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Electrophysiological aspects of benzodiazepine antagonists, Ro 15-1788 and Ro 15-3505

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1 The comparative action of two specific benzodiazepine antagonists, Ro 15-1788 and Ro 15-3505 was examined in six healthy volunteers. Medication was given i.v. in a double-blind cross over pattern, and EEG was recorded throughout each experimental session. Ten minutes after the injection of one of the antagonists or placebo, midazolam was injected in incremental doses until first signs of drowsiness appeared in the EEG.
2 The EEG was computer analyzed, using adaptive segmentation and time-dependent clustering. Continuous power profiles for various frequency bands, as well as power ratios for the physiological frequency bands (e.g. sigma/alpha power ratio) were generated.
3 It has been found that sigma/alpha power ratio was the most sensitive parameter detecting early effects of midazolam on the EEG signal, thus enabling a semi-quantitative titration of the antagonists by midazolam.
4 Ro 15-1788 in doses of 5 mg i.v. was counteracted on average by 7.3 mg midazolam.
5 From the EEG analysis it has been found that Ro 15-3505 was at least 4-5 times more potent than Ro 15-1788.

Keywords benzodiazepines Ro 15-3505 Ro 15-1788 electrophysiology

Introduction

There have been in recent years a significant increase in the use of benzodiazepines in anaesthetic practice, in premedication, induction, and maintenance of anaesthesia (e.g. flunitrazepam, midazolam). The benzodiazepines produce their main pharmacological and therapeutic effects on the central nervous system by interacting with a specific benzodiazepine receptor (Möhler *et al.*, 1980), this receptor is a membrane protein localized in synapses utilizing GABA as a transmitter (Costa *et al.*, 1975). Recently (Hunkeler *et al.*, 1981), a group of imidazodiazepines (Ro 15-1788 and Ro 15-3505) have been shown to inhibit specifically the central action of the benzodiazepines, by competitive interaction at the receptor level. Such selective benzodiazepine antagonists have a highly potential use in controllability of anaesthesia and the post-operative period, and as antidotes in cases of overdosage of benzodiazepines.

The aim of the present study was to examine electrophysiologically the comparative action of two benzodiazepine antagonists, Ro 15-1788 and Ro 15-3505 in opposing the action of midazolam, a short acting benzodiazepine hypnotic used in anaesthesia. The evaluation was based on computer analysis (Gath & Bar-On, 1980; Gath *et al.*, 1983) of the EEG tracings recorded during the clinical trial.

Methods

Trial protocol

Six healthy male volunteers participated in the

clinical trial. The volunteers were informed about the substances being investigated and gave their written consent for the participation. The study was approved by the Technion ethical committee. The mean age of the volunteers was 23.3 ± 1.5 years with a range of 21–25 years. Pertinent subject information is given in Table 1.

The trial was conducted in a double-blind cross-over randomized pattern, each subject going through five experimental sessions, Ro 15-1788 + placebo, Ro 15-1788 + midazolam, Ro 15-3505 + placebo, Ro 15-3505 + midazolam and placebo + midazolam, with 3 days interval between consecutive sessions.

During the experimental session the subjects were in a recumbent position, and were instructed to keep awake throughout the session. At the beginning of each session a venfion catheter was inserted into the cubital vein, and 5 mg of Ro 15-1788, or 1.5 mg of Ro 15-3505, or placebo (the vehicle) was injected i.v. EEG recording (C_3–A_2; P_3–A_2; P_4–A_1) was carried out throughout the whole session, starting 10 min prior to the first injection (antagonists or placebo). Ten minutes after the first injection midazolam or placebo was injected i.v. in incremental doses (solution of midazolam 0.5 mg/0.1 ml) until first signs of drowsiness appeared in the EEG, but nevertheless not exceeding 2 ml (equivalent to 10 mg midazolam). The subjects were not aware of the time of midazolam injection. The whole session lasted around 3 h, and the signals recorded on FM magnetic tape recorder for further analysis.

Data analysis

The EEG signal was sampled on a PDP 11/55 minicomputer. Adaptive segmentation was carried out on the EEG signal, using the autoregressive model (Gersch, 1970; Makhoul, 1975; Bodenstein & Praetorius, 1977; Gath & Bar-On, 1980), dividing the signal into short quasi-stationary segments of uneven length, homogenous in their spectral contents.

