International Clinical Psychopharmacology, 1986 **1**, 24–35

Evaluation of the Sedative Properties of PK 8165 (Pipequaline), a Benzodiazepine Partial Agonist, in Normal Subjects

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The sedative properties of two doses (50 and 150 mg) of a benzodiazepine partial agonist, PK 8165 (pipequaline), were compared to diazepam 10 mg and placebo in 12 normal volunteers. The assessment, performed before drug intake and 2 and 5 hours after drug intake, included a battery of visual analogue scales and standardized computerized tests (labyrinths, series of digits, colour test, and Zazzo test).

Results showed that, at low dose, PK 8165 is not only devoid of any sedative effect but presents psychostimulating properties. In contrast, diazepam 2 hours after intake and PK 8165 150 mg, 5 hours after drug intake induced a significant decrease in performance compared to placebo.

This study suggests that small doses of PK 8165 merits further development as an anxiolytic/psychostimulating drug devoid of sedative properties.

Introduction

The various clinical effects of benzodiazepines have been related to the presence of saturable, stereospecific and high affinity binding sites in the central nervous system (Squires and Braestrup, 1977; Möhler and Okada, 1977). Indeed, high correlations exist between the affinity of the various benzodiazepine compounds for these central receptors and their anticonvulsant and anticonflict properties in animals, as well as with their effective doses in humans. Moreover, the recent demonstration of benzodiazepine antagonists (e.g. Ro 15-1788), devoid of own pharmacological activity but which can completely prevent or antagonize benzodiazepine effects, as well as benzodiazepine inverse agonists (e.g. bêta-carbolines) which bind to benzodiazepine receptors but exert the opposite effect (anxiogenic and proconvulsant) favor the physiological role of benzodiazepine receptors in the modulation of anxiety (Insel *et al.*, 1984).

Until now, the anxiolytic and sedative properties of benzodiazepine compounds could apparently not be dissociated. However, some recently synthetized quinoline derivatives appear to present a dissociation of anticonflict and anticonvulsant properties in animals, suggesting a possible anxiolytic effect without untoward sedation in humans (Le Fur *et al.*, 1981; Gee *et al.*, 1983). Among this group PK 8165 (Fig. 1) seems especially promising. PK 8165 binds to benzodiazepine receptors with an affinity between diazepam and chlordiazepoxide. The minimal anticonflict dose of PK 8165 is close to chlordiazepoxide. In contrast, the sedative dose in mice is at least 4

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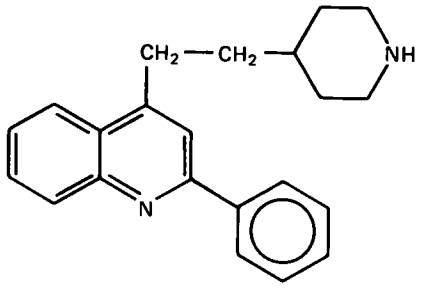

PK 8165

FIG. 1. Structural formula of PK 8165 (pipequaline).

times higher than chlordiazepoxide; moreover, PK 8165 is devoid of anticonvulsant activity (Le Fur, 1982).

Furthermore, in contrast to benzodiazepines, PK 8165 does not decrease the cGMP level of the Purkinje cells of the cerebellum, suggesting that PK 8165 could act on a subclass of benzodiazepine receptors not coupled to GABAergic receptors or on a GABA insensitive allosteric site of benzodiazepine receptors.

Therefore, the purpose of the present study was to confirm the lack of sedative properties of PK 8165 in human volunteers. Thus, we compared two doses of PK 8165 to a standard benzodiazepine, diazepam, and placebo, using visual analogue scales and automated assessment of vigilance. Indeed, on one hand, visual analogue scales are particularly sensitive to the subjective assessment of sedative effects and, on the other hand, automated testing involving micro-computers represents an accurate and sensitive method to measure changes in performance objectives (Sampson, 1983).

Subjects and Methods

The study was performed in 12 healthy volunteer members of the medical and paramedical staff of the University of Liège, Belgium. Subjects were 7 women and 5 men, with ages ranging from 26 to 38 years (mean age = 30·0 ± 14·2). Subjects with any drug intake during the previous 2 months were excluded from the study. Moreover, subjects were not allowed to use medication throughout the study period and were asked to keep their regular habits during this period. The subjects were fully informed of the aim and procedure of the study and gave their consent.

