PHYSIOLOGY

Gaseous Superoxide Potentiation of the Effect of Nonnarcotic Analgesics in Low Doses

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 135, No. 3, pp. 253-256, March, 2003
Original article submitted July 23, 2002

The action of inhalation of gaseous superoxide on the effects of low doses of nonnarcotic analgesics was studied on volunteers in the little finger compression test. After administration of placebo, inhalation of gaseous superoxide produced a negligible transient decrease in pain tolerance threshold. Inhalation of gaseous superoxide potentiated the effects of threshold doses of novalgin and aspirin and prolonged their action, but did not modulate the analgesic effect of diclofenac. It is assumed that the potentiating effect of superoxide on the action of analgesics is related to inhibition of monoamine oxidases leading to accumulation of monoamines in the brain.

Key Words: *superoxide; pain sensitivity; nonnarcotic analgesics; humans*

It was established that negatively charged oxygen ions modulate activity of CNS, various peripheral systems, and humoral processes [10]. Gaseous superoxide (GS) is used in clinical practice in patients with bronchial asthma and other respiratory diseases [6]. At the same time, it is interesting to study other beneficial effects of negatively charged oxygen ions, specifically, potentiation of antinociceptive effect of analgesics. A possible mechanism mediating the effect of GS on nociception is its action on monoamine oxidase (MAO) and modulation of the serotoninergic system [3]. Studies on animals demonstrated perspectives of using GS for potentiation of the effects of narcotic and nonnarcotic analgesics [5]. Our aim was to study the effect of GS on the action of low doses of some nonsteroid nonnarcotic analgesics in humans.

MATERIALS AND METHODS

Double-blind experiments with placebo preparations and placebo inhalators were carried out on 12 volunteers (6 males and 6 females) aging 20-40 years. A Pro-Inhal medical inhalator was used as a source of GS. At room temperature (18-20°C) and relative humidity 60-65%, GS production was 0.25 μmol/min at a distance of 1 cm from the electrode (measured by reduction of cytochrome *c* and nitroblue tetrazolium) [7]. Similar device (placebo inhalator) not producing GS was used in controls.

Pain sensitivity was measured with an Ugo Basile algesimeter. Nociceptive stimuli were gradual compression of the left little finger. The examinees reported the tolerable pain threshold (TPT). The recorded parameters were measured in arbitrary units: 1 arb. unit corresponded to 20 g compression force. During the experiments, the examinees did not see the shifts of load in the algesimeter and did not hear the moment of its turning on.

At the start of each experimental series, the initial TPT was determined. Then after 15-min inhalation of GS or placebo, the examinee received one of the following peroral preparations: placebo, sodium diclofenac (0.5 mg/kg, Berlin-Chemie AG), novalgin (metamizol, 5 mg/kg, Hoechst AG), or aspirin (3 mg/kg, Bayer). The doses of these preparations were approximately $^1/_2$ single analgesic dose administered during

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0007-4888/03/1353-0218$25.00 © 2003 Plenum Publishing Corporation

pain syndromes. Inhalation was resumed for another 15 min after intake of the drugs. TPT was measured immediately after inhalation and then every 30 min over the following 3 h.

Each examinee passed 8 tests: 2 times with each of 4 drugs (with GS- or placebo-inhalators), the interval between successive tests was no less than 1 week. The means and SEM were calculated for each time point individually in each series. The significance of TPT changes in a series was assessed relatively to individual baseline values and to the changes in other series with the use of corresponding Student's, Mann—Whitney, and Fisher's tests.

RESULTS

In different experimental series, the initial value of TPT varied from 15 to 21 rel. units. In females this index was lower than in males by 3-5 rel. units. Individual pain sensitivity slightly decreased from test to test over 2 months. However, these changes did not affect TPT dynamics.

During the entire recording period, no significant changes in TPT were observed in the control (placebo preparation+placebo inhalation) series. GS-inhalation with placebo preparation produced a slight decrease in TPT compared to baseline values, but the difference was significant only immediately after the end of inhalation. There were no differences from the control series (Fig. 1, *a*). Thus, GS-inhalation insignificantly increased pain sensitivity.

