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EFFECT OF BENZODIAZEPINES ON NEUROPATHOLOGICAL SYNDROMES OF SPINAL ORIGIN

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Creation of a generator of pathologically enhanced excitation (GPEE) in a system of propriospinal interneurons causes generalized spinal myoclonia [5], whereas in a system of nociceptive posterior horn neurons it causes a pain syndrome of spinal origin [5]. Since benzodiazepines depress hyperactivity of structures of the CNS [16, 19, 20] and, in particular, activity of GPEE in different parts of the brain [5], it was decided to study the effects of these substances in the above-mentioned neuropathological syndromes.

To determine the effectiveness and to study the mechanisms of action of these substances it was necessary to test the effect of different benzodiazepines on the same syndrome, the action of the same preparation on different syndromes (myoclonia and the pain syndrome), and also on the same clinical syndromes but evoked by GPEE of different nature, and created by means of different substances. In this investigation diazepam (seduxen), phenazepam and clonazepam were used.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats weighing 200-220 g. A syndrome of spinal myoclonia was induced by creating a GPEE in a system of propriospinal connections with the aid of tetanus toxin (TT), which disturbs various types of inhibition [7, 9, 12, 14]. TT (40 mld for mice) was injected into the calf muscles, from which it travels along the regional neural pathway to the anterior horns of the lumbosacral segments, after which it spreads within the nearest segments of the spinal cord [4]. The vascular pathway of spread of TT was blocked by intravenous injection of antitoxin (0.025 AU). The model of the syndrome and the method of its creation were fully described previously [1, 3]. The GPEE was activated by the method adopted previously [4, 5] by stimulation (pinching the skin of the toes) of the limb into which TT was injected. To record biopotentials, bipolar needle electrodes were used. Electrical activity (EA) was recorded in the spinal and sacral muscles on both sides and the posterior group of thigh muscles of both limbs. The electromyogram (EMG) was recorded on an 8-channel RM 86M encephalogram (Nihon Kohden, Japan). In a special series of experiments the spinal cord was divided at the level T2-T3 24 h before EA was recorded.

A pain syndrome of spinal origin was induced by forming a GPEE in the system of posterior horns of the spinal cord [6] by means of substances disturbing various types of inhibition (TT, strychnine, and penicillin) and substances inducing depolarization of neuron membranes (KCl and ouabain) [2]. The substances were deposited in 1% agar, a slab of which (measuring 10 × 4 × 1.5 mm) was applied to the dorsal surface of the spinal cord on one side in region

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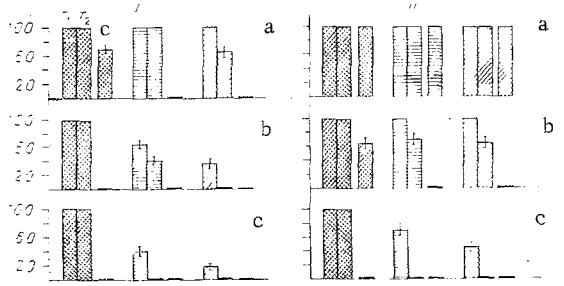

Fig. 1. Effect of benzodiazepines on EA of muscles during generalized myoclonia of spinal origin. Ordinate, amplitude of EA (in percent of initial). a, b, c) Dose of benzodiazepines 0.5, 1, and 2 mg/kg respectively. T_1) Tonic EA in muscles of left hind limb (GPEE region); T_2) tonic EA in spinal muscles on left side (region of muscles remote from GPEE); C) clonic EA. I) Animals with intact spinal cord, II) animals with divided spinal cord. Cross-hatched columns — seduxen, horizontally shaded columns — phenazepam, obliquely shaded columns — clonazepam.

L2-L6 (the agar slab method). The spinal cord was exposed under ether anesthesia. Clinical observations were made on the animals' behavior, and actograms and phonograms were recorded before and after application of the substances chosen for testing, which were injected intraperitoneally at the height of development of the syndromes in the following doses: diazepam 1-15 mg/kg, phenazepam and clonazepam 0.25-5 mg/kg. The drugs were dissolved in a mixture of propyleneglycol and ethyl alcohol (70 and 30% respectively). In control experiments only the solvent was injected into the animals. The latent period of the pain syndrome and its duration were estimated from the time of application of the substances inducing the pain syndrome.

