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Antianxiety Effect of Ethyl Loflazepate, CM6912, Assessed by Frontal Midline Theta Activity in Man

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Abstract The theta activity which appears in the frontal midline area during performance of mental tasks has been designated as Fm θ. In the present study, the potency of CM6912 as an anxiolytic was investigated using Fm θ as compared with diazepam. Sixteen male university students were given placebo, diazepam 5 mg, CM6912 2 mg and 4 mg, in a double-blind, crossover design. Scores were made on the state anxiety scale of Spielberger's State Trait Anxiety Inventory (STAI), EEGs were recorded before and during performance of an arithmetic addition. The test was repeated twice, before and 1 hr after drug administration. Placebo increased Fm θ ; however, diazepam, CM6912 4 mg and 2 mg only slightly influenced Fm θ. Scores of state anxiety were reduced by diazepam, placebo and CM6912 4 mg, and only slightly by CM6912 2 mg. Task performance was increased by placebo, but was not affected by diazepam and both doses of CM6912. These results suggest that CM6912 possesses a rather mild anxiolytic effect, and that Fm θ might be a useful tool in predicting the clinical efficacy of anxiolytic drugs in normal humans.

Key words: benzodiazepines, diazepam, CM6912, antianxiety effect, frontal midline theta activity (Fmθ)

Introduction

It is a very difficult problem to predict the clinical effects of anxiolytic or hypnotic drugs from data obtained from laboratory animals and normal human beings. We have previously presented a new procedure for solving this problem, which involves a particular kind of EEG activity[1].

The distinct theta rhythm appearing in the frontal midline area during performance

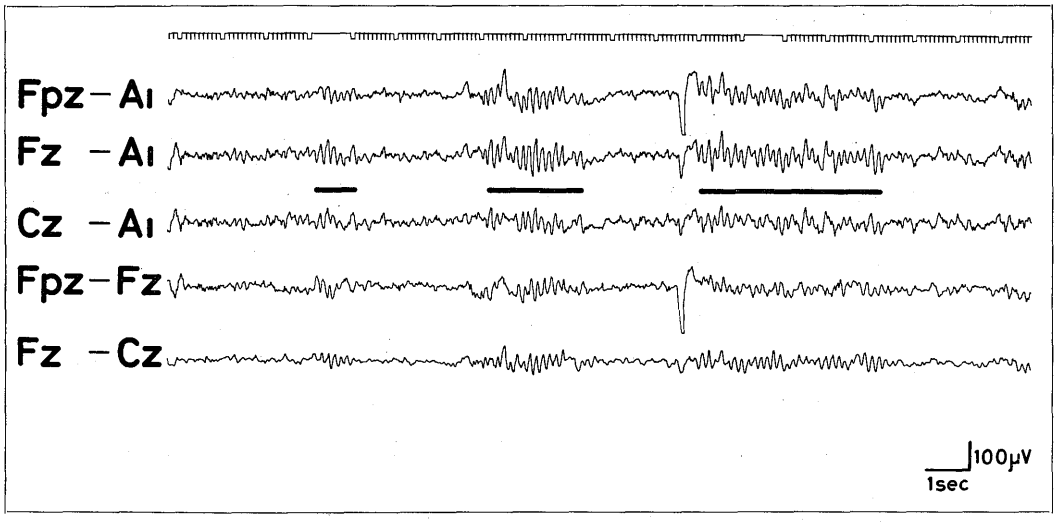

Fig. 1 A sample of EEG during performance of arithmetic addition. Fmθ is underlined.

of mental tasks has been called "Fmθ"[2]. We have reported that the appearance of Fmθ (Fig. 1) is closely related to the personality of the subject; Fmθ is more likely to appear in extraverted, less neurotic and less anxious subjects[3]. On the other hand, Fmθ is less likely to appear or does not appear at all in introverted, more neurotic and more anxious subjects[3]. In addition, when placebo, diazepam, amobarbital and methylphenidate were given to subjects who had failed to show any appearance of Fmθ, we found that Fmθ appeared following drug administration and we proposed that relief from anxiety might be involved in the appearance of Fmθ[4]. These studies suggest that Fmθ could be a useful tool in predicting the clinical effects of anxiolytic drugs in normal humans.

