CORRELATION BETWEEN THE ANXIOLYTIC EFFECT OF DIAZEPAM AND CHANGES IN EEG POWER SPECTRA IN RATS

N. N. Bogdanov and T. A. Voronina

UDC 615.214.22.015.4:
616.831-073.97].076.9

KEY WORDS: diazepam, EEG, psychotropic effect

Despite much research, correlation between changes in brain electrical activity observed under the influence of benzodiazepines and the psychotropic effects of these drugs remains unclear. Traditionally research workers have paid most attention to an increase in high-frequency EEG activity in man and animals [7, 9, 10, 12]. Meanwhile, in a few investigations on animals, more importance has been attached to a decrease in frequency of the hippocampal Θ-rhythm [8]. The absence of any clear ideas about the neurophysiological mechanisms of the effects of benzodiazepines is an obstacle in the way of using electroencephalographic methods for the experimental and clinical study of tranquilizers.

The aim of this investigation was to study specific features of the effect of diazepam on the EEG and to compare them with the action of a neuroleptic and psychostimulant and to discover any possible correlation between the effects of benzodiazepines at behavioral and neurophysiological levels.

EXPERIMENTAL METHOD

Experiments were carried out on male noninbred albino rats and male cottontail rats (*Sigmodon hispidus*, from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR) weighing 180-220 and 100-120 g respectively, under unrestrained conditions. Fourier spectral analysis of the EEG of the sensomotor cortex and dorsal hippocampus of the rats was carried out by means of a Ber—Fourier Analyzer ("O.T.E. Biomedica", Italy), 15, 30, 45, 60, 90, 120, 150, and 180 min after injection of the substances (epoch of analysis 4 min 8 sec). The method was described in detail previously [3]. The anxiolytic action of the substances was evaluated by a technique based on the use of a conflict situation, created by opposition of drinking and defensive motivations [2]. The substances tested, namely diazepam (1, 5, and 10 mg/kg), amphetamine (1 and 5 mg/kg), and chlorpromazine (1 mg/kg), were injected intraperitoneally. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Tests on the control group of noninbred animals showed that the Fourier power spectra (PS) of the EEG of the sensomotor cortex of quiet, conscious rats consist of unimodal distributions with a maximum of dominant activity in the 6-7 Hz range (Fig. 1, I, 1). After intraperitoneal injection of physiological saline, the PS were virtually unchanged (Fig. 1, II, a). A similar picture also was characteristic of PS of the dorsal hippocampus.

Diazepam in a dose of 1 mg/kg shifted the peak in the Θ-band of the cortical and hippocampal PS by 0.5-1 Hz toward the region of lower frequencies. The maximal effect was observed 30 min after injection. No marked changes in power of the individual bands of PS were present (Fig. 1, II, b; Table 1).

A considerable change in PS of the cortex and hippocampus, characterized by a shift of the peak in the Θ-band by 1-1.5 Hz into the region of low frequencies and by an abrupt "fanwise" increase in power of the high-frequency bands of PS (13.5-32 Hz), was observed 15 min after injection of diazepam in a dose of 5 mg/kg (see Fig. 1, II, c; Table 1). The structure of PS in most animals showed a marked change 30-60 min after injection of the drug: a peak appeared in the 12-16 Hz band and the power of the Δ-, α-, and β-bands, and also the total power of PS, increased.

Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 8, pp. 199-202, August, 1989. Original article submitted October 10, 1988.