Methods

In four different sessions, at 2-week intervals, the subject took either PK 8165 50 mg, PK 8165 150 mg, diazepam 10 mg or placebo in double-blind conditions, with randomization of the order.

At 9.00 a.m., the patient had a baseline assessment which comprised a battery of visual analogue scales (Norris, 1971; Bond and Lader, 1974), composed of 15 100-mm lines, on which he had to indicate a mark, according to his current condition, between 15 pairs of opposite conditions (Table 1). Each score was calculated by measuring the distance (in mm) in each line from the "left" end.

TABLE 1. *Visual analogue scales*

Alert	Drowsy
Calm	Excited
Strong	Feeble
Muzzy	Clear-headed
Well-coordinated	Clumsy
Lethargic	Energetic
Contented	Discontented
Mentally slow	Quick witted
Tense	Relaxed
Attentive	Dreamy
Incompetent	Proficient
Happy	Sad
Antagonistic	Amicable
Interested	Bored
Withdrawn	Gregarious

Then, the subject had a computerized assessment of performance with the following tests:

4 labyrinths: the first 3 were of increasing difficulty, the last 1 having to be memorized before the test;

20 series of 9 digits to reorder arithmetically;

a colour test where the position and the colour of vertical bars painted in series of 10 had to be memorized;

a Zazzo test where 50 geometric forms appearing successively on the screen to be identified as similar or not to one of two reference forms (Zazzo, 1960).

Subjects were asked to respond as correctly and as fast as possible.

After this first assessment, the volunteers took the drug with 100 ml of water and were reassessed according to the same procedure 2 hours and 5 hours after drug intake.

Before each of the subsequent assessments, subjects were interviewed about the presence of possible subjective changes.

Data analysis

According to the procedure to analyze cross-over designs defined by Hills and Armitage (1979), we first compared the baseline evaluation of each subject on the four sessions.

Then we analyzed the differences between baseline and following assessments during each session. Therefore all results describe variations from the baseline instead of raw data; this transformation gives exactly the same results as covariance analysis using baseline as a covariate.

Finally, an analysis of variance (ANOVA) for repeated measurements was completed to point out direct effects (depending on the drugs and on the time-sequence within a session) and drug/session interaction. Some specific hypotheses were studied by contrasts between placebo and diazepam, placebo and PK 8165 50 mg, and PK 8165 50 and 150 mg; using BMDP software (Dixon, 1983).

In case of non homoscedasticity of variances, the degrees of freedom of the ANOVA procedures were decreased by the Huynh-Feldt method (1976).

In the digit and Zazzo tests, the learning effect was measured by the covariance between the logarithms of time used to reorder one sequence of digits and the logarithm of the sequence. A positive result corresponded to an increase of time from beginning to end, thus indicating tiredness, a negative result corresponded to a decrease of time, thus indicating learning.

Results

1. *Baseline*

The comparisons between all the baseline parameters did not show any statistically significant differences, demonstrating a similar condition of the subjects at the beginning of each of the four sessions.

2. *Visual Analogue Scales*

Four scales exhibited significant changes both 2 hours after diazepam intake and 5 hours after PK 8165 150 mg where subjects rated themselves more drowsy ($F(3,33)=4{\cdot}56$, $p<{\cdot}01$], more feeble [$F(3,33)=4{\cdot}92$, $p<{\cdot}05$], more lethargic [$F(3,33)=2{\cdot}48$, $p<{\cdot}05$] and more sad [$F(3,33)=2{\cdot}68$, $p<{\cdot}06$ trend]. The changes over time in the visual analogue scale "alert-drowsy" are represented in Fig. 2.

Moreover, subjects were more muzzy 2 hours after diazepam intake ([$F(3,33)=2{\cdot}94$, $p<{\cdot}05$] and more dreamy 5 hours after PK 8165 150 mg intake [$F(3,33)=2{\cdot}60$, $p<{\cdot}07$ trend].

In contrast, after PK 8165 50 mg, subjects rated themselves more strong, clear-headed (Fig. 3), energetic and quick-witted [$F(1,11)=4{\cdot}83$, $p<{\cdot}05$] both on the second and the third assessment.

The other visual analogue scales did not exhibit significant difference among drugs.

3. *Computerized Psychometric Tests*

a. Labyrinths In the easiest labyrinth, the mean time per movement decreased under both doses of PK 8165 [$F(1,11)=5{\cdot}77$, $p<{\cdot}05$] while there was no difference between placebo and diazepam (Fig. 4).

