A STUDY OF THE SPECTRUM OF PSYCHOTROPIC ACTION OF MEBICAR

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In the course of an extensive program of search for new physiologically active substances among polynitro heterocyclic compounds of the bicyclic bis-urea class, a new psychotropic drug, mebicar, recommended for clinical use as a tranquilizer, has been discovered and studied [4].

The object of this investigation was a deep study of the spectrum of psychotropic action of mebicar and elucidation of some aspects of the mechanism of its action.

EXPERIMENTAL METHOD

The spectrum of emotional-behavioral reactivity was assessed in chronic experiments on seven cats under group interaction conditions [5]. An emotional state of fear or alarm was induced by stimulation of the hypothalamic emotiogenic zones through previously implanted electrodes (150 Hz, duration 1 msec). The intensity of stimulation used (50-150 μA) was such that during the period of stimulation (30-60 sec) slight behavioral manifestations were induced, but after the end of stimulation an emotional after-state continued for 4-15 min. The significance of the responses was qualified and assessed quantitatively (on a five-point system) on the basis of evaluation tables [2]. The electrical resistance of the skin was recorded continuously by the method described previously [1]. The aggressive behavior of the rats (50 animals) was assessed from the duration of the "boxing posture" and of fighting induced by electrodermal stimulation (0.3 mA), when the animals were placed in pairs in cages with an electrode floor. The investigative behavior of the mice was assessed by the method in [8]. The concentrations of nonradrenalin (NA) and adrenalin in the brain stem of the rats (24 animals) were measured fluorometrically [6] on the EF-3M instrument, improved in accordance with the scheme in [3]. The serotonin concentration (18 animals) was determined by a spectrophotofluorometric method [7] in the modification described in [9]. The significance of differences was determined by Student's t-test and by the Wilcoxon–Mann–Whitney nonparametric U-test.

EXPERIMENTAL RESULTS

The tranquilizing action of mebicar was clearly demonstrated against the background of an experimentally induced (by stimulation of the dorsomedial zones of the hypothalamus) negative emotional state of fear-alarm type. Data on the action of mebicar (compared with diazepam) are summarized in Table 1. In doses of 120-150 mg/kg (intraperitoneally) mebicar prevented the development of behavioral manifestations of fear and sharply reduced those of alarm both during and after hypothalamic stimulation. The spectrum of emotional-behavioral reactivity was restored close to its initial level. The animals were a little inhibited and preferred to sit or lie immobile. Investigative activity and initiative were depressed. However, no disturbance of motor activity (ataxia, muscle relaxation) and no sedative effect were observed. Responses to in-group interaction and to test reactions remained adequate.

Changes in the electrical resistance of the skin constitute an additional test reflecting changes in the emotional stress of animals and the tranquilizing effect of drugs (Fig. 1). The initially low level of skin resistance of the animals while exhibiting signs of fear and alarm in the experimental chamber increased after administration of mebicar (120-150 mg/kg) from 74.62 ± 21.63 to 129.44 ± 28.75 kΩ. This correlated with behavioral manifestations of

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TABLE 1. Effect of Mebicar and Diazepam (in equipotential doses) on Spectrum of Animal's Emotional Behavior in a Simulated State of Fear-Alarm

Spectrum of emotional behavior	Intensity of emotional reactivity (mean values in points)					
	initial	after hypothalmic activation		initial	after hypothalamic activation	
		aft. prelim. admins. of 120-150 mg/kg mebicar	without the drug		aft. prelim. adminis. of 120-150 mg/kg mebicar	without the drug
Fear	1,4	0,5*	3,2*	1,0	1,0*	2,6*
Alarm	1,0	0,16*	2,8*	1,0	0,7*	2,8*
Negativity	0,4	1,14*	3,4*	0	0,7*	3,0*
Conflict	2,0	0,09*	1,5*	1,6	0,6	1,3
Aggressiveness	1,0	0	0	1,4	0,5	0,8
Manifestation of positive emotions	1,2	0	0	1,0	1,3*	0
Friendly contacts	1,8	1,7	0,4	1,4	2,3	0,7
Investigative behavior	1,6	1,3*	0	2,5	2,0*	1,0*
Motor activity	1,8	1,1	2,0	2,2	1,8*	1,0*

*Differences significant at $P < 0.05$.

