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The effect of cyclosporin A on morphine tolerance and dependence: involvement of L-arginine/nitric oxide pathway

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Abstract

Cyclosporin A is known to decrease nitric oxide (NO) production in nervous tissues. The effects of systemic cyclosporine A on the induction and expression of morphine tolerance and dependence, acute morphine-induced antinociception, and the probable involvement of the L-arginine/nitric oxide pathway in these effects were assessed in mice. Cyclosporin A (20 mg/kg), N^G-nitro-L-arginine methyl ester (L-NAME) (10 mg/kg) and a combination of the two at lower and per se non-effective doses (5 and 3 mg/kg, respectively) showed a similar pattern of action, inhibiting the induction of tolerance to morphine-induced antinociception and increasing the antinociception threshold in the expression phase of morphine tolerance. These agents also inhibited the expression of morphine dependence as assessed by naloxone-precipitated withdrawal signs, while having no effect on the induction of morphine dependence. L-Arginine, at a per se non-effective dose (60 mg/kg), inhibited the effects of Cyclosporin A. Moreover, acute administration of Cyclosporin A (20 mg/kg) or L-NAME (10 mg/kg) enhanced the antinociception induced by acute administration of morphine (5 mg/kg), while chronic pretreatment with Cyclosporin A (20 mg/kg) or L-NAME (10 mg/kg) for 2 days (twice daily) did not affect morphine-induced antinociception. The inducible nitric oxide synthase inhibitor, aminoguanidine (100 mg/kg), did not alter morphine antinociception, tolerance or dependence. In conclusion, decreasing NO production through constitutive nitric oxide synthase may be a mechanism through which cyclosporin A differentially modulates mophine tolerance, dependence and antinociception.

Keywords: Cyclosporin A; NO (nitric oxide); L-Arginine; L-NAME (N^G-nitro-L-arginine methyl ester); Opioid dependence-tolerance; Antinociception; (Mouse)

1. Introduction

The mechanisms involved in the development and expression of opioid tolerance and withdrawal remain unclear despite a great deal of research. Tolerance may be defined as a reduction in sensitivity to an agent following repeated exposure, while dependence is generally thought of as the absolute requirement for the agent to maintain normal physiological function (Taylor and Fleming, 2001). There is evidence that opioid tolerance and dependence should be regarded as distinct biphasic phenomena each consisting of two distinctive, namely induction and expression, phases (Wüster et al., 1985; Taylor and Fleming, 2001). Some early reports have shown that cyclosporin A, a lipophilic undecapeptide used clinically as a potent immunosuppressor agent (Borel et al., 1996), modulates opioid-induced dependence (Dafny et al., 1985; Dougherty et al., 1986a, 1987; Dougherty and Dafny, 1988; Berthold et al., 1989; McVaugh et al., 1989) and alters the acute antinociceptive effects of morphine (Thompson et al., 2000). However, these reports concern only one of the phases of morphine dependence, with insufficient attention to the phenomenon of tolerance to morphine-induced antinociception. Moreover, the mechanisms of these effects have not been clarified. Early studies have tried to relate such apparently neural effects of cyclosporin A to its modulatory properties on the immune system (Dougherty et al., 1986a,b, 1987; McVaugh et al., 1989). However, it has been shown that protein receptors of cyclosporin A, immunophilins, are far more abundant in the nervous system than in the immune system (Steiner et al., 1992; Dawson et al., 1994; Sabatini et al., 1997). Subsequently,

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some important functions of cyclosporin A and other immunophilin-binding ligands in the central nervous system (CNS) have been described that include neurotransmitter release, neurotrophic influences and protection against glutamate-induced neurotoxicity (Steiner et al., 1996; Kikuchi et al., 1998; Ruiz et al., 2000, Lyons et al., 1994). It has been shown that, in the nervous system, cyclosporin A reduces the catalytic activity of neuronal nitric oxide synthase (nNOS) and subsequently causes the inhibition of nitric oxide (NO) release (Dawson et al., 1993; Sharkey and Butcher, 1994; Rao et al., 1996a; Snyder et al., 1998). This mechanism has been implicated in some of the important functions of cyclosporin A in the nervous system (Sabatini et al., 1997; Ruiz et al., 2000; Sanchez-Lozada et al., 2000).

