Involvement of dopamine D₂ and 5-HT₁A receptors in roxindole-induced antinociception

Ipe Ninan & S K Kulkarni*

Pharmacology Division, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh 160 014, India

Received 23 March 1998; revised 27 November 1998

Roxindole, a DA D₂ receptor agonist (2-16 mg/kg) produced dose-dependent increase in percentage antinociception. The effect which was blocked by DA D₂ antagonist (-)sulpiride (50 mg/kg) and 5-HT₁A receptor antagonist (-)pindolol (5 mg/kg). Roxindole (4 and 8 mg/kg) reversed both naloxone (20 mg/kg)-induced hyperalgesia and reserpine (2 mg/kg)-induced hyperalgesia. This reversal was sensitive to blockade by both (-)sulpiride (50 mg/kg) and (-)pindolol (5 mg/kg). The present study suggests that roxindole-induced antinociception is mediated by postsynaptic DA D₂ and 5-HT₁A receptors.

Roxindole (EMD 49980) is a representative of a new class of dopaminergic drugs, i.e., the indolyl butylamines, which lack structural similarities with either rigid dopamine (DA) analogs or ergot derivatives. Roxindole exhibits potent, long lasting and highly selective agonistic actions at presynaptic D₂ receptors without stimulating postsynaptic D₂ receptors even at high doses. This selectivity makes roxindole a pre-eminent candidate for a novel type of antipsychotic drug without neurological side effects which limit the usefulness of conventional D₂ receptor blockers. Within the past few years, several new putative, selective DA autoreceptor agonists have been developed, among them are the trans-fused analog of 3-PPP, (-)-HW 165, the transdihydroergoline terguride and the amino-ergolines SDZ 208-912 (ref. 3-5). Interest in these putative DA autoreceptor agonists derives largely from their potential use as non-classical antipsychotic drugs. Roxindole, besides its affinity for D₂ receptors, has affinity for the 5-HT₁A receptor also.

Considerable clinical evidence suggests that DA receptor-mediated mechanisms may play an important role in modulating pain in humans. A number of animal studies have shown that DA is involved in the regulation of nociception. However, the data reported are contradictory and DA agonists have been shown to produce either antinociception or hyperalgesia or to have both effects. However, some DA agonists have been reported to suppress the severe pain associated with various states of disease affecting the nervous system, such as: the thalamic syndrome, herpes zoster and Parkinson's disease, and a recent study showed that 8-OH DPAT inhibits nociceptive responses by stimulating postsynaptic 5-HT₁A receptors.

The present study was undertaken to investigate the role of D₂ and 5-HT₁A receptors in the antinociception produced by roxindole in naive and reserpinned mice.

Materials and Methods

Animals—Balb/C mice (bred in Central Animal House facility of Panjab University), weighing 20-25 g, were housed under a standard 12 h light/12 h dark cycle. The animals were acclimatized to the laboratory conditions with free access to food and water for 24 h before the experiment. All experiments were undertaken between 09.00 and 13.00 h.

Technique—The nociceptive threshold was determined as the tail-flick latencies elicited in response to noxious radiant heat. Baseline latencies to tail-flick withdrawal from the radiant heat source (3-4 s) were established. A cut off time of 10 s was observed to prevent any injury to the tail. A minimum of three trials, at 2 min intervals, were recorded for each animal before the test. Antinociception was calculated according to the following formula: % antinociception = 100×(test latency - baseline latency) / (10 - baseline latency).

Drugs—Roxindole mesylate (EMD 49980, Merck KGaA Biomed Res CNS, Germany), (-) sulpiride
hydrochloride (Research Biochemicals Inc, MA, USA), naloxone hydrochloride (Sigma, USA), (-) pindolol (Ranbaxy, India) and reserpine (Loba Chemicals, India) were used in the study. The drug solutions were made in distilled water, except reserpine and (-) pindolol. Reserpine was dissolved in a drop of glacial acetic acid while pindolol was dissolved in a drop of dilute HCl, volume made up with distilled water and pH adequately adjusted. All the drugs were administered intraperitoneally in a constant volume of 1 ml/100 g of body weight. The selection of doses was based on previous reports from our laboratory. Each group comprised 5-10 mice.

