Effect of L-Arginine in Central Spinal Pain Syndrome

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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 (L₅₋₇) with benzyl penicillin sodium salt that blocks GABAergic inhibition in the application area [6]. Unilateral laminectomy was performed under ether anesthesia at the Lw-L₅ 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.

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...
(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

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**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) 200 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).