Administration of novalgin against the background of placebo inhalation significantly increased TPT 90 min after the end of inhalation compared to the control and baseline (Fig. 1, *b*). The combined use of GS-inhalation with novalgin produced a significant

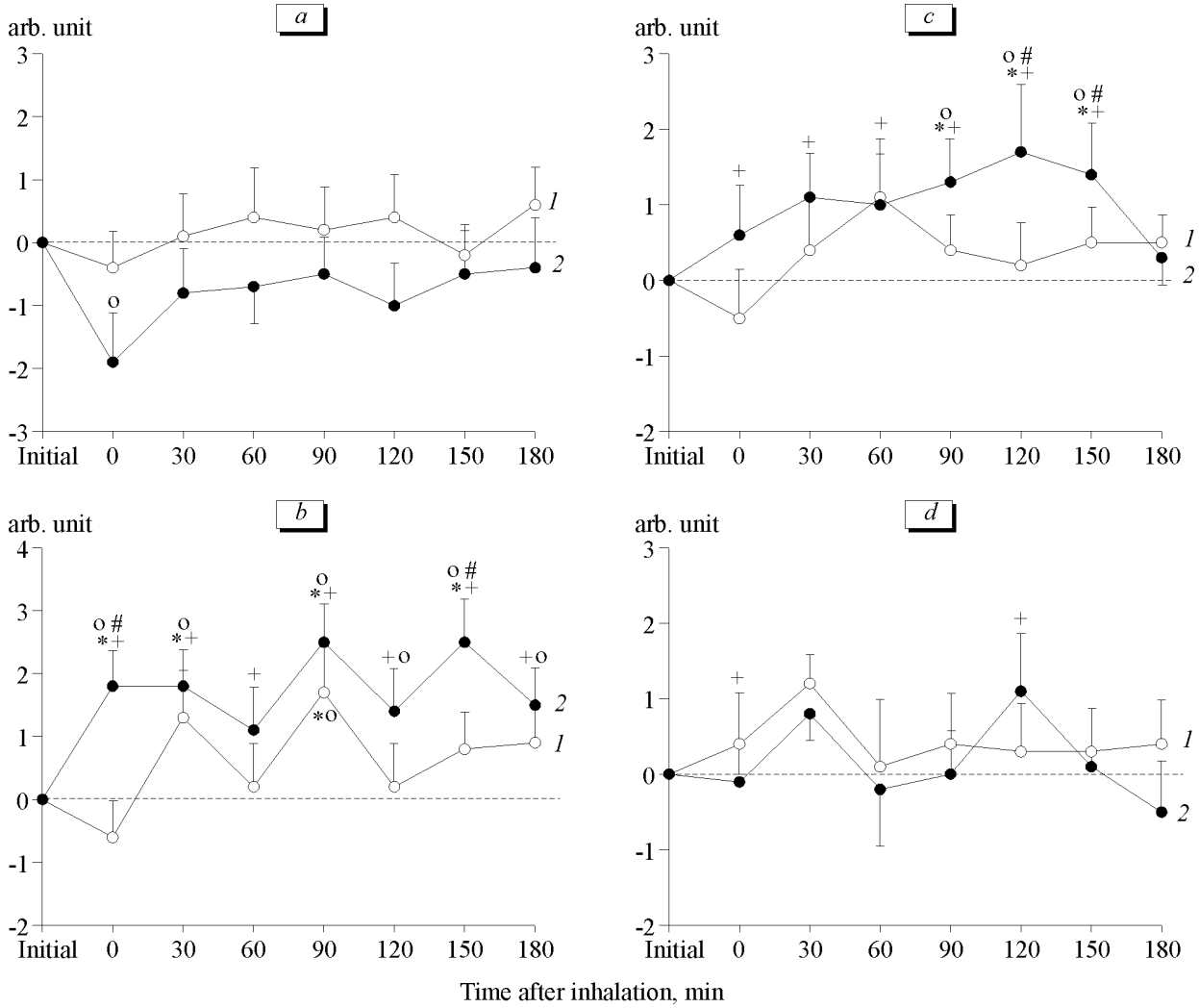

Fig. 1. Pain sensitivity measured in the little finger compression test under the effect of placebo inhalation (*1*) and gaseous superoxide inhalation (*2*) in control (*a*) and after administration of novalgin (*b*), aspirin (c), and diclofenac (*d*). Ordinate: tolerable pain threshold compared to the baseline (*AM*±*SEM*). $p<0.05$: in comparison with: *placebo inhalation+placebo preparation; $^{+}$gaseous superoxide inhalation+placebo preparation; ^{o}baseline; $^{\#}$placebo inhalation+test preparation.

and stable increase in TPT throughout the entire observation period. Moreover, a significant difference of TPT in comparison with that measured after novalgin+placebo inhalation was observed immediately after inhalation and also 150 min later. Therefore, when applied against the background GS inhalation, the drug produced its analgesic effect as early as 15 min after intake (or 15 min after the end of inhalation). This effect persisted for at least 3 h. Thus, GS-inhalation accelerates and prolongs the effects of novalgin and potentiates its action.

Pain sensitivity in subjects receiving aspirin with placebo inhalation did not change significantly from the control and baseline values, although a tendency to a rise of TPT compared to baseline value was observed 60 min after the end of inhalation (p=0.06). Aspirin given against the background og GS inhalation increased TPT compared to baseline and control values over 90-150 min and compared to placebo preparation+GS inhalation throughout the entire observation period (Fig. 1, *c*). Moreover, in this series a significant increase in TPT compared to series with aspirin+placebo inhalation was observed on minutes 120 and 150. Therefore, GS inhalation potentiates the analgesic effect of aspirin.

