Effects of Bemitil on the Activity of Noradrenergic and Serotonergic Brainstem Neurons and on EEG of Awake Cats

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In experiments on five awake cats, we studied the effects of bemitil, a drug possessing psychostimulatory, antidepressive, and actoprotector properties (peroral introduction, 50 mg/kg), on the activity of neurons of the aminergic cerebral systems. Eleven noradrenergic (NA-ergic) neurons of the *locus coeruleus* (LC) and 11 serotonergic (ST-ergic) neurons of the *nuclei raphe* (NR) were examined. A control experimental series was carried out on 8 NA-ergic neurons of the LC and 8 ST-ergic neurons of the NR. Bemitil was found to exert opposite effects on the impulse activity of NA-ergic and ST-ergic brainstem neurons; it suppressed impulsation of LC neurons and increased the spiking frequency of NR neurons within certain time intervals after its introduction. Analysis of EEG showed that bemitil decreased the spectral power of the delta and theta activities, which was accompanied by behavioral relaxation.

Keywords: aminergic systems, *locus coeruleus*, *nuclei raphe*, noradrenergic and serotonergic neurons, brainstem, bemitil.

INTRODUCTION

Increased emotional and informational loadings are negative consequences of scientific and technical progress. This, in turn, in many cases results in the formation of neurotic disorders, which usually should be corrected with pharmacological treatment. At present, such a preparation as bemitil (2-ethylthiobenzimidazole hydrobromide, Fig. 1) attracts considerable attention of neuropharmacologists. Bemitil is a derivative of imidazole and demonstrates psychostimulatory, antidepressive [1], and actoprotector [2] properties; a tranquilizing effect of this drug has also been described [3]. Despite the fact that bemitil began to be used in clinical practice relatively long ago, information on the pharmacodynamics of this preparation and its neuropharmacological effects is still limited and contradictory. It was found that the spectrum of the psychotropic influences of bemitil includes either soft psychostimulating and antiasthenic or tranquilizing effects, and their patterns depend on the dose of the preparation. Together with weakening of asthenia, the levels of anxiety, emotional lability, and irritability (these are the main symptoms of neurotic disorders) decrease, and sleep disorders are smoothed [4].

It is logical to believe that aminergic brain systems, which exert generalized effects on the state of other cerebral systems, are important targets for the action of pharmacological preparations used for treatment of neurological and mental disorders. This was the reason for our study, where we observed the effects of bemitil on the impulse activity (IA) of noradrenergic and serotonergic (NA-ergic and ST-ergic, respectively) brainstem neurons and on the mass electrical activity of the brain (EEG) of the cat under conditions close to free behavior of the experimental animals.

METHODS

Experiments were carried out on five awake cats of both sexes weighing 2-4 kg. Preliminary surgery was made under general anesthesia (Nembutal, 40 mg/kg, i.p.). In the course of operation, a directing cannula (stainless steel) was stereotaxically implanted; its tip

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was positioned 5 mm above the localization of the *locus coeruleus* (LC). The cannula was introduced in the brain at a 21 deg angle with respect to the frontal plane and at a 29 deg angle with respect to the sagittal plane; this allowed us to record the activity of neurons of both the LC and of the *nuclei raphe* (NR). The neuronal activity was recorded from zones with the following stereotaxic coordinates: P –1, L 1…3, H 7…10 (LC) and P –1…–2, L 2…0, H 4.5…9.0 (dorsal and superior central NR), where NA-ergic and ST-ergic neurons, respectively, are localized. The neurons under study were classified as aminergic units according to the relative low frequency of the background IA (below 8 sec^{-1}), the polyphasic pattern of the action potentials (APs), their long duration (2.5-5.0 msec), and corresponding localization of the units in the brainstem [5, 6] (Figs. 2 and 3).