In the second labyrinth, of higher difficulty, the only difference was an increase of the mean time per movement at the second assessment after diazepam as compared to placebo [$F(1,11)=6{\cdot}19$, $p<{\cdot}05$]. However, in the third and most difficult labyrinth as well as in the last one (which had to be memorized), there was no significant difference among drugs in the mean time per movement.

The number of errors was not significantly different on all drugs for the 2 first labyrinths (the easiest). In contrast, the number of errors decreased slightly with diazepam and PK 8165 in the third and most difficult labyrinth [$F(1,11)=6{\cdot}77$, $p<{\cdot}05$] while it increased on the second assessment with diazepam and on the last assessment with PK 8165 150 mg in the last labyrinth [$F(1,11)=3{\cdot}19$, $p={\cdot}08$ trend].

b. Digit Test The assessment of learning showed significantly different results among drugs [$F(3,33)=3{\cdot}39$, $p<{\cdot}05$]. Both placebo and PK 8165 50 mg showed an important learning effect within a given session which did not significantly differ. In

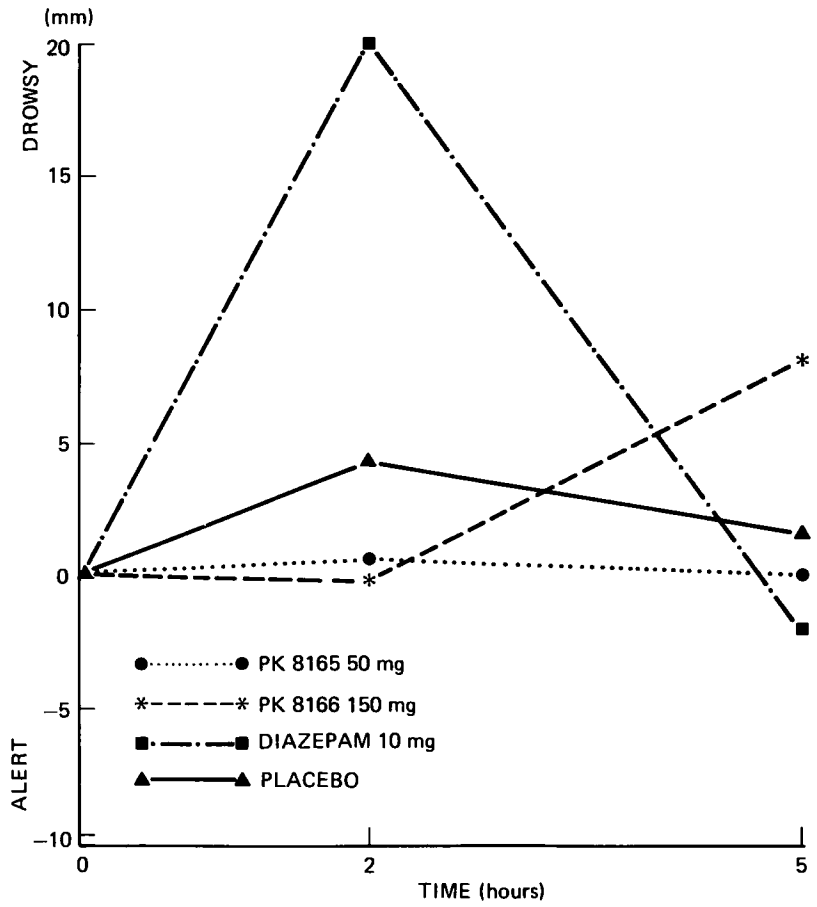

FIG. 2. Changes over time on the visual analogue scale "alert-drowsy" after PK 8165 50 mg, PK 8165 150 mg, diazepam 10 mg and placebo.

contrast, practically no learning was present after diazepam. No difference existed between both doses of PK 8165 and placebo on the second assessment, but PK 150 mg induced an effect parallel to diazepam on the third assessment (Fig. 5).

