Effect of New Anxiolytic CM-346 on Bioelectrical Activity of Cerebral Cortex in MR and MNRA Rats

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The effect of 2-[2-(morpholin)ethylthio]-5-ethoxybenzimidazole (CM-346) on the EEG of MR and MNRA rats was compared with that of diazepam. Both CM-346 and diazepam reversed stress-induced changes in bioelectrical activity after intraperitoneal injection of 0.9% NaCl. In MR and MNRA rats both drugs decreased the EEG spectral power in the high-frequency θ-band (7.5-8.2 Hz). In MR rats, CM-346 increased the power of the low-frequency θ-rhythm (4.8-5.8 Hz) and the dominant activity peak (6.0-7.2 Hz) and decreased the spectral power in the 19-20 Hz band. The data suggest that CM-346 exerts antistressor and anxiolytic effects in animals with passive reactions to emotional stress.

Key Words: *tranquillizer; emotional stress; EEG; MR and MNRA rats*

Previously we have shown that CM-346 (2-[2-(morpholin)ethylthio]-5-ethoxybenzimidazol) exerts an anxiolytic effect on MR rats and BALB/c mice exhibiting freezing reactions in the open field and conflict tests but does not change active behavior of NMRA rats and C57Bl/6 mice in the same tests [6,7].

The aim of this study was to determine the EEG-correlates of the anxiolytic effect of CM-346.

MATERIALS AND METHODS

Experiments were carried out on adult male MR (n=9) and MNRA (n=9) rats weighing 180-200 g, bred from Winston-Salem breeding stock at the Laboratory of Biological Models, Russian Academy of Medical Sciences (Svetlye Gory). Twenty-four intracranial steel electrodes were implanted under Nembutal anesthesia (50 mg/kg) for recording of biopotentials from all regions of convexital cortex. The active and reference (in the occipital bone) electrodes were fixed with dental acrylic. The EEG recorded in freely moving rats in a light- and sound-proof chamber was processed by specially developed SYNCHRO-EEG software after fast Fourier transform. The signals were sampled at 128 Hz within ten 4-sec epochs. Our software allowed us to evaluate 840 parameters characterizing spatial organization of cortical bioelectrical activity (BA) and to determine the difference between their pre- and postinjection values using Student's t test.

The main parameters (spectral power, spatial synchronization, coherence, spatial disorganization, fronto-occipital and interhemispheric asymmetries) were determined for 22 "coherence structures" (CS), i. e. narrow EEG frequency bands with high biopotential synchronization [9]. Their frequency classification is presented in Table 1.

Water solutions of CM-346 (20 mg/kg) and NaCl (0.9%), as well as the reference drug diazepam (10 mg/kg) dissolved in Tween-80 were injected intraperitoneally in a volume of 1 ml.

The EEG was recorded before and 5, 20, 35, 50, 80, and 95 min after injections. In the control rats receiving no injections the EEG was recorded according to the same schedule.

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RESULTS

In MR rats, intraperitoneal injection of 0.9% NaCl significantly modified many BA parameters (Fig. 1). The number of altered parameters considerably exceeded that in the control rats receiving no injection. This effect can be considered as an EEG correlate of pain and emotional stress caused by manipulations and irritation of the peritoneum, the most abundant with receptors.

CM-346 and to a lesser extent diazepam reversed the effect of injection decreasing the number of modified parameters to the control level. In MNRA rats, the effects of 0.9% NaCl and the two tranquillizers were considerably less pronounced compared to MR rats.

In MR rats, CM-346 significantly increased the EEG spectral power in CS 4 and 5 (4.75-7.25 Hz, the low-frequency bands of θ-rhythm), and decreased it in CS 6 and 7 (7.5-9.75 Hz, high-frequency θ-rhythm) (Fig. 2, *a*). In MNRA rats, CM-346 similarly decreased the spectral power in CS 6, but increased it in CS 4 without affecting CS 5.