The present study was undertaken to ascertain the clinical potency of CM6912 as an anxiolytic using Fmθ as compared with diazepam. CM6912 is a new benzodiazepine derivative and is generally regarded as an anxiolytic agent[5].

Materials and Methods

Subjects

Originally, 42 male university students had an EEG recorded during the performance of an arithmetic addition test for 5 min on 3 consecutive days. Each test was started at 13:00 hours each day. We selected 16 subjects who failed to show any appearance of Fmθ over these 3 days. Their ages ranged from 20 to 25 years (mean age : 23.2 years), and their resting EEGs were normal.

Mental Task

The subjects performed the Uchida-Kraepelin test[6], a test of continuous arithmetic addition. They were requested to add each set of overlapping sequential pairs of single digit numbers. In the present study, the test paper was lengthened to a band-paper 4 cm high and 240 cm wide on which 8 lines containing 464 numerals each were printed. Thus, the continuous addition could be executed without changing a line.

Psychological Test

Spielberger's State Trait Anxiety Inventory (ST AI)[7] was used. This test consisted of separate self-report scales for measuring two distinct anxiety concepts; state anxiety (STAI-Ⅰ) and trait anxiety (STAI-Ⅱ). The higher the score on each scale, the stronger is the anxiety level.

EEGs

EEGs were recorded before and during performance of the Uchida-Kraepelin test from disc electrodes placed on Fpz, Fz, Cz (10-20 electrode system) in both unipolar and bipolar fashion using the electroencephalograph (EEG-4214) with a paper speed of 1.5 cm/sec, a time constant of 0.3 sec, and a sensitivity of 7 mm=50 μV. In this study, with the exception of the 60 Hz high-cut filter, no filter was used. Resting EEGs were recorded simultaneously on an analog tape recorder (FRC-1402N) and analyzed for a requirement of a power spectrum by a digital computer (HR-1000) using the Fast Fourier Transform. Each 10-sec epoch was analyzed with 102.4 points/sec, which permitted the analysis of high frequencies up to 50 Hz. Time samples of 60 sec were used.

Drugs

CM6912, the chemical structure of which is ethyl 7-chloro-5-(o-fluorophenyl)-2, 3-dihydro-2-oxo-1H-1, 4-benzodiazepine-3-carboxylate, is a benzodiazepine derivative. Two doses of CM6912, 2 mg and 4 mg; diazepam 5 mg; and placebo were used. Tablets containing each dose of the drug or placebo were prepared identically. Drug doses were determined by reference to the data in animal studies[5] and human studies[8].

Experimental Procedure

The subjects, with scalp electrodes attached, were instructed to perform the Uchida-Kraepelin test as rapidly and accurately as possible. Subjects then completed the STAI-I and rested for 3 min with eyes closed. They then performed the arithmetic addition task for 5 min. Subsequently, subjects completed the STAI-II and were given one of the drugs or placebo in the form of a double-blind, crossover design. One hour after drug administration, the tests were repeated. Seven days were provided as a washing out period.

EEGs were recorded continuously throughout the entire procedure, except for the 60-min rest period following drug administration. Only theta activity recorded from the Fz electrode during performance of the Uchida-Kraepelin test was defined as Fm θ. Criteria for Fm θ were: (1) rhythmical-sinusoidal configuration, (2) markedly higher amplitude as compared with background activity, and (3) duration exceeding 1 sec.

Statistical Analysis

Student's t-test was used for statistical significance testing.

Results

Resting EEGs

In the frontal area (Fig. 2), the power value increased in a high frequency area, i.e., β band. Placebo increased the power of 1 and 9 Hz, and diazepam increased the power of 9 Hz, while both CM6912 2 mg and 4 mg increased the power of 1 Hz. Power values between 2 and 8 Hz were unchanged or decreased after administration of all the drugs.

All drugs showed higher values of the power of the following frequencies when compared with placebo: 10-23 Hz (diazepam 5 mg) and 15-23 Hz (CM6912 4 mg). However, the power of middle-fast β activity of CM6912 2 mg showed a similar spectrum to that of placebo. When compared with placebo, the value of 9 Hz and 35-50 Hz was decreased by CM6912 2 mg; and the value of 5-10 Hz and 31-50 Hz was decreased by CM6912 4 mg; moreover the value of 2-10 Hz was lower or not different under both diazepam and CM6912 treatment.