NO is suggested to play a role in pain perception (Meller and Gebhart, 1993; Sousa and Prado, 2001) as well as in the modulation of opioid antinociception, tolerance and dependence (Kolesnikov et al., 1992, 1993; Adams et al., 1993; Kimes et al., 1993; Babey et al., 1994; Dambisya and Lee, 1995, 1996). The inhibition of nitric oxide synthase has been shown to reduce the intensity of the naloxone-precipitated withdrawal syndrome (Adams et al., 1993; Cappendijk et al., 1993; Kimes et al., 1993; Thorat et al., 1994) and the development (Kolesnikov et al., 1992, 1993; Babey et al., 1994; Elliot et al., 1994) and expression (Dambisya and Lee, 1996) of tolerance to morphine-induced antinociception. Furthermore, acute (Przewlocki et al., 1993; Brignola et al., 1994; Dambisya and Lee, 1995) or chronic (Babey et al., 1994; Dambisya and Lee, 1996) modulation of NO synthesis differentially alters the antinociceptive effects of opioids. Thus, in the present study we assessed the effects of cyclosporin A on the development and expression of morphine tolerance and dependence in mice and examined the probable role of the L-arginine/nitric oxide mechanisms in these effects. In addition, we examined the effects of acute and chronic pretreatment with cyclosporin A on acute antinociception induced by morphine and the possible involvement of L-arginine/nitric oxide pathways in these effects. For this purpose, we used the NOS substrate, L-arginine, the non-specific NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) and the specific inducible nitric oxide synthase inhibitor, aminoguanidine.

2. Methods

2.1. Animals

Male NMRI mice weighting 22–30 g (Institute Pasteur of Iran) were used. The animals were housed 10 per cage in a room maintained at 22 ± 2 °C with an alternating 12-h light–dark cycle. The animals had free access to food and water. 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 8–10 animals.

2.2. Drugs

Drugs used were cyclosporin A (Sandimmune, Sandoz, Switzerland), N^G-nitro-L-arginine methyl ester (L-NAME), L-arginine, morphine sulfate (Sigma, UK), aminoguanidine (Sigma, USA) and naloxone hydrochloride (Tolid-daru, Iran) which were dissolved in saline. Morphine was administered subcutaneously (s.c.), and all other drugs were administered intraperitoneally (i.p.) in a volume of 10 ml/kg of body weight. The doses used were based upon previous studies (Dambisya and Lee, 1996; L'opez-ongil et al., 1996; Dehpour et al., 1998; Al-Shabanah et al., 2000; Nahavandi et al., 2001).

2.3. Induction and assessment of morphine tolerance and dependence

Morphine dependence and tolerance were induced in mice by a repeated-injection schedule described by Dambisya et al. (1991). The mice were on a regimen of morphine in graded doses twice daily for 2 days as follows: Day 1, 30 mg/kg, a.m. and 45 mg/kg, p.m.; Day 2, 60 mg/kg, a.m. and 90 mg/kg, p.m. On the third day, the animals were assessed for both tolerance and dependence as described by Way et al. (1969). The loss of the antinociceptive effects of morphine in the tail-flick test (D'Amour and Smith, 1941) was used to assess the degree of tolerance. Tail-flick latency was assessed 45 min after an injection of 10 mg/kg of morphine (Dambisya and Lee, 1996). The animals were briefly restrained 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 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 10 s. Analgesia was also measured qualitatively as a doubling or more of baseline values for each mouse (Kolesnikov et al., 1993). Physical dependence was assessed following the administration of a 2 mg/kg dose of naloxone. Immediately after the injection of naloxone, the animals were observed on a platform (40 cm long, 25 cm wide and 45 cm high) for 30 min and the number of jumps was recorded. The percentage loss of body weight at 1 h after naloxone administration was also measured. Antinociception following acute morphine administration (5 mg/kg) was assessed in the tail-flick test as described above.

2.4. Treatment

For the assessment of the effects of various agents on the induction of morphine tolerance and dependence, test drugs including either L-NAME (3 and 10 mg/kg), aminoguanidine

(100 mg/kg), L-arginine (200 mg/kg), cyclosporin A (5 and 20 mg/kg), cyclosporin A (5 mg/kg) plus L-NAME (3 mg/kg), cyclosporin A (5 mg/kg) plus aminoguanidine (100 mg/kg), cyclosporin A (20 mg/kg) plus L-arginine (200 mg/kg), or saline (10 ml/kg) were administered 30 min before each morphine injection during the induction, with none given on the test day. For assessment of the effects of the various drugs on the expression of morphine tolerance and dependence, animals that had received only morphine in the induction phase were used and the same doses of the drugs mentioned were administered only on the test day, 30 min prior to acute morphine injection.