A mobile electrode (silver microwire, 8-12 μm thick, in glass insulation) was used for recording the neuronal activity. The end of the microwire was obliquely sharpened in such a manner that it looked like an injection needle. EEG was recorded bilaterally from the sites above the frontal, parietal, and temporal cortical regions. For this purpose, small hollows were made in the skull bones using a dental burr; electrodes (0.5 mm^2, electrolytically covered with gold) were inserted into these hollows and overflowed with acrylate. EEG records were subjected to standard spectral analysis; the following frequency components were classified: 1-3 Hz (delta), 4-7 Hz (theta), 8-13 Hz (alpha), 14-30 Hz (beta), and 31-48 Hz (gamma rhythm) [7]. All signals were amplified and processed using a standard equipment set for recording and analysis of EEG and IA of single neurons. Under conditions of parallel recording of the IA from neurons of the aminergic systems and of the mass electrical activity from the neocortex (Fig. 2), we measured the initial levels of the activity, then either perorally introduced bemitil (50 mg/kg) added to dry fodder or simulated its introduction giving a fodder portion with no drug (placebo control). After feeding the animal with fodder containing bemitil or without this agent, 3-min-long fragments of a IA of aminergic neurons and those of EEG were recorded with 2-min-long intervals (i.e., the activity samples were recorded each 5 min) over 1 h. Bemitil is known to be rapidly absorbed in the gastrointestinal tract (period of absorbing of 50% of the dose, about 45 min [8]). Thus, the time segment of observation in our experiments included the period of a rise in the concentration of bemitil in the blood; the final stage of observation, probably, corresponded to attaining a quasistationary concentration of the above agent.

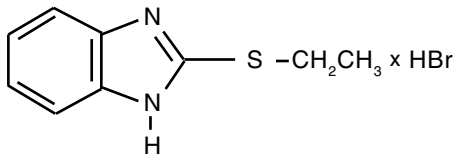

Fig. 1. Structural formula of bemitil (2-ethylthiobenzimidazole hydrobromide).

Data on the frequency of AP generation and EEG indices obtained within the period of action of bemitil were compared with the control. Action potentials were converted into standard rectangular impulses; their recording was performed using a software "Neuron-EEG" specially developed for the above-described experimental technique (computer programmer, E. Zinchenko).

After the experiment, the animals were euthanized by nembutal injected in a lethal dose. For controlling the localization of the recording zones, a nichrome needle electrode was inserted instead of the recording microelectrode, and 2 mA current was passed for

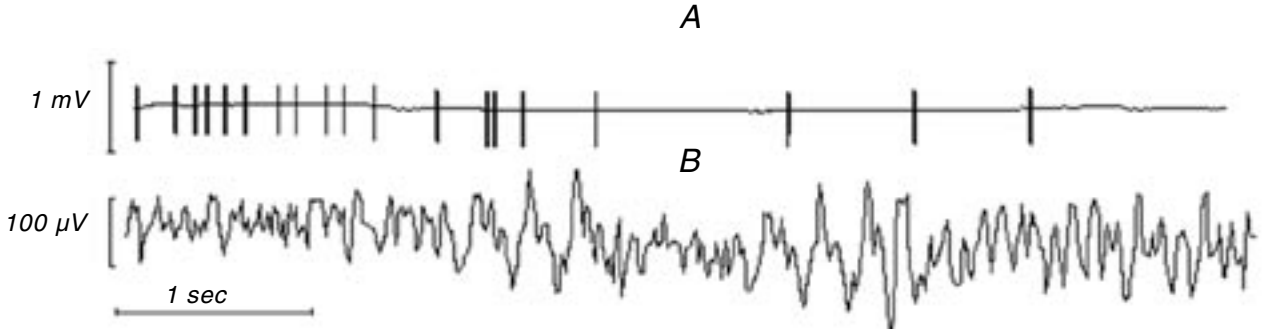

Fig. 2. An example of parallel recording of the impulse activity of a noradrenergic neuron of the *locus coeruleus* (A) and mass electrical activity of the neocortex (B).

