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Cyclo-oxygenase-inhibitors increase morphine effects on mesolimbic dopamine neurons

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Abstract

The effect of cyclo-oxygenase inhibitors, indomethacin and nimesulide, on the action of i.v. morphine on dopamine neurons projecting to the nucleus Accumbens was studied using standard extracellular recording techniques coupled with antidromic identification in unanesthetized rats. The i.v. administration of either nimesulide (3 mg/kg) or indomethacin (3.5 mg/kg) per se did not affect the firing rate of mesolimbic dopamine cells. In contrast, the subsequent administration of morphine (0.25–2 mg/kg i.v.) potently increased the firing rate of mesolimbic dopamine neurons in cyclo-oxygenase inhibitor-pretreated rats as compared with saline-pretreated rats. The maximal enhancement of basal firing rate at the highest dose of morphine tested was 92%, 80% and 47%, for nimesulide-, indomethacin-, and saline-treated rats, respectively. Our results indicate that the effect of morphine on mesolimbic dopamine cells is potentiated by blocking cyclo-oxygenase activity and suggest that the modulation of cyclo-oxygenase pathway in dopamine cells might be involved in the cellular mechanisms of the rewarding actions of morphine.

Keywords: Dopamine; Electrophysiology; Morphine

It has been proposed recently that cyclo-oxygenase inhibitors, such as indomethacin and acetylsalicylic acid, potentiate the suppressing effect of opioids on presynaptic γ-amino butyric acid (GABA) neurotransmission in periaqueductal Grey neurons, which is involved in the analgesic effects of opioids (Vaughan, 1998). Similarly, morphine in vitro hyperpolarizes GABA interneurons in the pars reticulata of the substantia nigra (Johnson and North, 1992) and reduces their spontaneous electrical activity in vivo (Finnerty and Chan, 1979). Such decreased synaptic input to dopamine cells leads to their disinhibition (Matthews and German, 1984), a μ-opioid receptor mediated effect, which appears to be important for the euphorigenic properties of morphine (Wise, 1987). Since the periaqueductal grey also plays a role in the reinforcing properties of systemic morphine (Cazala, 1990), and this effect is blocked by naloxone, it is tempting to speculate that the neural substrates of both the reinforcing and analgesic properties of morphine are at least partially overlapping (Franklin, 1998). In order to verify this hypothesis, we studied the effect of intravenous morphine on the firing rate and pattern of antidromically identified mesolimbic dopamine neurons after pre-treatment with two cyclo-oxygenase inhibitors, either nimesulide or indomethacin, using standard extracellular recordings coupled with antidromic identification in unanesthetized rats.

Experiments were carried out in non-anesthetized rats because general anesthetics modify both the firing rate and pattern of dopamine neurons and their response to pharmacological agents, including morphine (Matthews and German, 1984). Male Sprague–Dawley albino rats (200–225 g) were used in all experiments. All subjects were kept on a 12 h/12 h light/dark cycle with food and water available ad libitum. Experimental protocols were approved by the Ethical Committee (EC) of the University of Cagliari and performed in strict accordance with EC regulations for the care and use of experimental animals (CEE N°86/609). Electrophysiological experiments were performed as already described (Diana et al., 1998). Dopamine neurons were identified according to well established electrophysiological characteristics including antidromic activation from the nucleus accumbens and collision of an antidromically elicited spike with spontaneously occurring

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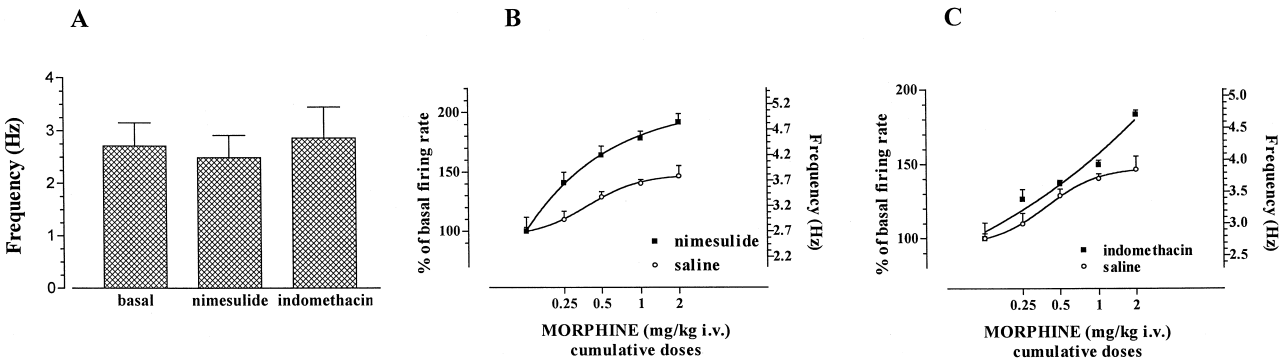

