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The involvement of nitric oxide in the antinociception induced by cyclosporin A in mice

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Abstract

Cyclosporin A (CsA) and other immunophilin-binding agents are known to inactivate neuronal nitric oxide synthase (nNOS). Nitric oxide (NO) is involved in the nociception at the spinal level. We evaluated the effect of acute intraperitoneal (ip) administration of CsA on the tail-flick response in mice and the involvement of NO and opioid receptors in this effect. CsA (5, 10, 20 and 50 mg/kg ip) induced a significant increase in tail-flick response. Nitric oxide synthase (NOS) inhibitor N^G-nitro-L-arginine (LNNA; 10, 40 and 80 mg/kg ip) significantly potentiated the CsA-induced (5 mg/kg) increase in tail-flick latency (TFL). While NOS substrate L-arginine (100, 200, 400 mg/kg ip) inhibited the CsA-induced (20 mg/kg) antinociception completely and in a dose-dependent manner. Concomitant administration of L-NNA and L-arginine blocked the inhibition exerted by the latter on the CsA-induced antinociception. The opioid receptor antagonist naloxone (4 mg/kg ip) did not alter the CsA effect. These results indicate that acute administration of CsA induces an antinociceptive effect that involves the L-arginine–NO pathway but is not mediated by opioid receptors. © 2002 Published by Elsevier Science Inc.

Keywords: Cyclosporin A; Antinociception; Tail-flick test; Nitric oxide; Naloxone

1. Introduction

Cyclosporin A (CsA), a lipophilic undecapeptide of fungal origin, is a potent immunosuppressor agent widely used to prevent rejection of transplanted organs (Borel et al., 1996). The immunosuppressive effects of CsA is due to its binding to its protein receptors, immunophilins (Schreiber and Crabtree, 1992). Recently, it has been shown that immunophilins are much more abundant in the nervous system than in the immune system (Steiner et al., 1992). Subsequently, some important actions of immunophilin-binding ligands in the nervous system have been revealed, which include neurotransmitter release, neurotrophic influences, regulation of intracellular Ca^{2+} release, as well as regulation of nitric oxide (NO) neurotoxicity (Steiner et al., 1996; Cameron et al., 1997; Snyder et al.,1998; Trajkovi'c et al., 1999). In the nervous system, CsA reduces the catalytic activity of neuronal nitric oxide synthase (nNOS) through inhibition of calcineurin-mediated dephosphorylation of nNOS, causing inhibition of NO release (Sharkey and Butcher, 1994; Rao et al., 1996; Sabatini et al., 1997).

NO is a potent mediator in the central and peripheral nervous system, which has shown pronociceptive activity in the spinal cord in a number of studies (Haley et al., 1992; Kitto et al., 1992; Meller and Gebhart, 1993). NOS inhibitors abolish facilitation of nociceptive tail-flick reflexes (Meller et al., 1992a,b), and in the spinal cord, the NO donor enhances the release of substance P and calcineurin gene-related peptide, both involved in the nociceptive transmission (Garry et al., 1994). Moreover, immunohistochemical studies have further confirmed the pronociceptive action of NO (Lee et al., 1992; Fiallos-Estrada et al., 1993; Herdegen et al., 1994). Thus, considering that CsA keeps the nNOS in its inactive state and decreases the release of NO, we examined the potential modulation of tail-flick response by acute administration of CsA in mice and evaluated the possible involvement of L-arginine–NO pathway in this effect. The involvement of opioid receptors in CsA effect was also examined.

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2. Methods

2.1. Animals

Male NMRI mice weighting 20–25 g (5–6 weeks old, Institute Pasteur of Iran) were used. Animals were housed six per cage in a room maintained at 22 ± 1 °C with an alternating 12-h light–dark cycle. The animals had free access to food and water. The animals were group housed during the testing procedures. All procedures were carried out in accordance with institutional guidelines for laboratory animal care and use. Each mouse was used only once and each treatment group consisted of seven animals.

