During stimulation of the expiratory site with coordinates of 2.5, 1.5, and 3 mm synaptic responses with a latent period of 0.7-4.0 msec were obtained in eight of the nine expiratory neurons of the ventral respiratory nucleus. The latent period in complete and late neurons was under 1.2 msec. During threshold stimulation evoked discharges of expiratory neurons were observed only in the phase of expiration, but with an increase in strength of the current, evoked activity was recorded throughout the period of the respiratory cycle. Only in one of the 19 inspiratory neurons of the ventral nucleus were evoked responses with a latent period of 6.4 msec obtained to stimulation of the expiratory site.

During stimulation of the structures described above in the gigantocellular nucleus, in some cases electrical responses of nonsynaptic origin were obtained, with a latent period of 0.3-0.4 msec, which correlated with the frequency of stimulation (up to 300 Hz).

The results thus indicate the existence of connections of a varied degree of complexity (including monosynaptic) between neurons of the inspiratory and expiratory sites of the gigantocellular nucleus and the ventral and dorsal nuclei. The latter, and also the marked facilitatory influences from these sites on the respiratory neurons of the lateral zone distinguish the sites described above [6-8] from others whose electrical stimulation switches the respiratory phases.

LITERATURE CITED

1. R. Sh. Gabdrakhmanov, Fiziol. Zh. SSSR, No. 10, 1514 (1972).
2. I. A. Keder-Stepanova and V. A. Ponomarev, Biofizika, No. 2, 324 (1965).
3. I. A. Keder-Stepanova, "Neuronal organization of the medullary respiratory center," Author's Abstract of Doctoral Dissertation, Moscow (1981).
4. M. V. Sergievskii, N. A. Merkulova, R. Sh. Gabdrakhmanov, et al., The Respiratory Center [in Russian], Moscow (1975).
5. M. V. Sergievskii and N. Ya. Kireeva, Byull. Éksp. Biol. Med., No. 12, 652 (1980).
6. M. V. Sergievskii, V. E. Yakunin, N. A. Gordievskaya, et al., in: Thalamo-Strio-Cortical Interrelations [in Russian], Vol. 2, Moscow (1981), p. 117.
7. V. E. Yakunin and N. Ya. Kireeva, Fiziol. Zh. SSSR, No. 2, 205 (1978).
8. V. E. Yakunin and M. V. Sergievskii, Byull. Éksp. Biol. Med., No. 3, 286 (1981).
9. V. E. Yakunin, V. A. Maiskii, N. N. Preobrazhenskii, et al., Neirofiziologiya, No. 2, 149 (1982).

ANALYSIS OF THE ACTION OF INCREASING ELECTRODERMAL STIMULATION ON VISCEROSOMATIC RESPONSES TO ELECTRICAL STIMULATION OF THE VENTROMEDIAL HYPOTHALAMUS

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KEY WORDS: hypothalamus; increasing electrodermal stimulation; naloxone; β-endorphin.

Among the various methods of increasing resistance to emotional stress, physical methods are achieving ever-increasing popularity: exposure to uhf electromagnetic fields, acupuncture, increasing electrodermal stimulation (EDS), etc. [1, 3, 4]. However, the mechanisms of action of physical factors on the course of emotional reactions still remain largely unexplained.

The aim of this investigation was to study the mechanism of the effect of increasing EDS on viscerosomatic responses to electrical stimulation of negative emotiogenic zones of the ventromedial hypothalamus (VMH). In