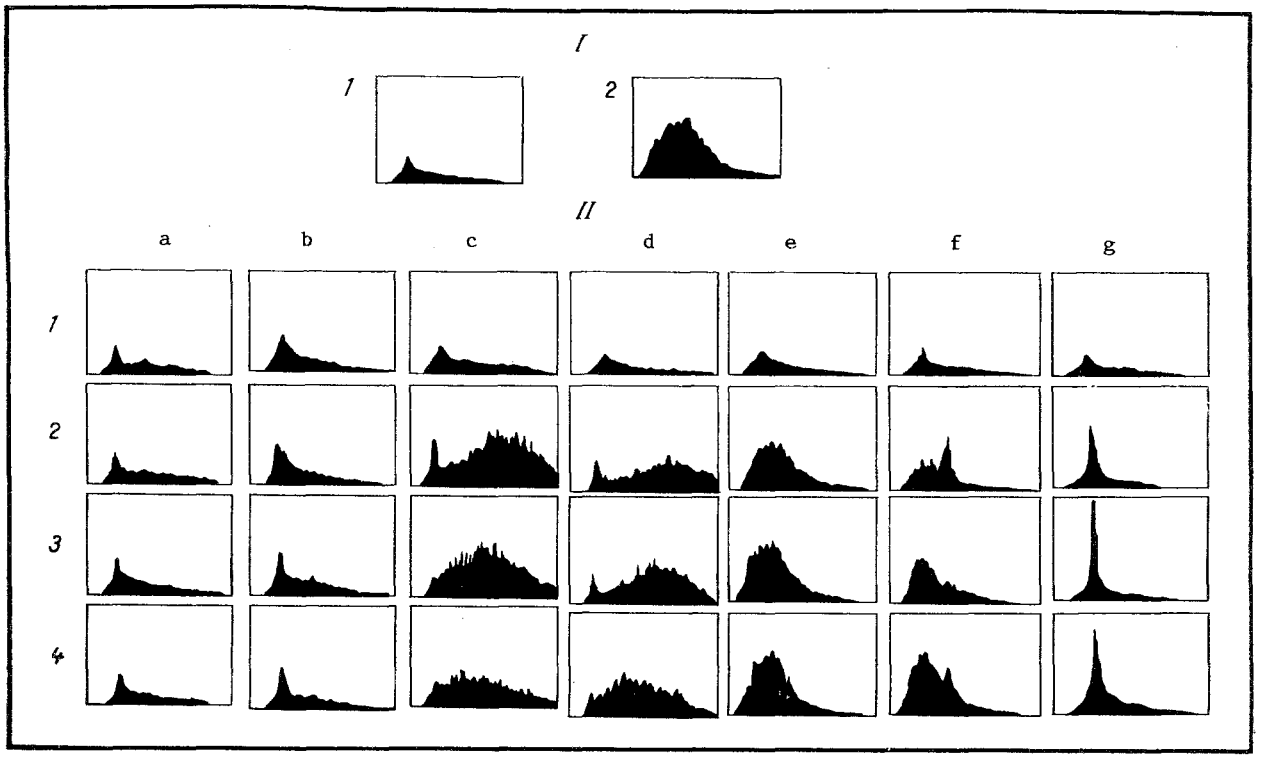

Fig. 1. Changes in PS of sensomotor cortex of noninbred rats at different levels of consciousness (I) and after injection (II) of physiological saline (a), diazepam in doses of 1, 5, and 10 mg/kg (b, c, e respectively), of chlorpromazine in a dose of 1 mg/kg (f), and amphetamine in a dose of 5 mg/kg (g) and in PS of sensomotor cortex of cottontail rats (d) under the influence of diazepam (5 mg/kg) during unrestrained behavior. I) PS of intact rat during quiet wakefulness (1) and slow-wave sleep (2); II) PS after injection of drugs: 1) before injection (background), 2-4) 15, 30, and 60 min respectively after injection (for a-f) and 30, 45, and 60 min after injection (for g). Calibration of abscissa of each frame: from 0 to 32 Hz; calibration of ordinate, 0-16 μV^2/Hz.

Under the influence of diazepam in a dose of 10 mg/kg, disturbances of the structure of PS of the sensomotor cortex and hippocampus were observed, with a marked increase in power mainly of the slow frequency bands (Fig. 1, II, e).

The next stage of the work was to discover how the effects of diazepam thus revealed correlate with effects of psychotropic drugs of other classes on the EEG.

Chlorpromazine (1 mg/kg), like diazepam in large doses (10 mg/kg), induced structural disturbances in PS with a sharp increase in power of slow frequency bands (the maximal effect was obtained 60 min after injection) (Fig. 1, II, f; Table 1). Considering that these EEG changes correlate with a marked sedative effect of diazepam and chlorpromazine, expressed as inhbition of the animals' spontaneous motor activity, and are close to the pattern of PS on lowering of the level of consciousness to slow-wave sleep (Fig. 1, I, 2) it can be tentatively suggested that these changes in PS are linked with sedation.

The action of the psychostimulant amphetamine in doses of 1 and 5 mg/kg was characterized by a sharp increase in power of the Θ-band with a simultaneous shift of the Θ-wave peak of PS by 1.00 ± 0.17 and 1.42 ± 0.08 Hz respectively in the region of higher frequencies (maximum of effect 45 min after injection of substance) (Fig. 1, II, g; Table 1). Thus changes in the Θ-band of PS of the cortex and hippocampus, induced by amphetamine, just as by other stimulants [6], are opposite in character to changes observed under the influence of diazepam (Fig. 2). When the effects of stimulants and tranquilizers are compared it must be recalled that there are two types of Θ-rhythm in rodents: the first type is cholinergic in nature, with a frequency of 5-7 Hz, and correlates with an immobile state of the animal, whereas the second, with a frequency of 7-12 Hz, is connected with motor activity [1, 4]. However, the increase in frequency of the Θ-rhythm after injection of amphetamine into cats [8], in the bioelectrical activity of whose brain the fast component of the Θ-rhythm is absent [1], suggests that the EEG changes examined in the present study are connected not only or, indeed, not so much with the motor excitation of the rat under the influence of amphetamine,