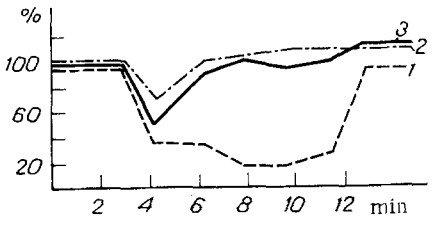

Fig. 1

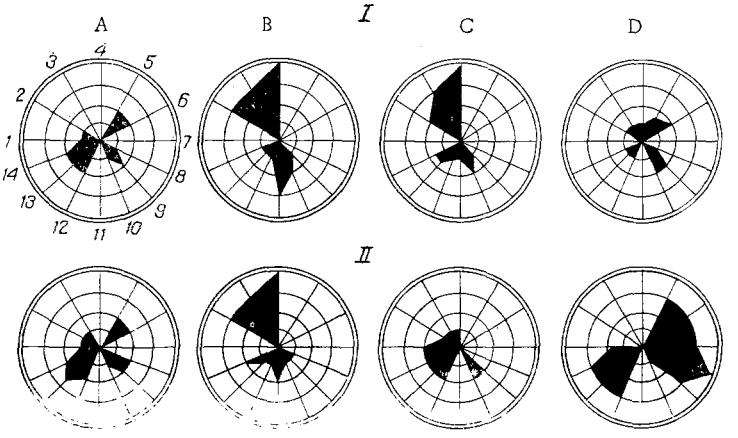

Fig. 2

Fig. 1. Effect of mebicar on magnitude and duration of PGR correlating with emotional excitation. Abscissa, time (in min); ordinate, change in electrodermal resistance (in % of initial level taken as 100%). 1) Dynamics of PGR in response to aversive stimulation; 2 and 3) the same, after preliminary administration of mebicar in doses of 120 and 150 mg/kg, respectively.

Fig. 2. Comparison of spectra of tranquilizing action of mebicar and diazepam in an experimentally induced state of acute fear-alarm. I) Mebicar, II) diazepam. A) Initial spectrum of animal's emotional state, B) emotional response of fear-alarm induced by electrical stimulation of the hypothalamus; C, D) action of mebicar in doses of 60 and 150 mg/kg (I) and of diazepam in doses of 1 and 2 mg/kg (II). Explanation of vectors: 1) aggressivesness, 2) fear, 3) alarm, 4) negativity, 5) investigative behavior, 6) manifestation of satisfaction, 7) play, 8) hunting, 9) friendly contacts with partners, 10) conflict in group, 11) inadequacy of response, 12) initiative and ability to compete, 13) motor activity, 14) orienting reaction.

the tranquilizing effect. The psychogalvanic reflex (PGR) to aversive stimulation was significantly modified after mebicar: The tonic phase of the PGR, which continued up to 10-15 min after aversive stimulation and corresponded to an after-change in the animal's emotional state, was replaced after administration of mebicar by a short (30-60 sec) phasic shift of electrodermal resistance, coinciding in duration with the animal's orienting reaction.

To detect the antiaggressive action of mebicar rats which demonstrated mutual aggressivesness for 2 min were selected. Control animals were given an intraperitoneal injection of bidistilled water, whereas the experimental animals were given mebicar (300-1000 mg/kg) 2 h before the investigation. Over the whole range of doses tested mebicar completely blocked aggressive behavior.

Unlike diazepam, mebicar had no activating action on behavior. No intensification of spontaneous motor activity, initiative, or investigative activity (cat) took place (Table 1). Mebicar did not potentiate manifestations associated with positive emotions and did not induce the "euphoric" action characteristic of diazepam [1]. The anticonflict effect of mebicar, by contrast with diazepam, was based not on the strengthening of friendly contacts with the partners, but on an increase in the adequacy of mutually determined behavior, thereby preventing conflicts from arising. No tendency toward a dominant form of behavior developed after administration of mebicar (Fig. 2).

Mebicar thus has an original spectrum of tranquilizing action which distinguishes it from the "standard" tranquilizer of the benzodiazepine series. These differences were also revealed by other tests. Mebicar (200-1500 mg/kg) produces a dose-dependent inhibition of investigative behavior in mice (assessed by the number of peeps through a hole in 5 min). After intraperitoneal injection of 1500 mg/kg mebicar the response was inhibited by 4.5 times after 20 min and by 13 times after 40 min ($P < 0.001$). In absolute activity, mebicar is significantly weaker than tranquilizers of the benzodiazepine series (the effect of mebicar in a dose of 750 mg/kg was equivalent ot 5 mg/kg of diazepam), but the toxicity of the compound was very low (LD_{50} for mebicar is 3800 ± 174 mg/kg and for diazepam 25.5 ± 0.8 mg/kg). Mebicar likewise has no muscle-relaxing action. In the "revolving rod" test diazepam, in a dose of 2.5 mg/kg, completely disturbed the ability of the mice to remain on the rod, whereas mebicar, even in a dose of 1500 mg/kg, not only did not disturb movement coordination, but actually increased the length of time the animals remained on the revolving rod (from 42.5 ± 5.3 sec in the control to 51 ± 6.2 sec in the experiment).