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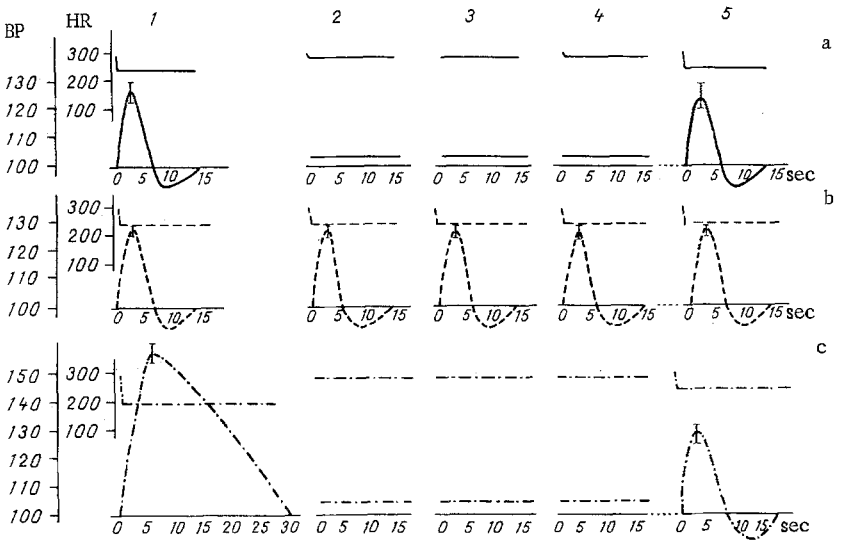

Fig. 1. Effect of increasing EDS on autonomic responses to electrical stimulation of VMH. a) Intact animals, b) animals receiving naloxone, c) animals receiving β-endorphin. 1) Background, 2, 3, 4) 20, 40, and 60 min of exposure to EDS respectively, 5) restoration of responses 180 min after beginning of experiment.

view of data in the literature on a connection between endogenous opiate peptides and mechanisms of autonomic responses to painful, emotional, and other stress-inducing factors [6-8], particular attention was devoted to the study of the role of opiate peptides in the physiological effects of increasing EDS on autonomic responses to stimulation of the emotiogenic centers of VMH.

EXPERIMENTAL METHOD

Acute experiments were carried out on 59 chinchilla rabbits weighing 2.5-3 kg. Stimulating bipolar electrodes were inserted into the animals in the region of the ventromedial nucleus of the left or right hypothalamus, taking coordinates from a stereotaxic atlas [8]. Individual threshold values of electrical stimulation of VMH evoking passive avoidance behavioral responses in the animals were determined. The parameters of stimulation were on average as follows: voltage 2-8 V, frequency 100 Hz, pulse duration 1.2 msec, duration of stimulation 3 sec. After testing the animals were fixed to a frame. Under procaine anesthesia a catheter, connected to the strain gauge transducer of a Mingograf-34 instrument (Sweden) was introduced into the femoral artery to record the blood pressure (BP). The ECG in standard lead II and respiratory movements also were recorded.

Increasing EDS was applied through needle electrodes inserted beneath the skin of the animals' hind limbs by means of a modified ESP-1 apparatus for electrophoresis-puncture [2]. The parameters of EDS used were: frequency of current 50 Hz, square pulses 1 msec in duration, strength of current gradually increased from 0 to 500 μA in the course of 20 min. The total duration of EDS was 1 h.

In the course of the experiments individual parameters of VMH stimulation, chosen so that each stimulation would cause BP to rise by 20-30 mm Hg without any marked motor response of the animal, were used. Viscerosomatic responses of the animals evoked by three consecutive stimulations of VMH, separated by intervals of 3 min, before, during, and after EDS were analyzed.

In the experiments of series I the effect of EDS on the dynamics of viscerosomatic responses to stimulation of VMH in immobilized rabbits was studied. In series II, 10 min before EDS, naloxone (Narcan, from Du Pont, USA) was injected in doses of 5-10 μg into the lateral ventricles through a specially implanted cannula. In series III the effect of intraventricular injection of β-endorphin (from ICM Biochemical, USA) in doses of 1-5 and 10 μg on the time course of the viscerosomatic responses to stimulation of VMH was studied. In series IV the effect of EDS on viscerosomatic responses to stimulation of VMH was studied after preliminary injection of β-endorphin into the lateral ventricles of the animals in doses of 5 to 100 μg in 30 μl of physiological saline.