2.2. Drugs

Drugs used were: CsA (Sandimmune, Sandoz, Switzerland), N^G-nitro-L-arginine (L-NNA; Fluka, Switzerland), and L-arginine, naloxone and morphine sulfate (Sigma, UK), which were dissolved in saline and were administered intraperitoneally (ip) at a volume of 10 mg/kg of body weight. Doses used were based upon previous studies (Sabatini et al., 1989; L'opez-ongil et al., 1996; Verhagen et al., 1998; Bhargava and Bian, 1998).

2.3. Assessment of antinociception

The antinociception was evaluated by the radiant heat tail-flick test (D'Amour and Smith, 1941). The animals were restricted by a restrainer with their tail positioned in an apparatus (type 812, Hugo Sachs electronics, Germany) for radiant heat stimulation on the dorsal surface of the tail. Tail-flick latency (TFL) was defined as the time interval between the application of a standardized beam focused on the tail and the abrupt removal of the tail from the nociceptive stimulus. The cut-off time was 8 s. The animals were allowed to acclimatize to the apparatus at least 2 min before eliciting a tail-flick, and the operator was unaware of the specific treatment group to which an animal belonged.

2.4. Experimental procedure

In the first experiment, the effect of acute administration of different doses of CsA (0.05, 5, 10, 20 and 50 mg/kg) on tail-flick response was assessed 15, 30, 45, 60, 75, 90 and 120 min after drug administration. In the second experiment, groups of mice received L-NNA (10, 40 and 80 mg/kg) 30 min before administration of either CsA (5 mg/kg) or saline and 90 min prior to tail-flick test. In the third experiment, groups of mice received L-arginine (100, 200 and 400 mg/kg) 30 min before either CsA (20 mg/kg) or saline and 90 min before testing. Two other groups received L-NNA (10 and 40 mg/kg) concomitant with L-arginine (200 mg/kg) 30 min before CsA (20 mg/kg) and 90 min before testing. In the fourth experiment, groups of mice received naloxone (4 mg/kg) 15 min before either CsA (20 mg/kg), saline or a comparison dose of morphine (4 mg/kg). The tail-flick response was assessed 45 min after morphine and 60 min after CsA or saline administration.

2.5. Statistics

All data are shown as the means ± S.E.M. Statistical comparison of groups in all experiments except the last one was done with one-way analysis of variance (ANOVA) followed by Tukey–Kramer posttest. Student's t test was used to compare groups in the last experiment examining the effects of naloxone combined with CsA, morphine or saline. A P value less than .05 was considered significant.

3. Results

3.1. The effect of acute administration of CsA on TFL

Fig. 1 shows that acute administration of CsA (5, 10, 20, 50 mg/kg) caused a significant increase in TFL [15 min, $F(4, 30) = 2.5$, $P > .05$; 30 min, $F(4,30) = 20.61$, $P < .0001$; 45 min, $F(4,30) = 17.31$, $P < .0001$; 60 min, $F(4,30) = 15.33$, $P < .0001$; 75 min, $F(4,30) = 17.41$, $P < .0001$; 90 min, $F(4,30) = 14.68$, $P < .0001$; 120 min, $F(4,30) = 13.65$, $P < .0001$]. However, a lower dose of CsA (0.05 mg/kg)

