Antinociceptive activity of a neurosteroid tetrahydrodeoxycorticosterone (5α-pregnan-3α-21-diol-20-one) and its possible mechanism(s) of action

P K Mediratta, M Gambhir, K K Sharma & M Ray

Department of Pharmacology, University College of Medical Sciences and GTB Hospital, Delhi 110095, India

Fax: 91-11-2299495; E-mail: dbmi@ucms.ernet.in/drpramod_k@yahoo.com

Received 9 April 2001; revised 2 July 2001

The present study investigates the effects of a neurosteroid tetrahydrodeoxycorticosterone (5α-pregnan-3α-21-diol-20-one) in two experimental models of pain sensitivity in mice. Tetrahydrodeoxycorticosterone (2.5, 5 mg/kg, ip) dose dependently decreased the licking response in formalin test and increased the tail flick latency (TFL) in tail flick test. Bicuculline (2 mg/kg, ip), a GABAA receptor antagonist blocked the antinociceptive effect of tetrahydrodeoxycorticosterone in TFL test but failed to modulate licking response in formalin test. Naloxone (1 mg/kg, ip), an opioid antagonist effectively attenuated the analgesic effect of tetrahydrodeoxycorticosterone in both the models. Tetrahydrodeoxycorticosterone pretreatment potentiated the antinociceptive response of morphine, an opioid compound and nimodipine, a calcium channel blocker in formalin as well as TFL test. Thus, tetrahydrodeoxycorticosterone exerts an analgesic effect, which may be mediated by modulating GABA-ergic and/or opioid-ergic mechanisms and voltage-gated calcium channels.

It is now well known that some of the steroids like progesterone can act on central nervous system (CNS) to produce a number of endocrine and behavioral effects. Further, enzymes involved in the biosynthesis of some of these steroidal hormones are found in cell specific areas in the brain. These steroids synthesized de novo in the brain are known as neurosteroids. Some of the important steroids characterized in the CNS include progesterone, allopregnanolone (5α-pregnan-3α-ol-20-one), tetrahydrodeoxycorticosterone (5α-pregnan-3α-21-diol-20-one), dehydroepiandrosterone and pregn-anolone sulfate. It has been suggested that neurosteroids may be playing a neuromodulatory role especially the allosteric bimodal regulation of GABA<sub>A</sub> receptor chloride channel<sup>2</sup>, inhibition of glycine chloride channel<sup>3</sup> and voltage gated calcium channels (VGCCs)<sup>4</sup> and potentiation of N-methyl-D-aspartate (NMDA) receptor responses<sup>5</sup>.

Increased levels of progesterone are reportedly associated with decrease in pain sensitivity<sup>6,7</sup>. However, the degree and mechanism by which progesterone exerts its antinociceptive effects are not clearly understood. Progesterone per se is devoid of binding capacity to GABA<sub>A</sub> receptors, but in the neuronal glia of the brain it is metabolized to allopregnanolone and tetrahydrodeoxycorticosterone<sup>8,9</sup> which are among the most potent of the known neurosteroids active at GABA<sub>A</sub> receptors<sup>2</sup>. Allopregnanolone has been shown to exhibit antinociceptive effect against an aversive thermal stimulus<sup>10</sup> and in a rat mechanical visceral pain model<sup>11</sup>. However, the role of tetrahydrodeoxycorticosterone in mediation of pain sensitivity has not been well defined. The present study investigates the effect of tetrahydrodeoxycorticosterone on tail flick latency test, a model of acute pain and formalin induced pain response, a model of tonic (continuous) pain.

**Animals**—Swiss albino male mice weighing 25-30 g were obtained from the Central Animal House of the Institution and housed in 12hr light-12 hr dark cycle at 22°±2°C with food and water available freely except 1hr before and during the experiment. The animals were maintained as per the ‘Guidelines for the Care and Use of Animals in Scientific Research’ prepared by Indian National Science Academy, Delhi<sup>12</sup> and the approval from the Institutional Ethical Committee was taken.

**Drugs and Chemicals**—Tetrahydrodeoxycorticosterone, naloxone, bicuculline methiodide (Sigma Chemical Co., USA), nimodipine (Cipla Ltd.) and morphine sulfate were used in the study. Tetrahydrodeoxycorticosterone was dispersed in 1% Tween 80 and diluted with saline, nimodipine was dissolved in 100% ethanol and morphine sulfate, naloxone and bicuculline were dissolved in distilled water. Formalin (0.5%; v/v) solution was prepared by
Adding normal saline to the stock solution of 4% formaldehyde in water.

Animals were randomly divided into different groups of 10 mice in each group. Tetrahydrodeoxy corticosterone was injected 1 hr, morphine, bicuculline and nimodipine 30 min, whereas naloxone 15 min, before performing the analgesic test. The drugs/vehicle were administered intraperitoneally (ip).

Tail flick latency test—The tail of the animal was placed in the slot of water circulated jacket of analgesiometer (TECHNO) which had a tungsten wire 1 mm below the tail surface. The heat was adjusted daily to provide normal reaction time of 2-4 sec. For drug studies a cut off time of 10 sec was used and if the mouse did not respond in 10 sec, it was removed and given a score of 10.

