LETTER TO THE EDITOR

GABAERGIC MECHANISMS IN MORPHINE ANALGESIA

Sir,

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There are conflicting reports regarding the role of GABA in morphine analgesia. While some investigators have reported that treatment with GABA_A receptor agonist muscimol and GABA transaminase inhibitors potentiates morphine analgesia (2,4,6), others have reported antagonism of morphine analgesia (8,10). In view of these conflicting reports and since the analgesic profile of THIP, a GABA_A receptor agonist, has some similarity with that of morphine (4), in the present study we have investigated on morphine analgesia, the effect of pretreatment with muscimol, a GABA_A receptor agonist, diazepam, a drug which enhances binding of GABA to GABA_A receptors and bicuculline, a GABA_A receptor antagonist (3). We have also investigated on morphine analgesia, the effect of pretreatment with sodium valproate, which increases brain GABA levels by inhibiting its degradation and reuptake (5), and isoniazid, which decreases GABA levels by inhibiting its synthesis (9).

Male albino mice (Haffkine strain) weighing between 30-40 g were used in this study. Antinociceptive activity was tested by employing a modified hot plate method (7). The hot plate apparatus (Techno) was maintained at 55°C and the end point was the first hindpaw lick. Mice reacting within 15 secs were first selected. Failure to respond within 45 secs was arbitrarily taken as the cut off point.

Mice divided into groups of 6 mice each. Each animal was used only once. Morphine sulphate (Bengal Immunity), muscimol (base; Sigma), isoniazid (Unichem) and sodium valproate (Torrent) were dissolved in physiological saline (0.9%). Bicuculline (base; Sigma) solution was prepared by adding 1M HCl and adjusting the final pH to 3. Diazepam (Ranbaxy) was dissolved in propylene glycol (10 mg/ml). All injections were given in a volume of 10 ml/kg.
Morphine sulphate was initially given sc in doses of 1.25, 2.5, 5 and 7.5 mg/kg and the animals were tested 30 min later. Two doses 1.25 mg/kg and 5 mg/kg producing low and high degree of antinociceptive effect respectively were then selected. Drugs affecting GABAergic mechanisms (or vehicle in control groups) were injected 15 min before morphine. Doses used were: muscimol 0.15 mg/kg ip; sodium valproate 115 mg/kg, ip; diazepam 0.5 mg/kg, ip; bicuculline 1 mg/kg, ip; and isoniazid 90 mg/kg, sc.

Results are presented as percentage of mice showing antinociceptive effect (mean ± S.E. of the 4 groups treated with each dose). The levels of significance between means were calculated using Student’s 't' test.

**Table I:** Effects of drugs affecting GABAergic mechanisms on the antinociceptive action of two doses of morphine.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Low dose of morphine (1.25 mg/kg, sc)</th>
<th>High dose of morphine (5 mg/kg, sc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscimol (0.15) ip</td>
<td>35.8 ± 5.8*</td>
<td>82.30 ± 3.4*</td>
</tr>
<tr>
<td>Sodium Valproate (115) ip</td>
<td>32.50 ± 5.8*</td>
<td>86.44 ± 4.5*</td>
</tr>
<tr>
<td>Diazepam (0.5) ip</td>
<td>37.50 ± 2.5*</td>
<td>78.20 ± 11.9*</td>
</tr>
<tr>
<td>Bicuculline (1.0) ip</td>
<td>17.50 ± 4.2*</td>
<td>46.20 ± 4.5**</td>
</tr>
<tr>
<td>Isoniazid (90) sc</td>
<td>42.40 ± 8.6**</td>
<td>12.50 ± 5.8**</td>
</tr>
</tbody>
</table>

P value as compared to morphine alone (*P<0.05; **P<0.01; + not significant). Numerals following drugs indicate their doses (mg/kg). n=6 in each group.

Table I compares the Influence of various drugs affecting GABAergic mechanisms on the antinociceptive effect of two doses of morphine - a low dose of 1.25 mg/kg, sc and a high dose of 5 mg/kg, sc. None of the drugs produced antinociceptive effect by itself. It can be seen that both muscimol and sodium valproate have significantly potentiated the antinociceptive effect of both the low and high doses of morphine. In case of diazepam, however, though it has increased the effect of both doses of morphine, this reaches significance only with the low dose. On the other hand, bicuculline has significantly antagonised the effect of the high dose of morphine without affecting the low dose. Isoniazid pretreatment antagonised the antinociceptive effect of morphine while it antagonised the antinociceptive effect of low and high dose of GABA to GABA receptor agonist, bicuculline.

Our observations, replicated the antinociceptive effect of (2), Buckett (4) and Contrera et al (1) of a GABAergic receptor agonist. However, diazepam and sodium valproate potentiated the antinociceptive effect of both doses of morphine, while bicuculline and isoniazid antagonised the antinociceptive effect of the high dose of morphine. Although the low dose of morphine showed antinociceptive effect alone, this effect was significantly potentiated by bicuculline.

In conclusion, we have demonstrated the influence of GABAergic mechanisms acting through inhibition of GABAergic neurotransmission on the antinociceptive effect of morphine.

1. Andree, T., D.A. Kendall (2), Buckett (4) and Contrera et al.
2. Biggio, G., D. Della Bona (2), Buckett (4) and Contrera et al.
dose. Isoniazid pretreatment has also exerted different effects on the two doses of morphine while it antagonised significantly the higher dose, it potentiated the lower dose.

Our observations, that both muscimol and sodium valproate, significantly potentiated the antinociceptive effect of morphine, concur with the observations of Biggio et al, (2), Buckett (4) and Contreras et al. (6). Furthermore, our observations, that muscimol, a GABA$_A$ receptor agonist, significantly potentiated the antinociceptive effect of both the low and high dose of morphine, and diazepam, a drug which enhances the binding of GABA to GABA$_A$ receptors, significantly potentiated the antinociceptive effect of the low dose of morphine, while bicuculline, a GABA$_A$ receptor antagonist, antagonised the antinociceptive effect produced by high dose of morphine, indicated that GABAergic mechanisms acting through GABA$_A$ receptors have a facilitatory influence on morphine analgesia. However, at present, we are unable to explain why diazepam failed to significantly potentiate the antinociceptive effect produced by high dose of morphine, why bicuculline had no effect on the antinociceptive effect of low dose of morphine and why isoniazid exerted different effects on the antinociceptive effect produced by the two doses of morphine.

In conclusion, we would like to state that our results suggest an involvement of the GABAergic system in the mediation of morphine analgesia.

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REFERENCES