The autocorrelation of the prediction error for the first 2 s of the signal ('fixed window') was compared to the autocorrelation of the prediction error for a window moving sequentially along the signal samples ('moving window'). A threshold coefficient, T, used for the segmentation procedure was defined as follows:

$$T = \sum_{K=0}^{M} \left[\frac{r_f(k) - r_m(k)}{r_f(o)} \right]^2$$

where $r_f(o)$ is the zero-lag error autocorrelation for the fixed window, and $r_f(k)$ and $r_m(k)$ are the k-lag error autocorrelations for the fixed and moving windows, respectively. Whenever this threshold, which is a measure of the spectral error between the real and predicted signal, exceeded a preset value, the segment was closed, and its features (the prediction coefficients, its total power and its length) stored on the disk for the next phase of the analysis.

These 'primary' segments, each a few seconds long, were submitted in the next phase of the analysis to time-dependent clustering, merging together consecutive segments with similar spectra, whenever the Euclidian distance between their respective spectra did not exceed a preset empirical threshold. The power spectrum of a 'secondary' segment was computed as a weighted sum of the spectra of each of the primary segments, considering the different lengths of the primary segments. The secondary segments were represented by the power in the various physiological frequency bands, delta, theta, alpha, etc.

Thus, unlike conventional spectral analysis carried out on predetermined EEG segments of constant length, adaptive segmentation which is based on the spectral error between the real and the predicted signal, results in segment boundaries determined by the signals inherent properties, in addition, calculation of the power

Table 1 Subjects' characteristics

Subject	*Sex*	*Age (years)*	*Weight (kg)*	*Height (cm)*	*Ro 15-1788 (mg/kg)*	*Ro 15-3505 (mg/kg)*
1	M	24	84	188	5.95×10^{-2}	1.19×10^{-2}
2	M	25	84	186	5.95×10^{-2}	1.19×10^{-2}
3	M	22	88	181	5.68×10^{-2}	1.14×10^{-2}
4	M	21	70	170	7.14×10^{-2}	1.43×10^{-2}
5	M	24	70	180	7.14×10^{-2}	1.43×10^{-2}
6	M	24	60	178	8.33×10^{-2}	1.67×10^{-2}
Mean		23.3 ± 1.5	76.0 ± 10.9	180.5 ± 6.4	$(6.70 \pm 1.02) \times 10^{-2}$	$(1.34 \pm 0.2) \times 10^{-2}$

Table 2 Amount of midazolam i.v. used to titrate benzodiazepine antagonists

Subject	*Ro 15-1788 5 mg* *Midazolam*		*Ro 15-3505 1.5 mg* *Midazolam*
	mg	*mg/kg*	*mg*
1	6	7.1×10^{-2}	> 10 mg
2	8	9.5×10^{-2}	> 10 mg
3	8	9.1×10^{-2}	> 10 mg
4	10	1.4×10^{-2}	10 mg
5	6	8.6×10^{-2}	> 10 mg
6	6	1.0×10^{-2}	> 10 mg
Average	7.3 ± 1.6	$(9.7 \pm 2.3) \times 10^{-2}$	> 10 mg

spectrum of the EEG signal through the autoregressive model gives a better estimate of the spectrum than that obtained by the FFT. A smaller standard error is achieved for the same spectral resolution without the necessity for further averaging (Bendat & Piersol, 1971; Blinowska *et al.*, 1981).

The output of the data analysis consisted of continuous power profiles for the various EEG frequency bands, as well as power ratio profiles (ratios of absolute power) for the physiological EEG frequency bands such as sigma/alpha, alpha/theta etc.

For a more detailed description of the methods of data analysis the reader is referred to Gath & Bar-On (1980), Gath *et al.* (1981, 1983).

Results

Titration of Ro 15-1788 with midazolam

It has been found that after injection of 5 mg of Ro 15-1788 doses of 6-10 mg of midazolam i.v. caused changes in the EEG signal in all six volunteers (Table 2). These changes were in the form of increase in the power content of the sigma frequency band (13.0–15.0 Hz), in addition to a decrease in the alpha power (8.0–13.0 Hz) and some increase in the theta-delta power (0.5–8.0 Hz).

