Role of ATP-sensitive potassium channels in the piracetam induced blockade of opioid effects

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The present study has been designed to investigate the effect of piracetam on morphine/ buprenorphine-induced antinociception in rats and effect of piracetam on morphine or minoxidil induced relaxation in KCl-precontracted isolated rat aortic ring preparation. Nociceptive threshold was measured by the tail flick test in rats. The cumulative dose responses of morphine or minoxidil were recorded in KCl-precontracted isolated rat aortic ring preparation. Piracetam attenuated buprenorphine-induced antinociception in rats. Piracetam significantly reduced the morphine and minoxidil induced relaxation in KCl precontracted isolated rat aortic ring preparation suggesting that piracetam interferes with opioid receptor and ATP-sensitive potassium channel (KATP) opener mediated responses in vitro. Thus, it may be suggested that piracetam attenuates opioid effects by an opioid receptor-KATP channel linked mechanism.

Keywords: ATP-sensitive potassium channels, Antinociception, Opioid receptor, Piracetam

Piracetam, a cerebroactive agent, is being used to treat various dementias for several years as it enhances or facilitates various learning and other cognitive functions1. Piracetam has also demonstrated to be clinically beneficial in controlling vertigo2 and myoclonus3. Opioid receptor agonists are the earliest known and clinically used analgesics4. Morphine and other opioid analogues like buprenorphine have been demonstrated to exert marked antinociceptive behavior in experimental animals5, 6. Piracetam has been shown to attenuate the opioid antinociception7. Besides, piracetam increases the intracellular ATP concentration in the nerve cell cytosol8 that may have an inhibitory effect over the ATP-gated potassium channels (KATP channels). Minoxidil, a selective KATP channel opener, when administered centrally has been shown to exert an antinociceptive effect9. Additionally, piracetam has been shown to block the centrally administered minoxidil induced antinoiceception in mice10. Therefore, the present study has been designed to investigate the effect of piracetam on morphine and buprenorphine induced antinociception in rats and also the effect of piracetam on morphine, an opioid agonist, and minoxidil, a selective KATP channel opener11, induced relaxation in KCl-precontracted isolated rat aortic ring preparation.

Materials and Methods

Age matched male wistar rats weighing 250 ± 15g maintained on standard laboratory diet (Kisan Feeds Ltd., Mumbai, India) and having free access to tap water were employed. They were housed in the departmental animal house and were exposed to 12 hr cycle of light and dark. The experimental protocol was approved by the institutional animal ethical committee and care of the animals was carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India (Reg. No. – 107/ 1999/ CPCSEA).

Drugs and chemicals—Minoxidil (Dr. Reddy's Laboratories Ltd., Hyderabad, India), morphine (Jackson Laboratories Ltd., Amritsar, India), buprenorphine (Unichem Laboratories Ltd., Mumbai, India) and piracetam (UCB India Ltd., Mumbai, India) were dissolved in normal saline immediately before use.

Measurement of the nociceptive threshold—Nociceptive threshold was measured by the tail flick test in rats12. The tail flick latency was considered as the time between tail exposure to radiant heat and tail withdrawal. Electrically heated nichrome wire was used as a source of radiant heat in the analgesiometer. The intensity of radiant heat was regulated in order to obtain pretreatment latency between 2 to 3 sec in the...
animals. A cut off latency time was fixed at 10 sec. Tail flick latency was expressed as a percentage of the maximum possible effect (MPE):

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\text{MPE} \% = \frac{(\text{post treatment latency} - \text{pre treatment latency})}{\text{(cut off time} - \text{pre treatment latency})} \times 100
\]

Peak time for morphine/buprenorphine was 30 min after administration. Thus, tail flick latency was observed at 30 min after morphine/buprenorphine administration.

**Experimental protocol**—The rats were divided into following 9 groups of 10 animals each:

Control group (Group I): Rats were administered saline (1 ml/kg, sc).

Morphine treated control group (Group II): Rats were administered morphine (8 mg/kg, sc).

Buprenorphine treated control group (Group III): Rats were administered buprenorphine (0.05 mg/kg, sc).

Low dose piracetam and morphine treated group (Group IV): Rats were administered piracetam (250 mg/kg, ip) 30 min prior to morphine injection (8 mg/kg, sc).

High dose piracetam and morphine treated group (Group V): Rats were administered piracetam (500 mg/kg, ip) 30 min prior to morphine injection (8 mg/kg, sc).

Low dose piracetam and buprenorphine treated group (Group VI): Rats were administered piracetam (250 mg/kg, ip) 30 min prior to buprenorphine injection (0.05 mg/kg, sc).

High dose piracetam and buprenorphine treated group (Group VII): Rats were administered piracetam (500 mg/kg, ip) 30 min prior to buprenorphine injection (0.05 mg/kg, sc).

**In vitro**: Morphine/Piracetam treated group (Group VIII): Aortic ring was isolated from the thoracic aorta region and the preparation was put up following which CRC of morphine alone and then piracetam followed by CRC of morphine on KCl precontracted preparation were taken.

**In vitro**: Minoxidil/Piracetam treated group (Group IX): Aortic ring was isolated from the thoracic aorta region and the preparation was put up following which CRC of minoxidil alone and then piracetam followed by CRC of minoxidil on KCl precontracted preparation were taken.