Reserpine (2 mg/kg) was administered 4 h prior

Table 1—Reversal of naloxone-induced hyperalgesia by roxindole and its modification by (-) sulpiride and (-) pindolol in mice

<table>
<thead>
<tr>
<th>No.</th>
<th>Treatment (mg/kg)</th>
<th>% Antinociception (Mean±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saline</td>
<td>0.88±00</td>
</tr>
<tr>
<td>2</td>
<td>Naloxone (20)</td>
<td>-36.27±4.26</td>
</tr>
<tr>
<td>3</td>
<td>Roxindole (4)+Naloxone (20)</td>
<td>21.60±1.63³</td>
</tr>
<tr>
<td>4</td>
<td>Roxindole (8)+Naloxone (20)</td>
<td>8.04±0.87⁴</td>
</tr>
<tr>
<td>5</td>
<td>Sulpiride (50)+Roxindole (4)+Naloxone (20)</td>
<td>0.16±0.10⁵</td>
</tr>
<tr>
<td>6</td>
<td>Sulpiride (50)+Roxindole (8)+Naloxone (20)</td>
<td>-5.68±0.38⁶</td>
</tr>
<tr>
<td>7</td>
<td>Pindolol (5)+Roxindole (4)+Naloxone (20)</td>
<td>-9.15±0.42⁸</td>
</tr>
<tr>
<td>8</td>
<td>Pindolol (5)+Roxindole (8)+Naloxone (20)</td>
<td>-10.4±0.06⁹</td>
</tr>
</tbody>
</table>

*P<0.001 as compared to group 2; *P<0.001 as compared to group 3; *P<0.001 as compared to group 4

Fig. 1—Antinociceptive effect of roxindole (2-16 mg/kg) and its modification by (-) sulpiride (50 mg/kg) and (-) pindolol (5 mg/kg) in mice. a,b,c,d P<0.001 as compared to roxindole (2, 4, 8 and 16 mg/kg) groups, respectively.


Table 2—Reversal of reserpine (4 hr post)-induced hyperalgesia by roxindole and its modification by (-) sulpiride and (-) pindolol respectively in mice

<table>
<thead>
<tr>
<th>No.</th>
<th>Treatment (mg/kg)</th>
<th>% Antinociception (Mean±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saline</td>
<td>0.88±0.00</td>
</tr>
<tr>
<td>2</td>
<td>Reserpine (2)</td>
<td>-18.30±0.51</td>
</tr>
<tr>
<td>3</td>
<td>Reserpine (2)+Roxindole (4)</td>
<td>12.46±0.78</td>
</tr>
<tr>
<td>4</td>
<td>Reserpine (2)+Roxindole (8)</td>
<td>23.34±1.36</td>
</tr>
<tr>
<td>5</td>
<td>Reserpine (2)+Sulpiride (50)+Roxindole (4)</td>
<td>-12.93±0.13</td>
</tr>
<tr>
<td>6</td>
<td>Reserpine (2)+Sulpiride (50)+Roxindole (8)</td>
<td>-11.04±0.78</td>
</tr>
<tr>
<td>7</td>
<td>Reserpine (2)+Pindolol (5)+Roxindole (4)</td>
<td>-13.09±0.74</td>
</tr>
<tr>
<td>8</td>
<td>Reserpine (2)+Pindolol (5)+Roxindole (8)</td>
<td>-10.41±0.42</td>
</tr>
</tbody>
</table>

*P<0.001 as compared to group 2; a P<0.001 as compared to group 3; a P<0.001 as compared to group 4.

and naloxone 15 min prior to the experiment. Roxindole was administered 30 min prior to the test. The DA receptor antagonists (-) sulpiride and 5-HT_{1A} antagonist (-) pindolol were administered 30 min prior to the agonists.

Statistical analysis—The data was analyzed using one way ANOVA followed by student's t-test and P<0.05 was considered statistically significant.

Results

Antinociceptive effect of roxindole and its reversal by (-) sulpiride and (-) pindolol—Roxindole (2-16 mg/kg) produced dose-dependent increase in percentage antinociception with higher doses (8 and 16 mg/kg) showing ceiling effects. Lower doses (0.01-1 mg/kg) of roxindole did not show antinociceptive effect. (-) Sulpiride (50 mg/kg) and (-) pindolol (5 mg/kg) blocked the antinociceptive effect of roxindole (2-16 mg/kg) (P<0.001) (Fig. 1). (-) Sulpiride (50 mg/kg) or (-) pindolol (5 mg/kg) per se did not show any effect on nociceptive response.