Figure 1 shows the effect of midazolam alone on the EEG (a session of placebo + midazolam). There is a marked increase in the sigma/alpha power ratio, with a latency of approximately 100 sec after the inejction of midazolam. Comparison of Figures 2 and 3 in the same subject (sessions Ro 15-1788 + placebo, and Ro 15-1788 + midazolam, respectively) demonstrates in Figure 3 an increase in the sigma/alpha power ratio after the injection of 6 mg midazolam, similar to the changes in Figure 1, but less pronounced. The effect of midazolam on the sigma/alpha power ratio lasted some 20 min. The decrease in the alpha/delta power ratio due to the effect of midazolam on the EEG signal lags after the increase in the sigma

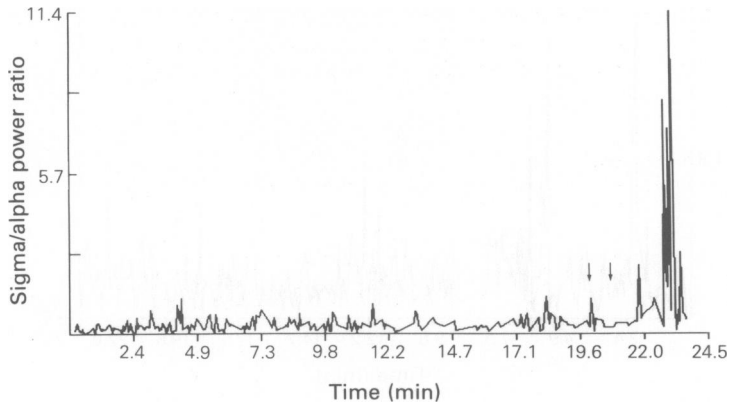

Figure 1 Sigma/alpha power ratio profile. Subject No. 5, session: placebo + midazolam. At 10 min placebo was injected. Starting at 20 min midazolam (arrows) was injected.

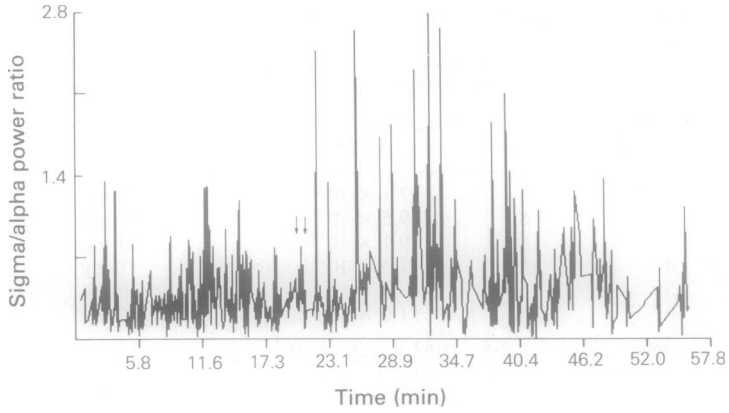

Figure 2 Sigma/alpha power ratio profile. Subject No. 5, session: Ro 15-177 + placebo. At 10 min the antagonist was injected. Starting at 20 min (arrow) placebo was injected.

power by approximately 80 s (Figure 4). Thus, the ratio between the sigma/alpha power was found to be the most sensitive parameter in detecting early changes in the EEG signal due to i.v. injection of midazolam, in the presence of 5 mg Ro 15-1788.

A summary of the results for the sigma/alpha power ratio, before and after the second injection (midazolam or placebo) for all the six subjects is given in Table 3.

Comparison between the cumulative power in the delta, theta, alpha, sigma and beta frequency bands calculated for EEG sections before the injection of Ro 15-1788 and 10 min after the inection (during sessions with Ro 15-1788 + placebo) did not reveal any significant changes (Figure 5).

Titration of Ro 15-3505 with midazolam

In five of the six volunteers given 1.5 mg of Ro 15-3505, no changes were detectred in the EEG signal after the inejction of 10 mg midazolam i.v., neither in the sigma/alpha power ratio nor in the power of the theta or delta frequency bands (Table 2). In one session (subject No. 4), increase in the sigma/alpha power ratio could be seen after the injection of 10 mg midazolam. However, this effect had a long latency of some 7 min (Figures 6 and 7).

A summary of the results for the sigma/alpha power ratio, before and after the second injection (midazolam or placebo), in the presence of 1.5 mg Ro 15-3505 is given in Table 3.

Comparison between the cumulative power

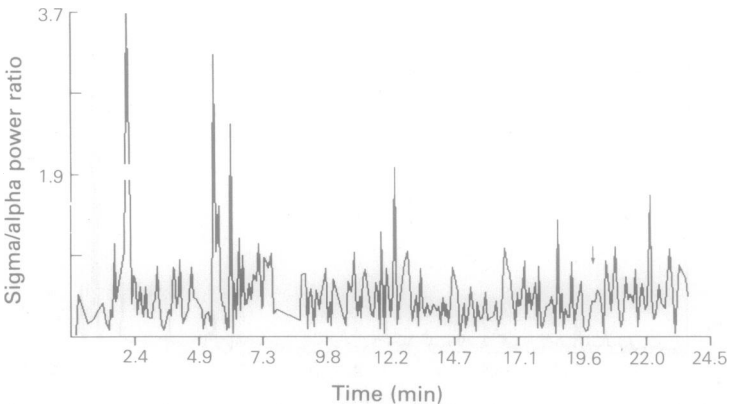

Figure 3 Sigma/alpha power ratio profile. Subject No. 5, session: Ro 15-1788 + midazolam. At 10 min the antagonist was injected. Starting at 20 min midazolam (arrows) was injected.