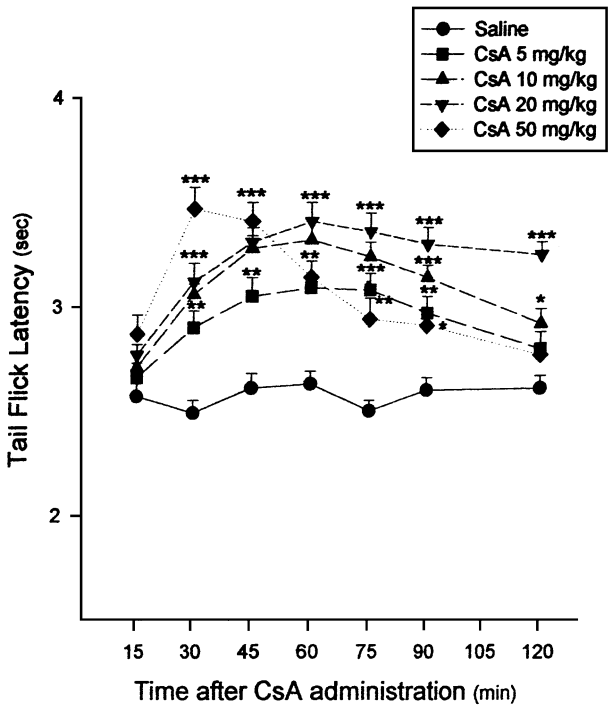

Fig. 1. The time course of the antinociception induced by acute administration of different doses of cyclosporin A (5, 10, 20 and 50 mg/kg ip). Data are shown as means ± S.E.M. of seven animals. * $P < .05$, ** $P < .01$ and *** $P < .001$ in comparison with the saline group.

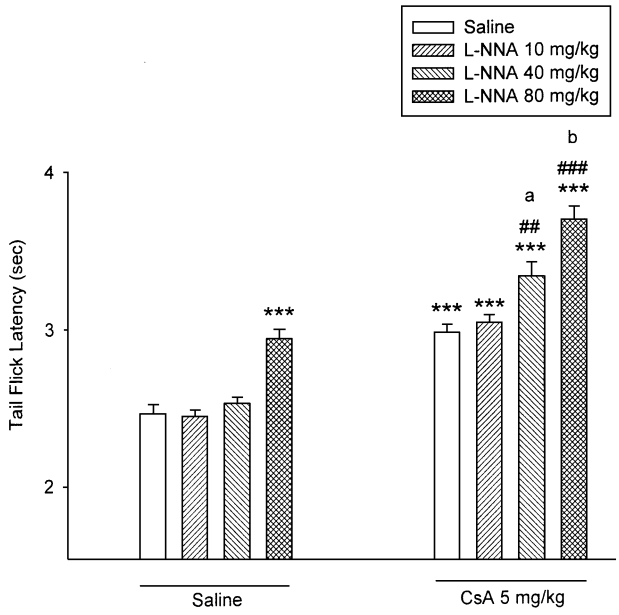

Fig. 2. The effect of of N^G-nitro-L-arginine (L-NNA; 10, 40 and 80 mg/kg ip) on the antinociception induced by acute administration of cyclosporin A (5 mg/kg ip). L-NNA was administered 30 min before CsA and 90 min before tail-flick test. Data are shown as means ± S.E.M. of seven animals. *** $P<.001$ in comparison with the saline/saline group. ### $P<.01$, ### $P<.001$ in comparison with the cyclosporin/saline group. [a] $P<.05$ in comparison with the CsA/L-NNA 10 mg/kg group. [b] $P<.01$ in comparison with the CsA/L-NNA 40 mg/kg group.

did not induce any significant change in TFL (data not shown). The acute effect of CsA was maximum with 20 mg/kg 1 h after administration, whereas the antinociception induced by a higher dose of CsA (50 mg/kg) declined after 30 min.

3.2. The effect of L-NNA on antinociception induced by CsA

Fig. 2 shows that the lower doses of NOS inhibitor, L-NNA (10 and 40 mg/kg), did not induce any significant change in TFL, while a higher dose (80 mg/kg) increased the latency ($P<.001$). Acute pretreatment with L-NNA significantly potentiated the antinociception induced by CsA (5 mg/kg) in a dose-dependent manner [$F(7,48)=50.75$, $P<.0001$].

