EFFECT OF BROtizOLAM ON THE AVERAGED PHOTOPALPEBRAL REFLEX IN MAN

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1 The photopalpebral reflex (PPR) is a useful method to assess level of arousal. Healthy males were given either brotizolam (0.0625, 0.125, 0.25 or 0.5 mg) or placebo within a double-blind, crossover design. Changes in PPR and subjective assessments were observed for 5 h after medication.

2 Prolongation of the latencies of PPR were dose dependent, and the amplitude tended to be reduced. These effects appeared within 30 min, and lasted about 4 h.

3 The dose-response curve of the maximum prolongation of the latencies was linear.

4 Sleepiness and slight ataxia were observed after drug ingestion. Sleepiness was correlated with the prolongation of the PPR latencies.

5 Brotizolam could be a potent hypnotic, with rapid onset and moderate duration of action, and it has no severe side-effects.

Keywords brotizolam hypnotic photopalpebral reflex

Introduction

It is important, yet very difficult, especially in the case of psychotropics, to predict the clinical effects of newly introduced drugs from pharmacological data obtained using laboratory animals and normal human beings. To facilitate the prediction of such clinical effects, several methods using normal human subjects have been proposed. The findings obtained in this way are considered to be as useful as, or more useful than, those obtained with animals for evaluating clinical efficiency in patients. However, the methods do appear to have some limitation in their predictability and a more useful method for predicting the clinical efficiencies of drugs is needed.

In previous reports, we have predicted the clinical effects of some anxiolytic and hypnotic drugs by analysis of changes in the averaged photopalpebral reflex (PPR) in healthy human adults. (Inanaga *et al.*, 1971; Tanaka *et al.*, 1974a,b, 1977a,b, 1978, 1979, 1981a,b; Mizuki *et al.*, 1978) The PPR is a record of the mean of summated reflexive contractions of the orbicularis oculi

muscle in response to periodically applied photic stimuli and it alters with a high degree of sensitivity according to changes in the level of arousal (Inanaga *et al.*, 1971; Tanaka *et al.*, 1974b; Sato & Tanaka, 1977). The peak latencies of PPR are prolonged and the amplitude reduced in drowsy states as defined by EEG recordings and the latencies are shortened and the amplitude increased in more highly aroused states. This suggests that PPR might be a useful index for evaluating drug effects on the level of arousal.

The dose-response curve is of importance in pharmacological studies regardless of whether the subjects are animal or human and is the basis of determining either drug action or drug interactions. However, most of the clinical studies already carried out using this concept have involved animal rather than human subjects. This is for several reasons. For example, it is difficult in humans to employ such a wide dose range as that used in animal studies when one must consider safety and ethical aspects, and it is also difficult in human

studies to determine the drug action as simply as can be done in animal studies which are carried out easily under either *in vivo* or *in vitro* conditions.

If it were possible to draw a dose-response curve by choosing several dosages of a certain drug which would be safe enough to use in a routine study and by employing a defined physiological method for measuring drug action, not only would the physiological method be very useful index for evaluating the drug effect, but also the drug effect on humans would become more evident.

To investigate these possibilities, we have tried to draw a dose-response curve based on the findings obtained in the PPR study in normal humans by employing four dosages of brotizolam, a new hypnotic, ranging from 0.0625 mg to 0.5 mg, which have been confirmed in Japan to be safe for use in normal human volunteers. Brotizolam is a triazolodiazepine derivative and its chemical structure is slightly different from that of conventional benzodiazepine derivatives (Figure 1).

Figure 1 Chemical structure of brotizolam.

Methods

Subjects

Ten male university student volunteers, aged from 20 to 23 years (mean age 20.9 years), were included in the study. They were first assessed as being physically and mentally healthy.

Drug

Brotizolam has potent taming, muscle relaxant and anti-pentetrazol-convulsion actions and potentiates the sleeping time induced by hexobarbitone (Shelley, 1979). By reference to the data deriving from an animal study and Phase I studies in human beings, four dosages of brotizolam, 0.0625 mg, 0.125 mg, 0.25 mg and 0.5 mg were chosen for use. Tablets containing the respective doses of brotizolam and identical placebo tablets were prepared.

