A non-sedating dose of diazepam improves symptoms of panic anxiety disorder in the rat

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Introduction

Benzodiazepines are frequently prescribed to treat symptoms of panic anxiety. Since there is no well established animal model of panic disorder, the anti-panic effect of benzodiazepines has essentially been seen in patients with panic disorder and also in healthy volunteers with experimental panic induced by systemic injections of cholecystokinin-tetrapeptide (CCK-4).

Objective and approach

The goal of the present study is to demonstrate the effect of non-sedating dose of diazepam against experimental panic induced in rodents.

First, the in-vivo efficiency of diazepam is evaluated in mouse and rat anxiety tests and its therapeutic window is then determined by the lack of significant side effects in the mouse open-field and rotarod tests.
Second, similar to the experimental setting in healthy volunteers, systemic administered CCK-4 or Yohimbine is used in rats to induce sign of panic behaviour as assessed in the Elevated Plus Maze.

Finally, anxiolytic dose (side effect free) of diazepam is assessed against experimental panic anxiety-induced in the rat.

Measures of anxiety in mouse and rat

Light dark box test in mouse

The LDB apparatus is consisted of a brightly illuminated and a darken boxes of equal size (19 cm long × 19 cm wide × 15 cm high) connected by a small tunnel. The animal's preference for the lit box is measured during 5 min. period.
An increase in the time spent as well as number of entries in the lit box reflects an index of anxiolytic activity of drugs. In contrast, a decrease in these parameters indicates an index of anxiogenic activity of drugs.

Elevated Plus Maze test in mouse and rat

The EPM apparatus is consisted of four elevated exploratory arms (21 cm long for the mouse and 45 cm long for the rat) which are all interconnected by a small platform (8 cm long x 8 cm wide for the mouse and 10 cm x 10 cm for the rat).
Two arms are open and two others are closed with walls (18 cm high for the mouse and 10 cm high for the rat). The animal's preference for the open arm is measured. An increase in the time spent as well as number of entries in the open arms reflects an index of anxiolytic activity of drugs. In contrast, a decrease in these parameters indicates an index of anxiogenic activity of drugs.

Measures of locomotor disturbance in mouse

Open-Field test

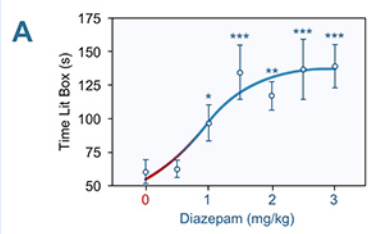
The Open-Field apparatus is consisted of an open arena of 50 cm long x 50cm wide. Activity of the mouse in the Open-Filed for 15 min. (travelled distance) is used to detect stimulant or sedative propriety of drugs that could confound the interpretation of results in anxiety tests.

Rotarod test

The rotarod apparatus is consisted of 3 cm diameter rod of 5 cm length rotating at 12 cycles per minute. The ability of mouse to remain on the rotating rod reflects its sensorimotor performance. Performance in this test is reduced by sedative drugs but increased by drugs having stimulant action.

Figure 1 :

Anxiolytic effect of diazepam anxiety tests in rats or mice
A : Light dark-box test in mice ; B : EPM test in mice ; C : EPM in rats.



In mice, diazepam (up to 3mg/kg) produced a dose-dependent increase in the time spent in the lit box, suggesting anxiolytic effect of diazepam. Similar pattern of result is obtained in mouse EPM.

In contrast, the effect of diazepam follows a bell-shaped curve with maximal effect between 1-1.5mg/kg in the rat EPM.
At higher doses (2 and 3mg/kg), the effect shows dramatic decline and diazepam becomes indeed ineffective.

Data are presented as mean ± sem of 10 animals. *, statistically significant as compared to the performance level of control animals (0 mg/kg diazepam).

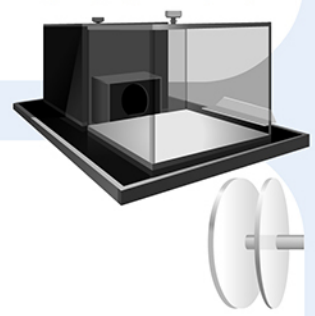
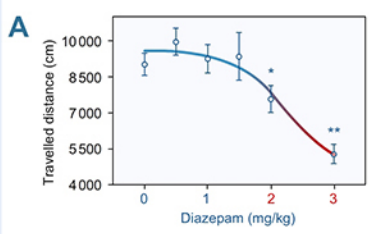


Figure 2 :

Side effect of diazepam in sensorimotor tests in mice
A : Open field test. B : Rotarod test.

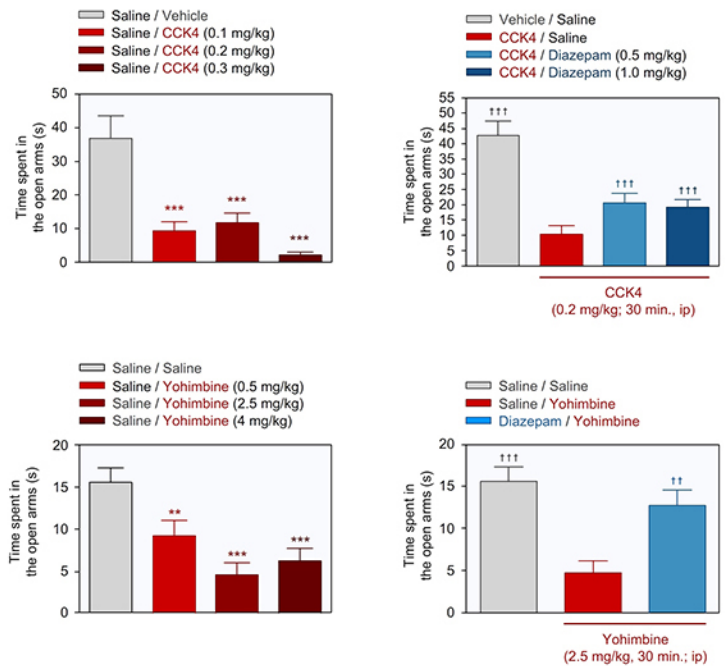


Data are presented as mean ± sem of 10 animals.
*, statistically significant as compared to the performance level of control animals (0 mg/kg diazepam).

Doses of diazepam greater than 1.5 mg/kg (i.e., 2 and 3 mg/kg) markedly affect the locomotor performance and the motor coordination of mice in open-field and in rotarod tests, respectively. Significant sedation is also observed at these doses.

Figure 3 :

Panicogenic effect of systemic CCK-4 or Yohimbine in rat EPM and reversal effect of non-sedating dose of diazepam.

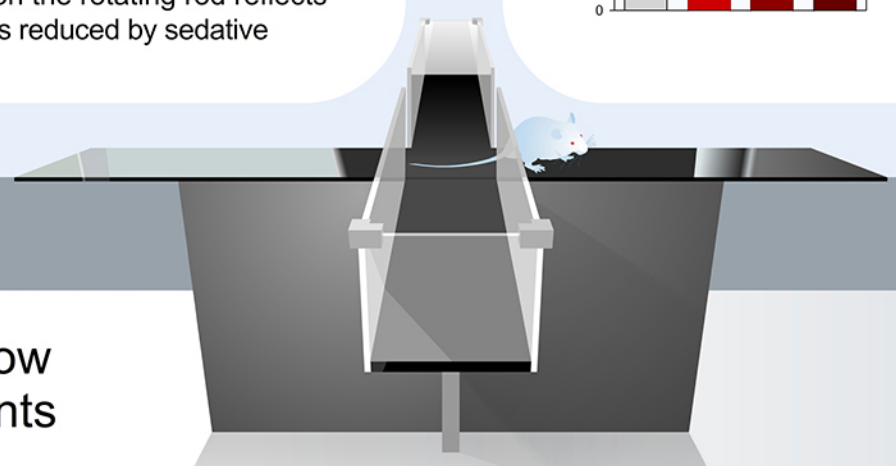


CCK-4 treatment produces a dramatic reduction of EPM performance, significantly below the anxiety level of control animals.

Similar pattern of result is also obtained with Yohimbine treatment.

Non-sedating dose of diazepam (1 mg/kg) normalises the anxiety status of CCK-4 and Yohimbine-treated rats.

Data are presented as mean ± sem of 10 animals.
*, statistically significant as compared to the performance level of vehicle-treated CCK-4 rats.



Summary of key findings

- Diazepam shows a very narrow therapeutic window in rodents
- CCK-4 and Yohimbine provoke panic anxiety in rat EPM and their use could be instrumental for the testing of panicolytic drug candidates
- Non-sedating anxiolytic dose of diazepam reverses CCK-4 and Yohimbine-induce panic anxiety in rat