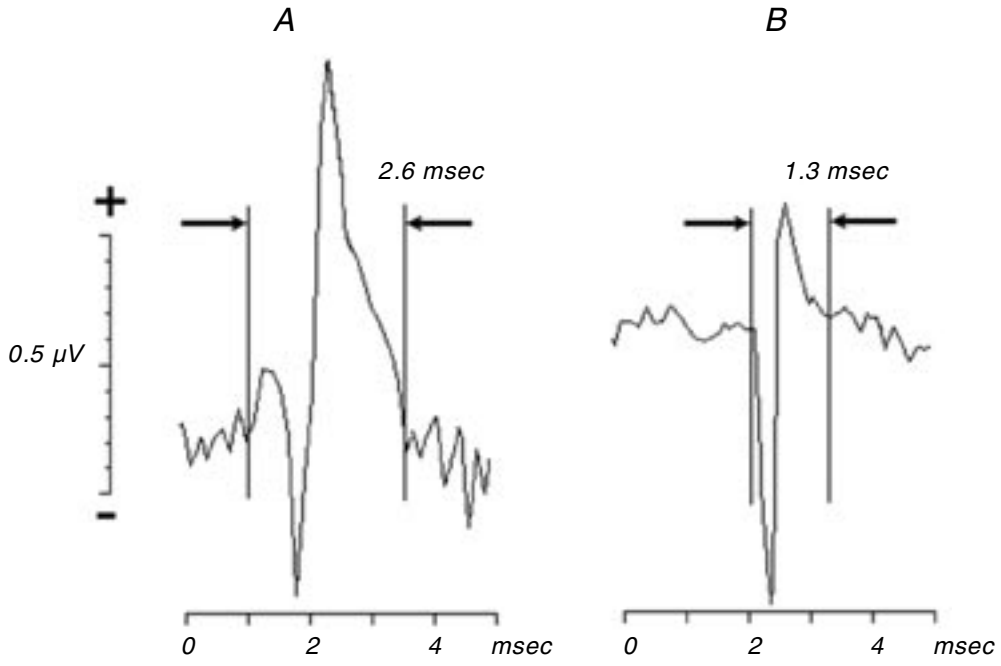

Fig. 3. Action potentials (APs) of putatively serotonergic (A) and non-serotonergic (B) neurons of the *nuclei raphe*. Arrows and vertical lines show measured values of the AP duration, msec.

1 min through it. Then the brain was fixed in formalin, and frontal sections were prepared using a freezing microtome. Localization of the coagulation zone was verified according to the stereotaxic coordinates [9] (Fig. 4).

Numerical data were statistically treated using standard computer software (Statistica). Indices of the IA were compared using a nonparametric test (Mann–Whitney).

RESULTS

In the course of the experiments, we recorded the activity of 22 putatively aminergic neurons (11 NA-ergic and 11 ST-ergic units). In the control experiments (placebo control), the examined group included 8 NA-ergic and 8 ST-ergic neurons.

In the control, the frequency of background IA of LC neurons showed no significant deviations from the initial value over the entire recording interval (60 min, Fig. 5). After introduction of bemitil, the discharge frequency of all studied LC neurons significantly ($P < 0.05$) decreased as early as in 5 min; later on, it dropped to about 60% of the initial value (on average in the studied group). Later on, the frequency of AP generation may vary somewhat, but in most neurons it did not exceed 60% of the initial value within the greater part of the observation period. Some trend toward recovery of the IA parameters could be found only at the end of the above interval (Fig. 5). The drop in the frequency of impulsation of NA-ergic neurons calculated for the entire observation period was statistically significant ($P < 0.05$). Changes were most clearly manifested approximately on the 45th min after introduction of bemitil, where the mean intragroup value of the frequency for NA-ergic neurons of the LC was 49.0 ± 9.4% (in the control, 111.0 ± 9.1%). In some neurons, however, the decrease in the spiking frequency reached 70% of the initial value. Within the entire period of action of the drug, we observed some suppression of the behavioral activity of the animals.

Generation of the background IA by ST-ergic neurons could also be considered in fact to be stationary within the observation period (Fig. 6); fluctuations of the frequency of APs in most cases were insignificant. The dynamics of the frequency of discharges of these neurons after peroral introduction of bemitil were more complex, as compared with those typical of NA-ergic neurons. Within the initial period (about 5-10 min after introduction), the frequency of impulsation of ST-ergic neurons increased somewhat

