Possible role of GABA₅-benzodiazepine receptor in anticonvulsant effects of Pasipay in rats

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Objective: To investigate the anticonvulsant effects of Pasipay, a commercially available preparation of hydro-alcoholic extract of Passiflora incarnata in rats.

Methods: The anticonvulsant effects of hydro-alcoholic extract of P. incarnata, Pasipay, were observed by intracerebroventricular injection of 0.125, 0.25, 0.55 and 1.5 μg Pasipay.

Results: Pasipay could dose-dependently affected minimal clonic seizures and generalized tonic-clonic seizures induced by pentylentetrazole, through increment in seizure onset significantly. Additionally, pretreatment with 5 nmol/L flumazenil could abolish the anticonvulsant effects of Pasipay on the onset of both seizures.

Conclusion: The results indicate that Pasipay has anticonvulsant effects in the brain, possibly through positive allosteric modulation of the GABA₅ receptor complex via interaction at the benzodiazepine site.

Keywords: Passiflora incarnata; anticonvulsants; diazepam; GABA₅ receptor; rats

γ氨基丁酸-苯二氮卓受体在西番莲提取物抗大鼠惊厥中的作用

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目的:探讨γ氨基丁酸-苯二氮卓受体在西番莲提取物抗大鼠惊厥中的作用。

方法:在大鼠脑室内分别注射0.125, 0.25, 0.55和1.5 μg西番莲提取物,观察其对大鼠的抗惊厥作用。

结果:西番莲提取物对戊四氮诱发最大阵发性痉挛和阵发性强直性抽搐的大鼠的保护作用存在剂量依赖关系。5 nmol/L氟马西尼可阻断西番莲提取物对最大阵发性痉挛和阵发性强直性抽搐的抗惊厥作用。

结论:西番莲提取物可能通过影响大脑内γ氨基丁酸-苯二氮卓受体的地西洋和受体结合位点来实现抗惊厥的作用。

关键词:西番莲; 抗惊厥药; 地西洋; GABA₅受体; 大鼠


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1 Introduction

*Passiflora incarnata* and other species are widely used in traditional medicine in many countries and *P. incarnata* is an official plant in the pharmacopoeias among most of these countries[13]. *P. incarnata* has shown antianxiety effects on animal models[8, 13]. In human studies, it was effective for the management of generalized anxiety disorder and the physical symptoms of opioid withdrawal[1-4, 32].

In our previous study, the anticonvulsant effects of Pasipay were proved with a pentylentetrazole (PTZ) model in mice by peripheral administration of it. PTZ, a selective blocker of the chloride channel coupled to the GABA<sub>A</sub> receptor complex, is the most popular chemoconvulsant used for the evaluation of antiepileptic drugs[32]. Pasipay (Iran Darouk Pharmaceutical Co., Iran), a commercially available preparation of hydro-alcoholic extract of *P. incarnata*, in the form of a tablet and drops, is used to treat nervous disorders, anxiety, insomnia, and muscular tension in Iran. The total flavonoid content of it is 4% (solution concentration by weight, w/w), which includes both vitexin and rutin[32].

In this study, the anticonvulsant effects of Pasipay in rats were examined by intracerebroventricular administration of Pasipay in the PTZ model. It was predicted to show anticonvulsive effects by central administration in this model, which may be due to several mechanisms[32]. Thus, the possible mechanisms underlying the actions of Pasipay in the central nervous system were elucidated and the probable involvement of the GABAergic system was assessed.

2 Materials and methods

2.1 Materials

2.1.1 Animal Male Wistar rats (250-300 g) were obtained from the Razi Institute (Karaj, Iran) and housed four per cage under standard laboratory conditions. They were kept at room temperature (21 ± 2)°C under a 12L:12D regime with free access to food and water. All animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) to minimize the number of animals used and their suffering.