For the assessment of the effects of acute pretreatment with different drugs on morphine antinociception, groups of mice received an injection of L-NAME (1, 10 or 30 mg/kg), aminoguanidine (100 mg/kg), L-arginine (60, 100 or 200 mg/kg), cyclosporine A (5, 10 or 20 mg/kg) or saline (10 ml/kg) 30 min before morphine (5 mg/kg) and were tested 45 min after morphine injection. For the assessment of the effects of chronic pretreatment with different drugs on acute morphine-induced antinociception, groups of mice were treated twice daily for 2 days with either L-NAME (10 mg/kg), aminoguanidine (100 mg/kg), L-arginine (60 mg/kg), cyclosporine A (20 mg/kg) or saline (10 ml/kg) and then were tested on the third day 45 min after the acute administration of saline or a dose of 5 mg/kg of morphine.

2.5. Statistical analysis

All data are shown as the means ± S.E.M. of value for corresponding parameters. Statistical comparison between groups in each experiment was done with one-way analysis of variance (ANOVA) followed by post hoc Student–Newman–Keuls test. In a few cases in which only two groups were to be compared, Student's *t*-test was used. A P value less than 0.05 was considered the limit of significance.

3. Results

3.1. Induction experiments

Table 1 shows the effects of various treatments on the development of morphine tolerance [$F(7,66)=9.53$, $P<0.001$]. L-NAME at the higher dose (10 mg/kg) attenuated the development of tolerance to morphine-induced antinociception, while L-arginine led to a higher degree of tolerance compared to the control group. Cyclosporin A at the higher dose (20 mg/kg) slightly attenuated the development of morphine tolerance (not statistically significant), and significantly inhibited the potentiating effect of L-arginine on development of tolerance. Furthermore, concurrent administration of non-effective doses of L-NAME (3 mg/kg) and cyclosporin A (5 mg/kg) during the induction period led to significant inhibition of morphine tolerance.

Table 1
The effects of cyclosporin A, L-NAME and L-arginine on the induction of morphine tolerance

Induction regimen	*n*	Tail-flick latency (s) (mean ± S.E.M.)	% Analgesia
Saline + morphine	10	6.28 ± 0.40	50
L-NAME 3 mg/kg + morphine	9	6.05 ± 0.38	44.44
L-NAME 10 mg/kg + morphine	9	9.11 ± 0.37[a]	88.88
Aminoguanidine 100 mg/kg + morphine	9	5.87 ± 0.53	55.55
L-Arginine 200 mg/kg + morphine	9	4.01 ± 0.26[b]	22.22
CsA 5 mg/kg + morphine	9	6.32 ± 0.33	44.44
CsA 20 mg/kg + morphine	9	6.9 ± 0.74	55.55
CsA 5 mg/kg + L-NAME 3 mg/kg + morphine	10	8.51 ± 1.64[b,c]	100
CsA 5 mg/kg + aminoguanidine 100 mg/kg + morphine	9	6.81 ± 0.81	55.55
CsA 20 mg/kg + L-arginine 200 mg/kg + morphine	9	7.11 ± 0.78[d]	44.44

The animals received induction regimens 30 min before each morphine injection during the induction period. All animals received only a test dose of morphine (10 mg/kg) on the test day.
[a] $P<0.01$ compared to saline/morphine group.
[b] $P<0.05$ compared to saline/morphine group.
[c] $P<0.05$ compared to CsA 5 mg/kg group.
[d] $P<0.01$ compared to L-arginine 200 mg/kg group.

The effects of these drugs on the induction of morphine-dependence are shown in Fig. 1 [Weight loss, $F(7,66)=2.68$, $P<0.05$; Jumpings, $F(7,66)=4.18$, $P<0.001$]. L-Arginine decreased the intensity of withdrawal signs, while neither L-NAME nor cyclosporin A induced any change in this regard. Concurrent administration of cyclosporin A (20 mg/kg) with L-arginine blocked the inhibitory effect of the latter on the development of morphine-dependence. Aminoguanidine did not exert any significant effect on the induction of morphine tolerance or dependence ($P>0.05$).

