EFFECT OF AURICULAR ELECTRICAL STIMULATION AND NALOXONE ON NOCICEPTIVE SENSITIVITY IN RABBITS

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Methods of analgesia not involving the use of drugs but based on reflex activation of antinociceptive systems are widely used in clinical practice. They include acupuncture, electroacupuncture, and transcutaneous electrical stimulation. Meanwhile it has been stated that reflex anesthesia is associated with a low level of analgesia or, sometimes, with the absence of analgesia altogether [3]. We know that analgesia arising during electrical stimulation is the result of activation of endogeneous antinociceptive systems, including the opioid system. To evaluate the role of endogeneous opioids as mediators of antinociceptive effects, it is possible to use naloxone, a blocker of opiate receptors, the hyperalgesic effect of which has been supposedly established. However, in some investigations an analgesic effect of naloxone has been clearly identified [8]. This prevents a consistent evaluation of the effect of naloxone on nociceptive sensitivity.

The aim of this investigation was to study nociceptive sensitivity in rabbits during stimulation of the pulp of the upper incisor teeth and the effect of auricular electrical stimulation (AES) and of naloxone (N) on it.

EXPERIMENTAL METHOD

Experiments were carried out on conscious, unanesthetized male chinchilla rabbits weighing 2-3 kg, lightly restrained. The animals had previously been scalped, the sites of incisions being infiltrated with 0.5% procaine. Electrodental stimulation (EDS) of the pulp of the upper incisors (single square pulses, 7-15 μA, duration 0.1-0.5 msec, generated by an SEN 3201 stimulator, Nihon Kohden, Japan), incuding a licking response, was used as the nociceptive stimulus. Evoked potentials (EP) in response to 10 stimuli applied in random order, were recorded in the somatosensory cortex from the surface of the skull in response to EDS, and averaged on an NTA-1024 amplitude-phase analyzer (Orion, Hungary). The time course of the amplitude of the negative-positive component of the EP with peak latency of 20-40 msec (NP_{20-40}) was used as indicator of the perceptual component of the nociceptive response [2]. The mean value of the amplitude of this component was determined in the course of 40-60 min before electrical stimulation, and taking its value to be 100%, the deviation from it was calculated both before and at various times after stimulation. The significance of changes was determined by Student's test. Using an "Analgedent" instrument [1] AES of symmetrical points of the concha auriculae was carried out. Cup electrodes were secured by clips to the base of the ear in the region of innervation with a branch of the trigeminal nerve, and the reference electrode was fixed in the region of the upper lip. AES was applied with a frequency of 15 Hz, a current of 40 μA, for 25 min. In some experiments N ("Sigma") was used in a dose of 0.2 mg/kg.

EXPERIMENTAL RESULTS

In response to single EDS, an EP with a latent period (LP) of the primary response of 10-12 msec and an amplitude up to 25 μV was recorded in the somatosensory cortex, followed by a second negative-positive wave with LP of between 20 and 40 msec (NP_{20-40}), whose amplitude increased with an increase in the strength of EDS. When the strength of EDS was suffi-

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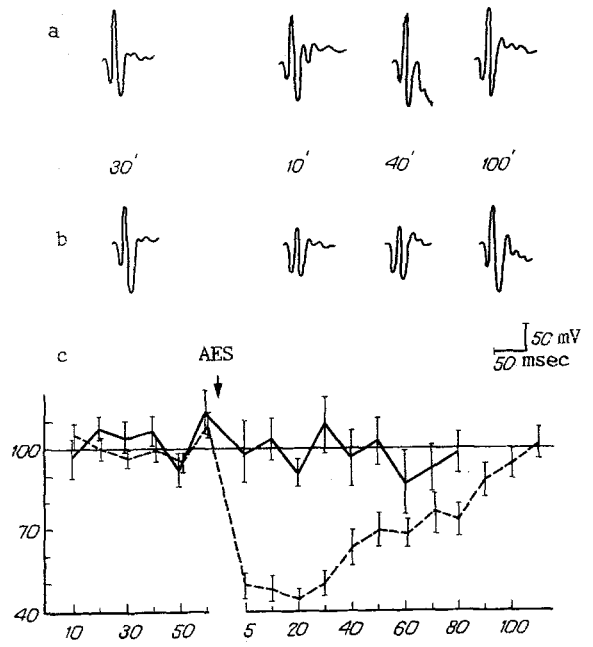

