NEW EVIDENCE OF A GABA-ERGIC COMPONENT IN THE MECHANISM OF ACTION OF BENZODIAZEPINE TRANQUILIZERS

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KEY WORDS: benzodiazepine tranquilizers; GABA-ergic component.

Many workers in recent years have stated that benzodiazepine tranquilizers potentiate the inhibitory effects of gamma-aminobutyric acid (GABA) in the cerebral cortex [5], cerebellum [8], and corpus striatum [10]. This property of the benzodiazepine tranquilizers has been analyzed at the neuronal [13] and receptor [9] levels. However, the role of the GABA-ergic mechanism in the realization of the inhibitory effects of the tranquilizers in the whole organism has so far received little study. Accordingly it was decided to study the effect of phenazepam [1], a tranquilizer of the benzodiazeine class, in conjunction with GABA-ergic agents with different types of action on one of the principal manifestations of nervous activity, namely impulse summation in the CNS. The phenomenon of impulse (excitation, nervous stimulus) summation in the CNS, which was discovered by Sechenov in 1863, lies at the basis of manifestations of brain activity such as memory, learning, behavior, and emotions. That this parameter is adequate for the study of the functional state of the CNS has been demonstrated in investigations of drugs of different classes [14].

Because of the lack of data on the effect of GABA-ergic agents on impulse summation, in the investigation described below a series of GABA-ergic analyzers and a representative of the benzodiazepine class (phenazepam) were studied from this aspect.

EXPERIMENTAL METHOD

Experiments were carried out on 25 male rabbits weighing 2.8-3.0 kg. The principle of the method elaborated by the writers is determination of the number of consecutive electrical stimuli of an assigned amplitude (in volts) and with a duration of 10 msec, applied at an interval of 0.5 sec to the skin of the rabbit's hind limb, to which a motor response in the form of flexion of the corresponding limb arises. During the experiment the animal is kept in a special hammock with four holes for the limbs.

As the writer showed previously phenazepam, injected intravenously in doses of 0.1 mg/kg and upward, has a depriming effect on impulse summation in the CNS, although in smaller doses (0.01-0.001 mg/kg) it potentiates impulse summation, reflecting its stimulant effect.

In the present investigation phenazepam was used in doses of 0.1 mg/kg or more, in which it potentiates GABA-ergic inhibition. The drug was injected intravenously as a solution made up in a mixture of polyethylene glycol and ethyl alcohol (4:1).

As drugs weakening the effect of GABA, i.e., GABA-negative drugs according to the classification suggested previously [7], we used thiosemicarbazide (TSC), a compound which prevents GABA formation by depressing glutamate decarboxylase activity, and bicuculline, which blocks postsynaptic GABA receptors. TSC was injected subcutaneously in a dose of 2-3 mg/kg or intravenously in a dose of 1 mg/kg, bicuculline in a dose of 0.2 mg/kg subcutaneously or 0.05 mg/kg intravenously, i.e., in the minimal doses in which they did not cause convulsions but, judging from the results of electrophysiological experiments [4, 5], they weaken the intensity of GABA-ergic inhibition. Considering the latent period of action of TSC (mean 70-90 min), it was injected 30 min before phenazepam. Bicuculline was injected when the effect of phenazepam had begun. Considering the data given above, showing that benzodiazepine tranquilizers potentiate GABA-ergic inhibition, i.e., that they have a GABA-positive effect according to the classification [7], another GABA-positive compound was studied, namely depakine (n-dipropyl acetate, valproate), which causes GABA to accumulate in brain tissue as a result of inhibition of the activity of α-ketoglutarate-GABA transaminase, which catalyzes inactivation of GABA [12]. Depakine was injected intraperitoneally with Tween-80 in doses of 100-200 and 300 mg/kg.