PPR recordings

The PPR recording procedure is as shown in Figure 2. The subject lies in a supine position with his eyes closed in a dimly illuminated, sound-proof recording chamber. One disc electrode is placed on the centre of the right upper palpebral and the other on the nasal part of the right lower palpebral. Photic stimuli are applied to the eyes by means of a stroboscope (Nihon Kohden) connected to a midjet analogue computer (ATAC 350, Nihon Kohden). In this study, one hundred single-flash stimuli were applied at a frequency of one per second from a distance of 10 cm. The potential changes of the orbicularis oculi muscle thereby evoked were conveyed via an EEG amplifier (EEG-4113, Nihon Kohden) to the analogue computer which averaged the sum of individual responses. The time constant for recording the PPR was 0.3 s. The averaged PPR responses were then magnified and recorded on an X-Y recorder. The analysis time of the records was 100 milliseconds.

Figure 2 Recording method of photopalpebral reflex.

The recorded pattern of the PPR is shown in Figure 3. The first deflection with a latency of 56–58 msec was designated as peak 1 (P_1) and the downward deflection at 73–76 ms as peak 2 (P_2). The amplitude was defined as the magnitude of the difference between P_1 and P_2 , and the latencies of the peak effects after the photic stimuli (0 ms) as P_1 latency and P_2 latency.

Test procedure

The control PPR was recorded at 13.00 h, i.e., 30 min after the same lunch was served for each subject. Immediately after recording the control PPR, one tablet of either brotizolam (containing one of the specified doses) or placebo was administered orally according to a double-blind, crossover design. Placebo and four dosages of

Figure 3 A schematic pattern of PPR. The first, upward deflection was designated peak 1 (P_1) and the downward peak 2 (P_2).

brotizolam were allocated to each subject according to the Latin square design.

After the drug or placebo ingestion, the PPR was recorded repeatedly at 0.5, 1, 1.5, 2, 2.5, 3, 4 and 5 h after medication. Each subject assessed his subjective impressions on a seven-point (0–6) rating scale derived from our department (Figure 8).

One week was allowed as a period for washing out the residual effects of the previous drug before the next test was carried out.

For the statistical analyses, an analysis of variance was employed.

Results

PPR changes

The time-course of changes in both latencies of the PPR is illustrated in Figures 4 and 5. After placebo administration, P_1 latency showed no significant alteration throughout a 5 h period (Figure 4).

Brotizolam at a dose of 0.0625 mg slightly prolonged P_1 latency with a peak prolongation at 2.5 h after administration. Although both 0.125 mg and 0.25 mg of brotizolam prolonged the latency more potently than did 0.0625 mg, a clearly defined dose-dependent relationship between these two doses was not observed. There seemed to be a slight difference in the prolongation of the latencies induced by the two doses; the prolongation induced by 0.25 mg appeared more rapidly and lasted for a longer time than did that induced by 0.125 mg. Brotizolam at a dose of 0.5 mg produced the most marked prolongation of P_1 latency of the four doses of the drug used. The peak effect of prolongation by the 0.5 mg dose was noted at 2.5 h after administration. The drug effect appeared relatively

Figure 4 Effects of various doses of brotizolam or placebo on P_1 latency of PPR. The ordinate indicates changes of the peak latency, expressed in terms of value obtained by subtracting the baseline value, prior to drug ingestion, from the value at a given time after administration. The change towards plus indicates a prolongation of the latency and that towards minus a shortening. The abscissa shows time after drug administration. $\times$ --- $\times$ placebo, $\triangle$ — $\triangle$ brotizolam 0.0625 mg, $\blacktriangle$ — $\blacktriangle$ brotizolam 0.125 mg, $\bullet$ — $\bullet$ brotizolam 0.25 mg.

rapidly but did not continue for very long, since the evident prolongations occurred within 0.5 h of administration of both 0.25 mg and 0.5 mg and the latency tended to recover to the control value at 5 h after either dose. Overall, these changes in P_1 latency appeared to be dose-dependent. Analysis of variance on P_1 latency revealed a significant difference for the effect of dosage; $F(4, 399) = 6.24$, $P < 0.01$.

P_2 latency exhibited a more evident dose-response relationship than did P_1 latency (Figure 5). As was the case for P_1 latency, placebo had virtually no effect on P_2 latency. The four dosages of brotizolam ranging from 0.0625 mg to 0.5 mg prolonged P_2 latency in such a way that the higher the dosage of the drug, the more potently did it prolong latency. The peak effect of prolongation was observed at 3 h after medication with the respective doses. As was the case for P_1 latency, the prolongation of P_2 latency caused by 0.25 mg and 0.5 mg of brotizolam appeared relatively rapidly and continued for a moderate period of time. Analysis of variance on P_2 latency showed a statistically significant difference for the effect of the dosage; $F(4, 399) = 5.21$, $P < 0.01$.