Similar changes in the power spectrum were observed in the central area. Fm θ did not appear in any subjects during the 3-min rest before the test.

Appearance of Fm θ

Fm θ appeared or increased in 9 out of 16 subjects, however, this activity decreased in one subject with the administration of diazepam. When placebo, diazepam 5 mg, CM6912 2 mg or 4 mg was administered, Fm θ appeared or increased in 6 subjects, 6 subjects, 6 subjects, and 4 subjects, respectively (Table 1).

The mean appearance time of Fm θ was longest with placebo, followed by diazepam 5 mg, CM6912 4 mg, and CM6912 2 mg (Fig. 3). The difference in appearance time of Fm θ between placebo and CM6912 2 mg reached marginal statistical significance ($P<0.10$).

STAI Scores

CM6912 2 mg minimally affected state

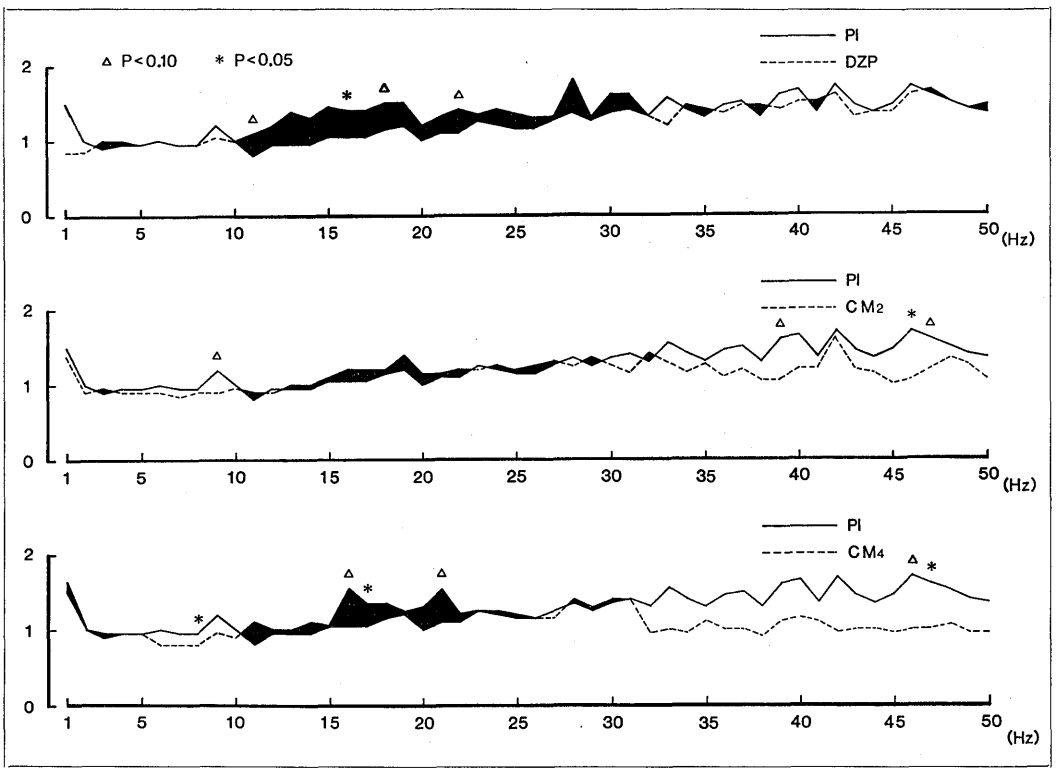

Fig. 2 The power spectrum of resting EEG from the frontal area.

The power spectrum was computed from 1 Hz to 50 Hz of unipolar leads in the Fz and Cz. The power spectrum of diazepam, CM6912 2 mg and 4 mg is compared with that of placebo, respectively. Pl, placebo; DZP, diazepam 5 mg; CM2, CM6912 2 mg; CM4, CM6912 4 mg. Ordinate: the values obtained by dividing the value after the drug administration by that before medication. Abscissa: frequencies of EEG. The shaded space represents the frequency where the value of each drug is higher than that of placebo, and the unshaded space represents the frequency where the opposite result was obtained.

anxiety scores following the administration (Fig. 3). However, diazepam 5 mg, placebo and CM6912 4 mg decreased anxiety scores, with diazepam 5 mg decreasing anxiety scores more clearly than placebo or CM6912 4 mg.

