

Anxiolytic Activity of Tenoten and Diazepam Depends on Conditions in Vogel Conflict Test

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We compared two modifications of Vogel conflict test and assessed anxiolytic activity of two drugs: diazepam (benzodiazepine anxiolytic) and tenoten (ultra-low doses of antibodies to S-100 protein) in both modifications of the test. It was found that the intensity of anxiolytic effect of the drugs depends on the conditions of Vogel test.

Key Words: *anxiety; antibodies to protein S-100; ultralow doses; tenoten; diazepam*

Vogel conflict test developed in 1971 [14] is one of the main tests for evaluation of anxiolytic activity of pharmacological agents recommended for the study of specific anxiolytic activity in Russia [3]. The test is based on conflict between drinking and defense motivations: after drinking deprivation every attempt to drink is punished by electric shock. Overcoming the fear of punishment manifests in increasing number of water intakes despite painful stimulation. Vogel conflict test is widely used in Russia and abroad and has several advantages over other tests. First, it includes simple quantitative method for assessing anxiolytic activity (the number of punished water intakes) allowing to screen and track changes in the effects of drugs depending on experimental conditions (doses, duration of administration, animals strain, etc.). Second, almost all anxiolytics are active in this test [1].

Behavioral techniques are often adapted to specific conditions of the laboratory for further validation in different research organizations. Vogel [14] developed a method in which Holtzman male rats were subjected to 48-h drinking deprivation and then during the 3-minute test every 20th water intake was punished by a mild electric shock. Later some versions of this method were developed, in which drinking motivation was not so strong. For example, drinking deprivation

could last for 18 or 24 h or animals received access to the water for 1 h over 4 days before the test [10,13]. Limited access to the water allowed extension of the total duration of deprivation, which increases stability and reproducibility of animal behavior. In some modifications of the test, the rats were previously trained to find drinking bottle, which was achieved by putting the animals in the test cage before the test [9,15]. Some authors before the drugs injections carried out a preselection of rats with initially low number of punished water intakes, but this approach introduced a systemic error and complicated the model without providing significant benefit [4,8].

In some experiments, authors reduced current strength compared to that in original Vogel test [14]. This modification, despite the increase in probability of errors, increased sensitivity of the test [6,9,10]. Increasing the test duration or frequency of punishment (for example, each water intake is punished) does not provide advantages over the original Vogel test [5,12].

In contrast to Vogel, who used Holtzman rats, many authors preferred other rat strains (Wistar, Sprague-Dawley) due to their availability. We found no systematic data on the differences in the sensitivity of the test depending on the use of rats of different lines. It should be noted that in almost all experiments, the body weight of the tested rats was higher than in the original Vogel research [14]. Interestingly, that according to some sources the use of rats with high body

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weight leads to the better repeatability and reproducibility of the results [11]. It is well known that quality of life have a great impact on the emotional state of rats and their response to stress. In different variations of the method, the rats were housed both individually and in groups. Unfortunately, the original article by Vogel [14] did not specifies exactly how the rats were kept (either individually or in groups).

The aim of this work was compare two modifications of Vogel conflict test with estimation of anxiolytic effects of two drugs: benzodiazepine anxiolytic diazepam and ultra-low doses of antibodies to protein S-100 (tenoten).

MATERIALS AND METHODS

In the original version [14], the test was performed on male Holtzman rats (mean body weight 170 g); the animals were subjected to 48-h drinking deprivation and then placed in a plexiglass chamber (38×38 cm) with a stainless steel rods floor. The cage had an outlet (5×7.5 cm) into a dark compartment with a drinking bottle. The current was switched on 2 sec after the animal began to drink, the current strength was 0.5 mA, and each 20th water intake was punished. The test was performed over 3 min, the tested drugs were injected 30 min before testing.

In this study we used two modifications of Vogel conflict test. One modification was developed at the V. V. Zakusov Institute of Pharmacology, Russian Academy of Medical Sciences and was recommended as the standard for the study of anxiolytic activity of pharmacological agents [3]. The experiments were done on outbred white male rats ($n=60$, 200-250 g) kept in groups of 5 animals per cage. On day 1 of the experiment, the animals were completely deprived of drinking water. After 24-hour deprivation, the rats were learned to get water from the drinking bottle located in the experimental chamber (27.5×27.5×45 cm) with the floor made of stainless steel rods [2]. On day 3 (after a 48-hour drinking deprivation), the rats were placed again into the experimental chamber. After 10 seconds of the first water intake, constant current (0.2 mA) was delivered on the nipple of a drinking bottle and on the electrode floor of the chamber. Each water intake was punished. The test was performed for 10 min; the tested drugs were injected 30 minutes before the start of the test.

Another modification of Vogel conflict test was developed and validated in the laboratory Porsolt & Partners Pharmacology (France) [7]. The experiments were performed on male Rj:Wistar (Han) rats ($n=100$, 160-227 g) housed 5 animals per cage, as in the previous experiment. After a 48-hour drinking deprivation rats were placed in transparent plexiglass chamber

(15×32×34 cm) with floor consisting of stainless steel rods (0.4 cm in diameter, spaced 1 cm apart). Posterior wall of the chamber was made of opaque plexiglass hiding the observer. A water bottle connected to one pole of shock generator (Apelex: type 011346) was placed in the center of the opposite wall 5 cm above the floor. The other pole of the generator was connected to the metal grid floor. The rat explored the cage until finding out the drinking bottle. Every time two seconds after the rat started drinking, it received an electric shock (1.7 mA). The test was performed for 3 min; the tested drugs were injected 60 minutes before the start of the test.