Figure 6 presents the evolution of mean time per digit in the 4 conditions. Significant differences were present among drugs [$F(3,33) = 4{\cdot}63$, $p < {\cdot}01$]. There was no significant change over time in this measure with diazepam, no difference between placebo and PK 8165 50 mg and a decrease at the second assessment followed by an increase on the last assessment with PK 8165 150 mg.

c. Colour Test In this memory test, there was also a difference in learning between drugs [$F(1,11) = 8{\cdot}54$, $p < {\cdot}05$]. In contrast to PK 8165 50 mg, placebo and diazepam caused tiredness.

d. Zazzo Test The number of errors exhibited significant changes [$F(3,33) = 2{\cdot}87$, $p < {\cdot}05$]. Placebo and PK 8165 50 mg induced the same changes with an increase of errors on the second assessment while PK 8165 150 mg showed a decrease in the number of errors on the second assessment followed by an evolution similar to placebo on the third assessment.

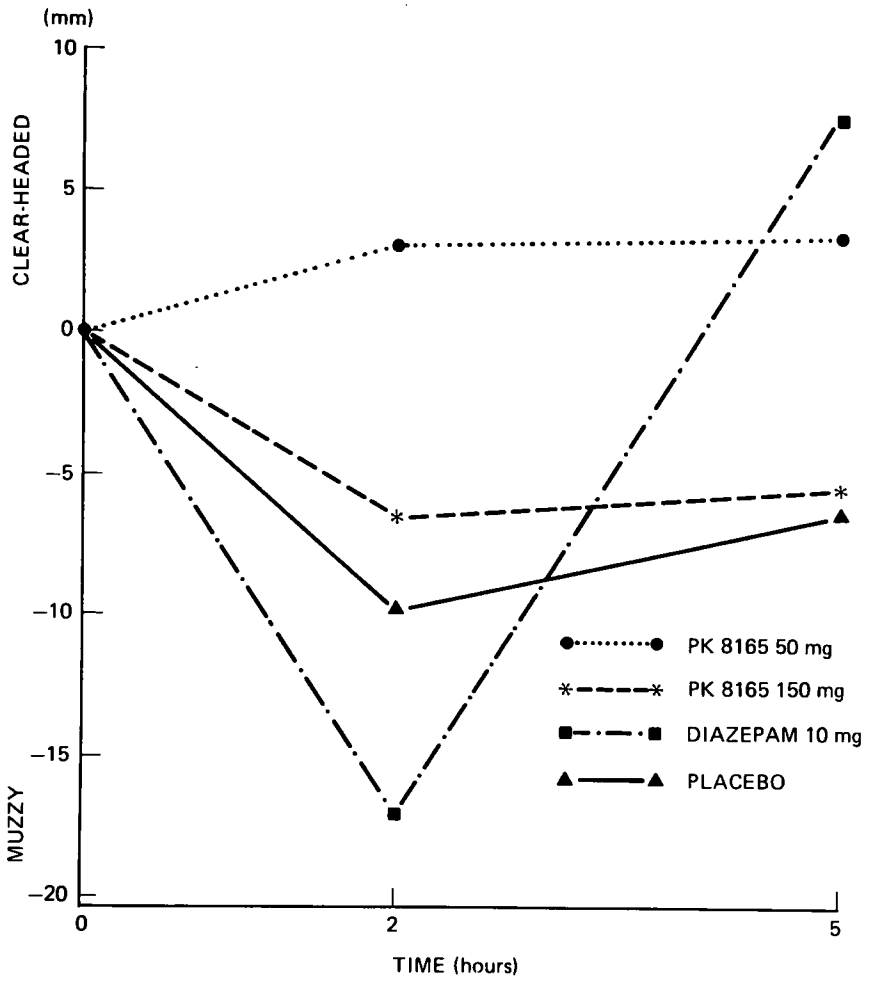

FIG. 3. Changes over time on the visual analogue scale "muzzy-clear-headed" after PK 8165 50 mg, PK 8165 150 mg, diazepam 10 mg and placebo.

4. *Subjective Side-effects*

The subjective effects mentioned by the volunteers are presented in Table 2.