Fig. 1. (A) Effect of nimesulide and indomethacin on the firing rate of mesolimbic dopamine neurons in non-anesthetized rats. The effect was observed at the highest dose administered for both nimesulide (3 mg/kg i.v.) and indomethacin (3.5 mg/kg i.v.). Data are expressed as means of firing rate ± SEM. $P > 0.05$ with respect to pre-drug level (Student's *t*-test). (B) Dose–response curves for the cumulative doses of i.v. morphine on the firing rate of antidromically identified mesolimbic dopamine neurons in non-anesthetized rats. Morphine is more efficacious in neurons recorded from nimesulide-treated rats as compared with controls. Data are expressed as percentages (on left *y*-axis) and as means of firing rate (on right *y*-axis) ± SEM. A logistic function was fitted to the concentration–response curves to determine EC_{50}. (C) Dose–response curves for the cumulative doses of i.v. morphine on the firing rate of antidromically identified mesolimbic dopamine neurons in non-anesthetized rats. Morphine is more efficacious in neurons recorded from indomethacin-treated rats. Data are expressed as percentages (on left *y*-axis) and as means of firing rate (on right *y*-axis) ± SEM. A logistic function was fitted to the concentration–response curves to determine EC_{50}.

action potentials. Firing rate and pattern analysis were performed as already described (Diana et al., 1998). Morphine hydrochloride (Salars, Como, Italy) was diluted in saline solution. Injection volumes were 1 ml/kg of body weight. The statistical significance of the data was evaluated by analysis of variance (ANOVA) for repeated measures, followed by Student–Newman–Keuls' test.

To determine the effect of i.v. nimesulide and indomethacin, we examined their effect on spontaneous electrical activity of mesolimbic dopamine cells. As shown in Fig. 1A, neither nimesulide (3 mg/kg i.v.) and indomethacin (3.5 mg/kg i.v.) affected the firing rate of mesolimbic dopamine neurons nor did they have any effect on the firing pattern. Interestingly, pretreatment with either nimesulide or indomethacin potentiated the morphine-induced increase of the firing rate of mesolimbic dopamine cells (Fig. 1B and C). Indeed, at the highest dose of morphine tested (0.25–2 mg/kg in a cumulative dose regimen), the maximal enhancement of frequency was of 92 ± 6.8 and 83.6 ± 2.8% for nimesulide- ($n = 9$, ANOVA for repeated measures, $F(1,4) = 26.36$, $P < 0.0001$) and indomethacin-treated ($n = 8$, ANOVA for repeated measures, $F(1,4) = 4.90$, $P = 0.03$) rats, respectively. In contrast, the maximal effect produced by i.v. morphine (0.25–2 mg/kg) in control rats ($n = 9$) was of 46.6 ± 8.7%. Furthermore, pre-treatment with nimesulide and indomethacin produced a four- and a two-fold increase in the potency of morphine, and shifted the lowest effective dose of morphine to 0.25 and 1 mg/kg, respectively (Nimesulide: $n = 9$, Student–Newman–Keuls' test $F = 18.14$, $P < 0.0001$; Indomethacin: $n = 8$, Student–Newman–Keuls' test $F = 11.97$, $P < 0.0001$).

The results obtained indicate that administration of cyclo-oxygenase inhibitors, nimesulide and indomethacin, potently stimulates the effect of morphine on the firing rate of dopamine neurons projecting to the nucleus accumbens.

Differences observed in efficacy and potency of nimesulide ($EC_{50} = 0.32$ mg/kg i.v.) and indomethacin ($EC_{50} = 1.0$ mg/kg i.v.) could have several explanations. It might be due to the selective and non-selective inhibition of the cyclo-oxygenase-2 enzymatic activity exhibited by nimesulide and indomethacin, respectively, or to a differing degree of diffusion into the brain, easier for nimesulide than indomethacin. Nevertheless, our results suggest that the inhibition of cyclo-oxygenase pathway causes a far greater increase in the response of dopamine neurons to morphine administration than that produced by morphine itself.

An important question is whether mesolimbic dopaminergic neuronal activation represents a disinhibitory response to the reduced synaptic input of GABA interneurons within the pars reticulata of the substantia nigra.

This problem is extremely relevant to interpretation of the role of cyclo-oxygenase pathway in the euphorigenic properties of morphine, similarly to those previously described in the periaqueductal (Vaughan, 1998), suggesting that the ''affective analgesia hypothesis'' (Franklin, 1998) could have overlapping neural substrates.

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