Effect of roxindole on hyperalgesia induced by naloxone and reserpine—Naloxone (20 mg/kg) produced a significant decrease in percentage antinociception. Roxindole (4 and 8 mg/kg) reversed the naloxone-induced hyperalgesia (P<0.001). Both (-) sulpiride (50 mg/kg) and (-) pindolol (5 mg/kg) blocked the reversal of hyperalgesia by roxindole (P<0.001) (Table 1).

Reserpine (2 mg/kg, 4 hr prior) pre-treatment significantly reduced percentage antinociception. The reserpine-induced reduction in the nociceptive threshold was reversed by roxindole (4 and 8 mg/kg) (P<0.001). Both (-) sulpiride (50 mg/kg) and (-) pindolol (5 mg/kg) blocked the reversal of hyperalgesia by roxindole (P<0.001) (Table 2).

Discussion

The present study demonstrates that acute administration of D_{2} autoreceptor selective agonist, roxindole produced an antinociceptive response in mice. Previously, Verma and Kulkarni (1993) have reported that DA agonists such as B-HT 920, bromocriptine and apomorphine produced antinociception. The antinociceptive response elicited by roxindole was blocked by (-) sulpiride, a D_{2} antagonist and (-) pindolol, a selective 5-HT_{1A} antagonist. The available literature suggests the involvement of postsynaptic D_{2} receptors in antinociception. Previous studies showed that D_{2} agonists do not show antinociceptive effect at doses which stimulate autoreceptors. Therefore, the possibility of involvement of presynaptic D_{2} receptors in this antinociceptive effect is rare. The antinociceptive effect produced by roxindole was blocked by both (-) sulpiride, a selective D_{2} antagonist and (-) pindolol, a 5-HT_{1A} antagonist. It is known that 5-HT_{1A} receptors control DA neurotransmission in several brain areas. The 5-HT_{1A} agonist, 8-OH DPAT increases locomotor activity in rats. It could be possible that roxindole can stimulate DA neurotransmission which inturn might be responsible for the antinociceptive effect. But some recent studies have shown that 5-HT_{1A} agonists including 8-OH DPAT and flesinoxan, have apparent inhibitory actions on dopamine neurotransmission.

The reversing effect of reserpine-induced hyperalgesia by roxindole was blocked by (-) sulpiride and (-) pindolol. In reserpinised animals, the consequences of any presynaptic receptor activation are eliminated because of the depletion of various neurotransmitters, and makes it possible to measure postsynaptically mediated effects. This suggests the partial agonistic action of roxindole at postsynaptic D_{2} receptors as well as 5-HT_{1A} receptors. Bartoszyk et al. (1996) reported that roxindole reverses reserpine-induced hypomotility. Both talipexole and roxindole have been reported to produce psychomotor
stimulation and exacerbation of psychosis\textsuperscript{27,28}. Recently, we have reported that clozapine, an atypical antipsychotic having partial agonistic action at D\textsubscript{2} receptors in DA depleted animals\textsuperscript{28}. Partial agonism of 5-HT\textsubscript{1A} receptors were recently proposed to contribute to the unique profile of clozapine as compared with classical neuroleptics\textsuperscript{29,30}. Similarly, oxindole reversed naloxone-induced hyperalgesia and this effect was reversed by both (-) sulpiride and (-) pindolol. Earlier, Verma and Kulkarni (1993) suggested that there existed an interlink between opioid and DAergic systems in the brain and showed that D\textsubscript{2} agonists reverse naloxone-induced hyperalgesia\textsuperscript{7}. Further, recent studies have shown that some possible interaction between 5-HT\textsubscript{1A}-opiod system in antinociceptive response\textsuperscript{31,32}. The present study therefore, suggests that oxindole-induced antinociception involved both postsynaptic DA D\textsubscript{2} and 5-HT\textsubscript{1A} receptors.

Acknowledgement
The Senior Research Fellowship (IN) of CSIR, New Delhi, is acknowledged. The authors also thank Merck KGaA, Biomed Res CNS, Germany for generously donating oxindole mesylate used in this study.

References