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Short communication

Role of nitric oxide in diabetes-induced attenuation of antinociceptive effect of morphine in mice

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Abstract

The study was designed to investigate the role of nitric oxide (NO) in the diabetes-induced decrease of the antinociceptive effect of morphine. The nociceptive threshold in diabetic and non-diabetic mice was measured in the tail-flick test. Streptozotocin (200 mg/kg i.p.) was administered to induce experimental diabetes in the mice. Four weeks after the administration of streptozotocin, the tail-flick test was performed and urinary nitrite concentration was estimated using Greiss reagent. Experimental diabetes markedly decreased the antinociceptive effect of morphine (10 μg in 5 μl/mice i.c.v.) and significantly increased the urinary nitrite concentration. Administration of aminoguanidine (12 mg/mice) markedly improved the antinociceptive effect of morphine and attenuated the increase in urinary nitrite concentration in diabetic mice. It may be tentatively concluded that an increase in NO formation may be responsible for the observed decrease in antinociceptive effect of morphine in diabetic mice.

Keywords: Diabetes; Nitric oxide (NO); Aminoguanidine; Morphine; Antinociception

1. Introduction

Experimental diabetes mellitus attenuates the antinociceptive effect of morphine in mice (Raz et al., 1988; Kamei et al., 1992, 1993a; Sood et al., 2000). Long-term treatment of diabetic mice with cyclosporine (inhibitor of interleukin-2 formation) is reported to restore the antinociceptive effect of morphine (Kamei et al., 1993b). This tentatively implicates the circulating cytokine in attenuation of the antinociceptive effect of morphine in diabetic mice. Cytokines are known to induce the expression of inducible nitric oxide synthase (iNOS) (Deng et al., 1993). L-arginine, a nitric oxide (NO) precursor, is reported to reduce the antinociceptive effect of morphine (Brignola et al., 1994). Constitutive nitric oxide synthase (cNOS) inhibitors are reported to potentiate the analgesic effect of morphine in the tail-flick test (Przewlocki et al., 1993; Machelska et al., 1997). Furthermore, cNOS inhibitors are demonstrated to attenuate the tolerance developed to the analgesic effect of morphine (Kolesnikov et al., 1992; Majeed et al., 1994; Bhargava and Zhao, 1996). Therefore, the present study was designed to investigate the role of NO in the diabetes-induced decrease in antinociceptive effect of morphine.

2. Methods

Swiss albino mice (20–30 g) of either sex were used. They were housed in an animal house provided with a 12-h light/dark cycle and free access to water and food. The animal experiments were conducted in accordance with guidelines of institutional ethics committee.

2.1. Experimental diabetes in mice

Diabetes was induced by a single intraperitoneal injection of streptozotocin (200 mg/kg) dissolved in 0.1 N citrate buffer (pH 4.5). Age-matched mice were injected with citrate buffer to serve as controls.

Blood samples were taken from the retro-orbital sinus 4 weeks after the administration of streptozotocin. Blood glucose was measured colorimetrically using the *o*-tolui-

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dine method (Cooper and McDaniell, 1970). Mice with a fasting blood glucose level of less than 14 mM were not included in the study.

2.2. Measurement of nociceptive threshold

The nociceptive threshold was measured by the tail-flick test (D'Amour and Smith, 1941). Tail-flick latency was considered as the time between tail exposure to radiant heat and tail withdrawal. An electrically heated nichrome wire was used as a source of radiant heat in the analgesiometer (INCO Pvt., Ambala, India). The intensity of radiant heat was selected so as to obtain a pretreatment latency between 2 and 4 S in both diabetic and non-diabetic animals. The maximum cut-off latency time was fixed at 10 S. Tail-flick latency was expressed as a percentage of the maximum possible effect (MPE):

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

Pretreatment latency refers to the control latency before drug administration, while post-treatment latency refers to the latency after drug administration.

2.3. Estimation of urinary nitrite

Each mouse was placed individually in a metabolic cage and its urine was collected for 24 h. The animals were allowed to drink water ad libitum before the study but were denied any water during the 24-h study period. NO is largely converted to nitrite in the presence of oxygen, water and haemoglobin (Imai et al., 1993). Urinary nitrite was estimated using Greiss reagent (Szabo et al., 1993) and served as an indicator of NO production: 2.0 ml of Greiss reagent (1% sulphanilamide and 0.1% naphthylethylene diamine in 5% phosphoric acid) was added to 2.0 ml of suitably diluted urine and O.D. was measured at 550 nm (spectrophotometer, Beckman DU 640B, Nyon, Switzerland). Nitrite concentration was calculated using a standard curve for sodium nitrite. Nitrite levels were expressed as the absolute amount excreted in 24 h.

