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Short communication

The role of nitric oxide in the development of opioid withdrawal induced by naloxone after acute treatment with μ- and k-opioid receptor agonists

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Abstract

The present study investigated the possible role of nitric oxide (NO) in the development of the withdrawal contractures of guinea pig isolated ileum after acute activation of μ- and k-opioid receptors. After a 4-min in vitro exposure to morphine (μ-opioid receptor preferring, but not selective, agonist), [D-Ala^2-*N*-methyl-Phe^4-Gly^5-ol-]enkephalin (DAMGO; highly selective μ-opioid receptor agonist), or trans(±)-3,4-dichloro-*N*-methyl-*N*-2(1-pyrrolidynyl)cyclohexyl-benzeneacetamide (U50-488H; highly selective k-opioid receptor agonist), the guinea-pig isolated ileum exhibited a strong contracture after the addition of naloxone. L-N^G-nitro arginine methyl ester (3–300 μM) injected 10 min before the opioid receptor agonists was able dose dependently to reduce the naloxone-induced contraction after exposure to μ- and k- opioid receptor agonists whereas D-N^G-nitro arginine methyl ester at the same concentrations did not affect it. The inhibitory effect of L-N^G-nitro arginine methyl ester on morphine, DAMGO and U50-488H withdrawal was dose dependently reversed by L-arginine (3–300 μM) but not by D-arginine. Finally, glyceryl trinitrate on its own (3–300 μM) significantly increased the naloxone-induced contraction after exposure to μ- and k-opioid receptor agonist and it was also able to reverse the inhibition of opioid withdrawal caused by L-N^G-nitro arginine methyl ester. These results provide evidence that NO has a role in the development of opioid withdrawal and that μ- or k- opioid receptors are involved.

Keywords: Opioid withdrawal; Ileum; (Guinea pig); Nitric oxide (NO)

1. Introduction

Opioid dependence can be induced and measured in vitro by using guinea-pig ileum (Lujan and Rodriguez, 1981; Collier, 1980; Collier et al., 1981; Chal, 1983, 1986). Tissues from untreated animals, after a brief exposure to opioids, show a strong naloxone-induced contracture (Valeri et al., 1990a,b,c; Morrone et al., 1990, 1993), indicating that cellular mechanisms of dependence may occur very rapidly following occupation of receptors and that these mechanisms are operative within the myenteric plexus (North and Karras, 1978).

The mechanisms involved in the development and expression of opioid dependence remain unclear despite a great deal of research. The characteristics of the opioid withdrawal syndrome suggest an involvement of excitatory neurotransmitters in drug-dependence phenomena. This is borne out by reports showing that antagonists of excitatory amino acid receptors suppress opioid withdrawal signs (Rasmussen et al., 1991; Trujillo and Akil, 1991; Koyuncuoglu et al., 1992). The finding that NO is produced postsynaptically in response to activation by central excitatory amino acids (Knowles et al., 1989; Garthwaite, 1991) raises the possibility that suppression of the withdrawal signs by NMDA receptor antagonists may be linked to inhibition of nitric oxide synthesis. The possible involvement of the nitric oxide-guanosine 3′,5′-cyclic monophosphate system (Bredt and Snyder, 1989) in these phenomena has been suggested by reports showing that nitric oxide synthase inhibitors abolish some aspects of the naloxone-precipitated withdrawal syndrome (Adams et al., 1993; Cappendijk et al., 1993; Dambisya and Lee, 1996), and that isosorbide, a NO donor, induces a quasi-morphine abstinence syndrome and exacerbates opioid withdrawal signs in dependent rats (Adams et al., 1993).

Although Dambisya and Lee (1996) reported that inhibition of NO attenuates the expression of both tolerance

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and physical dependence in vivo, in the present study we considered the possible involvement of NO on opioid dependence in vitro. Furthermore, we investigated whether the interaction NO-opioid dependence takes place through μ- and/or k-opioid receptors by using morphine (μ-opioid receptor preferring, but not selective, agonist),[D-Ala^2-*N*-methyl-Phe^4-Gly^5-ol-]enkephalin (DAMGO; highly selective μ-opioid receptor agonist) and trans(±)-3,4-dichloro-*N*-methyl-*N*-2(1-pyrrolidynyl)cyclohexyl-benzeneacetamide (U50-488H; a highly selective k-opioid receptor agonist).

In the present work we considered the effect of L-N^G-nitro arginine methyl ester, D-N^G-nitro arginine methyl ester, L-arginine, D-arginine and glyceryl trinitrate at concentrations of 3–300 μM on morphine, DAMGO and U50-488H withdrawal in order to verify the possible involvement of a specific opioid receptor in the interaction NO-opioid dependence.