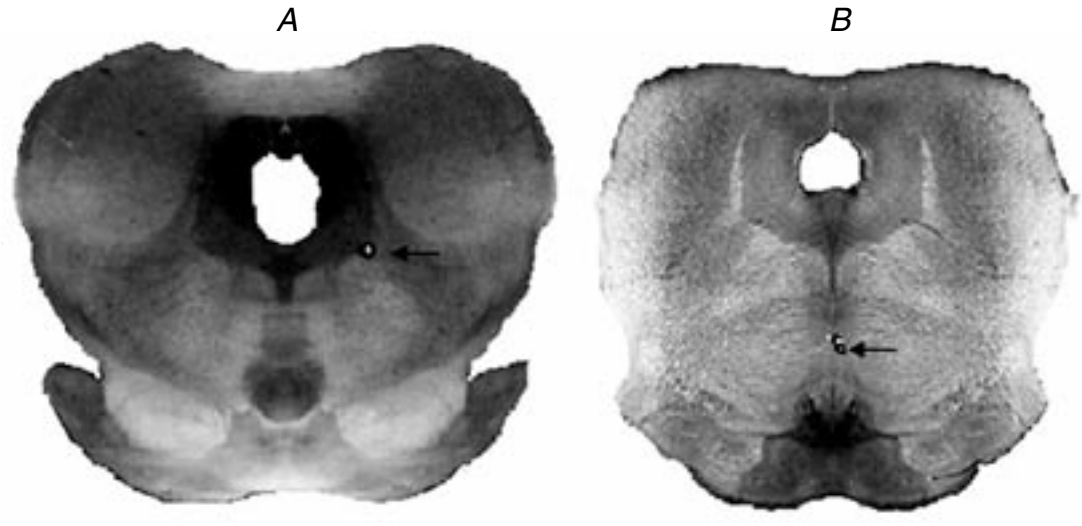

Fig. 4. Sections of the brainstem through the region of localization of noradrenergic neurons of the *locus coeruleus* (A) and serotonergic neurons of the *nuclei raphe* (B). Regions of localization correspond to regions of recording from these cells. Arrows indicate electrocoagulation labels.

(usually to 120%, as compared with the control); then, within a 15 to 25 min interval, the frequency dropped somewhat. These deviations, however, were not statistically significant because of the great variability of changes in separate cells. Later on, from the 30th min, we observed a clear long-lasting increase in the frequency of spiking of ST-ergic neurons, and this shift was preserved up to the end of the period of observation. Within an interval 45 to 60 min, these changes provided a significant intragroup shift ($P < 0.05$). On the 45th min after introduction of the preparation, the increment of the activity of ST-ergic neurons localized in the RN was about 50%, and in some neurons the frequency demonstrated a twofold increase. It should be noted that this shift coincides with the period of absorption of 50% of the drug and a maximum rise of its concentration in the blood.

Therefore, our experiments showed that peroral introduction of 50 mg/kg bemitil exerts in general opposite influences on the IA of NA-ergic and ST-ergic neurons of the aminergic cerebral systems.

We studied in detail the dynamics of indices of the mass electrical brain activity analyzing EEG recorded from the contralateral, with respect to localization of the cannula, loci. It should be mentioned that in EEG samples recorded ipsilaterally rather similar changes of all frequency EEG components after introduction of bemitil could be observed, but their pattern was less clear. It seems probable that this was related to the impairment of the studied aminergic neuronal systems capable of exerting constant tonic influences on the forebrain structures [10]; this probably resulted from insertion of the cannula and movements of the recording microelectrode. Introduction of bemitil was followed by certain fluctuations of the spectral powers of alpha, beta, and gamma EEG components, but in general deviations of these parameters from the control were insignificant. As to low-frequency EEG rhythms, as early as from the 5th min of observation and throughout its entire duration the spectral power of delta EEG activity decreased (within the studied group, usually to about 72-80% of the initial value) (Fig. 7). Significant changes (a decrease with respect to the control, $P < 0.05$) in the power of this EEG frequency range appeared on the 5th min of observation (intragroup means 104.0 ± 23.2% in the control, as compared with the initial value, and 82.0 ± ± 8.5% after introduction of bemitil); they were also obvious on the 10th min (102.0 ± 21.5% and 79.0 ± ± 7.1%), on the 35th min (101.0 ± 19.8% *vs* 72.0 ± ± 5.8%), and on the 40th min (102.0 ± 22.0% *vs* 74.0 ± ± 6.0%). Thus, the spectral power of the delta activity decreased by 20-30% within the above time sections, as compared with the control.