In MR rats, CM-346 decreased the spectral power CS 15 (19-20 Hz) of β_1-band. MNRA rats showed such effects since they had significantly lower ectral power and no peak in this CS before injection ig. 2, *b*).

Thus, except for CS 6, CM-346 induced qualitatively different effects on BA in MR and MNRA rats.

As seen in Table 2, both CM-346 and diazepam suppressed the high-frequency θ-rhythm spectral power (CS 6; 7.5-8.25 Hz). It can be suggested that this suppression is the main EEG correlate of anxiolytic activity [11]. This suggestion is supported by the fact that anxiogenic drugs increase the frequency of θ-activity [1].

Many researchers consider inhibition of θ-rhythm and shift of the dominant activity peak towards low frequencies down to the δ-band as the principal EEG correlate of anxiolytic effects of tranquillizers [3-5, 10]. We found neither increase in the low-frequency θ-band (CS 4), nor downward shift in the dominant activity peak from CS 5 after diazepam injection in rats of both strains. In MNRA rats, diazepam only decreased the spectral power of dominant activity (CS 5).

θ-Rhythm is the principal EEG rhythm in rodents. It arises from septo-hippocampal influences on the cortex. It is generally accepted that enhancement of θ-rhythm correlates with improvement of orienting and exploratory behavior, attention, and memory. Therefore, the increase in the peak of dominant θ-activity under the influence of CM-346 can be considered as a positive effect of the new drug, which distinguishes it from other tranquillizers, in particular, benzodiazepines. Impairment of attention and memory are the side effects of most tranquillizers limiting their application in individuals of certain professions. The fact that CM-346 does not shift the dominant activity peak to the low-frequency θ- and δ-bands, associated with CNS inhibition, indicates that it is probably devoid of hypnogenic activity and can be referred to as a "day tranquillizer".

TABLE 1. Numbers of CS and Corresponding EEG Frequency Bands

CS	Frequency, Hz
1	0.50-1.75
2	2.00-3.00
3	3.25-4.50
4	4.75-5.75
5	6.00-7.25
6	7.50-8.25
7	8.50-9.75
8	10.00-11.00
9	11.25-12.25
10	12.50-13.50
11	13.75-14.75
12	15.00-16.00
13	16.25-17.25
14	17.50-18.75
15	19.00-20.00
16	20.25-21.25
17	21.25-22.50
18	22.75-24.00
19	24.25-25.25
20	25.50-26.50
21	26.75-28.00
22	28.25-30.00

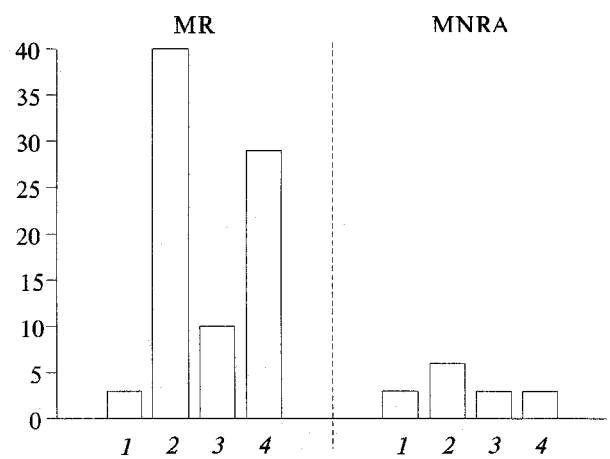

Fig. 1. Bioelectrical activity of cerebral cortex in MR and MNRA rats in comparison with control. *1*) intact; *2*) NaCl; *3*) CM-346; *4*) diazepam.