EXPERIMENTAL RESULTS

In animals fixed to the frame stimulation of VMH for 3 sec evoked pressor-depressor vascular responses in 56% of cases. The amplitude of BP was increased by 27.0 ± 2.1 mm Hg with a latent period of 1.4 ± 0.2 sec. BP fell 5.2 ± 1.4 sec after the beginning of stimulation of VMH by 10.2 ± 3.8 mm below its initial level, but returned to that level 11.2 ± 4.6 sec after the beginning of stimulation. Stimulation of VMH was accompanied by an increase in the respiration rate by 37.0 ± 12.2 cycles/min with a latent period of 0.6 ± 0.4 sec, and by slowing of the heart rate (HR) by 50.0 ± 7.0 beats/min with a latent period of 2.0 ± 1.4 sec. In 44% of experiments stimulation of VMH evoked only pressor vascular responses. After rising by 27.0 ± 3.6 mm Hg, BP returned to its initial level in 5.4 ± 1.6 sec. The recovery time of respiration and HR varied from 6 to 30 sec.

The use of increasing EDS in 12 of 23 experiments (52%) completely blocked the viscerosomatic responses to stimulation of VMH (Fig. 1a). In six animals the threshold of stimulation of VMH was raised from 1 to 4 V. In seven animals (32%), during and after EDS the amplitude of the pressor responses to stimulation of VMH fell to 10.8 ± 1.6 mm Hg (by 62% compared with the background), the latent period increased to 1.9 ± 0.7 sec (by 42%), the duration of the response was reduced to 6.2 ± 2.0 sec (by 63%), and the bradycardia disappeared. In two experiments (8%) EDS evoked motor responses of the animals to stimulation of VMH. In two experiments no changes were observed.

In 83.3% of cases the hypothalamic responses were restored to the background level 2-3 h after cessation of EDS, but in 16.7% of cases recovery of the responses was not observed for 3 h.

After preliminary injections of naloxone into the animals increasing EDS in 5 of 7 experiments (70%) did not change the responses to stimulation of VMH (Fig. 1b). In two animals (30%) injection of naloxone did not affect the blocking effects of EDS. In control experiments (without EDS) administration of naloxone in the same doses did not affect the thresholds or intensity of autonomic responses to stimulation of VMH.

In doses of 1-5 μg β-endorphin (10 animals) caused both depression of viscerosomatic responses to stimulation of VMH (30%) and also their facilitation (20%). In 50% of cases no change was observed. Injection of β-endorphin in a dose of 10 μg (5 animals) caused complete blocking of the viscerosomatic responses to stimulation of VMH in 100% of cases.

β-Endorphin in doses of 50-100 μg potentiated and prolonged the viscerosomatic responses to stimulation of VMH in all experiments (14 animals) within 2 min after injection: The amplitude of BP was increased by 2-3 times in response to stimulation of VMH, the latent period of the responses was shortened by 1-1.5 times, and the duration of the responses was increased by 3-5 times compared with background values (Fig. 1c). The greatest changes were observed 20 min after injection of β-endorphin: The general level of BP was raised by 5-10 mm Hg, marked bradycardia to 200-150 beats/min was observed, with extrasystoles, muscular tremor, and paroxysmal breathing. The threshold of stimulation of VMH was lowered from 3 to 1 V. The effects of β-endorphin were observed for 3-4 h.

In ten experiments 20-40 min after injection of β-endorphin EDS was applied and, as before, it completely blocked the viscerosomatic responses to stimulation of VMH. The threshold of EDS was unchanged under these circumstances. After EDS the autonomic parameters were restored to the level observed before injection of β-endorphin.

In four experiments 10 min before injection of β-endorphin the animals were given an injection of naloxone, which abolished for 30 min the effects of β-endorphin and, in particular, its effect on respiration and HR.

The experiments show that increasing EDS has a predominantly blocking effect on viscerosomatic responses to stimulation of negative emotiogenic zones of VMH. Both opiate peptides and their receptors participate in the mechanism of the viscerosomatic responses to stimulation of VMH and the action of EDS on them.