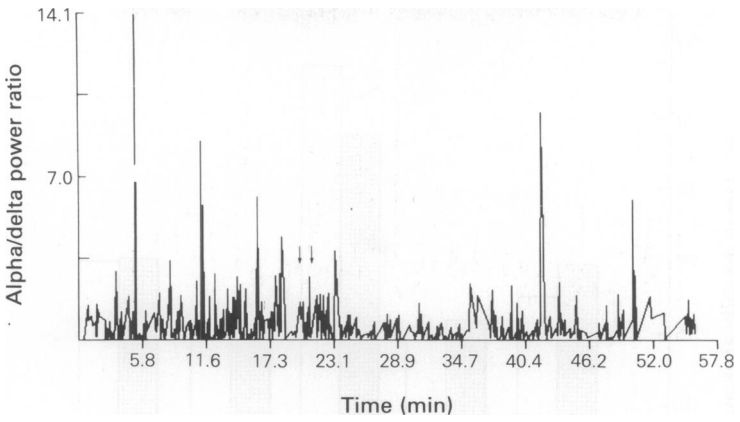

Figure 4 Alpha/delta power ratio profile. All the other details as in Figure 3.

in the various physiological frequency bands for EEG sections prior to and after the injection of the antagonist did not show any significant differences.

Discussion

In animal experiments (Möhler *et al.*, 1981) Ro 15-1788 has been shown to be a selective benzodiazepine antagonist, with binding potency to the central receptors similar to that of diazepam. Ro 15-1788 transiently abolished the increase in power in the beta frequency range in the rat electrocorticogram caused by flunitrazepam (Polc *et al.*, 1981).

In humans, clinical studies have demonstrated Ro 15-1788 to antagonize the central benzodiazepine effects of a variety of benzodiazepines given orally or intravenously (Darragh *et al.*, 1981a, b; Bonn & Lauven, 1982; Laurian *et al.*, 1984). Using a conventional power spectral analysis (Bonn & Schwilden, 1982; Laurian *et al.*, 1984), it has been shown that the antagonist reversed the increase in power in the slow and fast frequency ranges caused by benzodiazepine medication.

In the present study we have tried to examine quantitatively the dose relationship between the benzodiazepine (midazolam) and the two antagonists, Ro 15-1788 and Ro 15-3505. Adaptive segmentation and time-dependent clustering (Gath & Bar-On 1980; Gath *et al.*, 1983) of the EEG recorded during the clinical trial have shown that the power ratio sigma/alpha was the most sensitive parameter detecting the early effects of midazolam on the EEG, thus enabling a semi-quantitative titration of the antagonists by midazolam. It is also interesting to note that the increase in power in the delta-theta frequency range attributed to midazolam was

Table 3 Sigma/alpha ratio, average results for all six volunteers. (10 min of EEG signal)

	Base line	*Before second injection*	*After second injection*
Ro 15-1788 + midazolam	0.325 ± 0.049	0.357 ± 0.047	*0.511 ± 0.141
Ro 15-1788 + placebo	0.376 ± 0.060	0.354 ± 0.067	0.347 ± 0.051
Placebo + midazolam	0.353 ± 0.021	0.385 ± 0.039	*0.936 ± 0.254
Ro 15-3505 + midazolam	0.329 ± 0.061	0.355 ± 0.039	0.387 ± 0.078
Ro 15-3505 + placebo	0.360 ± 0.033	0.336 ± 0.060	0.306 ± 0.067