The mean trait anxiety scale score for all subjects in the present study was 48.3. This is indicative of a higher level of anxiety than that reported in a sample of 253 college students by Spielberger et al.[7].

Uchida-Kraepelin Test

Placebo increased the performed tasks, while diazepam 5 mg and both doses of CM 6912 failed to affect the task performance (Fig. 3).

There were no differences between drugs in terms of the number of errors committed.

Discussion

CM6912 is a new anxiolytic-sedative compound with a chemical structure of the ben-

Table 1 Appearance Time of Fm θ

		Drug			
		P1	DZP	CM2	CM4
Subjects	A	16.1	5.0	1.1	9.1
	B	0	0	0	0
	C	0	0	0	0
	D	0	0	0	0
	E	13.4	−1.8	6.6	4.5
	F	2.6	0	0	0
	G	0	0	3.6	1.1
	H	0	7.0	1.3	0
	I	0	0	0	0
	J	3.2	2.5	1.0	0
	K	0	0	0	0
	L	6.5	4.3	1.1	2.9
	M	0	0	0	0
	N	0	0	0	0
	O	3.6	5.5	0	0
	P	0	2.1	0	0

Appearance time of Fm θ obtained by subtracting the value before the drug administration from that after medication, respectively. Ordinate: the subjects are arranged from A to P. P1, placebo; DZP, diazepam 5 mg ; CM2, CM6912 2 mg: CM4, CM6912 4 mg.

zodiazepine derivatives. CM6912 shows a profound anticonvulsant effect, but has weaker muscle relaxant and anaesthesia-potentiating effects than those of diazepam[9),10)]. Some reports concerned with its antianxiety effect suggest that CM6912 has a weaker effect than that of diazepam[9)] while others indicate that it has a stronger effect than that of diazepam[10)]. Moreover, CM6912 possesses a longer duration of action as shown in normal humans[11)]. Breimer et al.[12)] reported that a short duration of action would be a favorable characteristic for a hypnotic, while a long duration of effect would favor an anxiolytic agent. Accordingly, these findings suggest that CM6912 could be considered as an anxiolytic rather than as a hypnotic agent, however, the potency of its antianxiety effect could not be established.

CM6912 4 mg showed an EEG profile wherein middle-fast β activity increased, while α and θ activities decreased. However, its effect on the β activity was weaker than that of diazepam. CM6912 2 mg decreased only α activity but failed to affect β activity. A well known anxiolytic, diazepam, rapidly increases β activity, decreases α activity, and slightly decreases δ activity in man[13)]. Saletu[14)] reported that although anxiolytic-sedatives decrease α activity and increase middle-fast β activity, those compounds possessing anxiolytic properties typically do not show significant increases in θ and δ activity. Drugs possessing a potent hypnotic effect exhibit an additional increase in slow wave activity. When considered in light of these findings, the EEG profile observed following CM6912 administration appeared to be more similar to that of an anxiolytic agent, whereas the potency of CM 6912 might be less than that of diazepam.

The effects of diazepam on the three indices used in this study (Fm θ, state anxiety scale, and arithmetic task performance) are consistent with previous results[4)], wherein a typical anxiolytic, diazepam 5 mg, increased Fm θ, decreased state anxiety scores, and slightly decreased or did not affect arithmetic task performance. Amobarbital 80 mg, a potent hypnotic agent, slightly increased the amount of Fm θ, slightly decreased anxiety scores, but markedly decreased the arithmetic task performance[4)]. CM6912 2 mg slightly increased the appearance of Fm θ and sligh -tly decreased state anxiety, while it did not affect the arithmetic task performance. CM 6912 4 mg slightly increased Fm θ and decreased state anxiety, while it did not affect the arithmetic task performance. Placebo increased the amount of Fm θ, slightly decreased state anxiety scores, and increased the arithmetic task performance. We reported previously that placebo might function as an anxiolytic drug without effects on the