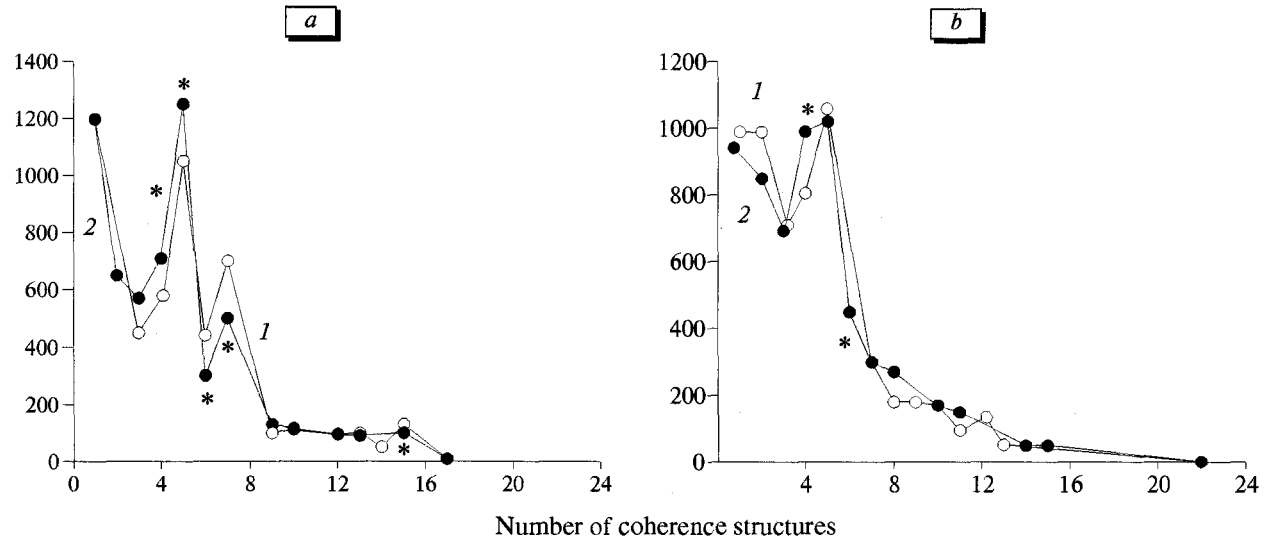

Fig. 2. Effect of CM-346 on the EEG spectral power in MR (*a*) and MNRA (*b*) rats. Ordinate: spectral power in standardized arb. units (mean value for 95 min postinjection). *1)* before injection; *2)* after C-346, **p*<0.05 in comparison with *1*, (Student's *t* test).

Diazepam increased the spectral power of α- and β_1-bands of the EEG (CS 8-13: 10.0-17.25 Hz) in both rat strains, while CM-346 exerted no such effect (Table 2). Diazepam-induced enhancement of high-frequency EEG components has been repeatedly reported. Some authors consider this effect as the principal EEG correlate of anxiolytic activity [12-14], while others believe that it reflects sedative [2] or myorelaxant [4] side effect of tranquillizers. We do not agree with the first opinion since we found no increase in α- and β-activity after CM-346 administration. If the second opinion is correct, CM-346 differs from benzodiazepines by the absence of side effects.

In contrast to CM-346, diazepam did not reduce the spectral power in the CS 15 (19-20 Hz) of β-band. The coherence of biopotentials in this subrange increased during psychomotor excitation in humans [9]. Thus, the inhibition of cortical activity in CS 15 under the influence of CM-346 suggests that this drug possesses antiaffective and antipsychotic properties, which are worthy of further investigation.

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TABLE 2. Effects of CM-346 and Diazepam* on EEG Spectral Power in MR and NMRA Rats

	EEG spectral power																					
	δ			θ			α				β_1					β_2						
	CS																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
MR rats																						
CM-346				+	+	—	—								—							
diazepam						—		+	+	+	+	+										
MNRA rats																						
CM-346				+		—																
diazepam					—	—	—		+	+	+	+	+									

Note. Significant increase (+) and decrease (—) of EEG spectral power (p<0.05). *Averaged over 95 min after intraperitoneal injection.

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