Discussion

The present study suggests that at low dose, PK 8165, a benzodiazepine partial agonist, is not only devoid of sedative activity but may exhibit psychostimulating properties. Indeed, in the most simple computerized tests (the first labyrinth), it increased the speed of response while in the most complex tests (e.g. colour tests), it prevented tiredness which appeared with the other drugs as well as with placebo. Moreover, subjects felt significantly more strong, clear-headed, energetic, and quick-witted with PK 8165 50 mg on the visual analogue scales. In contrast, both diazepam and high dose of PK 8165 induced a significant decrease in vigilance, but with a different time schedule: this effect appeared 2 hours after diazepam intake and 5 hours

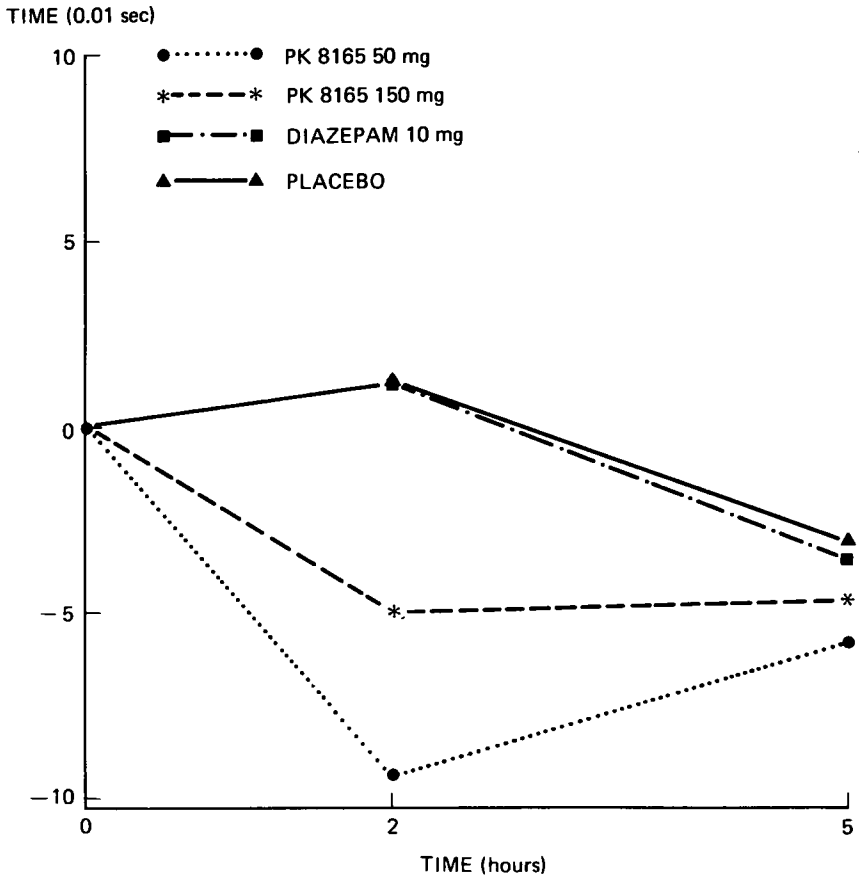

FIG. 4. Changes over time on the total time for the first labyrinth after PK 8165 50 mg, PK 8165 150 mg, diazepam 10 mg and placebo.

after PK 8165 intake. It was well demonstrated by the higher number of errors in the labyrinth which had to be memorized, the lack of learning effect and the increased mean time per response in the digit test as well as by the worsening in four visual analogue scales: feeble vs strong, drowsy vs alert, lethargic vs energetic, and sad vs happy.

These results confirm that at low dose, a benzodiazepine partial agonist can exert stimulating properties, suggesting a predominance of antagonist over agonistic properties. However, with increasing dosage, the agonistic properties may become predominant, leading to a profile similar to that of diazepam on the measures employed in the present study.

However, caution is warranted in interpreting the worsening in ratings observed with high dose of PK 8165 as actual sedative activity. Indeed, three volunteers exhibited significant side-effects (ataxia, visual disturbances) which may have interfered with the assessment of vigilance. However, the analysis of the data after excluding these 3 subjects still suggests a trend for sedative effects 5 hours after PK 8165 150 mg.

The difference in the schedule of decrease in performance after diazepam 10 mg and PK 8165 150 mg corresponds very well to pharmacokinetic data which show a