3.2. Expression experiments

Table 2 shows the effects of various acute pretreatments on the expression of tolerance to morphine-induced antinociception [$F(7.61)=14.32$, $P<0.001$]. L-NAME at the higher dose (10 mg/kg) significantly increased the antinociception threshold in morphine-treated animals. In a similar way cyclosporine A, 20 mg/kg, increased the antinociception threshold compared to morphine-treated animals which received saline on the third day. This effect of cyclosporine A was significantly decreased by concurrent administration of a per se non-effective dose of L-arginine. Moreover,

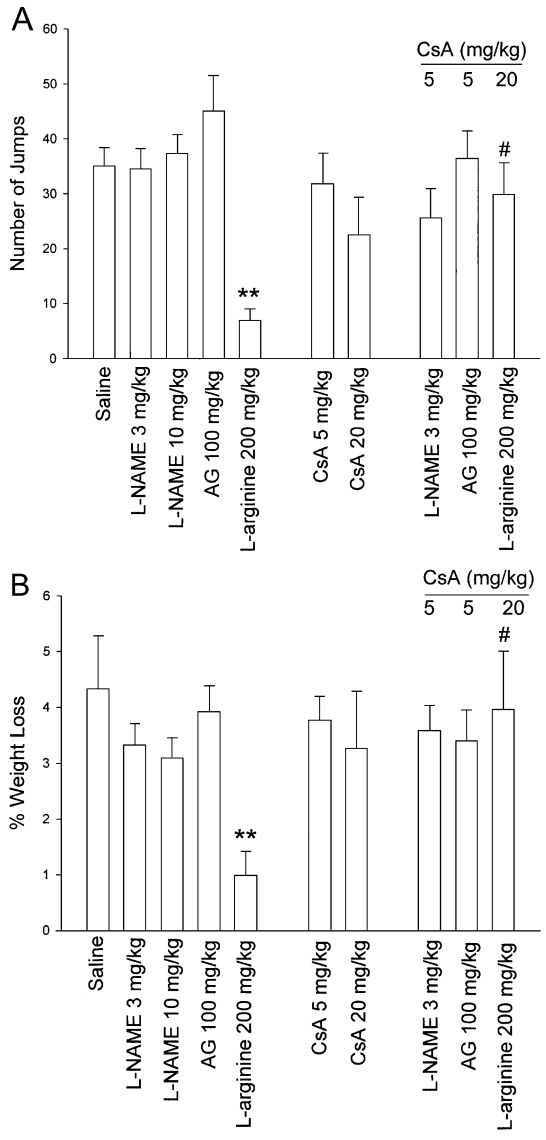

Fig. 1. The effects of various drugs on the induction of physical dependence on morphine as assessed by naloxone-precipitated withdrawal (A) jumping and (B) diarrhea. Drugs were administered 30 min prior to each morphine injection during induction phase. A test dose of 10 mg/kg of morphine without drug pretreatment was used on the test day. Data are expressed as means ± S.E.M. for 8–10 mice. CsA = Cyclosporin A, AG = aminoguanidine. ** $P<0.01$ compared to saline control group. # $P<0.05$ compared to L-arginine (200 mg/kg) group.

concurrent administration of non-effective doses of L-NAME (3 mg/kg) and cyclosporine A (5 mg/kg) led to a significant increase in antinociception threshold.

The effects of the various drugs on the expression of physical dependence on morphine are shown in Fig. 2. Acute pretreatment with either L-NAME (10 mg/kg), cyclosporine A (20 mg/kg) or a combination of lower and per se non-effective doses of two drugs (cyclosporine A, 5 mg/kg plus L-NAME 3 mg/kg) significantly reduced the severity of the withdrawal signs [Weight loss, $F(7,61)=7.03$, $P<0.001$; Jumpings, $F(7,61)=8.84$, $P<0.001$]. L-Arginine per se did not affect the severity of withdrawal signs but reversed the inhibitory effect of cyclosporine A (20 mg/kg) on the expression of morphine-dependence. Once more, aminoguanidine did not exert any significant effect on the expression of morphine tolerance or dependence ($P>0.05$).

3.3. Acute morphine antinociception

Table 3 shows the effects of various acute pretreatments on the antinociception induced by acute administration of morphine (5 mg/kg). Control groups received the same doses of the drugs plus saline. Acute administration of high doses of L-NAME (30 mg/kg) [$F(3,28)=4.68$, $P<0.001$] or cyclosporine A (20 mg/kg) [$F(3,28)=3.03$, $P<0.05$] induced mild antinociception, while L-arginine at 200 mg/kg showed pronociceptive properties [$F(3,28)=3.26$, $P<0.05$]. Per se non-effective doses of L-NAME (10 mg/kg) [$F(2,21)=4.69$, $P<0.05$] or cyclosporine A (10 mg/kg) [$F(2,21)=5.44$, $P<0.05$] potentiated while L-arginine [$F(2,21)=3.94$, $P<0.05$] decreased the acute morphine-induced antinociception. Aminoguanidine did not alter the nociceptive threshold either per se [$F(2,21)=0.58$, $P>0.05$] or in the presence of morphine [$F(2,21)=1.13$, $P>0.05$]. Lower doses of L-NAME (1 mg/kg) and cyclosporine A (5 mg/kg) showed an additive effect of potentiating morphine antinociception [$F(3,28)=6.17$, $P<0.01$], while aminoguanidine (100 mg/kg) had no such effect when combined with a low dose of cyclosporine A [$F(3,28)=1.23$, $P>0.05$]. On the other hand, the potentiation induced by cyclosporine A