Statistical analysis of the dynamics of changes of the theta activity allowed us to find significant

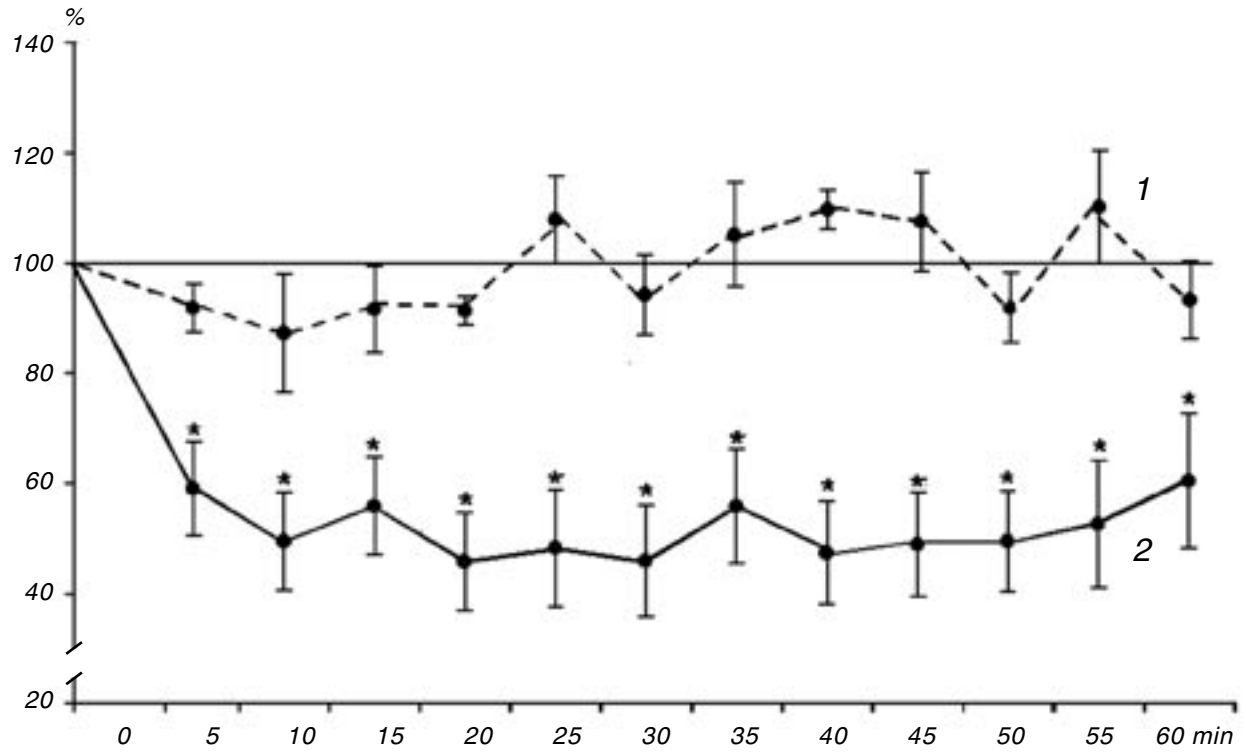

Fig. 5. Dynamics of the activity of the studied group of noradrenergic neurons of the *locus coeruleus* of an awake cat in the control (1) and after introduction of bemitil (2). Moments of peroral introduction of bemitil or of placebo correspond to zero time moments. Abscissa) Time, min; ordinate) normalized values of the frequency of the impulse activity averaged within the studied group, %; the initial level of the impulse activity is taken as 100%. Asterisks show cases of significant differences from the control ($P < 0.05$).

($P < 0.05$) changes only on the 5th min (control, 103.0 ± ± 15.7%; bemitil, 91.0 ± 10.2%) and on the 10th min (98.0 ± 16.8% *vs* 88.1 ± 9.0%) (Fig. 8). Within other time intervals, there were only trends toward decreases in the spectral power of this rhythm with respect to the control. We should mention that the spectral power of the theta activity practically returned to the control values on the 25th and 45th min.

DISCUSSION

The main result of our study is the demonstration of differentiated effects of bemitil (introduced *per os*) on brainstem NA- and ST-ergic neurons of the cat. Application of this drug results in opposite changes in the activity of the above neurons of the aminergic systems. Spiking of NA-ergic units is suppressed, while impulsation of ST-ergic neurons is intensified within definite time intervals. In addition, we found that bemitil noticeably influences low-frequency spectral components of the mass electrical activity of the cerebral cortex (EEG).

