Effect of L-Arginine in Central Spinal Pain Syndrome

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 127, No. 2, pp. 160-163, February, 1999
Original article submitted April 23, 1998

When applied in combination with penicillin (2000 U) to the dorsal surface of the spinal cord, L-arginine in a low concentration of 100 nmol had a pronociceptive effect, while being applied in concentrations of 65-130 μmol with penicillin or injected intramuscularly before penicillin (15,000 U) L-arginine exhibited an analgesic effect. The opposite effects of L-arginine as the precursor of NO and of opioid dipeptide kyotorphin are demonstrated.

Key Words: *L-arginine; nitric oxide; central spinal pain syndrome; kyotorphin*

The central spinal pain syndrome (CSPS) is a neurogenic pain syndrome caused by the formation of an aggregate of hyperactive nociceptive neurons in the dorsal horn capable to generate long-lasting spontaneous ongoing discharges of nerve impulses [1]. Clinical manifestations of the neurogenic pain syndrome include allodynia, hyperalgesia, and spontaneous pain. In many respects these symptoms result from disturbed inhibitory control [1], increased ionotropic glutamate and activation of NDMA receptors [10] and enhanced production of NO, a new neuronal messenger [3] involved in both central and peripheral nociception [5]. Our aim was to study the effect of L-arginine (L-Arg) on the development of CSPS.

MATERIALS AND METHODS

Experiments were carried out on 154 outbred albino rats (200-220 g). CSPS was provoked in the left spinal dorsal horns (L_{IV}-L_{VI}) with benzyl penicillin sodium salt that blocks GABAergic inhibition in the application area [6]. Unilateral laminectomy was performed under ether anesthesia at the L_{IV}-L_{VI} level. Penicillin (PC) was dissolved in liquid agar. An 1×0.4×10-mm agar plate with 2,000 or 15,000 U PC was applied to the dorsal surface of the left part of the spinal cord. L-Arg (100 nmol and 1, 20, 65, and 130 μmol). Sodium nitroprusside (100 and 200 nmol) and glutamate (150 μmol per rat) were applied to the spinal cord either individually or in various combinations. In special series, L-arg (130 μmol) and sodium nitroprusside (1 μmol) were injected intramuscularly 10 min before and 20 min after application of PC in a dose of 15,000 U. The following parameters were scored on a 3-point scale: incidence and duration of spontaneous pain attacks, response to stimulation of the pain projection area (tactile and noxious mechanical stimulation of the hind limb), motor activity, vocalization, allodynia, and hyperesthesia or analgesia of the pain projection area.

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RESULTS

Ten minutes after PC application to the spinal cord in a dose of 15,000 U the rats began to lick and gnaw femur and toes of the left hind limb, run from one place to another trying to spare the pain-projected limb and shriek. These symptoms indicate the development of CSPS. The intensity of these manifestations sharply increased during 10 min. The nocifensive reaction could be provoked by a light touch within or outside the pain projection area, which attests to the development of allodynia and hyperesthesia (Fig. 1, *I, a*). The duration of CSPS was 2.5-3 h. Application of 2,000 U PC or 100-200 nmol L-Arg had no effect on rat behavior (Fig. 1, *I, b* and *II, a*). When the dose of L-Arg was increased to 20-130 μmol, a dose-dependent analgesia of the left hind limb developed (Fig. 1, *II, b-d*). The duration of analgesia increased from 3 (20 μmol) to 5-6 h (130 μmol). Application of L-Arg in doses of 100 nmol — 20 μmol in combination with PC

0007-4888/99/0002-0144$22.00

(15,000 U) did not modify the development of CSPS; in doses of 65-130 μmol L-Arg decreased the incidence of spontaneous pain attacks and reduced motor activity and vocalization. Analgesia of the pain projection area developed, allodynia disappeared, although diffuse hyperesthesia persisted (Fig. 1, *III, a, b*). Intramuscular injection of L-Arg (65-130 μmol 10 min prior to PC application) moderated the signs of CSPS: analgesia of the pain projection area developed, allodynia and hyperesthesia were absent (Fig. 1, *III, c*). L-Arg (65-130 μmol) administered against the background of developed pain syndrome (20 min after PC application) decreased the incidence and duration of spontaneous pain attacks, but did not affect motor activity and vocalization. The rats also had allodynia and hyperesthesia. Analgesia of the pain projection area did not develop (Fig. 1, III, *d*).

