Effects of Ca-Regulating Drugs on the Formation of a Generator of Pathologically Enhanced Exitation in the Spinal Dorsal Horn System

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The emergence of a generator of pathologically enhanced excitation (GPEE) in the pain sensitivity system is one of the principal mechanisms in the development of pain syndrome [2]. This generator represents an aggregation of hyperactive neurons producing a potent stream of impulses capable of overcoming regulation and inhibition control mechanisms in the CNS and of inducing a pain syndrome. Increased Ca influx into neurons [9,12, 13] and a reduced level of extracellular Ca [8] contribute much to the neuronal hyperactivation process. For this reason studies of the effects of Ca-regulating drugs on pain syndrome development are of considerable importance.

The present research was undertaken to investigate the effects of the calcium channel blokers nimotop and OF 5015, an agent synthesized at the Pharmacology Laboratory of the Research Chemical and Pharmaceutical Institute (Russia), as well as of calcium gluconate of the development of the pain syndrome.

MATERIALS AND METHODS

Experiments were carried out with outbred white rats weighing 200-220 g. A pain syndrome of spinal origin [2,3] was induced by creating a GPEE in the dorsal horn of the lumbar region of the spine with penicillin. For this purpose penicillin in a dose of 40,000 MU in 1 ml was mixed with liquid 1% agar and a plate of solidified agar was applied to the dorsal surface of the spine at the L_4-L_5 level. Laminectomy was carried out under ether anesthesia. OF5015 was used in doses of 50 and 75 mg/kg, Ca gluconate in doses of 150 and 250 μg/kg. OF5015 and Ca gluconate were administered orally with Tween-80, nimotop intramuscularly and intracisternally. For local drug action on the site of the developing GPEE the agents were added to the liquid agar with penicillin: OF5015 in doses of 25 and 35 mg/kg, nimotop in doses of 75 and 125 μg, and Ca gluconate in doses of 5, 10, 20, and 40 mg per ml agar. The effect of OF5015 was compared to that of analgin. Analgin was administered orally and applied to the spine in the same doses as OF5015; both drugs are classified as non-narcotic analgesics, analgin being a reference preparation of this group. Ten animals were used in each experimental series. The pain syndrome was assessed on a three-point scale [4].

RESULTS

Pain syndrome signs were manifested 10-15 min after application of an agar plate with penicillin on

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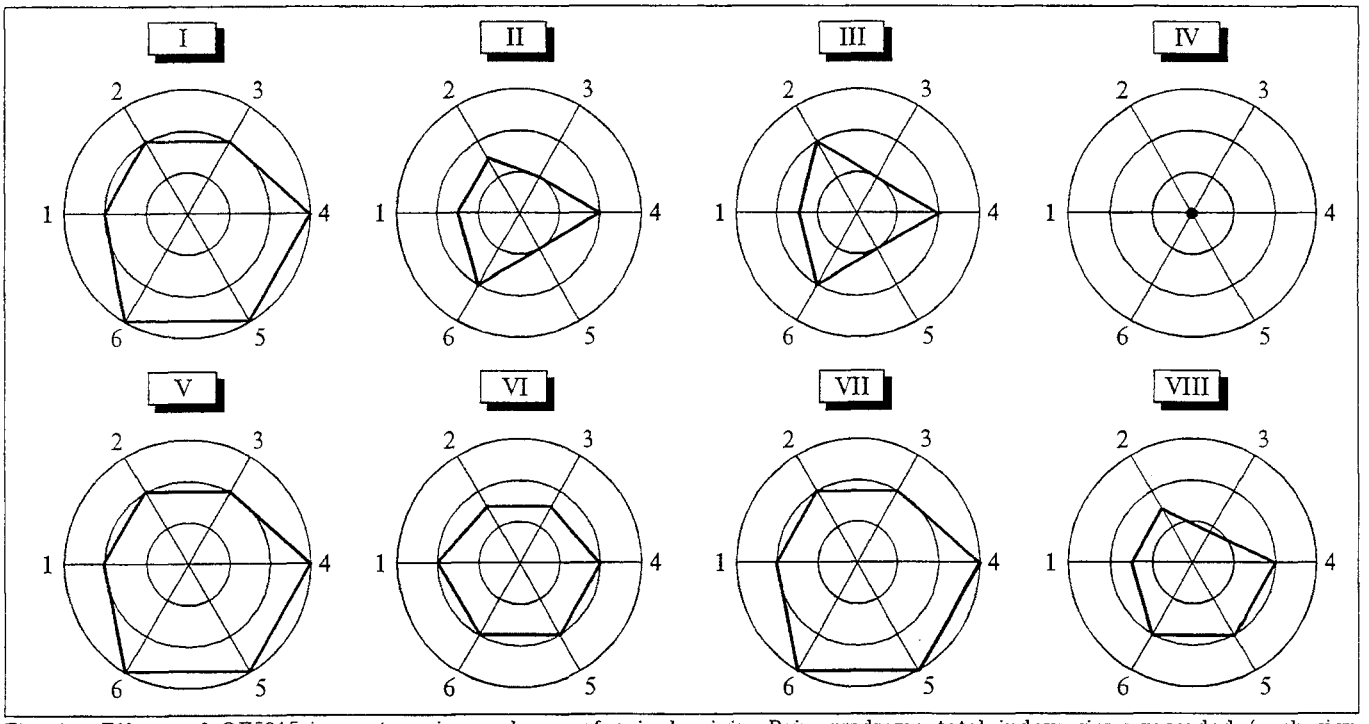