TABLE 1. Changes in Absolute Power of Fourier Power Spectra of Sensomotor Cortical EEG of Noninbred Albino Rats and Cottontail Rats under the Influence of Diazepam, Amphetamine, and Chlorpromazine (in % compared with background)

Substance (dose, in mg/kg, in parentheses)	Time of maximal effect, min	Bands of spectrum, Hz					Total power	Δθ	Shift of peak (max), Hz	$\frac{\Delta\theta}{\theta}$
		0,25—4,5 δ	5,0—9,0 θ	9,5—13,0 α	13,5—20,0 β_1	20,5—32,0 β_2				
Diazepam (1)	30	—16	+1	+9	+9	+10	+15	+5	← 1,0	+3
Diazepam (5)	15	+55*	+33*	+68*	+195*	+318*	+145*	+44*	← 1,5	+15*
Diazepam (5) – cottontail rats	30	+150*	+7	+18	+326*	+325*	+154*	+39*	← 2,5	+47*
Amphetamine (1)	45	—3	+41*	—4	—14	—1	+10	+154*	→ 1,0	+48*
Amphetamine (5)	45	+8	+120*	+15	—21*	—32*	+34*	+235*	→ 1,5	+52*
Chlorpromazine (1)	30—60	+137*	+117*	+84*	+86*	+19	+90*	(+84)	—	—8

Legend. ΔΘ) Peak in Θ-band (natural band 1.5 Hz), can be distinguished only formally under the influence of chlorpromazine. Arrows in "shift of peak" column indicate direction of shift: to the left—toward low frequencies, to the right—toward higher frequencies. *p < 0.05.

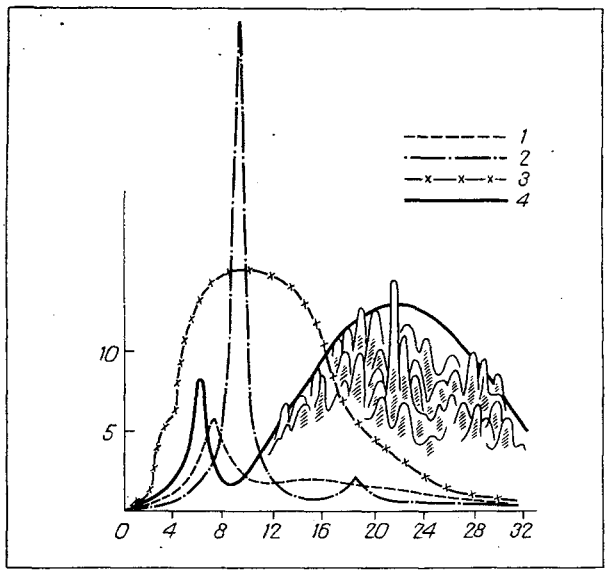

Fig. 2. Diagram showing changes in PS of sensomotor cortex of noninbred rat (1) under the influence of amphetamine (2), chlorpromazine (3), and diazepam (5 mg/kg, 15 min after injection) (4). Abscissa, frequency bands of PS (in Hz); ordinate, power of PS (in μV^2/Hz).

but probably reflect the general level of excitation of the animal's CNS. The importance of these changes is emphasized by the existence of mutually exclusive effects of stimulants and tranquilizers at other levels. Contrary to benzodiazepines, for instance, amphetamine reduces the number of punishable takings of water in conflicting situations [5] and, according to clinical observations, it can induced a state of dysphoria in man with the development of anxiety [11]. It follows from the facts described above that changes in the Θ-band of PS observed under the influence of diazepam are specific for the benzodiazepines and may perhaps play an important role in their anxiolytic action. In that case, a direct relationship must evidently exist between the degree of the effect at the EEG level (a change in frequency of the Θ-rhythm) and at the behavioral level (the number of punishable takings of water in a conflicting situation). The existence of such correlation is determined primarily by the fact that diazepam, in a dose as low as 1 mg/kg, has a distinct anxiolytic action in the conflicting situation test, increasing the number of punishable takings of water to 6.3 ± 0.43 (control 1.86 ± 0.22, $p < 0.05$), but not inducing sedation under these circumstances. PS of the cortex and hippocampus of the animals under these circumstances were characterized by changes virtually only in the Θ-band. With an increase in the dose of diazepam the anxiolytic effect was strengthened and the peak of the Θ-band of PS shifted toward lower frequencies.