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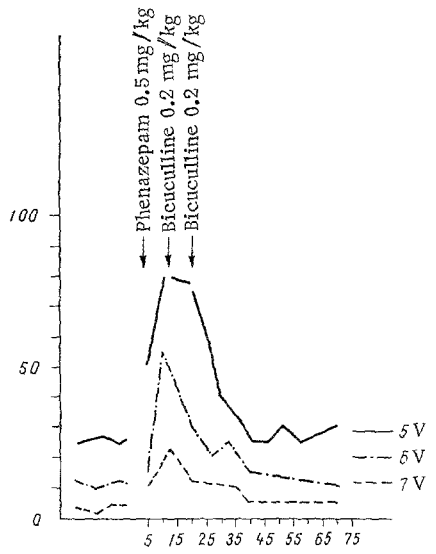

Fig. 1

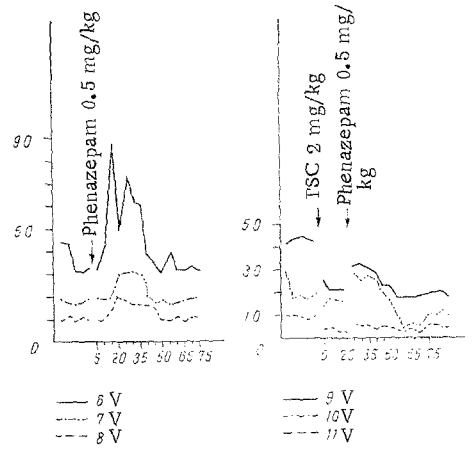

Fig. 2

Fig. 1. Effect of phenazepam and bicuculline on impulse summation in rabbit CNS. Abscissa, time (in min); ordinate, number of stimuli evoking flexor response of hind limb. Arrow indicates injection of drugs.

Fig. 2. Effect of TSC on effect of phenazepam as shown by impulse summation test. Legend as to Fig. 1.

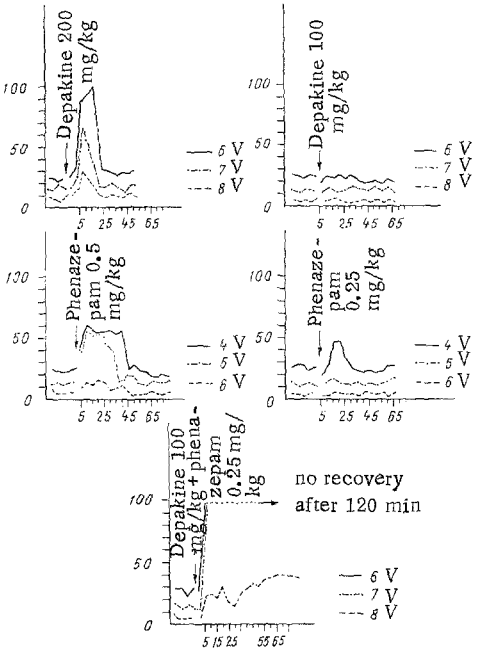

Fig. 3. Effect of depakine on effect of phenazepam as shown by impulse summation test. Legend as to Fig. 1.

EXPERIMENTAL RESULTS

Phenazepam in a dose of 0.1-0.3 mg/kg raised the threshold of the motor response; in some animals only the threshold of the response to stimuli of minimal amplitude was raised, and in some rabbits, when the effect of phenazepam was stronger, impulse summation was completely suppressed whatever the amplitude of the stimuli used. With an increase in the dose of phenazepam to 0.5-1.0 mg/kg, summation of subthreshold stimuli was inhibited in all animals. Bicuculline weakened the depriming effect of phenazepam relative to impulse summation (Fig. 1); usually the response to stronger stimuli was restored to begin with, followed by that to minimal stimuli. In the doses mentioned above TSC prevented the development of the effect of phenazepam on impulse summation (Fig. 2). TSC and bicuculline had a similar antagonistic effect on impulse summation when weakened by diazepam. Depakine, in a dose of 300 mg/kg, completely suppressed impulse summation and usually it did not recover before the end of the experiment (the duration of which was limited to 2 h in order to avoid fatiguing the rabbit). Preliminary injection of TSC completely prevented this effect of depakine. Bicuculline quickly abolished the effect of depakine on impulse summation. Depakine in a dose of 150-200 mg/kg caused short-term (15-20 min) weakening of impulse summation, but in a dose of 100 mg/kg it caused no change whatever in impulse summation. Phenazepam in a subthreshold dose (0.25 mg/kg), combined with an ineffective dose of depakine (100 mg/kg) caused marked weakening of impulse summation, which showed no tendency to recover even at the end of 2 h (Fig. 3).