**Isolated rat aortic ring preparation**—The rats were decapitated, thoracic aorta was removed, cut into a ring of 4-5 mm and mounted in organ bath containing Krebs-Henseleit solution (NaCl, 119 mM; KCl, 4.7 mM; NaHCO₃, 25 mM; MgSO₄, 1.0 mM; glucose, 11.1 mM; KH₂PO₄, 1.2 mM and CaCl₂, 2.5 mM) bubbled with carbonated oxygen (95% O₂ and 5% CO₂) and maintained at 37°C. The preparation was allowed to equilibrate for 90 min under 1.5 g tension. The isometric contractions were recorded with a force displacement transducer (Ft-2147) connected to Physiograph (INCO, Ambala, India).

The cumulative dose responses of morphine (3.5 × 10⁻⁶ to 2.24 × 10⁻⁴ M) or minoxidil (5 × 10⁻⁵ to 3.2 × 10⁻³ M) were recorded in KCl (8 × 10⁻² M & 2 × 10⁻² M for morphine and minoxidil respectively) precontracted preparation. For the piracetam treated groups, piracetam (2.82 × 10⁻² M) was added 10 min prior to the repetition of the cumulative dose response procedure performed for morphine and minoxidil. Time matched KCl precontracted rat aortic ring preparations were also put up to serve as control. The concentrations of various drugs/chemicals were selected in line with certain previous findings.

**Statistical analysis**—Statistical analysis for the results of the tail-flick test was done using multifactorial ANOVA followed by Tukey-kramer test. Whereas, data for isolated aortic ring preparation were statistically analyzed using Repeated Measures ANOVA (RM-ANOVA) followed by Newman-Keul’s test. A value of \(P<0.05\) was considered to be statistically significant.

**Results**

**Effect of piracetam on morphine induced antinociception in rats**—Morphine (8 mg/kg, sc) produced significant increase in % maximum possible effect (MPE) as compared to the control group. Piracetam (250, 500 mg/kg, ip) significantly and dose dependently attenuated morphine-induced antinociception in rats measured in terms of increase in % MPE (Fig. 1).

**Effect of piracetam on buprenorphine induced antinociception in rats**—Buprenorphine (0.05 mg/kg, sc) produced significant increase in % MPE as compared to the control group. Piracetam (250, 500 mg/kg, ip) significantly attenuated buprenorphine-induced antinociception in rats measured in terms of
increase in % MPE. The attenuation was found to be dependent on the dose of piracetam (Fig. 2).

**Effect of piracetam on morphine induced relaxation in KCl-precontracted isolated rat aortic ring preparation**—Morphine (3.5 \times 10^{-6} to 2.24 \times 10^{-4} M) produced relaxation of KCl (80 \times 10^{-3} M) precontracted isolated rat aortic ring preparation in a dose dependent manner. Piracetam (2.82 \times 10^{-2} M) significantly attenuated the morphine induced relaxation in KCl precontracted isolated rat aortic ring preparation (Fig. 3).

**Effect of piracetam on minoxidil induced relaxation in KCl-precontracted isolated rat aortic ring preparation**—Minoxidil (5 \times 10^{-5} to 3.2 \times 10^{-3} M) produced relaxation of KCl (20 \times 10^{-3} M) precontracted isolated rat aortic ring preparation in a dose dependent manner. Piracetam (2.82 \times 10^{-2} M) significantly attenuated the minoxidil induced relaxation in KCl precontracted isolated rat aortic ring preparation (Fig. 4).
Discussion

The results of the present study demonstrate that morphine/buprenorphine-induced antinociception was dose-dependently attenuated by piracetam. Morphine and its analog buprenorphine are well known potent opioid analgesics\(^\text{14}\). Piracetam has been shown to attenuate the antinociceptive effects of opioid analgesics and this supports our findings\(^\text{5}\). In the present investigation piracetam blocked the relaxant effect of morphine on KCl-induced precontraction in isolated rat aortic ring preparation. Morphine has been shown to relax the vasculature possibly through the µ opioid receptors present on the endothelial lining\(^\text{15}\). Piracetam at higher concentrations has been shown to exert inhibitory influence on opioid receptors\(^\text{16}\). Yoshii \textit{et al.}\(^\text{17}\) have reported that nefiracetam, an analog of piracetam, has an inhibitory effect over the opioid receptor-G protein complex by preferentially binding to Gi3\(\alpha\) associated with opioid receptors thereby inhibiting the association of G proteins with ion channels. Therefore, piracetam induced reversal of morphine/buprenorphine antinociception may be mediated through its modulatory role on opioid receptors. K\(\text{ATP}\) channels are one of the end effectors in the opioid transduction pathway\(^\text{18-20}\). Minoxidil, a selective K\(\text{ATP}\) channel opener\(^\text{11}\), when administered centrally have been shown to induce antinociception in rats\(^\text{9}\). Piracetam has been noted to increase the intracellular ATP concentration in the nerve cell cytosol\(^\text{8}\), which may have an inhibitory effect over the K\(\text{ATP}\) channels. Additionally, piracetam has been shown to block the centrally administered minoxidil induced antinociception in mice\(^\text{10}\). Thus, piracetam induced blockade of K\(\text{ATP}\) channels might be mediating its blockade of opioid effects. This contention is further supported by present observation where piracetam was also observed to block the relaxant effect of morphine/minoxidil on KCl-induced contraction in the isolated rat aortic ring preparation.

On the basis of above discussion, it may be concluded that piracetam attenuates opioid effects possibly by an ATP-sensitive potassium channel and opioid receptor linked signal transduction systems. However further investigation is required to validate the link between these two receptor systems in mediating piracetam induced antagonism of opioid effects.

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