2. Materials and methods

2.1. Animals

Adult male guinea-pigs (200–250 g) purchased from Charles River, Italy, were used in the experiments. Animal care and use followed the directions of the Council of the European Communities (1986). The animals were housed in colony cages (4 guinea-pigs each) with free access to food and water. They were maintained in a climate- and light- controlled room (22 ± 1°C, 12/12 h dark/light cycle) at least 7 days before testing.

2.2. Preparation of guinea-pig isolated ileum

The animals were killed by CO_2 and bled. The terminal portion of the ileum, discarding the 10 cm nearest the caecum, was kept in a Petri dish with Tyrode solution (mM: NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2 \cdot 6H_2O$ 1.05, $NaH_2PO_4 \cdot H_2O$ 0.4, $NaHCO_3$ 11.9, glucose 5) for 30 min and then washed free of faecal matter. Segments, 2–3 cm long, from the same animal were placed between platinum electrodes and connected to a 85/2/50 model MARB Stimulator (Ditta MARB, Chiesina Uzzanese, Pistoia, Italy). A force-displacement transducer and unirecord model polygraph were used for measurement of isotonic contractions (Ugo Basile, Milano, Italy). A resting tension of 0.5 g was applied. The baths were maintained at 37°C and continuously bubbled with a mixture of 95% O_2 and 5% CO_2.

2.3. Acute opioid dependence in guinea-pig isolated ileum

The methods used to elicit acute opioid dependence and the typical contracture responses of the ileum to repeated challenges with opioid receptor agonist and naloxone were the same as described previously (Capasso and Sorrentino, 1997).

2.4. Experimental procedure

The administration of L-N^G-nitro arginine methyl ester, D-N^G-nitro arginine methyl ester, L-arginine, D-arginine and glyceryl trinitrate was performed according to the following schedule: (a) 3 acetylcholine responses; (b) electrical stimulation (10–20 min); (c) opiate administration (morphine, DAMGO or U50-488H) in the absence of electrical stimulation (4 min) and addition of naloxone with subsequent contraction (first opioid withdrawal); (d) washout and acetylcholine response; (e) electrical stimulation (30 min); (f) L-N^G-nitro arginine methyl ester, D-N^G-nitro arginine methyl ester, L-arginine, D-arginine and glyceryl trinitrate (3–300 μM) without electrical stimulation, added 10 min before morphine, DAMGO or U50-488H followed by naloxone (second opioid withdrawal); (g) washout and acetylcholine response; (h) electrical stimulation (30 min); and (i) final control opioid withdrawal (third opioid withdrawal).

Under our experimental conditions, to induce a strong contracture, opioid receptor agonists and naloxone were administered at the following concentrations: morphine (10^{-5} M) + naloxone (10^{-5} M); DAMGO (10^{-6} M) + naloxone (10^{-6} M); U50-488H (10^{-7} M) + naloxone (10^{-5} M).

Each experiment was performed with at least 6 to 9 isolated preparations from different animals.

2.5. Drugs

All drugs used in the experimental sessions were purchased from Sigma (St. Louis, MO, USA) with the exception of morphine HCl, which was purchased from Carlo Erba (Milan, Italy). Drugs were dissolved in distilled water.

2.6. Parameter evaluation

Four parameters (naloxone contracture in the pre-drug period, acetylcholine responses, electrically stimulated contraction and naloxone contracture in the post-drug period) were evaluated as previously described (Capasso and Sorrentino, 1997).

2.7. Statistical analysis

Results are expressed as means ± S.E.M. and were tested for statistical significance using Student's *t*-test for