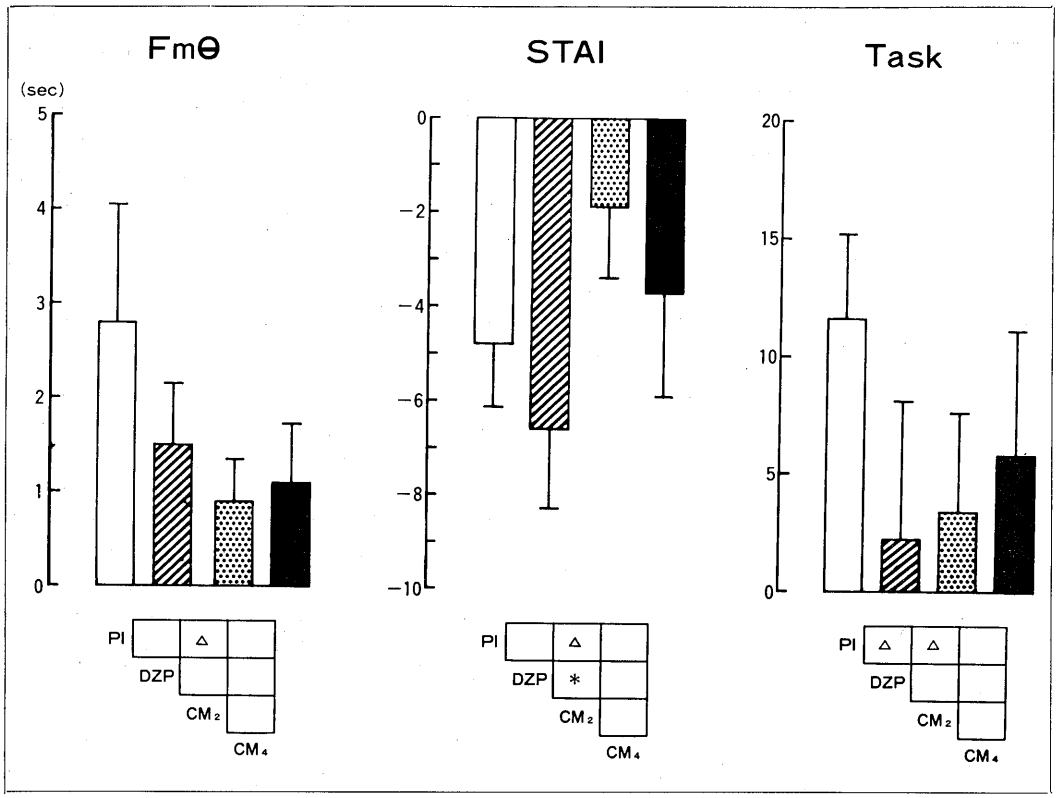

Fig. 3 Effects of the drugs on appearance time of Fmθ, STAI scores and arithmetic task performance.

The ordinate indicates the mean ± SEM of the values obtained by subtracting the value before the drug administration from that after medicatien. ☐ : placebo (Pl); ▨ : diazepam 5 mg (DZP); ▩ : CM6912 2 mg (CM2); ■ : CM6912 4 mg (CM4). Statistical significance levels are shown at the foot of the figure. △P<0.1; * P<0.05.

arousal level in anxious normal humans[4]. In general, benzodiazepines lower the arousal level of humans[15]. We also pointed out that the arithmetic task performance reflects the arousal level of the subject[4]. Therefore, the present results indicate that the behavioral effects of CM6912 are similar to those of diazepam but not to those of barbiturates. However, the effects of CM6912 on Fmθ and state anxiety were weaker than those of diazepam. Moreover, the effect of both doses of CM6912 on the arithmetic task performance was also weaker than that of diazepam. The reason for this might be that CM6912 possesses a mild antianxiety effect. Another possibility would be that the drug action of CM6912 occurs within 1 hr but peaks at 2.5-3 hr after[8], that is, the 60-min rest period after CM6912 administration in this study is not enough to affect the three indices. However, the present data suggest that CM6912 possesses an anxiolytic effect rather than a hypnotic effect in man, and that CM 6912 has a mild anxiolytic effect. This interpretation is also supported by the resting EEG results.

Although more definitive approaches such as investigating antianxiety effects of CM 6912 in anxious patients are necessary, the present study indicates that CM6912 could be applied to neurotic, psychosomatic and aged patients, and that Fm θ could be a useful tool in predicting the clinical efficacies of anxiolytic drugs in normal humans.

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