Subthreshold doses of L-Arg (100 nmol) and PC (2,000 U) produced allodynia: 40 min after application, tactile stimulation of femoral surface provoked nocifensive reaction. Diffuse hyperesthesia developed after 90 min: the nocifensive response could be evoked from any part of the body. Allodynia and hyperesthesia persisted for 2 h. Combined application of L-Arg (20 μmol) and PC (2,000 U) induced neither allodynia nor hyperesthesia.

To determine the role of nitric oxide in the L-Arg-induced analgesia, we compared the effects of L-Arg and sodium nitroprusside, a non-enzymatic NO precursor. When applied to the spinal cord in a dose of

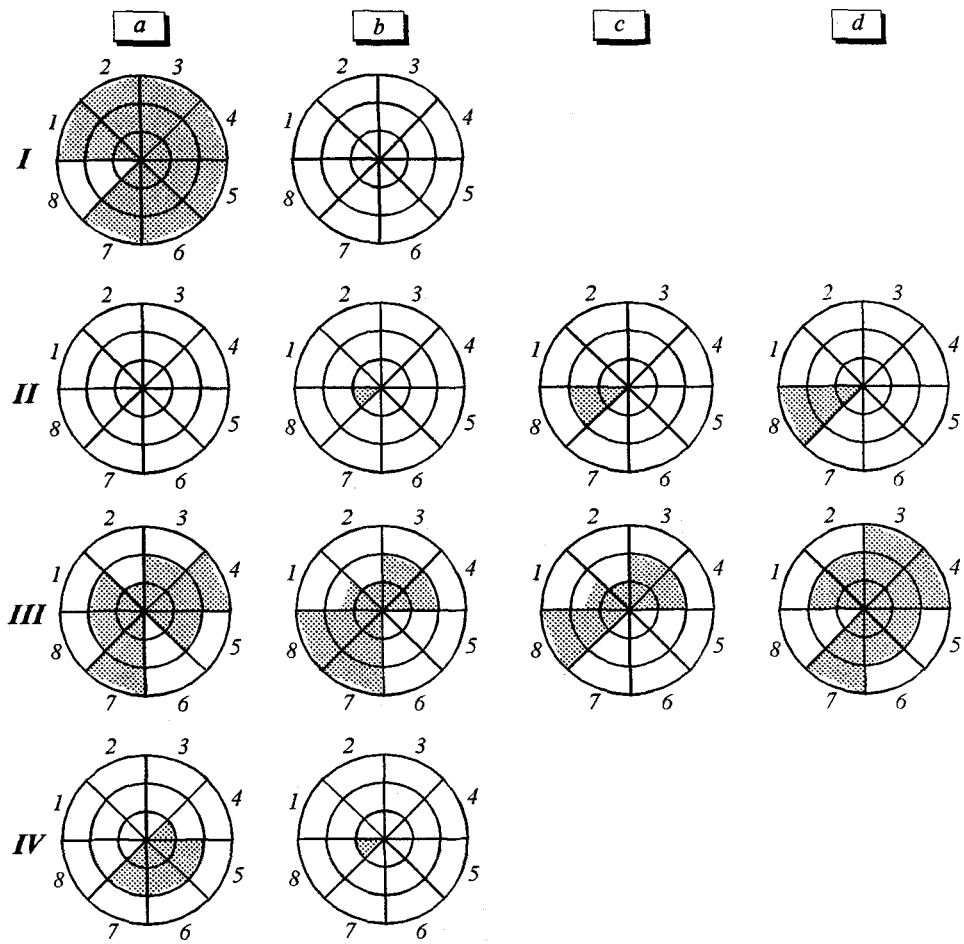

Fig. 1. Effects of L-arginine (L-Arg) on central spinal pain syndrome. *I*) Penicillin (PC) in doses of (*a*) 15,000 and (*b*) 2,000 U. *II*) L-Arg in doses of (*a*) 100-200 nmol, (*b*) 20 μmol, (*c*) 65 μmol, and (*d*) 130 μmol. *III*) L-Arg in doses of (*a*) 65 μmol and (*b*) 130 μmol in combination with PC (15,000 U); L-Arg (130 μmol) injected intramuscularly 10 min prior to (*c*) and 20 min after PC (*d*). *IV*) L-Arg in doses of (*a*) 100 nmol and (*b*) 20 μmol applied with PC (2,000 U). Here and in Fig. 2: the score of the pain syndrome signs are shown by the internal (*1*), middle (*2*), and external (*3*) circles. The sectors are: *1*) the incidence of pain attacks (1 point corresponds to 1 attack per 3 min, 2 points: 1 attack per 