EXPERIMENTAL RESULTS

The Spinal Myoclonia Syndrome. General convulsions consisting of tonic and clonic phases occurred 96 h after injection of TT into the calf muscles in the animals in response to stimulation of the limb into which TT was injected (the side of formation of the GPEE), and also spontaneously. The tonic phase was recorded on the EMG in the form of a prolonged burst of EA, consisting of high-amplitude and low-frequency discharges; the clonic phase was expressed as synchronized potentials, the frequency and amplitude of which depended on the severity of the syndrome. After high division of the spinal cord the syndrome could be manifested more intensively, and in this case muscles with a spinal innervation were involved in the process.

In the animals with an undivided spinal cord diazepam, depending on the dose, reduced the discharge frequency or completely suppressed clonic EA (Fig. 1, I). Tonic EA began to decline only after injection of large doses of the drug (5-10 mg/kg). EA was reduced first in the spinal muscles on the left and right sides and sacral muscles on the right side, i.e., in regions most remote from the GPEE, and only in the largest doses (10-15 mg/kg) did diazepam reduce tonic EA in the region of GPEE. The effects of phenazepam and clonazepam were similar to those of diazepam but they occurred in response to much smaller doses of the drug (Fig. 1, I).

The efficacy of the benzodiazepines was reduced after division of the spinal cord. Under the influence of diazepam in the chosen doses only clonic EA was suppressed, while tonic was preserved (Fig. 1, II). Unlike diazepam, phenazepam and clonazepam suppressed not only clonic, but also tonic EA (Fig. 1, II). If the effects of the benzodiazepines are compared it can be concluded that the tonic component of seizure activity is more resistant than the clonic component; clonic EA is suppressed comparatively easily, even by the use of

relatively small doses of the drugs. These findings suggest that the functional apparatus of the GPEE is heterogeneous: It consists of structures which produce tonic and clonic activity and which differ in sensitivity to benzodiazepines. The inhibitory effect of benzodiazepines is also realized after division of the spinal cord, evidence of their influence on GPEE in the lumbosacral segments of the spinal cord. The great strength of the effects of diazepines when connections of the spinal cord with supraspinal levels are preserved indicates that descending inhibitory influences participate in the mechanism of action of the benzodiazepines.

Pain syndromes evoked by depolarization of posterior horn neurons (after application of KCl and ouabain) were completely suppressed by benzodiazepines. Phenazepam and clonazepam were most effective (Fig. 2, I, d, II, d). These last two drugs also were more effective in pain syndromes induced by TT, strychnine, and penicillin. In a pain syndrome induced by penicillin, which was not suppressed by diazepam in these doses, phenazepam and clonazepam caused considerable weakening, and sometimes even complete abolition of the pain syndrome (Fig. 2, I, c, II, c).

It follows from the data described above that inhibitory effects of the benzodiazepines on pain syndromes depended on the nature of the GPEE created in the posterior horns, which gave rise to the pain syndromes. This fact is evidence that benzodiazepines act on generator mechanisms of pain syndromes. It cannot be connected only with the intensity of the pain syndromes determined by the power of excitation produced by the GPEE, for dependence of the effect of benzodiazepines, not on the intensity of the pain syndrome, but on the nature of the GPEE is most clearly in evidence, although the severity of the pain syndromes likewise is unquestionably important. Benzodiazepines are most effective in pain syndromes created by depolarizing agents, i.e., in cases when inhibitory mehcanisms are preserved in neuron populations of GPEE. When inhibitory processes were disturbed by TT, which disturbs the release of inhibitory mediators (GABA and glycine [7, 12, 14]), or by strychnine, which blocks glycine receptors on the postsynaptic membrane [13], all the benzodiazapines tested evoked an analgesic effect to some degree. The only difference in the action of the drugs was observed in the pain syndrome evoked by a "penicillin" GPEE. Penicillin blocks GABA receptors on the post- and presynaptic membrane [10, 17]. It can also have a direct depolarizing action [15, 18]. The effects of diazepam, which increases the affinity of GABA for its specific receptors and which can presumably act presynaptically, leading to GABA release from terminals [16, 20, 21], were therefore expressed relatively more weakly in the presence of a "penicillin" GPEE. However, phenazepam and clonazepam [8, 11], which are much more active than diazepam against all types of GPEE studied, have a marked effect also in the case of the "penicillin" GPEE.

According to existing views benzodiazepines are not analgesics, and the analgesic effects of these drugs which have been described are difficult to explain. This view is valid in connection with their action on physiological pain and the response to ordinary nociceptive stimulation. Under these conditions benzodiazapines do not give an analgesic effect. The situation is radically changed when the effect of these drugs on pathological pain — pain syndromes caused by the formation of a GPEE in the nociceptive system — is considered. By suppressing GPEE activity benzodiazepines also suppress pain syndromes and they thus behave as analgesics.