No significant changes in TPT were observed in series with combined use of diclofenac and placebo-inhalation. The potentiating effect of GS was not observed when diclofenac was used against the background GS-inhalation (Fig. 1, *d*). However, there was a significant decrease in pain sensitivity compared to series with GS-inhalation and placebo drug immediately after the end of inhalation and 120 min later. Therefore, diclofenac prevented the hyperanalgesic effect of GS-inhalation.

Thus, our findings confirm the hypothesis on possible potentiating action of GS on the effect of low doses of nonnarcotic analgesics. A significant improvement of the efficiency of novalgin and aspirin by GS-inhalation was demonstrated. In previous experiments on animals we showed that GS potentiates the effects of opioid analgesics. In this case, GS potentiated the effects of threshold and subanalgetic doses of the drugs [5]. On the whole, our studies demonstrated that inhalation of GS potentiates the effects of opioid and nonopioid analgesics. Inefficiency of diclofenac+GS combination can be explained by the chosen experimental model (somatosensory pain), which is characterized by the absence of inflammation focus. In this model the effects of diclofenac are not pronounced [2].

We previously showed that the effects of negatively charged oxygen ions on CNS are related to inhibition of MAO in the basal ganglia [4]. Serotonin and catecholamines play an important role in nociception: they inhibit transmission of nociceptive traffic at various levels [8,11]. Numerous studies showed that nonnarcotic and nonsteroid analgesics exert both peripheral (inhibition of prostaglandin synthesis) and central effects [1,9], their central effects being mediated primarily via monoaminergic mechanisms [12]. It was also shown that the decrease in serotonin and dopamine content in the brain attenuates opioid-induced analgesia [8], while MAO inhibitors potentiate analgesia induced by morphine [11]. Thus, it can be concluded that the potentiating effect of GS on antinociceptive effects of nonnarcotic and narcotic analgesics is determined by inhibition of MAO and accumulation of monoamines in the brain.

Further study of the effects of GS on pain sensitivity (specifically, on the models of pathological pain in animals) can extend clinical use of GS and provide deep insight into the mechanisms of its physiological effects in humans and animals.

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