1 min, 3 points, 2-3 attacks per 1 min); *2*) duration of a pain attack (1 point — 5 sec; 2 points — 10 sec; 3 points — 15-20 sec); *3*) motor activity (1 point — 1-2 short runs during a pain attack; 3 points — persistent running with jumping); *4*) vocalization (1 point — a weak short squeak; 3 points — a long squeal during the entire attack); 5) responses to provocative stimulation (in points); *6*) duration of allodynia (1 point — 20 min; 2 points — 40-60 min; 3 points — 2.5-3 h); *7*) duration of hyperesthesia (1 point — 30 min; 2 points — 90-120 min; 3 points — more than 3 h); *8*) analgesia in the pain projection area (1 point — weak response to mechanical stimulation, duration of analgesia up to 2 h; 2 points — a weak response to very strong mechanical stimulation, duration of analgesia 3-4 h; 3 points — no response to stimulation of any modality, duration of analgesia 5-6 h).

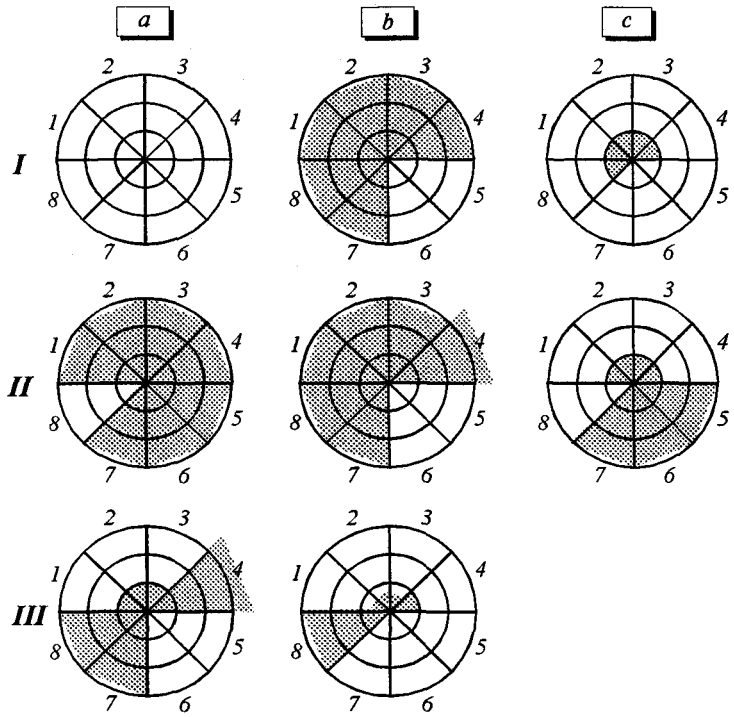

Fig. 2. Effects of sodium nitroprusside and glutamate in central spinal pain syndrome. *I*) Sodium nitroprusside in doses of 100 (*a*) and 200 nmol (*b*); glutamate in a dose of 150 μmol (c). *II*) Sodium nitroprusside in doses of 100 (*a*) and 200 nmol (*b*) in combination with penicillin (15,000 U); sodium nitroprusside (100 nmol) in combination with PC (2,000 U) (*c*). *III*) Sodium nitroprusside (200 nmol, *a*) and glutamate (150 μmol, *b*) in combination with L-arginine (65 μmol).