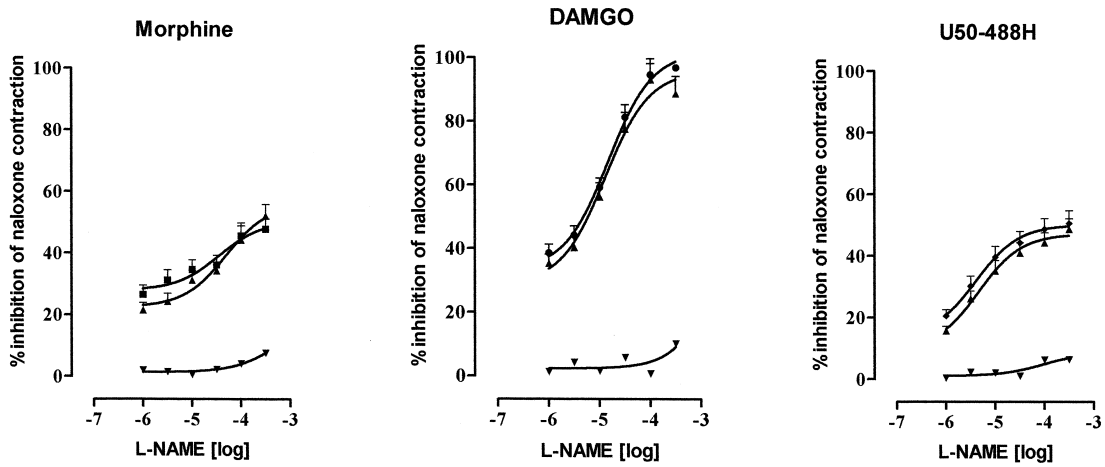

Fig. 1. The inhibitory effect of L-N^G-nitro arginine methyl ester on morphine (solid square), DAMGO (solid circle) and U50-488H (solid rhomb) withdrawal is inhibited by L-arginine (solid down triangle) but not by D-arginine (solid up triangle). L-arginine and D-arginine concentrations were equimolar to L-N^G-nitro arginine methyl ester (3–300 μM). The concentrations of L-arginine and D-arginine were increased in a fixed ratio to L-N^G-nitro arginine methyl ester. Drugs were injected 10 min before the opioid receptor agonist. Results are expressed as means ± S.E.M., $n = 9$.

paired data when results before and after treatments on the same preparation were compared.

3. Results

L-N^G-nitro arginine methyl ester (3–300 μM) added 10 min before the opioid receptor agonists dose dependently reduced the naloxone-induced contraction after exposure to μ- and k-opioid receptor agonists (Fig. 1) whereas D-N^G-nitro arginine methyl ester, at the same concentrations, did not affect it (data not shown). L-N^G-nitro arginine methyl ester almost completely inhibited DAMGO withdrawal whereas morphine withdrawal and U50-488H withdrawal were inhibited by only about 50% (Fig. 1).

The inhibitory effect of L-N^G-nitro arginine methyl ester (3–300 μM) on morphine, DAMGO and U50-488H withdrawal was dose dependently blocked by L-arginine (3–300 μM) but not by D-arginine (3–300 μM) (Fig. 1). The concentrations of L-arginine and D-arginine were increased in a fixed ratio to L-N^G-nitro arginine methyl ester.

After washout, the responses to acetylcholine and electrical stimulation were not affected by L-N^G-nitro arginine methyl ester treatment whereas the final control opioid withdrawal response was still reduced (data not shown).

Finally, glyceryl trinitrate alone (3–300 μM) significantly increased the naloxone contraction elicited after exposure to μ- and k-opioid receptor agonists (data not shown) whereas it was able to reverse the inhibition of opioid withdrawal elicited by L-N^G-nitro arginine methyl

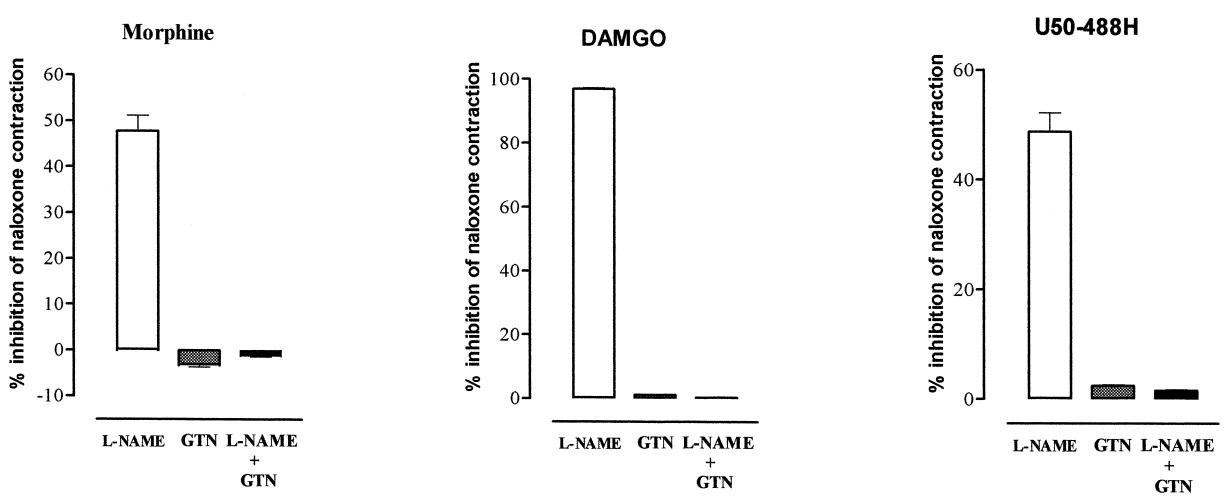

Fig. 2. Effect of glyceryl trinitrate (0.3 μM) on the inhibition induced by L-N^G-nitro arginine methyl ester (300 μM) of morphine or DAMGO or U50-488H withdrawal. Drugs were injected 10 min before the opioid receptor agonist. Results are expressed as means ± S.E.M., $n = 9$.

ester at a concentration which did not modify the naloxone contraction alone (0.3 μM) (Fig. 2).