Fig. 1. Time course of amplitude of NP_{20-40} component of somatosensory cortical EP in response to EDS: a) animals resistant to AES; b) animals sensitive to AES; c) as percentages of average background values before and after AES, in rabbits resistant (continuous line, n = 16) and sensitive to AES (broken line, n = 9). Abscissa, time (in min); ordinate, value of NP_{20-40} (in %).

cient to induce a licking response, the amplitude of the component NP_{20-40} of EP averaged 100 ± 8 μV, varying in different animals from 80 to 120 μV. When background values of the amplitude of NP_{20-40} were recorded, its oscillations in some animals were not significant for 40-60 min. Immediately after AES for 25 min a decrease in amplitude of NP_{20-40} to 52 ± 8 μV, i.e., on average to 50% of its initial level, was recorded in 16 rabbits (64%), in response to EDS with the same characteristics. This decrease reflected the analgesic effect of AES and continued for 60-70 min after discontinuation of AES, and this was followed by a gradual increase in its value to the 90th minute (Fig. 1). On the basis of this time course of the perceptual component of the nociceptive response these animals were described as sensitive to AES. In nine rabbits (36%), after similar AES, a decrease in amplitude of the NP_{20-40} component in response to EDS was not observed for 80 min after the discontinuation of AES and no change in nociceptive sensitivity was recorded in them. These animals were described as resistant to AES (Fig. 1).

In series II 15 rabbits whose sensitivity to AES had been established previously were used. After recording of the background values of amplitude of the NP_{20-40} component of EP in response to EDS, they were subjected to AES with the same parameters. Immediately after AES a decrease in amplitude of the component (AC) by 45-48% was recorded. At the 20th minute after AES the animals were given an intravenous injection of N in a dose of 0.2 mg/kg. After 10 min an increase in AC of NP_{20-40} to the background values was observed (Fig. 2). Consequently, injection of N reversed the analgesic effect of AES in rabbits sensitive to AES.

In series III, 13 rabbits resistant to AES were used. Background values of AC of NP_{20-40} of EP in response to EDS also were recorded in these animals, after which they were subjected to AES, but no subsequent changes in AC were noted. However, when these rabbits were given an intravenous injection of N in a dose of 0.2 mg/kg 20 min after AES, a distinct fall of AC of NP_{20-40} on average by 40% of its initial level was recorded, and it lasted 40-45 min (Fig. 2). Consequently, the analgesic effect in rabbits resistant to AES arose after injection of N, as reflected in the value of the parameter studied.

In the last series 10 animals resistant to AES were used. The original values of AC of NP_{20-40} of EP in response to EDS were recorded, after which N was injected intravenously

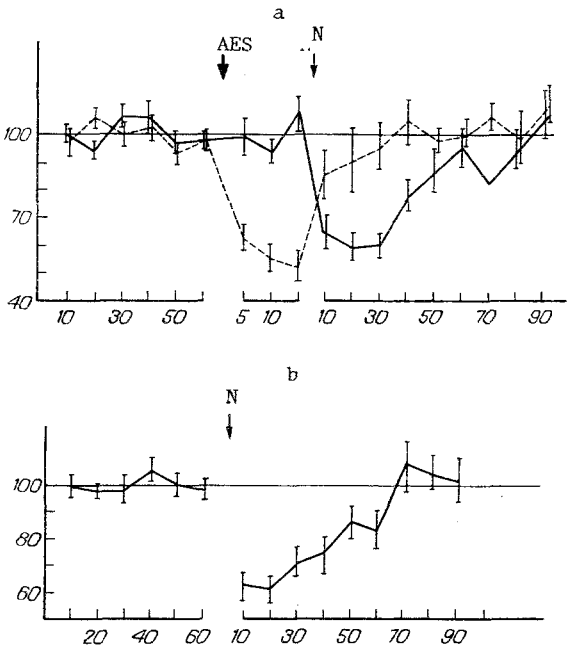

Fig. 2. Dynamics of change in amplitude of NP_{20-40} of the EP during EDS (in % of background values) before and after AES and administration of N in rabbits resistant (n = 13) and sensitive (n = 15) to AES, and similar changes in amplitude of NP_{20-40} of EP during EDS in rabbits resistant to AES (b, n = 10). Legend as to Fig. 1. Arrow indicates time of injection of N.

in a dose of 0.2 mg/kg. A decrease in the value of the perceptual component of the nociceptive response by 42-45% of the background values was recorded 10 min after the injection, and this lasted 40-45 min. Recovery took place after the 60th minute of recording (Fig. 2). In other words, administration of N without previous AES leads to a distinct analgesic effect in animals resistant to AES. The use of the same scheme in three experiments on rabbits sensitive to AES showed that injection of N leads to an increase in AC of NP_{20-40} from 107 ± 1.6 to 142 ± 1.9 μV, i.e., a hyperalgesic effect was observed.