The clearest correlation between the EEG and behavioral effects was observed when the effect of diazepam was compared in noninbred albino and cottontail rats (Fig. 1, II, c, d; Table 1). In the conflicting situation test diazepam

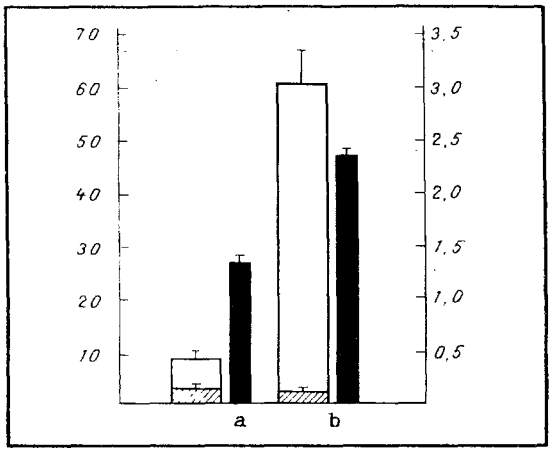

Fig. 3. Correlation between effects of diazepam (5 mg/kg) at the EEG level (shift of peak in the Θ-band of PS of the sensomotor cortex) and at the behavioral level (number of punishable takings of water in the conflicting situation test) in noninbred (a) and cottontail (b) rats. Unshaded columns—number of punishable takings of water (experiment), oblique shading—the same (control), columns shaded black—value of shift of peak of Θ-band of PS toward lower frequencies. Ordinate: on left, number of punishable takings of water, on right, value of shift of peak of PS (in Hz).

in a dose of 5 mg/kg increased the number of punishable takings of water by the albino rats to 8.73 ± 0.93 and in the cottontail rats to 60.75 ± 6.36 ($p < 0.01$). In the control there was virtually no difference between these species of rats: 3.33 ± 0.23 (albino rats) and 2.60 ± 0.35 (cottontail). In PS of the sensomotor cortex the shift of the peak frequency into the Θ-band under the influence of diazepam (5 mg/kg) was 1.33 ± 0.09 in noninbred rats and 2.33 ± 0.09 Hz in cottontail rats toward lower frequencies ($p < 0.05$) (Fig. 3). The difference between the background spectra was not significant (Fig. 1, II, c, d).

The results suggest that the decrease in frequency of the Θ-rhythm observed in the rat brain under the influence of diazepam is a most important effect of benzodiazepines at the EEG level, and it is evidently most closely connected with the anxiolytic action of preparations of this class. So far as the increase in β-activity (12-32 Hz) is concerned, although its genesis and physiological importance have not been adequately studied, it seems unlikely that such gross changes in brain electrical activity would be selectively linked with the anxiolytic effect.

LITERATURE CITED

1. T. L. Bennet, Functional Role of Brain Electrical Processes [in Russian], Moscow (1977), pp. 232-239.
2. T. A. Voronina, Yu. I. Vikhlyaev, T. L. Garibova, et al., Phenazepam [in Russian], Kiev (1982), pp. 87-169.
3. T. A. Voronina, S. V. Krapivin, and N. N. Bogdanov, Vest. Akad. Med. Nauk SSSR, No. 2, 17 (1987).
4. B. H. Bland, Prog. Neurobiol., 26, No. 1, 1 (1986).
5. L. Cook and A. B. Davidson, The Benzodiazepines, New York (1973), pp. 327-345.
6. W. Dimpfel, M. Supler, B. Nickel, and U. Tibes, Neuropsychobiology, 15, 101 (1986).
7. C. L. Ehlers and J. M. Havstad, Psychopharmacol. Bull., 18, No. 3, 43 (1982).
8. F. Gekiere, G. Allegre, F. Brindeau, and P. Borenstein, Pathol. Biol., 28, No. 1, 63 (1980).
9. A. Glatt, T. Duerst, B. Mueller, and H. Demieville, Neuropsychobiology, 9, No. 2-3, 163 (1983).
10. W. M. Herrmann, Electroencephalography in Drug Research, New York (1982), pp. 249-351.
11. M. R. Mandel and G. Klerman, Principles of Psychopharmacology, New York (1978), pp. 537-551.
12. J. Yamamoto, Jpn. J. Pharmacol., 37, 227 (1985).