The results show that phenazepam and diazepam, like depakine, in the doses stipulated weaken impulse summation in the CNS, and that this effect is abolished by GABA-negative drugs; meanwhile depakine potentiates the depriming effect of phenazepam on impulse summation. To test the specificity of the phenomena described above diphenylhydantoin, an anticonvulsant with no GABA-positive properties [13], was used. Unlike benzodiazepine and depakine, diphenylhydantoin did not weaken impulse summation. Evidence that the changes in impulse summation described above are connected with GABA-ergic inhibitory mechanisms and not with a nonspecific change in the level of excitability of the brain is given by the fact that bicuculline, in the present experiment, did not abolish the weakening of summation caused by chlorpromazine (0.1 mg/kg) and that caffeine (1 mg/kg) did not abolish the effect of phenazepam.

The data described above can be interpreted from the standpoint of "postactivation potentiation," defined as a "prolonged increase in the transmitting power of the synapses" [11]. After-hyperpolarization is a factor which prevents this facilitation. Considering that GABA can hyperpolarize the postsynaptic membrane, and also that benzodiazepines increase the sensitivity of the postsynaptic membrane to GABA [3] and delay its inactivation [2, 5], it can be tentatively suggested that these effects are the cause of the ability of GABA-positive drugs to weaken impulse summation as was found in the present experiments. The fact that phenazepam has a selective effect on impulse summation, the similarity between phenazepam and diazepam, on the one hand, and depakine on the other, the antagonism of the drugs mentioned above with bicuculline and TSC, and the synergism between depakine and phenazepam are evidence that the effects of the benzodiazepines described above are connected with potentiation of GABA-ergic inhibitory processes.

In addition to data obtained previously by the analysis of the effect of benzodiazepines on a model of conflict behavior [6] and of isolation stress, the present investigation of a fundamental manifestation of nervous activity such as impulse summation thus yielded fresh evidence of the participation of a GABA-ergic component in the mechanism of the effects of benzodiazepines.

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EFFECT OF ENKEPHALINS ON CENTRAL REGULATION OF THE HEMODYNAMICS

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Recent investigations have revealed a high density of opiate receptors and a high concentration of enkephalins in brain structures concerned with the central regulation of the hemodynamics [6, 16]. The role of endogenous opioid regulators in the control of the cardiovascular system has virtually not been investigated. Isolated observations [7, 13] have been made on anesthetized animals, although anesthetics are known to modify the processes of central regulation of the hemodynamics considerably [3], and part of the anesthetic effect is mediated through an opiate mechanism and is abolished by naloxone [4].

The aim of the present investigation was to analyze the hemodynamic effects of leu- and met-enkephalins in unanesthetized animals. The structure of baroreceptor reflexes and responses of the cardiovascular system to stimulation of hypothalamic emotiogenic zones was assessed.

EXPERIMENTAL METHOD

Experiments were carried out on eight unanesthetized cats under free behavior conditions. Aortic and venous catheters were introduced into the animals 3-5 days before the beginning of the experiments, and a cannula was introduced into the fourth ventricle with coordinates P = 11, L = 0, H = −5. Monopolar nichrome electrodes 150 μ in diameter were introduced with coordinates A = 11-14, L = 1-1.5, and H between −5 and +3. In the course of the experiment the arterial blood pressure (BP) was measured on an EMT-34 electromanometer (from Elema, Sweden), the signal from which was led to an integrating RC circuit with time constant of 2 sec, and from it to an Shch1413 digital voltmeter. Momentary values of the heart rate (HR) were determined by a digital pulsotachometer, triggered by the pulse wave of BP. To record motor activity the animal was placed on the platform of a strain gauge with four semiconductor Yu-12A tensometric resistors. The baroreceptor reflex was tested by intravenous injection of phenylephrine at the rate of 40 μg/kg/min. The sensitivity of the reflex was determined as the ratio of the increase in the intersystolic interval in milliseconds to the increase in the systolic BP in mm Hg. The parameters of hypothalamic stimulation were 100 Hz, 2 msec, 2-6 V, for 10 sec. All parameters obtained in analog form were recorded on the Mingograph-81 and in digital form on a type 3512 (East Germany) digital printer.

Met- and leu-enkephalins synthesized in the laboratory of peptide synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, were dissolved in sterile physiological saline in a concentration of 100-200 μg (volume of fluid injected 100-200 μl) by means of a microsyringe. Opiate receptors were blocked with naloxone (from Endo Laboratories) injected intracisternally in doses of 50 to 100 μg.

EXPERIMENTAL RESULTS

Injection of met- and leu-enkephalins in a dose of 100 μg into the fourth ventricle caused transient hypertension and tachycardia after 10-15 sec. BP returned to normal after 1-2 min but HR fell (Fig. 1, 2). BP 15-

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