4. Discussion

The results of our experiments provide further evidence of the role of NO in the development of opioid withdrawal. In fact, the ability of L-N^G-nitro arginine methyl ester to reduce and of glyceryl trinitrate to increase opioid withdrawal suggest that during opiate withdrawal NO may be released after nitric oxide synthase activation. This relationship between NO and opioid withdrawal is further supported by data showing that nitric oxide synthase inhibitors abolish some aspects of the naloxone-precipitated withdrawal syndrome (Adams et al., 1993; Cappendijk et al., 1993; Dambisya and Lee, 1996), and that isosorbide, a NO donor, induces a quasi-morphine abstinence syndrome and exacerbates opioid withdrawal signs in dependent rats (Adams et al., 1993).

Although the role of NO in the expression of opiate withdrawal has already been described in vivo (Dambisya and Lee, 1996), this is the first paper which evaluates the effect of NO in a model of dependence in vitro and whether the NO-opioid dependence interaction takes place at a specific opioid receptor. The results of our experiments indicate that both μ- and k- opioid receptors are involved in the NO effect during opioid withdrawal since L-N^G-nitro arginine methyl ester reduced and glyceryl trinitrate increased both μ and k opioid withdrawal. However, it is of interest to note that L-N^G-nitro arginine methyl ester almost completely inhibited the DAMGO withdrawal whereas morphine and U50-488H withdrawal were inhibited by only about 50%. These results suggest an important functional interaction between NO and opioid withdrawal primarily at the μ-opioid receptor level. This may be related to the different intracellular biochemical mechanisms mediating the inhibitory actions of opioids on myenteric neurons because μ-opioid receptor agonists increase potassium conductance, whereas k-opioid receptor agonists reduce Ca^{2+} conductance (North, 1986).

However, although it seems that the effect induced by k-opioid receptor agonists is mainly due to the excitation of cholinergic neurons, as μ opioid receptor agonists, it is not known whether these two opioid receptor agonists activate the same neurons, and whether the sequence of biochemical and neuronal events leading to the development of dependence and its symptoms is different for the two opioid receptor agonists (Valeri et al., 1990c).

It has been reported that there is a strong link between NO and prostaglandins. In fact, the nitric oxide synthase inhibitor L-N^G-nitro arginine methyl ester reduces in parallel both NO and prostaglandin generation: this effect is reversed by L-arginine, the precursor for the NO synthesis, but not by D-arginine (Di Rosa et al., 1996). Moreover, both sodium nitroprusside and glyceryl trinitrate enhance the production of prostaglandins, suggesting that NO stimulates prostaglandin biosynthesis through a direct interaction with cyclooxygenase enzymes (Di Rosa et al., 1996).

Also, the lipooxygenase inhibitor, nordihydroguaiaretic acid, has been reported to inhibit NO^{2-} accumulation, suggesting that lipooxygenase metabolites may upregulate NO production (Imai et al., 1994). Recently, we have demonstrated that arachidonic acid metabolites are involved in the development of opiate withdrawal since both cyclooxygenase and lipooxygenase inhibitors reduced morphine withdrawal (Capasso and Sorrentino, 1997). Taken together, our data support the possibility that the reduction of opiate withdrawal induced by L-N^G-nitro arginine methyl ester is related to its ability to inhibit cyclooxygenase activity whereas the ability of glyceryl trinitrate to increase opiate withdrawal is related to its ability to stimulate cyclooxygenase activity.

It is possible that other neurotransmitters may be involved in the withdrawal contracture. It has been shown that a large proportion of the contracture is due to acetylcholine release because it is reversed by atropine or hyoscine (Tsou et al., 1982; Chal, 1983).

In our experiments we excluded the possibility of a direct action of L-N^G-nitro arginine methyl ester on pre- and postsynaptic acetylcholine receptors because electrical stimulation and acetylcholine responses were not modified in the guinea pig ileum after L-N^G-nitro arginine methyl ester treatment.

In conclusion, the results of the present study confirm the involvement of NO in the expression of opiate dependence and suggest that there is a link between NO, prostaglandins and opiate withdrawal.

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