Figure 5 Effects of various doses of brotizolam or placebo on P_2 latency. $\times$ --- $\times$ placebo, Δ — Δ brotizolam 0.0625 mg, $\blacktriangle$ — $\blacktriangle$ brotizolam 0.125 mg, $\circ$ — $\circ$ brotizolam 0.25 mg, $\bullet$ — $\bullet$ brotizolam 0.5 mg.

The amplitude of PPR tended to decrease after administration of brotizolam, and the decrease appeared to occur dose-dependently when the dosages ranging from 0.0625 mg to 0.25 mg were administered. No dose-response relationship was observed however, between 0.25 mg and 0.5 mg (Figure 6). As far as amplitude was concerned, no statistically significant difference for the effect of dosage was obtained by analysis of variance, since intragroup variation was so large; $F(4, 399) = 2.45$, $P > 0.05$.

Figure 7 shows the dose-response curve for each latency based on the value of the maximum prolongation of the latencies in each person (regardless of time after ingestion) for the respective doses of the drug or placebo administered. For both curves, 0 mg of brotizolam represents the value obtained when placebo was given; slight prolongations of both the latencies were observed following placebo ingestion.

When we considered the maximum prolongation of P_1 latency, the dose-response curve was almost linear from 0.0625 mg to 0.5 mg as shown in Figure 7. Similarly, the dose-response curve based on the maximum prolongation of P_2 latency was linear for the same dose range.

Changes in self-assessments

Vagueness, sleepiness and slight ataxia were most frequently observed, but these subjective symptoms

Figure 6 Effects of various doses of brotizolam or placebo on the amplitude of PPR. $\times$ --- $\times$ placebo, Δ — Δ brotizolam 0.0625 mg, $\blacktriangle$ — $\blacktriangle$ brotizolam 0.125 mg, $\circ$ — $\circ$ brotizolam 0.25 mg, $\bullet$ — $\bullet$ brotizolam 0.5 mg.

were slight as is shown in Figure 8. Although 0.5 mg of brotizolam caused more subjective symptoms than did other dosages, they were still so slight as to score less than 2 on the 7 point scale. Only for the highest dose was a dose-response curve for subjective assessments obtained.

PPR changes and self-assessments

Table 1 indicates the correlation coefficients between the parameters of the PPR and subjective assessments. Both latencies were positively correlated with each other and negatively correlated with the amplitude of the PPR. Items of subjective assessment which correlated positively with the prolongation of the latencies and negatively with the amplitude were sleepiness, vagueness and slight ataxia. These three items correlated positively with each other and a high correlation coefficient was obtained between slight ataxia and vagueness. The item which showed the highest correlation with prolongation of the latencies was sleepiness.

Discussion

For more than two decades, many benzodiazepine derivatives have been used widely as anxiolytic or

Figure 7 The dose-response curve for P₁ latency (a) and P₂ latency (b). The curve was drawn according to the maximum prolongations of the latencies, regardless of time after medication, when the respective doses of the drug or placebo were administered.

hypnotic agents in most fields of medicine. Brotizolam, a triazolodiazepine derivative which has a structure slightly different from that of the benzodiazepines, shows very similar pharmacological profiles.

When compared with diazepam, a typical potent benzodiazepine derivative, brotizolam is more potent in taming effect (suppression of isolation-induced fighting in mice) and anti-pentetrazole convulsion effect, equivalent or slightly weaker in muscle relaxant effect, and weaker in inhibition of open-field locomotion. Brotizolam possesses a potent hypnotic effect as evidenced by EEG studies and has proved highly effective in increasing the frequency of lever pressing in the conflict situation, which might be related to anxiolytic action in humans.

Such results obtained in animal studies suggest that brotizolam could be used for its anxiolytic, hypnotic, anticonvulsant and muscle-relaxant effects in clinical situations. Brotizolam is thus similar in nature to the benzodiazepine derivatives despite the slight difference in their chemical structures. Incidentally, in spite of the fact that many benzodiazepine derivatives are shown by animal studies to have differing pharmacological profiles, it is often difficult to see how these differences are reflected by their pharmacological actions in human beings. Because such variations exist between animal and human studies, it is important that data from each should be used as feedback for the other.