It has been mentioned that, at present, information on the mechanisms of the effects of bemitil on the CNS is contradictory and scarce, and we can propose only some general considerations with respect to the observed phenomena.

Earlier, the effects of bemitil were tested on identified neurons of the mollusc *Helix albescens* [11]. Intracellular recording from such cells showed that bemitil in optimum concentrations (10^{-4} M) decreased the frequency of generation and the amplitude of APs. Higher concentrations of the drug (10^{-2} M) provided suppression of both inward sodium and calcium currents and outward potassium currents, and generation of APs was blocked.

Probably, there is another mechanism underlying the effect of bemitil, which is not related to direct effects of this agent on ion channels in the membranes of nerve cells. If we take into account the molecular mass of the tested preparation and even if we suppose that its entire amount was absorbed and evenly distributed within the body mass, the concentration of bemitil in the blood of our experimental animals should not

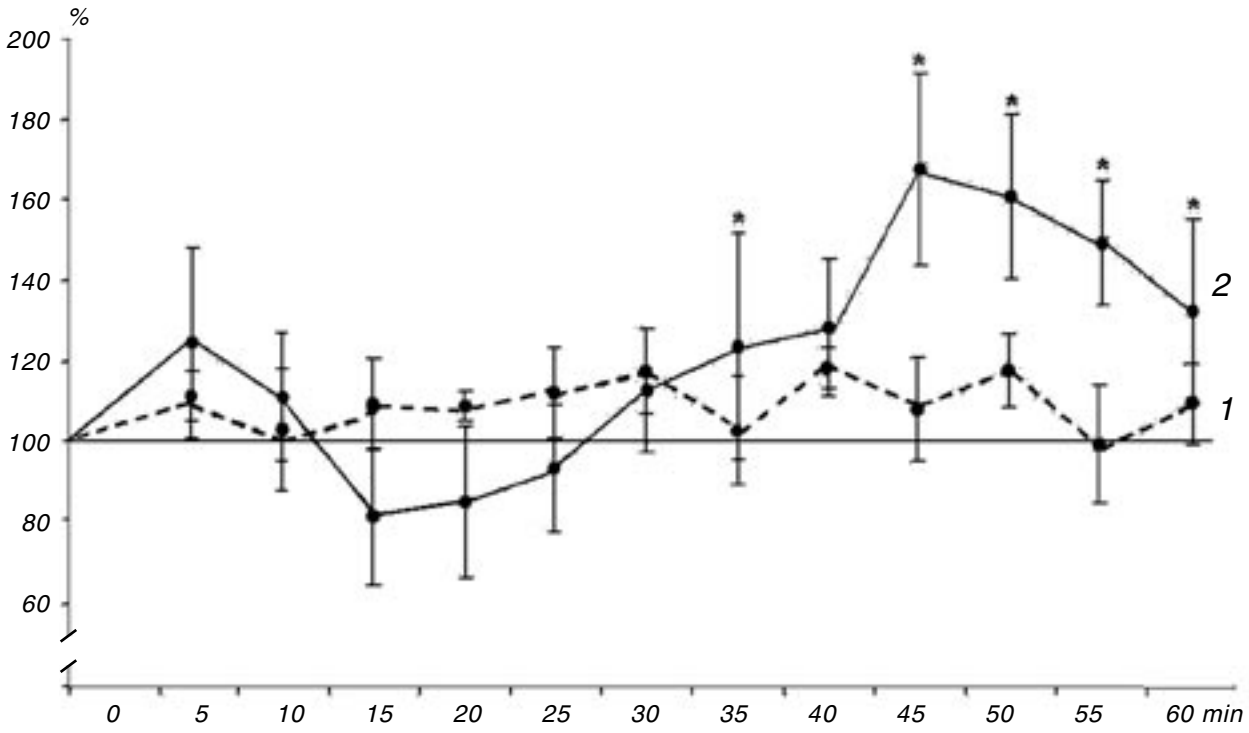

Fig. 6. Dynamics of the activity of serotonergic neurons of the *nuclei raphe* in the control (1) and after introduction of bemitil (2). Designations are similar to those in Fig. 5.