3.3. The effect of L-arginine on antinociception induced by CsA

Fig. 3 shows that NOS substrate L-arginine (100, 200 and 400 mg/kg) had a pronociceptive effect per se ($P<.001$). Pretreatment with L-arginine reversed the antinociceptive effect of CsA (20 mg/kg) in a dose-dependent manner [$F(7,48)=60.40$, $P<.0001$]. While 200 mg/kg L-arginine completely inhibited the CsA-induced antinociception ($P<.001$), the higher dose of the substrate (400 mg/kg) induced significant pronociception in CsA-treated animals ($P<.05$). Fig. 4 shows that concomitant administration of L-NNA (10 and 40 mg/kg) with L-arginine (200 mg/kg) reversed the L-arginine-induced inhibition of CsA (20 mg/kg) antinociception [$F(3,24)=35.31$, $P<.0001$]. The higher dose of L-NNA (40 mg/kg) completely restored the antinociceptive effect of CsA.

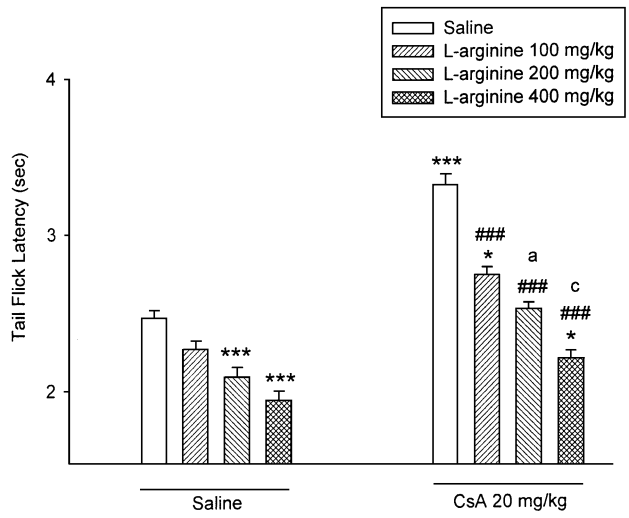

Fig. 3. The effect of administration of L-arginine (100, 200 and 400 mg/kg ip) on the antinociception induced by acute administration of cyclosporin A (20 mg/kg ip). L-Arginine was administered 30 min before CsA and 90 min before the tail-flick test. Data are shown as means ± S.E.M. of seven animals. * $P<.05$, *** $P<.001$ in comparison with the saline/saline group. ### $P<.001$ in comparison with the cyclosporin/saline group. [a] $P<.05$ in comparison with the CsA/L-arginine 100 mg/kg group. [c] $P<.001$ in comparison with the CsA/L-arginine 200 mg/kg group.

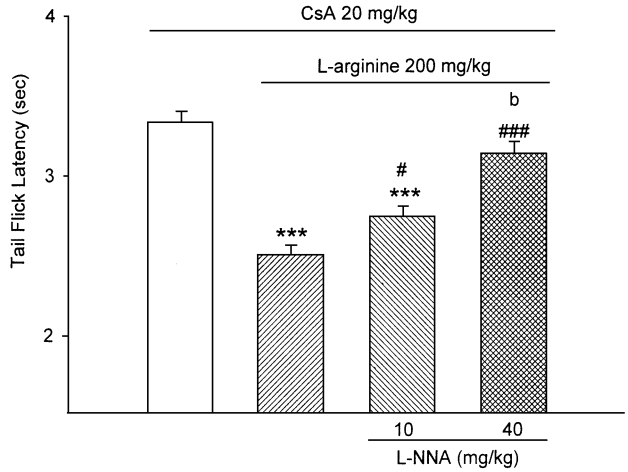

Fig. 4. The effect of concomitant administration of N^G-nitro-L-arginine (L-NNA; 10 and 40 mg/kg ip) with L-arginine (200 mg/kg ip) on the antinociception induced by acute administration of cyclosporin A (20 mg/kg ip). L-NNA and L-arginine were administered 30 min before CsA and 90 min before the tail-flick test. Data are shown as means ± S.E.M. of seven animals. *** $P<.001$ in comparison with the cyclosporin group. # $P<.05$, ### $P<.001$ in comparison with the cyclosporin/L-arginine group. [b] $P<.01$ in comparison with the L-NNA 10 mg/kg group.