2.1.2 Plant material Pasipay, hydro-alcoholic extract of *P. incarnata* was obtained from Iran Darouk Pharmaceutical Co. (Tehran, Iran) which was prepared from the standardized extract of leaves, flower and fruit of *P. incarnata*. The total flavonoid content in hydro-alcoholic extract related to the dried plant material was 4% (w/w), including vitexin and rutin.

2.1.3 Chemicals PTZ and flumazenil were purchased from Sigma. The other drugs used in this investigation were diazepam (Chemidaru, Iran), ketamine (Rottexmedica, GmbH, Germany), and xylazine (Loughrea Co, Galway, Ireland). PTZ was dissolved in physiologic saline. Flumazenil was dissolved in 0.8% (volume per volume, v/v) Tween 80.

2.2 Methods

All drugs were freshly prepared for each experiment and administered intracerebroventricularly (i.c.v.) in a volume of 5 µl, apart from PTZ, which was administered intraperitoneally (i.p.). Rats were anesthetized with ketamine (60 mg/kg i.p.) and xylazine (6 mg/kg i.p.), and were placed in a stereotaxic apparatus (Stoelting, USA). The surface of the skull was exposed and the head was oriented. Accordingly, the skull sutures bregma and lambda were at the same vertical levels. Stereotaxic coordinates based on Paxinos and Watson’s atlas of the rat brain were; anterior-posterior (AP), −0.92 mm from bregma; medial-lateral (ML), 1.6 mm from midline; and dorsal-ventral (DV), 3.5 mm from the skull surface[32]. Chronic i.c.v. cannulation was induced according to a previous protocol[40].

2.2.1 Administration methods The rats were divided into six groups, and there was ten animals in each group. The first group was given PTZ (90 mg/kg, i.p.) 30 min after the administration of vehicle (normal saline plus Tween 80, i.c.v.) as control. In the rest of the five groups, Pasipay (0.125, 0.25, 0.5, 1.5 µg, i. c. v.) and diazepam (10 µmol/L, i. c. v.) were given 30 min before PTZ administration (90 mg/kg, i.p.). The animals were placed individually in plastic boxes and observed immediately after PTZ injection for a period of 60 min. Latency to first minimal clonic seizure (MCS, the first myoclonic twitch), sudden involuntary jerking of the whole body, incidence of MCS, latency to first generalized tonic-clonic seizure (GTCS, the full tonic extension of both forelimbs and hindlimbs), incidence of GTCS and protection percentage against mortality were evaluated during PTZ administration[10].

2.2.2 Anticonvulsant effects In order to investigate the probable involvement of GABA<sub>A</sub>-benzodiazepine receptors, the effects of a selective GABA<sub>A</sub>-benzodiazepine receptor antagonist, flumazenil, on the anticonvulsant activity of Pasipay were also studied. Ten rats in each group were selected. In the first group, rats received flumazenil (5 nmol/L) 10 min before normal saline (5 µL, i.c.v) (30 min before the injection of PTZ). In the second group, the animals were given flumazenil (5 nmol/L) 10 min before the administration of Pasipay (1.5 µg) (30 min before the injection of PTZ). In the third group, the animals received flumazenil (5 nmol/L) 10 min before the administration of diazepam (10 µmol/L) (30 min before the injection of PTZ). The anticonvulsant activity of Pasipay and diazepam in
rats pretreated with flumazenil was assessed and compared with vehicle, flumazenil, Pasipay and diazepam treated groups. After recording the effects of the various drugs, the animals were injected with 2 μl crystal violet and deeply anesthetized. The brains were removed and fixed in 10% formaldehyde solution. For the histological examination of the cannula and needle placement in the lateral ventricle region, 100 μm-thick sections were taken and the cannula track was examined in each rat. Only those animals whose cannulas were exactly placed in the left ventricle were used for data analysis.

2.2.3 Statistical analysis The data were expressed as x̄ ± s. Fisher’s exact probability test, as well as analysis of variance (ANOVA), followed by the multiple comparison test of Tukey-Kramer were used for statistical evaluation. P value < 0.05 was considered significant.

