# Behavioral Effects of Penicillin in a Test for Anxiety in Rats

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The use of benzylpenicillin in a wide range of doses to test its influence on the behavior of rats in the elevated-plus maze revealed its activating effects on various behavioral parameters in doses between 10,000 and 150,000 U/kg, indicating that this penicillin exhibits a degree of anxiolytic activity.

**Key Words:** *benzylpenicillin; behavior; anxiety*

Some antibiotics, including the penicillins, have been the subject of recent research to explore the possibility of utilizing physiologically active properties not directly related to their antimicrobial activity. We found previously [2] that systemically administered benzylpenicillin (BP) exerted, in various doses, a rapid and marked antiulcerogenic effect in acutely stressed rats, and that a similar dose-dependent effect was produced by penicillamine, a BP fragment devoid of antimicrobial activity [7]. These findings suggested to us that BP and some of its analogs possess central neurotropic antistress properties that require closer examination [2]. The present investigation was undertaken to study BP actions using the elevated-plus maze as a multipurpose behavioral test for anxiety.

## MATERIALS AND METHODS

In our experiment designed to examine the effects of BP on behavior, we modified [4] the elevated-plus maze proposed [8] as a model for the study of anxiety (the maze was raised to a height of 0.8 m above the floor). A total of 180 random-bred male white rats weighing 200-250 g were used, divided into groups of 10 to 15 animals. Forty-five minutes before testing, the rats of the control group were injected intraperitoneally with 0.4 ml of physiological saline and the rats of the test groups, with 0.4 ml of the sodium salt of BP in physiological saline. A single standard dose (SD) of BP was taken to be 10,000 U/kg, which is close to the daily therapeutic dose for man. Doses ranging from 1 to 40 SD were used. After BP or saline injection, rats were placed in an isolated chamber for 40 min and then transferred to the platform of the maze, where the following four behavioral parameters were recorded during 5 min: number of entries into the open arms of the maze, number of rearings, number of head dips from the ends of the closed arms, and the total number of crossings of the platform center.

The data were processed on a computer using the SOLO software package and the nonparametric Wilcoxon-Mann-Whitney test.

## RESULTS

In the elevated-plus maze model, intraperitoneal administration of BP in various doses altered the behavioral reactions of the rats. With doses in the range of 1 to 15 SD, the number of platform center crossings and the number of entries into the open maze arms both increased in a dose-dependent manner by 150-200% within 60 min postinjection, while the number of rearings was 2 to 3 times higher than in the control animals (Fig. 1). The doses most effective in these respects proved to be 5 and 10 SD (Table 1). Higher doses (30-40 SD)

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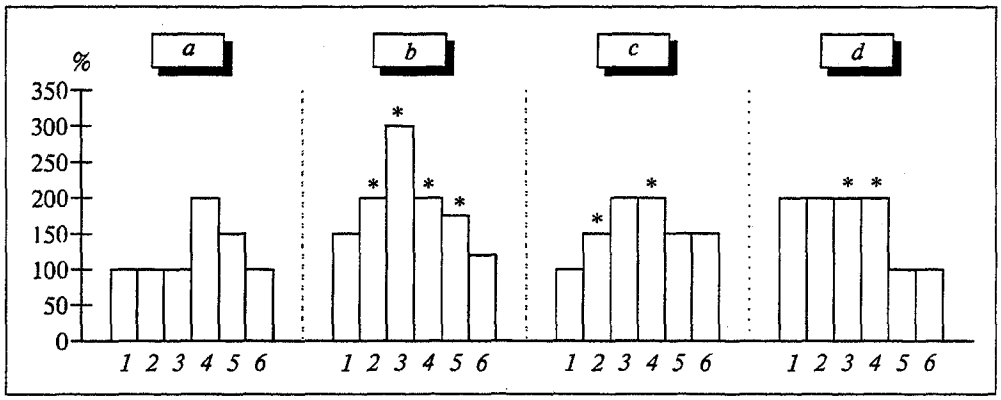


**Fig. 1.** Effects of different BP doses on behavioral parameters of rats in the elevated–plus maze (as expressed in % of the control values taken as 100%): *a*) head dips; *b*) rearings; *c*) platform center crossings; *d*) entries into open maze arms. Fifteen rats were tested for each dose. *$p<0.05$ in comparison with the control group. BP doses: *1*) – 1 SD; *2*) – 5 SD; *3*) – 10 SD; *4*) – 15 SD; *5*) – 30 SD; *6*) – 40 SD.

were less effective: the levels of both motor activity (rearings and platform center crossings) and exploratory behavior (head dips and entries into the open arms) were lower (Fig. 1). For some behavioral parameters (e.g., rearings), the dependence on the BP dose was extreme in that the effect disappeared after doses between 30 and 40 SD. A notable feature was similar dose-response curves for such independent behavioral parameters of maze behavior as the number of rearings and the number of platform center crossings (Fig. 1).

BP is known to be neurotropic in various doses [3]; in particular, it nonselectively blocks gamma-aminobutyric acid (GABA) receptors [6], exhibits marked epileptogenic activity [1], and, when injected into the cerebral cortex directly in a small dose, can affect the behavior of animals by eliciting rapid hyperactivation [5].

The elevated-plus maze model is a convenient and appropriate tool for evaluating behavioral responses of animals and has been widely employed for measuring the level of anxiety and detecting the anxiotropic action of various drugs [8]. The enhancement of motor activity and the activation of exploratory behavior observed with this model are commonly interpreted as resulting from a reduction in the general level of anxiety [4]. Our results using the elevated-plus maze indicate that in certain doses BP does influence behavior leading to increased activity and, consequently, to reduced anxiety (Fig. 1). It should be noted that these results agree well with those of other authors who have reported an ability of BP, following intracerebral administration, to exert a general activating influence on the behavior of animals in the elevated-plus maze [5]. The mechanism of this effect is not clear, however. It may be that BP acts by inhibiting the GABA-ergic system. In discussing the putative mechanisms of such action by BP, the important role played by GABA in stress and the involvement of the GABA-ergic system in anxiety mechanisms in various behavioral tests [9,10] should be taken into consideration. Possibly, there are other rapidly activable central mechanisms of BP action. It is important to emphasize that in our present experiment the greatest anxiolytic effect of BP was observed in doses at which in our previous studies it was found to display pronounced antiulcerogenic activity in acutely stressed animals [2,7]. We may therefore

**TABLE 1.** Effect of Different BP Doses on Behavioral Parameters of Rats in the Elevated–Plus Maze (15 Rats in Each Group)

| Parameter | BP dose (No. of SD) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 5 | 10 | 15 | 30 | 40 |
| *Control rats* | | | | | | |
| Head dips | 2±0.4 | 2±0.4 | 1±0.3 | 2±0.1 | 2±0.4 | 3±0.5 |
| Rearings | 2±0.4 | 2±0.3 | 1±0.3 | 4±0.8 | 3±0.4 | 5±0.9 |
| Platform center crossings | 2±0.4 | 2±0.3 | 4±0.7 | 3±0.5 | 2±0.3 | 2±0.4 |
| Entries into open maze arms | 2±0.2 | 1±0.2 | 2±0.3 | 1±0.3 | 2±0.3 | 2±0.3 |
| *Test rats* | | | | | | |
| Head dips | 2±0.5 | 2±0.4 | 1±0.2 | 4±0.4 | 3±0.5 | 3±0.6 |
| Rearings | 3±0.9 | 4±0.7 | 3±1.7 | 6±0.9 | 5±1.2 | 6±1.0 |
| Platform center crossings | 2±0.6 | 3±0.4 | 8±1.0 | 6±1.2 | 3±0.4 | 3±0.6 |
| Entries into open maze arms | 2±0.4 | 2±0.3 | 4±0.6 | 2±0.2 | 2±0.4 | 2±0.5 |

conclude that in certain doses BP can produce positive anxiolytic neurotropic effects that are probably related to the previously discovered stress-mitigating properties of this penicillin.

## REFERENCES

1. I. V. Batueva, N. P. Veselkin, and R. Veskov, *Neirofiziologiya*, **24**, № 2, 151-160 (1992).
2. A. V. Kaluev, G. E. Samonina, and I. P. Ashmarin, *Byull. Eksp. Biol. Med.*, **118**, № 8, 130-133 (1994).
3. A. B. Chernomordik, *Klin. Med.*, № 2, 102-105 (1980).
4. S. T. File and C. Fernandes, *Anxiety*, **1**, № 1, 8-12 (1994).
5. E. Horn, K. Esseling, and R. Wagner, *Pharmacol. Biochem. Behav.*, **40**, № 2, 351-357 (1991).
6. A. Horne, K. Hadingham, A. Macaulay, *et al.*, *Brit. J. Pharmacol.*, **107**, № 3, 732-737 (1992).
7. A. V. Kalueff, G. E. Samonina, and I. P. Ashmarin, *Neuropsychopharmacology*, **10**, № 3 (Suppl., Pt. 2), 272 (1994).
8. R. C. Montgomery, *J. Comp. Physiol. Psychol.*, **48**, 254-260 (1955).
9. D. J. Sanger, *Life Sci.*, № 36, 1503-1513 (1985).
10. F. Sherif and L. Oreland, *Behav. Brain Res.*, **63**, 11-15 (1994).

# Effects of Enkephalins on Bile-Secreting Function

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Various doses of leu- and met-enkephalins injected into the portal vein of rats inhibited predominantly the secretory function of the liver. In most instances, the changes in bile secretion were observed to coincide in direction, time of occurrence, and magnitude with those in the secretion of bile acids.

**Key Words:** *enkephalins; liver; bile secretion*

The endogenous opioid peptides enkephalins play an important part in regulating the functional state not only of the central nervous system but also of many visceral organs and systems, including the cardiovascular, digestive, and excretory systems [3,7,8]. There are reasons to believe that the functional state of the liver is also regulated to some extent by enkephalins, as is indicated by their liver-protecting action [4], their influence on metabolic processes in liver cells [2], and the presence of opiate receptors on the plasma membrane of these cells [1]. However, there is no direct experimental evidence that enkephalins are involved in the regulation of a major liver function, namely bile secretion. The purpose of the present work was to examine how enkephalins might influence the bile-secreting function of the liver.

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## MATERIALS AND METHODS

The study was conducted on male white rats with a cannulated common bile duct and involved acute tests. The intensity of bile and bile acid secretions was measured as described earlier [6]. Leu-(LE) and met-(ME) enkephalins, received from the Institute of Organic Synthesis of the Latvian Academy of Sciences, were infused via the portal vein in doses of 0.1, 1, and 10 μg per 100 g body weight. The infusion rate was 50 μl/min and the infusion time, 30 min.

## RESULTS

The intensity of bile secretion during and after the intraportal infusion of LE and ME varied depending on the type and dose of the peptide. When LE was infused in the dose of 0.1 μg, bile secretion decreased throughout the test period, whereas the dose

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