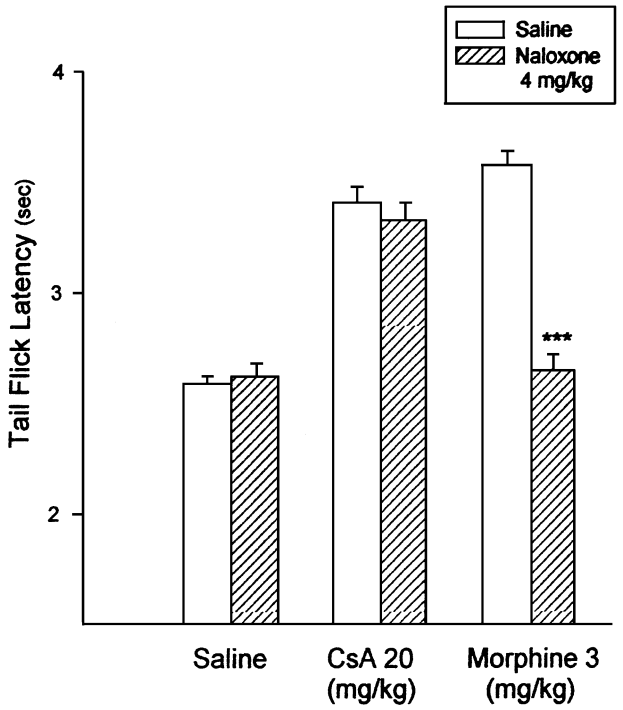

Fig. 5. The effect of naloxone (4 mg/kg ip) on anticociception induced by acute administration of cyclosporin A (20 mg/kg ip). Naloxone was administered 15 min before CsA, saline or a comparison dose of morphine (4 mg/kg ip) The tail-flick response was measured 60 min after CsA or saline and 45 min after morphine injection. Data are shown as means ± S.E.M of seven animals. *** $P<.001$ in comparison with the corresponding saline group.

3.4. The effect of naloxone on antinociception induced by CsA

Fig. 5 shows that acute administration of opioid receptor antagonist naloxone (4 mg/kg) prior to CsA (20 mg/kg), did not alter the antinociception induced by CsA ($P>.05$). Naloxone per se did not change the tail-flick response but completely reversed the antinociception induced by a comparison dose of morphine (4 mg/kg, $P>.05$).

4. Discussion

In the present study, the acute administration of CsA induced significant increase in TFL. This effect was dose-dependently potentiated by NOS inhibitor L-NNA. Pretreatment with NOS substrate, L-arginine, completely reversed the antinociception induced by CsA, and this reversal was inhibited by L-NNA. Blocking opioid receptors by naloxone did not alter the effect of CsA.

The surprising finding that levels of immunophilins in the nervous tissues are up to 50 times greater than those in tissues of the immune system provided the first suggestion for a neural role of CsA and other immunophilin-binding ligands (Steiner et al., 1992). Further research revealed that in the nervous system, the complex of CsA with its protein receptor binds to and inhibits calcineurin, which, in turn, leads to an increase in phosphorylated levels of nNOS. This, in turn, reduces the catalytic activity of nNOS and leads to important neural changes such as protection against stroke-induced damage and regulation of NO neurotoxicity by CsA and other immunophilin-binding ligands such as FK 506 (Dawson et al., 1993; Sharkey and Butcher, 1994; Rao et al., 1996). Moreover, impaired NO activity has been suggested as a mechanism involved in the CsA-induced unwanted side effects on cardiovascular and renal tissues (Diederich et al., 1992; Marumo et al., 1995; Stephan et al., 1995). Meanwhile, previous studies have shown the involvement of L-arginine–NO pathway in nociceptive processes (Haley et al., 1992; Kitto et al., 1992; Meller and Gebhart, 1993). NO mediates the *N*-methyl-D-aspartate (NMDA)-produced facilitation of tail-flick reflexes (Kitto et al., 1992; Meller et al., 1992a), while NOS inhibitors attenuate the formalin-induced nociception and change the electrophysiological activity of dorsal horn neurons accordingly (Haley et al., 1992; Kitto et al., 1992).