2.4. Experimental design

There were nine groups of mice. Each group consisted of five animals.

2.4.1. Morphine-treated groups

Morphine (dissolved in 5 μl distilled water) was administered to mice by intracerbroventricular injection under light ether anaesthesia as described by Haley and McCormick (1957). There were three groups of age-matched non-diabetic mice and one group of diabetic mice. Mice from each non-diabetic group were given 5, 7.5 or 10 μg of morphine, respectively, before the tail-flick test. Each mouse in the diabetic group was injected with 10 μg of morphine before the tail-flick test.

2.4.2. Aminoguanidine and morphine-treated groups

Each non-diabetic mouse was given aminoguanidine (12 mg/mice i.p.) every 12 h for three consecutive times. Thirty minutes after the last dose of aminoguanidine, the non-diabetic mice were divided into three groups. Each group was injected with 5, 7.5 or 10 μg morphine, respectively, before the tail-flick test. There were two groups of diabetic mice. Diabetic mice of each group were given aminoguanidine, 6 or 12 mg/mice i.p., respectively, every 12 h for three consecutive times . Thirty minutes after the last dose of aminoguanidine, diabetic mice had tail-flick latency measured immediately, 5, 15, 30, 45, 60, 90 and 120 min after morphine administration. Intergroup comparisons were carried out on the basis of peak %MPE, which was noted 5 min after morphine injection. The observer recording tail-flick latency was not aware of the nature or schedule of drug treatments.

2.5. Drugs

Streptozotocin was obtained from Pharmacia and Upjohn, Kalamazoo, USA and was dissolved in 0.1 N citrate buffer. Morphine was supplied by Jackson Labs, Amritsar, India. Aminoguanidine was obtained from Lancaster Synthesis, Mumbai, India. The solutions of these drugs were prepared freshly before use.

2.6. Data analysis

All the results are expressed as means ± S.E.M. One-way analysis of variance (ANOVA) followed by the Studentised range test were employed to calculate the statistical significance for multiple comparisons between groups. The level of significance (α) was fixed at $P < 0.05$.

3. Results

3.1. Effect of experimental diabetes and aminoguanidine treatment on antinociceptive effect of morphine

Streptozotocin-induced diabetes significantly attenuated the morphine-induced increase in %MPE as compared to that in non-diabetic mice (Fig. 1). Administration of aminoguanidine did not alter the antinociceptive effect of various doses of morphine in non-diabetic mice (Fig. 1). However, aminoguanidine in a high dose markedly improved the decreased antinociceptive effect of morphine in diabetic mice. Aminoguanidine in a low dose partially

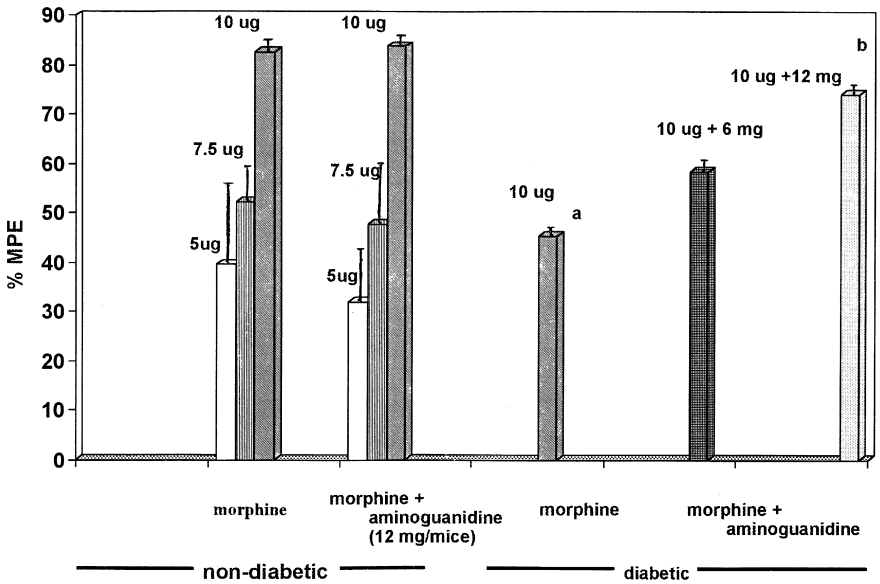

Fig. 1. Effect of morphine and aminoguanidine on percent maximal possible effect (%MPE) in non-diabetic or control and diabetic mice. Morphine was administered i.c.v. just before subjecting the mice to tail-flick testing. Aminoguanidine was administered three times at 12-h intervals and morphine was administered i.c.v. half an hour after the last dose of aminoguanidine. (a) $P < 0.05$ vs. morphine in non-diabetic mice; (b) $P < 0.05$ vs. morphine in diabetic mice.