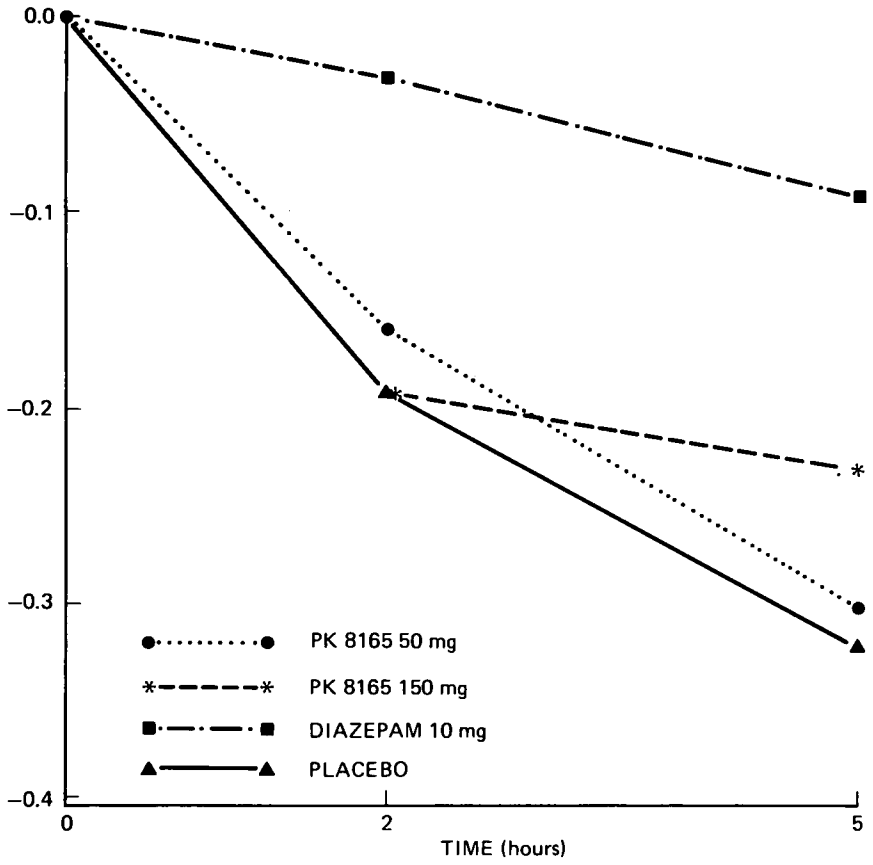

FIG. 5. Changes over time in learning for the digit test after PK 8165 50 mg, PK 8165 150 mg, diazepam 10 mg and placebo.

plasma peak 0·88 hour after diazepam intake (Kaplan and Jack, 1983) and 3–4 hours after intake of PK 8165 (unpublished data).

However, pharmacoelectroencephalogram studies in humans of both 50 and 150 mg of PK 8165 have shown changes in power spectrum occurring 2 hours after drug intake which did not significantly increase thereafter (Thébault *et al.*, 1984), suggesting that clinical activity of PK 8165 may appear earlier than predicted by plasma pharmacokinetics. These data confirm the difficulty of interpreting central activity from plasma studies (Ansseau *et al.*, 1984*b*, 1984*c*).

Interestingly, in this study, diazepam appeared to prevent learning, especially in the tasks which need recollection of the material presented. These results can be interpreted by the amnesic properties of benzodiazepines which primarily disrupt acquisition processes (see Petersen and Ghoneim, 1980 for review).

On the methodological level, the sensitivity and the discriminant power of Visual Analogue Scales and the automated assessment procedure were demonstrated in the present study.

Visual analogue scales are particularly sensitive to assess short-term changes in subjective feelings and have been widely used as indicators of the time-effect curve of analgesic and sedative drugs (Bond and Lader, 1974; Ansseau *et al.*, 1984*a*). Moreover, they are easy for the subjects to grasp, quick to fill out and do not require