When considering the effects of benzodiazepines as a whole the first point to note is that they were effective in both experimental neuropathological syndromes studied. The reason is that the pathogenetic basis of both syndromes created in the spinal cord is the GPEE. The specific character of the syndromes depends on the location of the GPEE in the CNS, i.e., on the system in which it appeared and which becomes hyperactive, acquiring the properties of a pathological system [5]. Benzodiazepines have a general inhibitory action on the GPEE irrespective of where it appeared, on the conduction of excitation produced by the GPEE, and on structures receiving this excitation, i.e., on all components of the pathological system (PS) produced by the GPEE. The GPEE itself, as a determinant structure [5], can persist in a weakened form when other components of the PS have already been blocked.

The fact that after high division of the spinal cord the effects of the various drugs tested were weaker than when the spinal cord was intact is evidence of a contribution of the descending inhibitory control activated by supraspinal structures in the action of benzodiazepines at the spinal level. It can be tentatively suggested that activation of the anti-

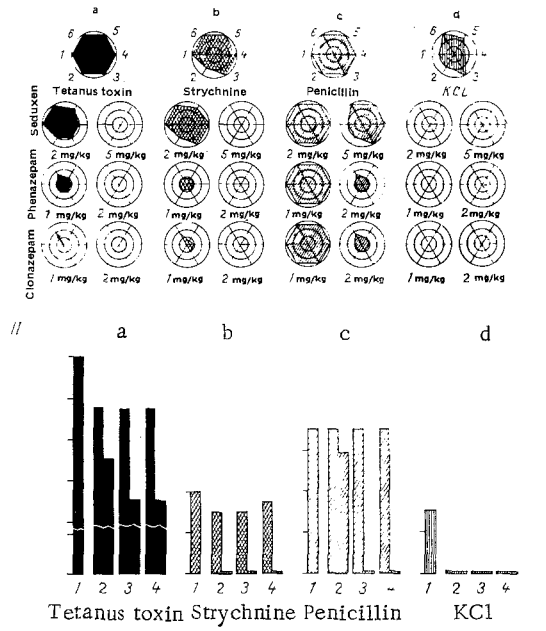

Fig. 2. Effect of benzodiazepines on pain syndrome of spinal origin. I) Changes in individual features of pain syndromes under the influence of benzodiazepines: 1) squeaking, 2) general running about, 3) local response, 4) duration of one episode, 5) frequency of episodes, 6) response to provocation. Each test (component of pain syndrome) represented graphically as a vector. Severity of features (i.e., degree of their suppression) given on a three-point scale: 0) absence of feature, 1) mild, 2) average, 3) severe degree of manifestation. Filled parts of circle along vectors denote degree of manifestation of individual features and of pain syndrome as a whole. Whole complex of features (complete circle) of pain syndromes evoked by TT (a), strychnine (b), penicillin (c), and KCl (d), characterizing severity and clinical picture of syndromes, shown above. II) Duration of pain syndromes in control animals (1) and under the influence of seduxen (2) in doses of 2 and 5 mg/kg (left and right sides of column respectively), phenazepam (3) — 1 and 2 mg/kg respectively, and clonazepam (4) — 1 and 2 mg/kg respectively.

nociceptive system takes part in the abolition of pain syndromes by benzodiazepines, as is the case with the action of certain analgesics [5].

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EFFECT OF β-ENDORPHIN ON G CELLS IN RATS WITH EXPERIMENTAL DUODENAL ULCER

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β-endorphin is an endogenous opioid substance that is distributed mainly in parts of the CNS responsible for pain sensation and emotions. Endorphins have recently been found in the gastrointestinal tract. We now know that the discovery of opioid peptides in the alimentary tract is not accidental, for they have a protective action on the gastric and duodenal mucosa in rats with cysteamine-induced duodenal ulcer [1] and they also inhibit gastric secretion [3] and affect the blood gastrin level [2].

The aim of the present investigation was accordingly to study the effect of β-endorphin on gastrin producing cells in the mucosa of the antral portion of the stomach in rats with experimental duodenal ulcer.

EXPERIMENTAL METHOD

Experiments were carried out on 40 male Wistar rats weighing 150-200 g. The rats were divided into four groups with 10 animals in each group. Rats of group 1 (control) received 0.2 ml of physiological saline, animals of group 2 received a single subcutaneous injection of 350 mg/kg of cysteamine hydrochloride, rats of group 3 received cysteamine and also β-endorphin in a dose of 300 nmoles/kg twice a day (4 times altogether), and animals of group

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