These results suggest that differences in sensitivity to AES in rabbits, like the opposite effects of N on nociceptive sensitivity, are determined by individual characteristics, most probably qualitative, of the endogenous opiate nociceptive system. Acupuncture with electrical stimulation low frequency (under 15 Hz) is known to activate the antinociceptive opioid system [6, 9]. In the absence of an analgesic effect of acupuncture, the concentrations of morphine-like substances in an extract of brain tissue were found to be lowered by 25 times [7]. Administration of morphine does not lead to a distinct analgesic effect in these animals, possibly indicating a deficiency of endogenous opioids and of receptors to them in acupuncture-resistant rabbits [2]. Meanwhile, in our experiments on animals sensitive to AES, stimulation activated this system and an analgesic effect developed, and was blocked by N. In animals resistant to AES stimulation did not lead to increased release of opiates, there was no analgesic effect, but such an effect appeared when N blocked the free opiate receptors. The results of experiments with N, without preliminary AES, suggest that definite insufficiency of the endogenous opioid system exists ab initio in these animals. This conclusion is confirmed indirectly by studies on man [4, 5]. These showed that N had an analgesic-like action on subjects sensitive to pain (possibly with an initially low level of endogenous opiates), whereas in insensitive subjects (a high level of opiates) it induced potentiation of nociceptive sensitivity. Consequently, the use of AES and N to obtain analgesic effects must take into account individual differences between subjects.

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CHARACTERISTICS OF SARCOLEMMAL ATPASE ACTIVITY OF LONGITUDINAL AND CIRCULAR MUSCULATURE OF THE CANINE ILEUM

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The enzyme Na,K-ATPase, responsible for energy-dependent electrogenic transport of Na^+ and K^+ across the plasma membrane, plays a fundamental role in the function of smooth-muscle cells [5, 10]. The catalytic properties of this enzyme from muscles of the uterus [8], blood vessesl [9], and stomach [12] have been investigated. Previously the present writers partially characterized the Na,K-ATPase activity of membrane preparations of the muscles of the canine ileum and discovered it to be specifically inhibited by acetylcholine (ACh) [1, 2]. On the whole, however, the Na,K-ATPase of smooth muscles remain incompletely studied because of the difficulty of isolating purified preparations of sarcolemma from this tissue, and the many stages involved in the process, and also because of the low activity of this enzyme in the preparations obtained, which is masked by the much higher Mg-ATPase activity [4, 12].

The aim of this investigation was to compare some properties of the ATPase activity of the sarcolemma of longitudinal and circular muscles of the canine small intestine, which have different mechanical and electrical characteristics [11, 15]. We also studied the effect of neurotransmitters ACh and serotonin (5-HT), which contract the smooth muscles of the ileum [7]. The experimental approach used in the work, consisting of a combination of a rapid method of isolating the sarcolemma and treating the membranes with sodium dodecylsulfate (SDS), enabled many of the difficulties associated with the study of smooth muscle Na,K-ATPase to be eliminated.

EXPERIMENTAL METHOD

Experiments were carried out on mongrel dogs weighing 5-8 kg, anesthetized with thiopental sodium (30 mg/kg). A segment of ileum (40-60 cm) was excised, the mucous membrane removed from it, and the circular and longitudinal muscles were then carefully isolated. This and subsequent procedures were carried out at 2-4°C. Preparations of the sarcolemma were isolated by the method in [13] in the following modification: 5 mM Tris-HCl, pH 7.4; 50 mM Na pyrophosphate; 1 mM dithiothreitol; 5 mM phenylmethylsulfonyl fluoride; 0.1 mM EDTA, 0.02% NaN_3. The tissue was homogenized in a homogenizer of "Polytron" type 5 times, for 5 sec each time, separated by intervals of 1 min. The homogenate was centrifuged in a stepwise sucrose density gradient (analytical version: 15, 30, 35, and 41%, practical version: 30 and 35%) in a bucket-rotor (VAC-602, East Germany) at 95,000g for 90 min. Membrane fractions floating between the homogenate and the 15% sucrose solution (F_1), 15% and

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