Our finding that systemic administration of CsA induces antinociception is in accordance with previous reports showing that CsA can cross the blood–brain barrier and accumulate in brain tissue (Boland et al., 1984). However, CsA concentration is reported to be three times lower in the brain than in serum and most tissues. This may explain the ineffectiveness of a lower dose of CsA (0.05 mg/kg) on tail-flick response. Interestingly, L-NNA dose-dependently potentiated the CsA-induced antinociception, while the NOS inhibitor per se did not affect the latency except at the highest dose used (80 mg/kg). This is in line with previous reports showing that inhibitors of NOS can attenuate nociceptive responses at high doses (Moore et al., 1991; Machelska et al., 1997) and are able to abolish facilitation of these reflexes (Meller et al., 1992a,b). Thus, reducing the activity of NOS in the nervous system may be suggested as a probable mechanism for CsA effect. This hypothesis is further supported by the observation of inhibitory effect of L-arginine on CsA-induced response. L-Arginine per se induced significant facilitation of tail-flick response that is in accordance with previous studies (Meller and Gebhart, 1993; Meller et al., 1996; Machelska et al., 1997). However, CsA-induced antinociception was strongly ($P<.001$) inhibited even by the lower dose of L-arginine (100 mg/kg) that was ineffective per se, and the higher dose of L-arginine induced significant pronociception in CsA treated mice ($P<.05$). It may be suggested that the balance between increased NO synthesis following L-arginine and decreased NOS activity due to CsA is responsible for determining the effect of their combined administration on nociception. Accordingly, the effect of combined treatment with L-arginine and N^G-nitro-L-arginine methyl ester (L-NAME) on morphine analgesia is dependent on the dose ratio of the two drugs (Brignola et al., 1994). Likewise, in the present study, concomitant administration of L-NNA with L-arginine blocked the inhibitory effect of

L-arginine on CsA-induced antinociception in a dose-related manner. Thus, inhibition of CsA response by L-arginine seems to be related to increased NO production following administration of substrate, an observation further supporting the modulatory effect of CsA on NO synthesis. However, three different forms of NOS have been identified that include inducible NOS (iNOS), endothelial NOS (eNOS) and nNOS (Moncada and Higgs, 1993). In the nervous system, nNOS is largely responsible for NO production (Bredt and Snyder, 1990), while iNOS is also reported to be present in normal adult brain and to contribute to the pathophysiology of many neuronal diseases (Licinio et al., 1999). In addition to its inhibitory effects on nNOS, CsA is also known to have inhibitory effects on iNOS while enhancing the activity of eNOS (Stroes et al., 1997; Sanchez-Lozada et al., 2000). Thus, the observed effects of CsA may involve NOS isoforms other than nNOS. Further studies using specific NOS inhibitors or measuring the nNOS activity in the spinal cord after CsA treatment are required to address this question.

We also examined the probable involvement of opioid receptor pathways in CsA-induced antinociception. It has been reported that NO and opioid pathways are connected in pain processing (Duarte and Ferreira, 1992; Machelska et al., 1997). However, opioid receptor antagonist naloxone did not alter the CsA effect showing that opioid pathways do not directly mediate this effect. This is the first report on the antinociception induced following the acute administration of CsA. Our data suggest the modulation of L-arginine–NO pathway as a probable mechanism involved in CsA-induced antinociception. However, opioid receptors do not mediate the observed effect. Further research on the effects of CsA and other immunophilin-binding ligands on nociception using different experimental paradigms is validated.

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