The main characteristic of brotizolam is its hypnotic action and it is possible to assess this

Figure 8 Changes in subjective assessments recorded on a seven point (0-6) rating scale. 0: initial (before ingestion), 2: slightly, 4: moderately, 6: markedly x---x placebo, Δ—Δ brotizolam 0.0625 mg, ▲—▲ brotizolam 0.125 mg, ○—○ brotizolam 0.25 mg, ●—● brotizolam 0.5 mg.

Table 1 Correlation coefficients between parameters of PPR and subjective assessments

	<i>P</i> ₁ latency	<i>P</i> ₂ latency	Amplitude	Sleepy	Vague	Ataxic
<i>P</i> ₁ latency	1.000	0.800	-0.329	0.477	0.412	0.341
<i>P</i> ₂ latency	0.800	1.000	-0.371	0.355	0.299	0.277
Amplitude	-0.329	-0.371	1.000	-0.262	-0.189	-0.185
Sleepy	0.477	0.355	-0.262	1.000	0.549	0.485
Vague	0.412	0.299	-0.189	0.549	1.000	0.649
Ataxic	0.341	0.277	-0.185	0.485	0.649	1.000

action by utilizing changes in PPR which are very sensitive to changes in the level of arousal (Inanaga *et al.*, 1971, Tanaka *et al.*, 1974b). This method was used for the present study as previously described.

The time-course study of the drug effect revealed that brotizolam prolonged both latencies of PPR dose-dependently in a dose range from 0.0625 mg to 0.5 mg. The dose-response curve drawn, based on the maximum prolongation of latencies, exhibited an evident and linear relationship between the prolongation of the latencies and the dosage of the drug used. The study showed that 0.0625 mg of brotizolam seemed to be effective in prolongation of the latencies. However, both latencies were prolonged more markedly when 0.125 mg of the drug was given. This indicates that 0.125 mg, rather than 0.0625 mg, is likely to be the minimum effective dose. When these actions are compared to those observed in previous reports on benzodiazepine derivatives it is shown that the effect of brotizolam on the level of arousal is sufficiently potent to be of therapeutic use.

The drug effect appeared very rapidly as shown by the fact that prolongation of the latencies was observed within 30 min of administration of 0.25 mg or 0.5 mg of brotizolam. The maximum effect was observed 2.5–3 h after medication and tended to recover gradually to the control level at 5 h. This indicated that the peak effect of the drug occurred 2.5–3 h after medication, (when the drug was administered orally 30 min after lunch) and that the duration of the drug effect was approximately 4 h. The characteristics of brotizolam are thus similar to those of flurazepam though the former is more potent than the latter.

In comparison to the effects shown on PPR, the drug effects on subjective assessments were not severe. Vagueness, sleepiness and slight ataxia occurred but only to a mild degree.

Brotizolam 0.5 mg caused the most marked alterations of subjective assessments, but a dose-relationship as clear as that observed for the PPR latencies was not apparent. This indicates that the PPR latencies are a more sensitive and reliable index than subjective assessments for evaluating drug effects.

Both latencies showed a high correlation

coefficient with each other ($r = 0.800$). The item of subjective assessment correlated positively and most highly with prolongation of the PPR latencies was sleepiness. This supports our previous view that PPR changes are highly sensitive to changes in the level of arousal, and that the test is useful in assessing physiologically the level of arousal.

Furthermore, the present study revealed that the dose-response curves could be drawn even for healthy human subjects by utilizing the PPR test and by employing several dosages within a range known to be safe for volunteers. This fact further supports the usefulness of the PPR test in the field of psychopharmacology. The test has several advantages as follows:

1. PPR is useful as a physiological index for measuring the effects of hypnotics; the drug effects can be measured objectively as physiological changes.
2. PPR is altered according to the arousal level with such sensitivity that it can be a good index for studying drug effect on the arousal level.
3. PPR permits quantification of drug responses.
4. PPR permits determination of the time at which the maximum effect of the drug occurs.
5. PPR permits determination of the time required for appearance of the drug.
6. PPR permits determination of the equipotent dosages of different drugs, when several drugs are tested at the same time.
7. Recording procedure and analysis method for obtained data are relatively straightforward.