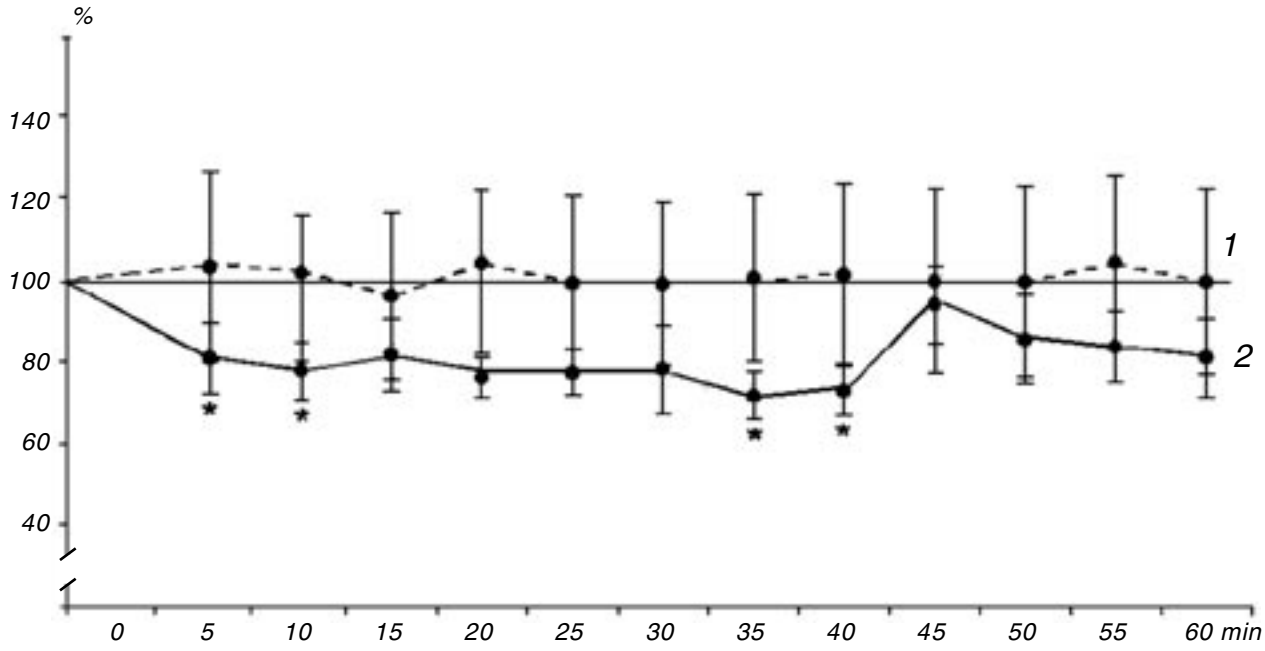

Fig. 7. Dynamics of changes in the spectral power of delta activity in the EEG of an awake cat after introduction of bemitil. Designations are similar to those in Fig. 5.