LITERATURE CITED

1. R. A. Burchuladze and Yu. P. Mironenko, in: Engineering Problems in Medicine [in Russian], Tol'yatti (1981), p. 164.
2. Yu. P. Mironenko and G. E. Meierkop, Inventor's Certificate No. 588676 (USSR) (1976).
3. V. I. Loshchilov, Yu. P. Mironenko, and T. S. Mel'nikova, in: Engineering Problems in Medicine [in Russian], Tol'yatti (1981), p. 156.
4. S. K. Sudakov, Abstract Lodged in the All-Union Institute of Scientific and Technical Information (1981).
5. H. M. Emrih and M. I. Milan, J. Psychosom. Res., 26, 101 (1982).

6. W.-S. Liu, Y. Tosaki, S. Gillmor, et al., Anesthesiology, 55, 246 (1981).
7. J. Rossier, E. D. French, C. Rivier, et al., Nature, 270, 618 (1977).
8. C. H. Sawyer, J. Everett, and J. D. Green, J. Comp. Neutrol., 101, 801 (1954).

EFFECT OF χ-CASEIN GLYCOMACROPEPTIDE ON GASTROINTESTINAL MOTILITY IN DOGS

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Casein, the principal dietary protein of newborn mammals, has features of alimentary specificity, elaborated as a result of prolonged evolution and expressed in a surprising adaptibility of the caseins to differences in the character of function of the gastrointestinal tract in the neonatal period [8]. Maximal vulnerability of native casein to attack by gastrointestinal proteinases [3, 9] and its ability to undergo curdling enable the newborn animals to digest this protein with minimal expenditure of energy and ensure the optimal rate of entry of protein into the intestine and absorption of amino acids [4, 10].

The writers have shown that physiologically active peptides, capable of affecting the circulation [5] and of inhibiting gastric secretion [11] can be formed from the caseins of cows' milk as a result of their limited proteolysis. It has also been shown that a peptide inhibiting gastric secretion in the newborn rat stomach can be formed in vivo by digestion of rat milk proteins [6].

The object of this investigation was to study the action of casein glycomacropeptide (an inhibitor of acid secretion by the stomach [11]) on motor activity of the fundal portion of the stomach and of the duodenum.

EXPERIMENTAL METHOD

χ-Casein was obtained by Zittle's method and subjected to brief (2 min) action of pepsin with an enzyme–substrate ratio of 1:100 (37°C, pH 5.8). Proteolysis was stopped and protein and large peptides precipitated with a 12% solution of TCA (final concentration). The precipitate was discarded and the filtrate dialyzed and lyophilized. The resulting peptide material, in a quantity of 400 mg in 10 ml, was applied to a Sephadex G-25 column (2.5 × 80 cm), equilibrated with 0.5 M NaCl solution. The filtration rate was 80 ml/h. Elution of the fractions from the column was recorded by means of an RÉPPS-1M recorder on the basis of the change in percentage absorption at 260 nm. The high-molecular-weight fraction, namely the glycomacropeptide, eluted in the void volume of the column, was desalted and lyophilized on a Sephadex G-10 column (2.5 × 80 cm).

Experiments were carried out on two dogs weighing 15 kg with fistulas to the gastric fundus and duodenum. Motor activity was recorded graphically using a balloon and mechanotron electromanometer, with RPCh-2 electromechanical recorder. An ink-writing x-y recorder also was used and gastric and duodenal motor activity was expressed in conventional units as a motor index by means of an electronic integrator. The concept of "motor index" was based on analysis of the work done by the stomach and duodenum in the course of 15-min time intervals. In control experiments on dogs the background feeding motor activity was recorded. For this purpose hungry dogs were fed with a test meal (75 g bread and 75 g meat), after which their gastric movements were recorded and the motor index calculated for a period of 3-5 h. Glycomacropeptide was injected intra-

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