3 Results

All rats in the experimental groups were positively verified with histological examination. All animals treated with vehicle 30 min before PTZ (90 mg/kg, i.p.) presented MCS and GTCS (Table 1). Microinjection of Pasipay (1.5 μg), 30 min before the injection of PTZ, significantly prolonged the latency of MCS compared to vehicle (P < 0.05) (Table 1). Moreover, Pasipay (0.55, 1.5 μg, i.c.v.) significantly prolonged the latency of GTCS compared to vehicle in a dose-dependent manner (P < 0.05, P < 0.01) (Table 1).

As shown in Table 1, Pasipay exhibited its protective effect against seizures in a dose-dependent manner. In addition, the survival rate of Pasipay increased dose-dependently (Table 1). However, protection against seizures and mortality were not observed in a significant manner.

Pretreatment with flumazenil (5 nmol/L, i.c.v.) 10 min prior to Pasipay abolished its protective effects for the incidence and latency of MCS (Table 1). Flumazenil also reduced the protective effects of Pasipay for the incidence as well as latency of GTCS (Table 1). Furthermore, diazepam (10 μmol/L, i. c. v.) significantly prolonged the latency of MCS and GTCS (P < 0.01) and it also decreased MCS and GTCS incidence (Table 1). Diazepam significantly increased the survival rate compared to vehicle (P < 0.01). In contrast to diazepam, pretreatment with flumazenil significantly decreased the anticonvulsant effects (Table 1).

Table 1 Effects of Pasipay on MCS and GTCS induced by PTZ in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>MCS (n)</th>
<th>MCS latency (x̄ ± s)</th>
<th>GTCS (n)</th>
<th>GTCS latency (x̄ ± s)</th>
<th>Survival rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>10</td>
<td>50.4 ± 2.3</td>
<td>10</td>
<td>81.3 ± 10.1</td>
<td>0</td>
</tr>
<tr>
<td>10 μmol/L Diazepam</td>
<td>8</td>
<td>268.0 ± 69.7&quot;**</td>
<td>4</td>
<td>447.4 ± 67.4&quot;**</td>
<td>60</td>
</tr>
<tr>
<td>0.125 μg Pasipay</td>
<td>10</td>
<td>48.9 ± 1.8</td>
<td>10</td>
<td>178.0 ± 43.7</td>
<td>0</td>
</tr>
<tr>
<td>0.25 μg Pasipay</td>
<td>10</td>
<td>62.0 ± 2.9</td>
<td>10</td>
<td>225.8 ± 64.4</td>
<td>0</td>
</tr>
<tr>
<td>0.55 μg Pasipay</td>
<td>10</td>
<td>110.0 ± 32.4</td>
<td>7</td>
<td>386.7 ± 59.3&quot;**</td>
<td>30</td>
</tr>
<tr>
<td>1.5 μg Pasipay</td>
<td>8</td>
<td>256.2 ± 66.4&quot;**</td>
<td>6</td>
<td>395.8 ± 101.7&quot;**</td>
<td>40</td>
</tr>
<tr>
<td>5 nmol/L Flumazenil</td>
<td>10</td>
<td>50.5 ± 4.5</td>
<td>10</td>
<td>85.5 ± 17.1</td>
<td>0</td>
</tr>
<tr>
<td>Flumazenil plus diazepam</td>
<td>10</td>
<td>63.0 ± 3.9</td>
<td>10</td>
<td>133.8 ± 18.3</td>
<td>0</td>
</tr>
<tr>
<td>Pasipay plus flavumazenil</td>
<td>10</td>
<td>47.5 ± 4.4</td>
<td>10</td>
<td>150.3 ± 18.9</td>
<td>0</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01, vs vehicle group.

4 Discussion

Our results demonstrated that central administration of Pasipay had dose-dependent effects on epileptic seizures induced by PTZ, through increment in seizure onset. However, seizure incidence and mortality were not significantly decreased. