AEDs) primarily acting via GABAergic mechanisms [sodium valproate (SVP) and gabapentin (GBP)] on MSO-induced convulsions in mice. The latter has not been evaluated for its effects on MSO-induced convulsions.

Eleven groups of Swiss strain male albino mice (20-25 g) were used for the study. The guidelines of the University Animal Ethics Committee were followed during the study. All animals were housed in cages kept at 23°C-30°C with a natural light-dark cycle. They had free access to standard pellet diet (Amrut Laboratory Rat and Mice feed, Navmahrashtra Chakan Oils Mills Ltd. Pune, India) and tap water.

Convulsions were induced by MSO following the method of Blizard and Balkoski. The convulsant dose of MSO employed by other workers ranged from 100-300 mg/kg, ip. In the present study, a median dose (200 mg/kg, ip) was used. This dose...
produced mortality in 100% of animals within 6 h of administration. As the pilot experiments revealed a latency of 2-3 h, observations were begun 2 h after MSO injection and continued for up to 4 h. The parameters included: (i) latency to abnormal dorsoflexion (abrupt head twitches with clonic jerking), (ii) latency to tonic hind limb extension (HLE), and (iii) mortality (%). MSO, THP and RAMH were procured from Sigma (USA), SVP from Torrent, India and GBP from Intas, India. All drug solutions were prepared fresh in distilled water except for RAMH, which was dissolved using pyrogen-free sterile water for injection. All treatments were administered by intraperitoneal route (ip) in a volume not exceeding 10 ml/kg except RAMH, which was given intracerebroventricularly (icv) following the method of Haley and McCormick. The drug was administered in a volume of 5 µl/mouse using a Hamilton’s microlitre syringe in conscious animal. The site of injection was 2 mm from either side of the midline on a line drawn through the anterior base of the ears.

The protective and sub-effective doses of SVP, GBP, THP and RAMH were as per earlier study. The pre-treatment timings were determined on the basis of reported time of peak action and pilot experiments: 1 h for SVP, GBP and THP and 15 min for RAMH. The data were analyzed by one-way ANOVA followed by Dunnett’s t-test except in case of % mortality where Chi square test with Yate’s correction was applied.

The results are summarized in Table 1. Neither THP (15 mg/kg, ip) nor RAMH (5 µg/mouse, icv) affected MSO-induced convulsions. The latencies to head twitches/ clonic jerks, tonic HLE and percent mortality were not significantly different from the control group. SVP (300 mg/kg, ip) and GBP (400 mg/kg, ip) offered protection against MSO-induced convulsions as evidenced by a significant prolongation of the latency to abnormal dorsoflexion [F (6, 29) = 25.66, P<0.01, ANOVA; P<0.001, Dunnett’s t test] and complete protection against mortality within 6 h of administration [P<0.001, Chi-square test]. The latency to tonic HLE was also prolonged but it was found to be statistically insignificant. The sub-effective dose (that offered no protection) was found to be 75 mg/kg, ip for both GBP and SVP. The combined treatment of THP (15 mg/kg, ip) with 75 mg/kg, ip of GBP and SVP revealed no significant differences from the control group or either drug alone.

Both experimental seizures (MES, PTZ) and AEDs modulate brain histamine levels. Consistent with these findings, a decrease in histamine content of amygdala and hypothalamus has been reported following kindling. A similar decrease in histamine levels has also been reported in genetically epilepsy prone rats. All these reports points toward an inverse relationship between brain histamine levels and epileptogenic activity. MSO is reported to deplete brain histamine levels by enhancement of its catabolism through a stimulatory action on histamine-N-methyl transferase activity. Since histaminergic agents that deplete brain histamine produces proconvulsant effects in experimental animals, whether this depletion of brain histamine by MSO

| Treatments | Dose (per kg) and route of administration | n | Latency (mean ± SE, sec) | Mortality with in 6 h (%)
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<tbody>
<tr>
<td>Distilled water</td>
<td>10 ml, po</td>
<td>6</td>
<td>123.33 ± 5.61</td>
<td>180.00 ± 6.23</td>
</tr>
<tr>
<td>THP</td>
<td>15 mg, ip</td>
<td>5</td>
<td>126.00 ±7.26</td>
<td>170.00 ± 8.00</td>
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<tr>
<td>RAMH</td>
<td>10 mg, icv</td>
<td>5</td>
<td>114.00 ± 4.56</td>
<td>184.00 ± 11.52</td>
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<tr>
<td>SVP</td>
<td>300 mg, po</td>
<td>5</td>
<td>299.00 ± 23.21*</td>
<td>364.00 ± 3.57</td>
</tr>
<tr>
<td>GBP</td>
<td>75 mg, po</td>
<td>5</td>
<td>132.00 ± 5.01</td>
<td>195.00 ± 20.19</td>
</tr>
<tr>
<td>400 mg, po</td>
<td>5</td>
<td>274.00 ± 24.26*</td>
<td>337.00 ± 20.76</td>
<td>0**</td>
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<tr>
<td>GBP</td>
<td>75 mg, ip</td>
<td>5</td>
<td>136.00 ± 6.06</td>
<td>206.00 ± 18.24</td>
</tr>
<tr>
<td>THP</td>
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<td>5</td>
<td>133.60 ± 6.95</td>
<td>200.00 ± 2.44</td>
</tr>
<tr>
<td>GBP</td>
<td>15 mg, ip</td>
<td>5</td>
<td>126.00 ± 8.17</td>
<td>196.00 ± 17.85</td>
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n = number of animals; HLE: Hind limb extension; SVP: Sodium valproate; GBP: Gabapentin; THP: Thioperamide; RAMH: R(α)-methylhistamine.

*P values: * <0.01; ** <0.001
contributes to its convulsant effect is not known. Conversely, agents that inhibit histamine-N-methyl transferase (e.g. metoprine etc) thereby enhancing brain histamine levels produce anticonvulsant effects in various animal models\(^8\). In the present study, no modulation of MSO-induced convulsions by histaminergic modulators THP and RAMH alone or by the combination of THP with AEDs was found. Therefore the convulsant action of MSO, which is attributed to its modulatory effect on GABA synthesis\(^9\), does not appear to be mediated via histaminergic mechanisms. MSO is also known to increase ACh levels in rat brain\(^10\). However, even the latter effect doesn’t contribute to its convulsant effects\(^16\).

In the present study, both SVP and GBP significantly protected mice against MSO-induced convulsions. While SVP is known to have such effects, protective action of GBP in the MSO model is reported for the first time. Elevation of cerebral GABA levels is known to protect against a range of experimentally induced seizures. Bilateral injections of gamma-vinyl GABA in various brain regions were reported to protect against MSO-induced convulsions\(^10\). Several studies indicate that GABA plays a role in the convulsive mechanisms of MSO\(^9,10\). Results of the present study for effects of SVP and GBP alone provide additional evidence for attributing the convulsant effects of MSO to GABAergic mechanisms.

To conclude, SVP and GBP exhibited a protective action against MSO-induced convulsions. The latter were not modulated by THP and RAMH alone or by the combination of THP with SVP or GBP. There is no evidence for the mediation of MSO-induced convulsions via histaminergic mechanisms.

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