* $P < 0.05$

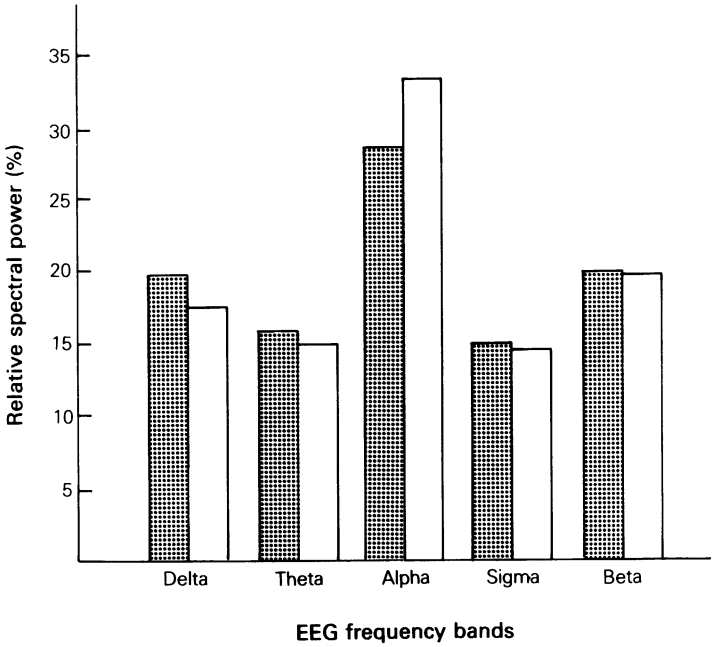

Figure 5 Comparison of the cumulative relative power in the various EEG frequency bands before (dotted histogram), and after (blank histogram) injection of Ro 15-1788. Subject No. 5, session: Ro 15-1788 + placebo. Analysis of 8 min long sections of the EEG.

shown to lag after the initial increase of power in the fast frequency range.

It has been found that an average dose of 7.3 mg midazolam counteracted the presence of 5 mg of Ro 15-1788 (both drugs given i.v.), whereas on average 10 mg of midazolam in the presence of 1.5 mg of Ro 15-1788 did not cause any significant EEG changes. Thus, on a milligram per milligram basis Ro 15-3505 has been demonstrated to be at least 4–5 times more potent than Ro 15–1788.

The findings in subject No. 4 during the session with Ro 15-3505 + midazolam (a somewhat late increase in the power ratio sigma/alpha) which could mean that 1.5 mg Ro 15-3505 has a shorter time effect than 5 mg Ro 15-1788 require confirmation by further studies.

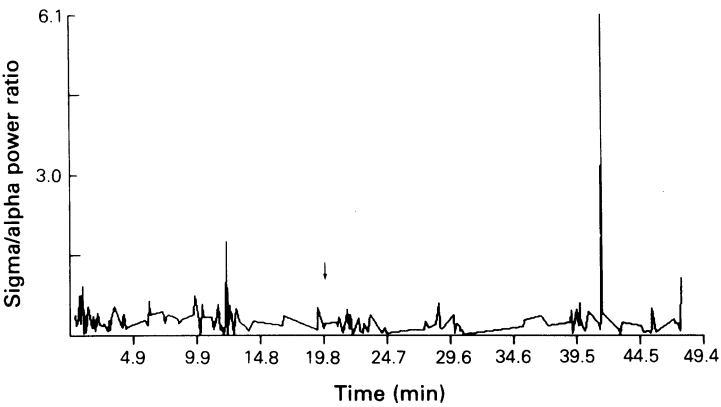

Figure 6 Sigma/alpha power ratio profile. Subject No. 4, session: Ro 15-3505 + placebo. At 10 min the antagonist was injected. Starting at 20 min (arrow) placebo was injected.

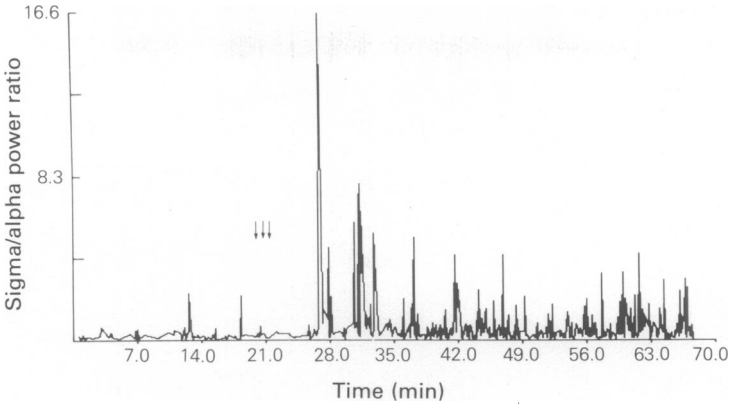

Figure 7 Sigma/alpha power ratio profile. Subject No. 4, sesssion: Ro 15-3505 + midazolam. At 10 min the antagonist was injected. Starting at 20 min midazolam (arrows) was injected.

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