Table 2
The effects of cyclosporin A, L-NAME and L-arginine on the expression of morphine tolerance

Acute pretreatment	n	Tail-flick latency (s) (mean ± S.E.M.)	% Analgesia
Saline	9	6.32 ± 0.54	44.44
L-NAME 3 mg/kg	9	6.15 ± 0.41	55.55
L-NAME 10 mg/kg	9	8.48 ± 0.47[a]	88.88
Aminoguanidine 100 mg/kg	8	6.35 ± 0.71	50
L-Arginine 200 mg/kg	8	6.12 ± 0.37	37.5
CsA 5 mg/kg	9	6.35 ± 0.39	55.55
CsA 20 mg/kg	9	9.76 ± 0.15[b]	100
CsA 5 mg/kg + L-NAME 3 mg/kg	8	8.81 ± 0.50[a,c]	87.5
CsA 5 mg/kg + aminoguanidine 100 mg/kg	8	6.94 ± 0.64	62.5
CsA 20 mg/kg + L-arginine 200 mg/kg	8	5.32 ± 0.41[d]	25

All the animals received morphine alone during the induction period. Pretreatment drugs were acutely administered 30 min before a test dose of morphine (10 mg/kg).
[a] $P<0.05$ compared to saline group.
[b] $P<0.001$ compared to saline group.
[c] $P<0.01$ compared to CsA 5 mg/kg group.
[d] $P<0.001$ compared to CsA 20 mg/kg group.

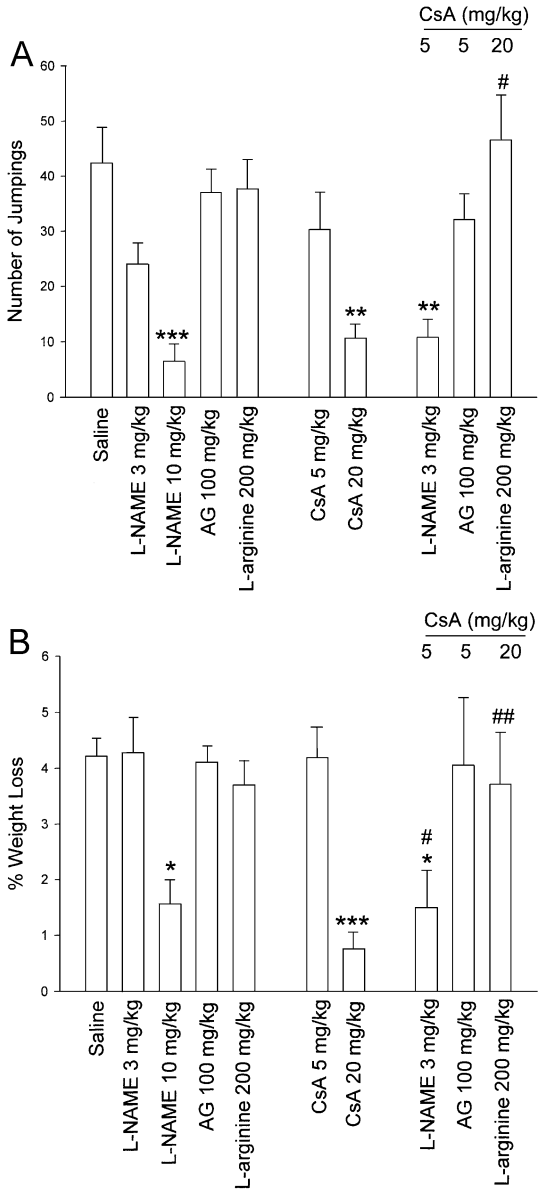

Fig. 2. The effects of various drugs on the expression of physical dependence on morphine as assessed by naloxone-precipitated withdrawal (A) jumping and (B) diarrhea. Animals were made dependent as described in Methods. Drugs were administered 30 min prior to a dose of 10 mg/kg of morphine on the test day. Data are expressed as means ± S.E.M. for eight to nine mice. CsA = Cyclosporin A, AG = aminoguanidine. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared to saline control group. $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared to corresponding cyclosporine A group.