PPR does, however, have some disadvantages. For example, the neuronal pathway of PPR is still uncertain. Also it becomes difficult to define the peak in the recorded PPR if its amplitude is suppressed strongly by a lowered arousal level induced by the drugs or other conditions. Large individual differences occur in the PPR test in the same way as in other psycho-pharmacological indices, although intra-individual differences in PPR are small. The PPR test, nevertheless, is considered to be a useful, new method to predict the clinical effects of anxiolytic or hypnotic drugs, especially the latter.

In conclusion, it is predicted from the present study that brotizolam could be a potent hypnotic

whose onset of action is rapid and duration of action moderate and which has no severe side-effects.

We are grateful to Nippon Boehringer Ingelheim Co., Ltd, for the generous supply of brotizolam.

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Die Wirkung von Brotizolam auf den gemittelten Photopalpebralreflex beim Menschen: Die Anwendungsmöglichkeit als Schlafmittel

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1 Der photopalpebrale Reflex (PPR) ist eine nützliche Methode zur Beurteilung des Vigilitätsgrades. Gesunde männliche Versuchspersonen erhielten in einer doppelblinden, crossover Versuchsanordnung entweder Brotizolam (0,0625, 0,125, 0,25 oder 0,5 mg) oder Placebo. Veränderungen im PPR und subjektive Beurteilung wurden über 5 Stunden lang nach der Medikation beobachtet.

2 Verlängerungen der PPR-Latenzzeit waren dosisabhängig und die Amplitude wurden geringer.

Diese Wirkungen traten innerhalb von 30 min auf und hielten ca. 4 Stunden an.

3 Die Dosis-Wirkungs-Kurve der maximalen Verlängerung der Latenzzeiten war linear.

4 Müdigkeit und leichte Ataxie wurden nach der Substanzeinnahme beobachtet. Die Müdigkeit war mit der Verlängerung der PPR-Latenzzeiten korreliert.

5 Bei Brotizolam könnte es sich um ein stark wirksames Schlafmittel handeln mit schnellem Wirkungseintritt und mäßiger Wirkungsdauer, das keine ernsthaften Nebenwirkungen aufweist.

Effet du brotizolam sur le reflexe photopalpebral moyen de l'homme: possibilité d'application comme hypnotique

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1 Le reflexe photopalpebral (RPP) est une méthode efficace d'appréciation du degré d'éveil.

Des personnes de sexe masculin en bonne santé ont absorbé, soit du brotizolam (0,0625, 0,125, 0,25 et

0,5 mg), soit un placebo dans un essai cross-over en double aveugle. Les modifications du RPP et les appréciations subjectives ont été relevées pendant 5 h après application.

2 Le prolongement des temps de latence du RPP a été dépendant de la dose et l'amplitude a eu tendance à se réduire. Ces effets sont apparus en l'espace de 30 min et ont duré environ 4 h.

3 La courbe dose/réponse du prolongement maximum des temps de latence a été linéaire.

4 De la somnolence et une légère ataxie ont été observées après la prise du médicament. La somnolence était corrélée avec le prolongement des temps de latence du RPP.

5 Le brotizolam pourrait être un hypnotique puissant, à démarrage d'action rapide et de durée d'action moyenne. Il n'a pas d'effets secondaires importants.

Efecto de brotizolam sobre el reflejo fotopalpebral promediado en el ser humano: posibilidades como hipnótico

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1 El reflejo fotopalpebral constituye un método útil para valorar el nivel de vigilia. Individuos sanos del sexo masculino recibieron ya sea brotizolam (0,0625, 0,125, 0,25 ó 0,5 mg) o placebo en el transcurso de un ensayo doble-ciego, cruzado. Durante 5 h después de la medicación se observaron las alteraciones del reflejo fotopalpebral y se efectuaron evaluaciones subjetivas.

2 La prolongación de los períodos de latencia del reflejo fotopalpebral fue dosis-dependiente y la amplitud tendió a reducirse. Estos efectos aparecieron en el plazo de 30 min y duraron alrededor de 4 h.

3 La curva dosis-respuesta de la prolongación máxima de los períodos de latencia fue lineal.

4 Después de la ingestión del fármaco se observaron somnolencia y ligera ataxia. Hubo correlación entre la somnolencia y la prolongación de los períodos de latencia del reflejo fotopalpebral.

5 Brotizolam podría ser un hipnótico potente, con comienzo rápido y duración moderada de la acción y ausencia de efectos secundarios graves.