Fig. 1. Effects of OF5015 in acute pain syndrome of spinal origin. Pain syndrome total index: signs recorded (each sign represented as a radius): *1*) frequency of attacks per min; *2*) duration of attack, sec); *3*) intervals between attacks, sec; *4*) response to provocation, points; *5*) vocalization (spontaneous, provoked); *6*) motor activity (spontaneous, provoked). Signs were assessed on a three-point scale: 0 – no signs, 1 – slight, 2 – moderate, 3 – serious degree of expression (each circle means one point, counting from the center). *I*) effects of OF5015 in a dose of 50 mg/kg and *II*) in a dose of 75 mg/kg orally; *III*) effects of OF5015 applied in an agar plate with penicillin to the spine in a concentration of 25 mg/ml and *IV*) in a concentration of 35 mg/ml; *V*) analgin effects, dose 50 mg/kg and *VI*) in a dose of 75 mg/kg orally; *VII*) effects of analgin applied in an agar plate with penicillin to the spine in a concentration of 25 mg/ml and *VIII*) 35 mg/ml.

the spinal cord, that is, immediately after the animals came out of narcosis. The local reaction was as follows: the animals licked the area of pain projection corresponding to the area of penicillin application to the lumbar segments, were noisy and excited, and developed hind paw flexion. Stimulation of any portion of the body, especially tactile irritation of the site of increased sensitivity (pain projection site), caused an acute paroxism of pain. Spontaneous pain attacks occurred as well. The frequency and intensity of these attacks attained the maximum 30-40 min later and remained unchanged for 2 h, after which the pain gradually abated over one hour. The total duration of the pain syndrome was 3 to 3.5 h. Oral administration of 50 mg/kg OF5015 in the course of pain syndrome development slightly reduced the duration of painful attacks and prolonged the intarvals between the attacks (Fig. 1, *I*); spontaneous attacks were somewhat less frequent. The effect of the drug could be observed right up to the end of the pain syndrome. Increasing the dose to 75 mg/kg resulted in an abatement (by 35-50%) of all pain syndrome signs (Fig. 1, *II*) and a markedly decreased incidence of spontaneous attacks. The duration of the syndrome was the same as in the controls, 3-3.5 h. OF5015 application to the spine at the site of the GPEE in a dose of 25 mg in 1 ml agar was associated with alleviation of the pain syndrome and prolongation of the intervals between attacks; the animals became less noisy (Fig. 1, *III*) and the intensity and frequency of spontaneous painful attacks decreased. If the dose of OF5015 was increased to 35 mg/ml agar the pain syndrome did not develop at all in 80% of cases (Fig. 1, *IV*) or was attenuated to 1-1.5 points in 20% of animals. No spontaneous painful attacks were observed.

Analgin in a dose of 50 mg/kg had the same effect as OF5015 (Fig. 1, *V*); an increase of the dose to 75 mg/kg negligibly enhanced its effect (Fig. 1, *VI*). Analgin application in a dose of 25 mg/ml agar resulted in a slight alleviation of the pain syndrome, equal to that attained by oral administration of the drug (Fig. 1, *VII*). An increase of the dose to 35 mg/ml agar enhanced the effect of analgin, although its efficacy was much inferior to that of OF5015 (Fig. 1, *VIII*).

Intraperitoneal injection of nimotop, whatever the dose, had no effect on the pain syndrome (Fig. 2, *a, I, II*). Intracisternal injection resulted in a somewhat lower incidence of painful attacks

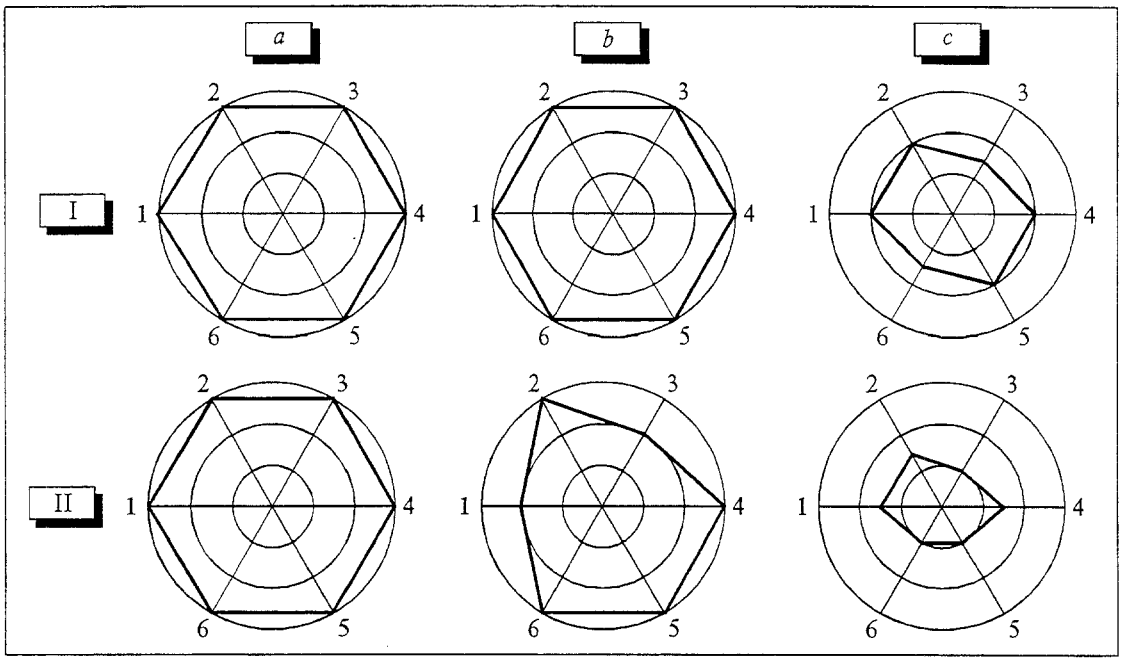

Fig. 2. Effects of nimotop in acute pain syndrome of spinal origin. *a*) nimotop injected intraperitoneally: *I*) in a dose of 150 μg/kg and *II*) 250 μg/kg; *b*) nimotop injected intracisternally: *I*) in a dose of 150 μg/kg and *II*) 250 μg/kg; *c*) effects of nimotop applied in an agar plate with penicillin to the spine: *I*) in a concentration of 75 μg/ml and *II*) 150 μg/ml. Other notation as in Fig. 1.

and a longer duration of the intervals between attacks for a dose of 250 μg/kg (Fig. 2, *b, I, II*). Nimotop application to the spine in a dose of 75 μg/ml agar resulted in a slight reduction of all pain syndrome signs (Fig 2, *c, I*). An increase of the dose to 125 μg/ml agar led to a noticeable alleviation of the pain syndrome: motor activity and vocalization were particularly decreased (Fig. 2, *c, II*), and the incidence of spontaneous painful attacks dropped as well.