(20 mg/kg) was decreased by L-arginine (60 mg/kg) co-treatment [$F(3,28)=5.22$, $P<0.01$].

Table 4 shows the effects of various chronic pretreatments on the antinociception induced by acute administration of morphine (5 mg/kg). L-NAME (10 mg/kg) and cyclosporine A (20 mg/kg) mildly increased the antinociceptive threshold ($P<0.05$), while aminoguanidine (100 mg/kg) or L-arginine (60 mg/kg) did not alter the threshold. However, the chronic administration of any of these agents

Table 3
The effects of acute cyclosporin A, L-NAME and L-arginine on the antinociception threshold after acute saline or morphine injection

Acute pretreatment	Acute treatment	*n*	Tail-flick latency (s) (mean ± S.E.M.)
Saline	saline	8	2.62 ± 0.07
L-NAME 1 mg/kg	saline	8	2.66 ± 0.09
L-NAME 10 mg/kg	saline	8	2.88 ± 0.17
L-NAME 30 mg/kg	saline	8	3.18 ± 0.12[a]
Aminoguanidine 50 mg/kg	saline	8	2.48 ± 0.06
Aminoguanidine 100 mg/kg	saline	8	2.59 ± 0.14
L-Arginine 60 mg/kg	saline	8	2.41 ± 0.11
L-Arginine 100 mg/kg	saline	8	2.29 ± 0.14
L-Arginine 200 mg/kg	saline	8	2.11 ± 0.11[a]
CsA 5 mg/kg	saline	8	2.87 ± 0.21
CsA 10 mg/kg	saline	8	2.95 ± 0.18
CsA 20 mg/kg	saline	8	3.45 ± 0.28[a]
Saline	morphine	8	4.41 ± 0.14
L-NAME 1 mg/kg	morphine	8	4.47 ± 0.18
L-NAME 10 mg/kg	morphine	8	5.11 ± 0.21[a]
Aminoguanidine 50 mg/kg	morphine	8	4.58 ± 0.13
Aminoguanidine 100 mg/kg	morphine	8	4.29 ± 0.14
L-Arginine 60 mg/kg	morphine	8	4.30 ± 0.21
L-Arginine 60 mg/kg	morphine	8	3.80 ± 0.13[a]
CsA 5 mg/kg	morphine	8	4.64 ± 0.17
CsA 10 mg/kg	morphine	8	5.39 ± 0.31[b]
CsA 5 mg/kg + L-NAME 1 mg/kg	morphine	8	5.32 ± 0.18[b,c]
CsA 5 mg/kg + aminoguanidine 100 mg/kg	morphine	8	4.68 ± 0.21
CsA 10 mg/kg + L-arginine 60 mg/kg	morphine	8	4.49 ± 0.18[d]

The animals received an acute injection of pretreatment drugs 30 min before saline or morphine (5 mg/kg) and 75 min before tail-flick test. Data are expressed as means ± S.E.M. for eight mice.

[a] $P<0.05$ compared to corresponding pretreatment saline group.
[b] $P<0.01$ compared to corresponding pretreatment saline group.
[c] $P<0.05$ compared to CsA 5/morphine group.
[d] $P<0.05$ compared to CsA 10/morphine group.

Table 4
The effects of chronic cyclosporin A, L-NAME and L-arginine on the antinociception threshold after acute saline or morphine injection

Chronic pretreatment	Acute treatment	*n*	Tail-flick latency (s) (mean ± S.E.M.)
Saline	saline	8	2.65 ± 0.09
L-NAME 10 mg/kg	saline	8	2.93 ± 0.13[a]
Aminoguanidine 100 mg/kg	saline	8	2.52 ± 0.08
L-Arginine 60 mg/kg	saline	8	2.41 ± 0.21
CsA 20 mg/kg	saline	8	3.10 ± 0.16[a]
Saline	morphine	8	4.51 ± 0.12
L-NAME 10 mg/kg	morphine	8	4.39 ± 0.17
Aminoguanidine 100 mg/k	morphine	8	4.66 ± 0.21
L-Arginine 60 mg/kg	morphine	8	4.38 ± 0.26
CsA 20 mg/kg	morphine	8	4.29 ± 0.23

The animals received chronic administration (twice daily for 2 days) of pretreatment drugs and were given saline or morphine 95 mg/kg) on the third day 45 min before tail-flick test. Data are expressed as means ± S.E.M. for eight mice.