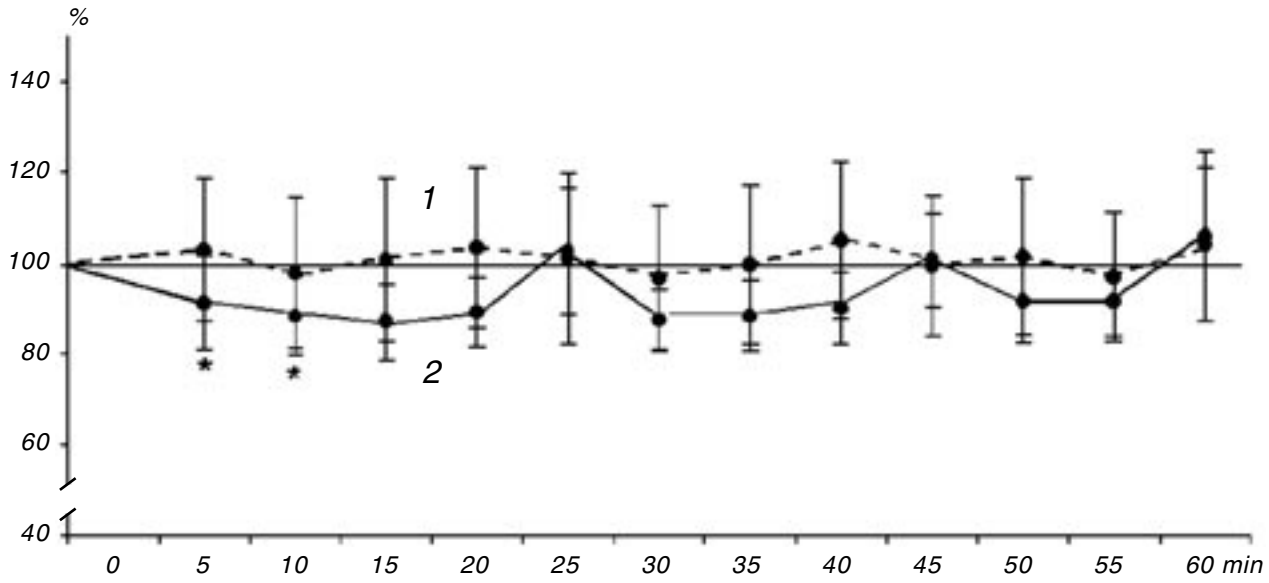

Fig. 8. Dynamics of changes in the spectral power of theta activity in the EEG of an awake cat after introduction of bemitil. Designations are similar to those in Fig. 5.

be higher than $3 \cdot 10^{-4}$ M. Thus, the supposition that the main part of the effects under conditions of our experiments resulted from specific influences on the noradrenaline and serotonin receptors of the CNS neurons seems more adequate.

It is known that discharges of NA-ergic cells are modulated by activation of the mosaics of the receptors on their somata and dendrites. Among these receptors, α_1 and α_2 adrenoreceptors, acetylcholine receptors, and opioid receptors play crucial roles. Acetylcholine and α_1 receptors mediate activation of NA-ergic cells, while α_2 adrenoreceptors and opioid receptors suppress their activity. Inhibition of NA-ergic cells is also provided by glycine and GABA receptors [12, 13]. The effects of serotonin are mediated by postsynaptic receptors of this amine localized on the dendrites and dendrite spines of target cells and also via presynaptic serotonin receptors localized on axon terminals and autoreceptors. Seven types of serotonin receptors and their 15 subtypes were classified in the CNS, and their activation provides a variety of effects [13, 14]. Mostly inhibitory effects on target cells are provided by 5-HT_1 receptors, while other types are responsible for mostly excitatory effects. Taking into account our experimental results, we believe that the above-described changes in the frequency characteristics of spiking of NA-ergic cells of the LC are related to stimulation of a "receptor cocktail" inhibiting the activity of these cells; among these receptors, presynaptic α_2 adrenoreceptors probably play a most significant role.

In turn, the activating effects of bemitil on the ST-ergic system can be related to the blockade of 5-HT_1 receptors; the respective influence on the frequency of discharge of ST-ergic neurons leads to modulation of the transmitter release by these cells. The ambiguity of the effects of bemitil within different time intervals after its introduction probably results from the formation of specific zones determined by the corresponding "mosaics" of ensembles of inhibited and activated neurons, and the peak of these changes provides noticeable intensification of the functional activity of the ST-ergic brain structures. These changes in the frequency characteristics of spiking of NA-ergic and ST-ergic neurons of the brain aminergic systems, which were induced by the action of 50 mg/kg bemitil, are accompanied by behavioral relaxation, which is inevitably reflected in changes in the spectral characteristics of EEG (decreases in the power of the delta and theta activities). These results agree with the observations of other authors [10, 15]. They reported that pharmacological stimulation of α_2 receptors suppresses impulsation of NA-ergic neurons and is accompanied by sedative (calming) effects. It is

also known that an artificial increase in the serotonin concentration in the rabbit neocortex partially blocks theta EEG rhythm, and this is interpreted as a manifestation of the inhibitory processes [16]. Probably, these data allow one to interpret the results of experiments on rats, where bemitil in the same dose (50 mg/kg) suppressed most behavioral physiological reactions of the animals. This was manifested in limitation of the motor and orientation/research activity and in corresponding changes in the emotional state of experimental animals [17].

The main general conclusions from our study can be formulated in such a way. Bemitil evokes specific changes in different neurotransmitter systems and to a considerable extent modulates the activity of NA-ergic and ST-ergic neuronal systems. This drug, when tested in a 50 mg/kg dose, is capable of evoking relatively "soft" tranquilizing effects, which can be manifested in a decrease in the level of anxiety and emotional stress; these effects result in behavioral relaxation of the animals.

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