100 nmol, sodium nitroprusside produced no nocifensive reaction (Fig. 2, *I, a*), but in a dose of 200 nmol it provoked spontaneous pain attacks (gnawing of the pain projection area on the hind limb, enhanced motor activity and vocalization). Pain attacks of varying intensity persisted for more than 7 h and were accompanied by pronounced hyperesthesia. Stimuli of different modalities applied to the pain projection area did not provoke the nocifensive response (analgesia of the pain projection area, Fig. 2, *I, b*). Addition of sodium nitroprusside in the subthreshold dose of 100 nmol to PC (15,000 U) had no effect on CSPS (Fig. 2, *II, a*), while in a superthreshold dose of 200 nmol it augmented vocalization, increased the duration of CSPS to 4-5 h, and produced analgesia in the pain projection area (Fig. 2, *II, b*). Combined application of sodium nitroprusside and PC in subthreshold doses (100 nmol and 2,000 U, respectively) induced allodynia, hyperesthesia, 40-60-min spontaneous pain attacks (0.5 point) accompanied by vocalization (3 point) persisting during 4 h (Fig. 2, *II, c*). Combined application of L-Arg (65 μmol) and sodium nitroprusside (200 nmol) considerably alleviated spontaneous pain attacks (to 1 point), but vocalization (>3 point), hyperesthesia, and analgesia of the pain projection area remained unchanged (Fig. 2, *III, a*). L-Arg (65 μmol) applied in combination with glutamate (150 μmol) reduced spontaneous pain attacks in comparison with glutamate alone and potentiated analgesia of the pain projection area (Fig. 2, *III, c*).

Thus, the effect of L-Arg depended on the dose: it produced a pronociceptive effect when applied in subthreshold doses in combination with PC, and an antinociceptive effect when applied or injected against the background of CSPS induced by PC, sodium nitroprusside, or glutamate.

Our findings agree with previous data [8] on the involvement of L-Arg in nociception. This substance is a source of NO that is a necessary link in the processes facilitating neuronal responses to activation of spinal NDMA receptors by glutamate [9,11]. Moreover, L-Arg is a precursor of kyotorphin, an endogenous antinociceptive dipeptide (Tyr-Arg), which promotes the release of met-enkephalin in the brain and spinal cord. It also acts as neurotransmitter and neuromodulator in the structures regulating pain sensitivity [13]. Synthesis of NO and kyotorphin from L-Arg is catalyzed by NO-synthase [12] characterized by high affinity for L-Arg [2] and kyotorphin synthase characterized by low affinity for L-Arg [15]. This probably explains the fact that the low doses of L-Arg produce nocifensive reaction, i.e., the effect of L-Arg is NO-dependent. This hypothesis is supported by the development of algesia in the experiments with sodium nitroprusside (a non-enzymatic NO precursor) and subthreshold doses of PC, as well as by enhancement of vocalization and prolongation of CSPS provoked by PC in combination with sodium nitroprusside. The analgesic effect of moderate and high doses of L-Arg is probably related to enhanced kyotorphin synthesis [7,8]. These data agree with observations

that intracervical or subcutaneous L-Arg induces strong analgesia that can be blocked by naloxone [8]. There are also clinical data that L-Arg relieves pain in postherpetic neuralgia, cervical and lumbar radiculitis, and rheumatoid arthritis [9,14]. We showed that application of L-Arg prior to PC produces a more strong analgesia than its injection against the background of PC-disturbed inhibition, probably due to inhibitory influence of preliminary application of L-Arg on the nociceptive neurons (for example, due to intensification of met-enkephalin synthesis), preventing their hyperactivation by PC. Analgesia developing in the projection area corresponding to L-Arg or sodium nitroprusside application may result from inhibition of peripheral nociceptors [7]. The mechanism of this analgesia is not clear, but presumably cGMP or kyotorphin may participate in it.

Thus, our findings suggest that nocifensive effect of low doses of L-Arg is mediated by the formation of NO potentiating the activating effect of glutamate on nociceptive neurons, while the antinociceptive effect of high doses of L-Arg results from the formation of kyotorphin.

The study was supported by Leading Scientific Schools Grant of President of Russia, project No. 96-15-97767.

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