[a] $P<0.05$ compared to corresponding pretreatment saline group.

for 2 days did not affect the antinociception induced by acute morphine administration ($P>0.05$).

4. Discussion

These data show that systemic administration of cyclosporine A during the induction phase attenuates the development of tolerance to morphine-induced antinociception but does not affect morphine dependence, while its administration in the expression phase increases the antinociception threshold and reduces the severity of withdrawal signs. These effects of cyclosporine A are in parallel with those of the nitric oxide synthase inhibitor, L-NAME, and lower doses of the two agents show potentiating effects in this regard. Moreover, modulatory effects of cyclosporin A on morphine-induced antinociception, tolerance and dependence are reversible by L-arginine. Acute but not chronic (twice daily for 2 days) administration of cyclosporine A and L-NAME also increased the acute antinociceptive effect of morphine.

Although most of the earlier researchers investigating the modulatory properties of cyclosporine A on morphine-induced effects had attributed these properties to immunosuppressive effects of cyclosporine A (Dougherty et al., 1986a,b, 1987; McVaugh et al., 1989), a direct central effect of cyclosporine A on the nervous system had also been postulated (Dougherty and Dafny, 1988; Berthold et al., 1989). In recent years, inhibition of the catalytic activity of nNOS through blocking the calcineurin-mediated dephosphorylation of nNOS has been suggested as an important mechanism through which cyclosporine A and FK506 exert their effects in nerve tissues (Liu et al., 1991; Sharkey and Butcher, 1994; Rao et al., 1996a; Snyder et al., 1998). Cyclosporine A and FK506 are also known to exert similar inhibitory effects on nitric oxide production in other tissues such as kidney (Rao et al., 1996b), liver (Kaibori et al., 1999), lung (Mathieu et al., 1997), heart (Suzuki et al., 1996) and vascular (Lee et al., 1999) and immune (Conde et al., 1995) tissues. Our data showing a complete analogy between the modulatory properties of NOS inhibitor, L-NAME, and cyclosporine A on morphine-induced effects support the hypothesis that inhibition of nitric oxide production by cyclosporine A may be responsible for these modulatory properties. Interestingly, low and per se non-effective doses of cyclosporine A (5 mg/kg) and L-NAME (3 mg/kg) exerted a significant potentiating effect on morphine tolerance and dependence, similar to the effect induced by the higher doses of each agent alone. Moreover, L-arginine reversed the inhibitory effects of cyclosporine A on the development and expression of tolerance to morphine-induced antinociception and lost its inhibitory effect on the development of morphine dependence in the presence of cyclosporine A. A similar reversal of cyclosporine A-induced effects by L-arginine has been shown in various tissues (Mathieu et al., 1997; Lee et al., 1999; Fiore et al., 2000; Fu et al., 2000). L-Arginine can also reverse the inhibitory effects of NOS inhibitors on the development of morphine tolerance (Babey et al., 1994) and their potentiating effects on acute morphine antinociception (Brignola et al., 1994). Thus, inhibition of the L-arginine/NO pathway may contribute to the modulatory properties of cyclosporine A on morphine-related effects. However, as that cyclosporine A inhibits both the inducible and neuronal isoforms of NOS (Conde et al., 1995; Rao et al., 1996a; Sanchez-Lozada et al., 2000) and also considering its modulatory effects on endothelial nitric oxide synthase (Stroes et al., 1997; Sanchez-Lozada et al., 2000), the site of this interaction needs to be further investigated. Interestingly, it has recently been suggested that inducible NOS (iNOS) plays an important role in central nervous processes (Licinio et al., 1999). However, in the present study the irreversible inhibitor of iNOS, aminoguanidine (Al-Shabanah et al., 2000), whether alone or in combination with cyclosporine A did not exert any significant effect, implying that previously reported interactions between cyclosporine A and iNOS are not involved in the modulation of morphine effects by cyclosporine A.