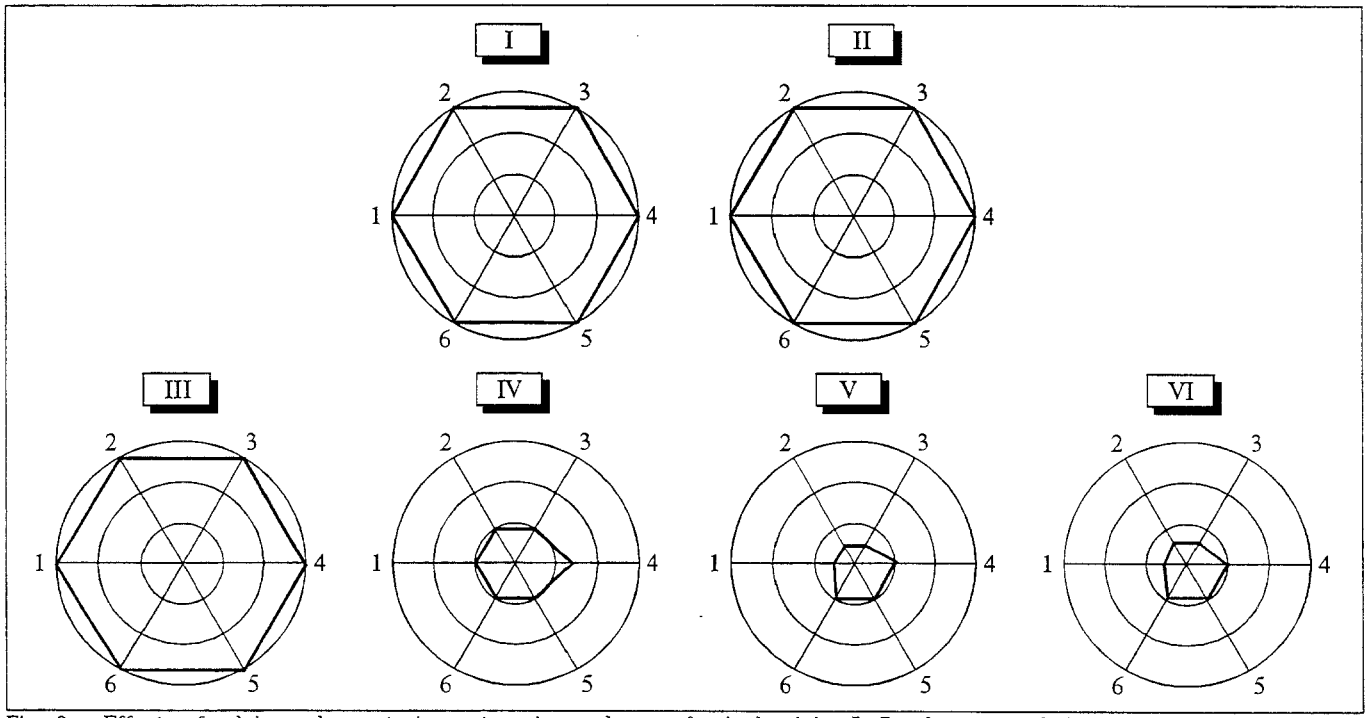

Fig. 3. Effects of calcium gluconate in acute pain syndrome of spinal origin. *I*) Ca gluconate administered orally in a dose of 100 mg/kg twice daily for 3 days before pain syndrome induction; *II*) oral administration in a dose of 100 mg/kg at peak of pain syndrome. Effects of Ca gluconate applied in an agar plate with penicillin to the spine in concentrations: *III*) 5, *IV*) 10, *V*) 20, *VI*) 40 mg/kg. Other notation as in Fig. 1.

Calcium gluconate in a dose of 100 mg/kg was administered orally twice a day for 3 days and then the pain syndrome was induced against such a background. In another series of experiments the drug was orally administered in the same dose at the peak of the pain syndrome. The drug was ineffective in both series of experiments (Fig. 3, *I, II*). In contrast to these results, calcium gluconate application to the spine resulted in a clear-cut alleviation of the pain syndrome, the degree of alleviation depending on the dose. The effect of the agent was maximal when it was administered in a dose of 20 mg/ml agar (Fig. 3, *V*). Further dosage build-up was not paralleled by an enhancement of its effect (Fig. 3, *VI*).

Hence, administration of the Ca^{2+} blocker OF5015 in sufficiently high doses (75 mg/kg) arrested the pain syndrome caused by the penicillin-induced generator of pathologically enhanced exitation in the dorsal horns of the spine. This result is in line with current data on the role of Ca^{2+} in neuronal epileptization [6,16]. Some authors report opiate activity of Ca channel blockers enhancing and prolonging the antinociceptive effect of opioids [7]. Ca^{2+} attenuates opiate effects due to inhibition of opiate binding to receptors [1,5,10]. By reducing calcium entry, calcium channel blockers may be influencing the endogenous opioid system.

Exposure of the site of the formed GPEE to calcium gluconate prevented the development of the pain syndrome. This effect may be due to the rise in the extracellular Ca^{2+} level, which may lead to suppression of neuronal hyperactivity [8]. It has been shown [15] that calcium injected intrathecally potentiates the antinociceptive effect of norepinephrine as indicated by the tail flick test in mice. We believed that the antinociceptive activity of Ca^{2+} is a result of adenosine released from the spine, which potentiates the effect of norepinephrine. Calcium antinociceptive activity may be a result of adenosine and norepinephrine synergism [15]. Some scientists [11] claim that the antinociceptive effect of high doses of calcium depends on supraspinal release of endogenous opiates either directly facilitating opioid peptide release at the segmental level or stimulating the neuronal route containing endogenous opioid components [17]. By facilitating the release of one or several neurotransmitters involved in antinociceptive mechanisms at the spinal level [14], calcium causes an antinociceptive effect.

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