Formalin test—The test was performed by injecting 0.2 ml of 0.5% formalin under the plantar surface of right hind paw of mouse. The left paw was injected with 0.2 ml of 0.9% saline and acted as control. The animal was then kept in an open mouse perspex cage and the time spent by the animal licking the injected paw was recorded. Two distinct periods of intensive licking, i.e. an early phase (0-5 min) and late phase (25-30 min) were observed and scored separately for assessing drug effect.

Statistical analysis—All results are expressed as mean ± SE. The data were analyzed using unpaired Student’s t test and a P value of less than 0.05 was considered as significant.

The results are summarized in Table 1. Tetrahydrodeoxy corticosterone (2.5, 5 mg/kg, ip) produced a dose dependent antinociceptive effect in both tail flick latency (TFL) test, a model for acute pain, and formalin induced pain response, a model for tonic continuous pain. There was a significant increase in the TFL and a decrease in time an animal spent in licking the formalin injected paw during early as well as late phase in formalin test. The increase in TFL produced by tetrahydrodeoxy corticosterone was effectively blocked by both bicuculline (2 mg/kg, ip), a GABA<sub>A</sub> receptor antagonist and naloxone (1 mg/kg, ip) an opioid antagonist. Thus, the analgesic effect of tetrahydrodeoxy corticosterone on TFL seems to be mediated via modulation of GABA<sub>A</sub> chloride channel complex as well as opioid-ergic pathways. Unlike its effect on TFL, the decrease in paw licking response by tetrahydrodeoxy corticosterone was antagonized significantly only by naloxone and not by bicuculline indicating thereby that the opioid-ergic mechanims may be playing a more important role in the antinociceptive effect of tetrahydrodeoxy corticosterone. Morphine (2.5, 5 mg/kg, ip) produced a dose dependent analgesic response in both TFL and formalin test. This antinociceptive effect of morphine was potentiated by pretreating the animals with tetrahydrodeoxy corticosterone (Table 1).

Table 1—Effect of tetrahydrodeoxy corticosterone (THDOC) and its interaction with GABAergic, opioidergic and a calcium channel blocker, nimodipine in tail flick latency and formalin tests

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tail flick latency (sec)</th>
<th>Licking response (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early phase (0-5 min)</td>
<td>Late phase (25-30 min)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>2.8 ± 0.3</td>
<td>132.2 ± 10.4</td>
</tr>
<tr>
<td>THDOC (2.5)</td>
<td>4.4 ± 0.8</td>
<td>90.6 ± 8.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>THDOC (5)</td>
<td>6.4 ± 0.8&lt;sup&gt;**&lt;/sup&gt;</td>
<td>62.4 ± 7.4&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>THDOC (5) + Bicuculline (2)</td>
<td>3.0 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>65.5 ± 9.4&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>THDOC (5) + Naloxone (1)</td>
<td>3.2 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>112.4 ± 7.2&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Morphine (2.5)</td>
<td>4.8 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>88.6 ± 8.4&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Morphine (5)</td>
<td>7.8 ± 1.8&lt;sup&gt;***&lt;/sup&gt;</td>
<td>50.4 ± 9.6&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>THDOC (2.5) + Morphine (2.5)</td>
<td>6.8 ± 0.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>58.8 ± 6.8&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nimodipine (20)</td>
<td>2.9 ± 0.6</td>
<td>85.4 ± 8.8&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nimodipine (40)</td>
<td>3.2 ± 0.4</td>
<td>58.6 ± 6.7&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>THDOC (2.5) + Nimodipine (20)</td>
<td>7.2 ± 1.6&lt;sup&gt;***&lt;/sup&gt;</td>
<td>52.4 ± 12.4&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>P</sup> values: *<sub>P</sub> < 0.05; **<sub>P</sub> < 0.01; ***<sub>P</sub> < 0.001
<sup>a</sup> Compared to vehicle-treated group
<sup>b</sup> Compared to THDOC-treated group
<sup>c</sup> Compared to respective THDOC/Morphine/Nimodipine-treated group
observation lends further credence to the hypothesis that opioid-ergic pathways are playing a significant role in mediating the analgesic effect of tetrahydrodeoxycorticosterone.

Neurosteroids have been shown to inhibit VGCCs. Further, various calcium channel blockers are reported to exhibit an antinociceptive effect. Hence, tetrahydrodeoxycorticosterone may be exerting an analgesic effect by inhibiting VGCCs also. In the present study nimodipine, a CCB per se decreased the paw licking response in formalin test but failed to modulate TFL. This corroborates earlier observations, which reported that various CCBs produced a significant analgesic response in formalin test but failed to do so in TFL test. The analgesic effect of nimodipine was potentiated by prior administration of tetrahydrodeoxycorticosterone suggesting thereby that inhibition of VGCCs may also be involved in mediating the antinociceptive effect of tetrahydrodeoxycorticosterone. It has been shown that various calcium channels like P, Q and L-type are coupled to opioid receptors and are inhibited by activation of the mu, delta or kappa opioid receptors. Results of the present study show that tetrahydrodeoxycorticosterone potentiated the analgesic response to morphine and nimodipine and the per se antinociceptive effect of tetrahydrodeoxycorticosterone was effectively antagonized by naloxone indicating thereby that tetrahydrodeoxycorticosterone may be exerting an analgesic effect via modulating opioid receptors linked to calcium channels. Thus, tetrahydrodeoxycorticosterone exhibited a significant antinociceptive response, which may involve modulation of GABA-ergic and/or opioidergic mechanisms and VGCCs.

References