Morphine and NO have been coupled in many physiological processes (Kolesnikov et al., 1993; Fecho et al., 1994; Gyires, 1994; Magazine et al., 1996; Bhargava and Bian, 1998; Stefano et al., 2000), and morphine has been shown to stimulate nitric oxide release in the various tissues, including rat median eminence, providing a role for NO in neurotransmitter release (Stefano et al., 1997; Prevot et al., 1998). In the present study, acute administration of cyclosporine A or L-NAME increased the morphine-induced antinociception in the tail-flick test. This is in accordance with previous reports showing enhancement of morphine antinociception following acute NOS inhibition (Dambisya and Lee, 1995; Machelska et al., 1997). However, chronic administration of L-NAME and cyclosporine A on a twice daily basis for 2 days did not affect the antinociceptive effect of morphine, showing that the potentiating effects of cyclosporine A and L-NAME on morphine antinociception decrease following chronic treatment. It seems that NO exerts a tonic pronociceptive effect (Meller and Gebhart, 1993), while chronic inhibition of its synthesis diminishes the antinociceptive responses to morphine (Dambisya and Lee, 1996). Potentiation of opioid antinociception by acute cyclosporine A, as shown in hot-plate test has been recently reported (Thompson et al., 2000). Our findings with the tail-flick test suggest a mechanism for cyclosporine A-induced modulation of morphine antinociception at the spinal level, which probably involves neuronal NOS.

Inhibitory effects of NOS inhibitors on the development and expression of morphine tolerance have been previously reported (Kolesnikov et al., 1992, 1993; Babey et al., 1994; Dambisya and Lee, 1996). It should be noted that acute enhancing effects of cyclosporine A or L-NAME on morphine antinociception can explain their effect to increase the antinociception threshold during the expression phase of

morphine tolerance. However, such a concern is not implied in the inhibitory effects of these agents on the induction of morphine tolerance. It should be noted that chronic administration of cyclosporine A and L-NAME did not affect the acute antinociceptive effect of morphine. Furthermore, cyclosporine A significantly reduced the intensity of withdrawal signs during the expression phase but did not affect the induction of morphine dependence. This effect of cyclosporine A has been previously reported (Dougherty et al., 1986a, 1987; Berthold et al., 1989; McVaugh et al., 1989), though usually linked to the immunomodulatory effects of cyclosporine A. Interestingly, some researchers have reported a similar pattern of differential modulation of morphine dependence by NOS inhibitors (Dambisya and Lee, 1996). However, it should be noted that there has been some controversy regarding the effects of NOS inhibitors on morphine dependence. Many researchers have shown attenuating effects of NOS inhibitors on the expression of different signs of morphine dependence (Kimes et al., 1993; Thorat et al., 1994; Vaupel et al., 1995), while some have reported no effect (Pataki and Telegdy, 1998). Dambisya and Lee (1996), using an experimental similar to that used in the present study, observed no effect of NOS inhibitors on the development of morphine dependence. Majeed et al. (1994) showed that repeated administration of NOS inhibitor along with morphine decreases some signs of morphine dependence. Moreover, chronic morphine administration leads to up-regulation of neuronal NO synthase immunoreactivity in dependent animals (Cuellar et al., 2000). Our data showed a similar effect for NOS inhibitor and cyclosporin A for inhibiting the expression but not the development of morphine dependence. The reason for this differential modulation of morphine tolerance and dependence has not yet been elucidated, though some authors have suggested that they are distinct phenomena (Wüster et al., 1985; Taylor and Fleming, 2001). The 2-day escalating dose regimen used for induction of tolerance and dependence in this study has been used and verified earlier (Way et al., 1969; Dambisya et al., 1991; Dambisya and Lee, 1996) but the short period of induction in this regimen may be a reason for the observed differential modulation of tolerance and dependence in the current study.

The observed effects of cyclosporine A may be linked to suppression of the NO-cyclic GMP pathways in the CNS that may also explain the reversibility of cyclosporine A-induced effects by L-arginine. Meanwhile, a recent report has suggested that cyclosporine A pretreatment may increase the entry of opiates into the brain through inhibiting the transmembrane protein, p-gylcoprotein (Thompson et al., 2000), a mechanism that may also play a role in cyclosporine A-induced modulation of morphine effects. This effect may play a part in the increased acute morphine antinociception and increased antinociception threshold in the expression phase induced by cyclosporine A. It cannot, however, explain the inhibitory effects of cyclosporine A on the development of morphine tolerance where increased entry of morphine into the brain by cyclosporine A will increase, not decrease, the degree of tolerance. In conclusion, cyclosporine A exerts differential modulatory effects on morphine-induced antinociception, tolerance and dependence, which may have potential clinical benefit. These effects may be due to a decrease in NO production induced by cyclosporine A through one or several mechanism(s) involving constitutive but not inducible isoforms of NOS.

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