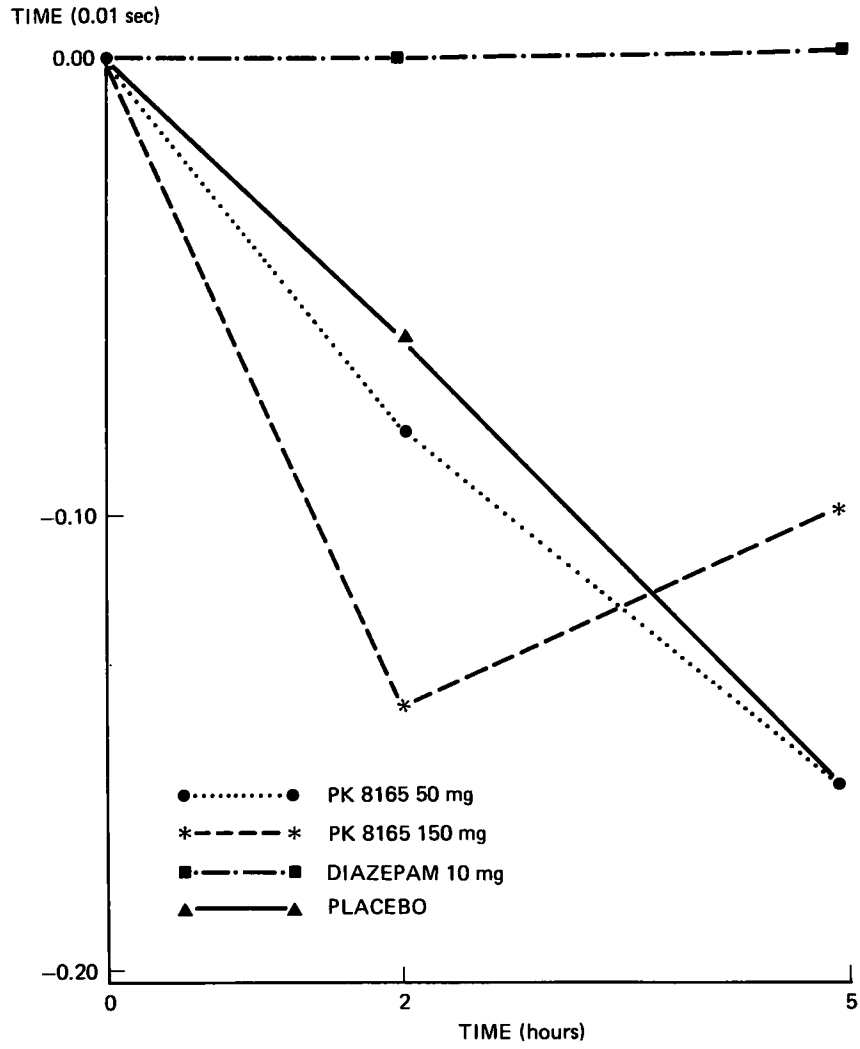

FIG. 6. Changes over time in mean time per response on the digit test after PK 8165 50 mg, PK 8165 150 mg, diazepam 10 mg and placebo.

much subjective motivation and, as the rater is not restricted to direct quantitative terms, permit as fine a discrimination as he/she wishes (Freyd, 1923). In addition, they reduce the difficulty of response sets and the artificial distribution of positive and negative responses (Aitken, 1969).

Computerized assessment also demonstrated good reliability and sensitivity. The lack of significant differences between the four baseline evaluations performed on each session reflects the reliability of the tests. On the other hand, the various statistically significant results among drugs and within each session demonstrate their high sensitivity and discriminant power. The benefits of micro computer-aided assessment include gains in accuracy and sensitivity, saving time and possibilities of expanding assessment services without additional cost (Sampson, 1983). Moreover, volunteers prefer this form of testing to more standard assessments (Beaumont, 1981; Space, 1981). Finally, this methodology permits significant conclusions to be obtained with a limited number of subjects.

TABLE 2. *Subjective effects following placebo, PK 8165 50 and 150 mg and diazepam.* The time of report after drug intake is given in parentheses

	Placebo	*PK 8165 50 mg*	*PK 8165 150 mg*	*Diazepam*
1	Cheerfulness (5)	—	—	Drowsiness (2)
2	—	—	Drowsiness (5)	—
3	—	—	—	—
4	—	Relaxation (2)	Relaxation (2) Drowsiness (5) Visual disturbances (5)	Relaxation (2)
5	Cheerfulness (2)	—	Tiredness (5)	Retardation (2) Headaches (2)
6	Drowsiness (2)	—	Drowsiness (5) Dizziness (5) Ataxia (5) Visual disturbances (5)	Drowsiness (2)
7	—	—	Drowsiness (2)	Memory disturbances (2) Ataxia (2)
8	Dizziness (2) Concentration difficulties (2)	—	—	Memory disturbances (2) Drowsiness (5)
9	—	—	Ataxia (2) Visual disturbances (2)	Drowsiness (2–5)
10	—	—	Drowsiness (2)	—
11	—	—	Confusion (2) Cheerfulness (2)	—
12	Confusion (2)	—	—	—

Acknowledgments

Our gratitude is due to G. Le Fur for his helpful comments on the manuscript, J. Fondarai (Marseille, France) for the first version of the psychometric software, and Ch. Gayetot for her technical assistance.

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