Comparison of the original test and the two modifications is summarized in Table 1.

J. R. Vogel in his original study (1971) made screening of anxiolytic activity of some drugs (diazepam, chlordiazepoxide, oxazepam, meprobamate, pentobarbital) in several doses. In our paper we presented the data only for the effect of diazepam in a dose of 2 mg/kg for comparison with the effect of this drug obtained in this study. In the modified test of the V. V. Zakusov Institute of Pharmacology, diazepam in a dose of 2 mg/kg showed a pronounced and stable anxiolytic effect that allowed using it as the reference drug. The anxiolytic effect of tenoten (2.5 ml/kg) was compared to that of diazepam. In the modified test from Porsolt & Partners Pharmacology, clobazam in a dose of 64 mg/kg was chosen as a validated reference drug; anxiolytic activity of tenoten (2.5, 5, 7.5 and 10 ml/kg) and diazepam (2 mg/kg) was tested.

For statistical analysis, the means (M) and standard deviations (m) for each group were calculated. Significance of differences between the groups was determined using Student's t test for independent variables.

RESULTS

In the classical version of Vogel conflict test, diazepam (2 mg/kg) 3.3-fold increased the number of punished water intakes ($p<0.05$ compared to the control) [14].

In the modified test of V. V. Zakusov Institute of Pharmacology (Table 2), diazepam in a dose of 2 mg/kg and tenoten in a dose of 2.5 ml/kg increased the number of punished water intakes 1.5-fold ($p<0.05$ in comparison with control).

In the modified test from Porsolt & Partners Pharmacology (Table 2), diazepam in a dose of 2 mg/kg and tenoten in a dose of 2.5 ml/kg increased the number of punished water intakes 1.2 times. However, the effects of both drugs were not statistically significant in comparison with control. When the dose of tenoten was increased to 7.5 ml/kg, the effect of the drug reached the level of statistical significance

TABLE 1. Comparison of the Original Vogel Conflict Test and Two Its Modifications

| Parameter | Original Vogel test [14] | Modification of V. V. Zakusov Institute of Pharmacology [3] | Modification of Porsolts Partners Pharmacology [7] |
|---|--------------------------------|---|--|
| Rat strain | Holtzman | Mongrel | Rj: Wistar (Han) |
| Water deprivation | 48 h | 48 h | 48 h |
| Location of water bottle | In a separate dark compartment | In the general compartment of the chamber | In the general compartment of the chamber |
| Previous learning (search for water bottle) | No | 24 hours before the test | No |
| Start of painful electrical stimulation | 2 sec after first water intake | 10 sec after first water intake | 2 sec after first water intake |
| Current strength | 0.5 mA | 0.2 mA | 1.7 mA |
| Frequency of painful electrical stimulation | Each 20-th water intake | Each water intake | Each water intake |
| Test duration | 3 min | 10 min | 3 min |
| Interval between drug administration and test start | 30 min | 30 min | 60 min |
| Maintenance conditions | No information | 5 rats per cage | 5 rats per cage |

(the number of punished water intakes increased by 1.5 times in comparison with the control, $p < 0.05$). The reference drug clobazam in a dose of 64 mg/kg significantly increased the number of punished water intakes by 1.8-2.1 times ($p < 0.05$ in comparison with the control).

Thus, anxiolytic activity of diazepam and tenoten depended on the doses of the drugs and conditions of the test. In one modification of Vogel conflict test, the doses of diazepam 2 mg/kg and tenoten 2.5 ml/kg were sufficient to achieve a statistical significance of the anxiolytic effect of both drugs; in another modification the dose of tenoten was to be increased (testing of higher doses of diazepam was not performed).

Differences in the effect of diazepam and tenoten depending on the test modification pose the problem of choosing the dose of test drugs for specific modification of the test, because the drugs in the same doses can produce significant effect in one modification and insignificant in another. Apparently, evaluation of anti-conflict activity of pharmacological substances in a dose range in comparison with the reference drug dose providing significant effect is more preferable.

Studies showed that Vogel conflict test in two models allows reliable screening of pharmacological preparations for anxiolytic activity in comparison with the effect of the reference drug exhibiting stable activity in a specific modification of the test.

TABLE 2. Comparison of Efficiency of Diazepam and Tenoten in Vogel Conflict Test and Two Its Modifications

| Drug | Number of punishing water intakes, abs. | Original Vogel test [14] | Modification of V. V. Zakusov Institute of Pharmacology [3] | Modification of Porsolts Partners Pharmacology [7] |
|--------------------|---|--------------------------|---|--|
| Control | 3.5 | 303.7±84.8 | 35.0±0.6 | 3.2±0.2 |
| Diazepam, 2 mg/kg | 11.5* | 456.3±86.1* | — | 3.9±0.3 |
| Tenoten, 2.5 ml/kg | — | 464.9±78.4* | 4.2±0.6 | — |
| Tenoten, 5 ml/kg | — | — | — | 4.4±0.5 |
| Tenoten, 7.5 ml/kg | — | — | — | 4.9±0.6* |
| Tenoten, 10 ml/kg | — | — | — | 3.9±0.6 |
| Clobazam, 64 mg/kg | — | — | 7.4±1.1* | 5.7±0.8* |

Note. * $p < 0.05$ in comparison with the control; "—" no data.

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