restored the analgesic effect of morphine in diabetic mice, but the results were not statistically significant (Fig. 1).

3.2. Effect of experimental diabetes and aminoguanidine treatment on urinary nitrite concentration

Experimental diabetes markedly increased the urinary nitrite concentration. Administration of aminoguanidine in low, as well as in high doses, produced no change in urinary nitrite concentration in control or non-diabetic mice (Table 1). On the other hand, amioguanidine treatment in low and high doses markedly attenuated the diabetes-induced increase in urinary nitrite concentration. Moreover, the urinary nitrite concentration in diabetic mice treated with a high dose of aminoguanidine (12 mg/mice) was comparable to the urinary nitrite concentration in control or non-diabetic mice (Table 1).

Table 1
Effect of streptozotocin-induced diabetes and aminoguanidine treatment on urinary nitrite concentration. Each value is the mean ± S.E.M. for five animals

Treatment	Urinary nitrite concentration (μM/24 h)
Non-diabetic mice	10.20 ± 0.65
Aminoguanidine (6 mg/mice)-treated non-diabetic mice	9.60 ± 0.46
Aminoguanidine (12 mg/mice)-treated non-diabetic mice	9.23 ± 0.60
Diabetic mice	20.40 ± 1.10[a]
Aminoguanidine (6 mg/mice)-treated diabetic mice	15.14 ± 1.60[a,b]
Aminoguanidine (12 mg/mice)-treated diabetic mice	12.65 ± 1.25[b]

[a] $P < 0.05$ vs. non-diabetic mice.
[b] $P < 0.05$ vs. diabetic mice.

4. Discussion

The results of the present study demonstrated a significant decrease in the antinociceptive effect of morphine in diabetic mice. These observations are consistent with earlier reports (Simon and Dewey, 1981; Raz et al., 1988; Sood et al., 2000). However, the mechanism of this decreased antinociceptive effect of morphine as a consequence of diabetes is not known. L-Arginine, a NO precursor, was reported to reduce the antinociceptive effect of morphine (Brignola et al., 1994), whereas cNOS inhibitors were reported to potentiate the analgesic effect of morphine (Przewlocki et al., 1993; Machelska et al., 1997). Therefore, it is possible that the observed decrease in antinociceptive effect of morphine in diabetic mice was due to an increased formation of NO. The marked increase in urinary nitrite concentration in diabetic mice now noted supports this contention. Aminoguanidine is a selective inhibitor of iNOS and a weak inhibitor of cNOS (Griffiths et al., 1993; Misako et al., 1993). The doses of aminoguanidine employed in the present study are reported to inhibit iNOS in mice (Corbett et al., 1993). Aminoguanidine was reported to prevent the diabetes-induced reduction in nNOS-containing neurons in the retina (Roufail et al., 1998). In the present study, aminoguanidine treatment of diabetic mice restored the decreased antinociceptive effect of morphine and simultaneously reduced the

increase in urinary nitrite concentration in diabetic mice. This suggests that aminoguanidine-sensitive iNOS and the consequently increased formation of NO may have decreased the antinociceptive effect of morphine in diabetic mice. cNOS is a calcium-dependent enzyme and iNOS does not require calcium for its activation (Fukuto and Chaudhuri, 1995). A similar calcium-independent decrease in antinociceptive effect of mu receptor agonists was noted by Ohsawa et al. (1998) in diabetic mice. On the other hand, iNOS may not be involved in the analgesic effect of morphine in non-diabetic mice, because aminoguanidine, a preferential inhibitor of iNOS, did not markedly affect the analgesic effect of morphine and urinary nitrite concentration in non-diabetic mice.

On the basis of the above, it can be concluded that the increased urinary nitrite concentration in diabetic mice may be due to the increased NO production. This increased NO production may be responsible for the observed decrease in antinociceptive effect of morphine.

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