The neurochemical mechanism of the psychotropic action of mebicar requires further study. Preliminary analysis reveals the complex action of the drug on mutually determined neuromediator processes. When injected intraperitoneally (1 h before investigation) mebicar lowers the NA level in the rat brain stem: in a dose of 500 mg/kg to 1.30 ± 0.05 μg/kg, in a dose of 1000 mg/kg to 0.99 ± 0.22 μg/g ($P < 0.001$) compared with an NA concentration in the control of 1.98 ± 0.14 μg/g. There was no significant change in the adrenalin concentration. The absence of effect in the apomorphine stereotype test (mebicar, given in a dose of 1500 mg/kg 40 min before injection of 20 mg/kg apomorphine, did not affect the duration of stereotypy: 75.4 ± 3 min in the control, 80.5 ± 1.5 min in the experiment; $P = 0.15$) suggests that mebicar has no significant effect on dopaminergic systems. In dose of 500 mg/kg (intraperitoneally, 1 h beforehand) mebicar increased the serotonin concentration in the brain stem by 31% ($P < 0.004$) and also in whole blood (by 60%; $P < 0.004$). With no anticholinesterase effect (in concentrations of 1×10^{-2} M—1×10^{-7} M mebicar did not change the acetylcholinesterase activity of hemolyzed sheep's erythrocytes), with no antinicotinic action (either in the nicotine toxicity test or in its effect on nicotine convulsions), and without changing the intensity of action of acetylcholine (1×10^{-7} M) on a segment of isolated rabbit's intestine, mebicar increased the sensitivity of central muscarinic cholinergic systems a little to the action of arecoline (intensification of tremor up to the level of clonic convulsions, without any increase in the duration of the convulsions).

Mebicar is very similar in its structure to certain natural metabolites produced by the body. It includes two urea fragments, methylated at their nitrogen atoms.

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EFFECT OF β-PHENYLETHYLAMINE ON SYNAPTOSOMAL AND GLIAL TRANSPORT OF LABELED NEUROTRANSMITTERS

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β-Phenylethylamine (β-PEA), an endogenous [5] sympathomimetic amine, has a powerful stimulating action on the CNS [7, 8, 12]. There are grounds for considering that β-PEA may participate in the regulation of monoaminergic synaptic transmission in certain brain structures, including the caudate nucleus [4, 6, 8]. For instance, *in vitro* experiments on synaptosomes of the caudate nucleus have shown that β-PEA moderately inhibits reverse transport of dopamine-^{3}H and stimulates its liberation [4]. The object of the present investigation was to study the role of β-PEA in the regulation of reverse transport into nerve endings of the caudate nucleus of other hypothetical mediators responsible for the activity of this structure (serotonin, glutamate, and GABA). For comparison, the effect of β-PEA was studied on synaptosomal and glial serotonin transport.

EXPERIMENTAL METHOD

Experiments were carried out on male albino mice weighing 180-250 g. The intensity of synaptosomal and glial transport processes was judged from the uptake of labeled mediators. The fraction of glial cells was isolated from the cerebral cortex of rabbits by Rose's method [10] with certain modifications described previously [2]. Total synaptosomal fractions from the caudate nucleus of the rat brain and from the rabbit cerebral cortex were obtained by Whittaker's method in the modification of Shevtsov et al. [3]. Uptake of GABA-^{3}H, glutamate-^{14}C, and serotonin-^{14}C was determined by incubating synaptosomes or glial cells (0.25 mg protein/ml) in medium containing 100 mM NaCl, 6 mM KCl, 10 mM glucose, 100 mM sucrose, 0.54 mM EDTA, 0.125 mM pargyline (a monoamine oxidase inhibitor), 1.14 mM ascorbic acid, and 30 mM Tris-phosphate buffer, pH 7.4, with continuous agitation (20 min, 37°C). β-Phenylethylamine (from Serva, West Germany) was converted into the hydrochloride and purified by repeated recrystallization. The following materials were used in the experiments: β-PEA hydrochloride

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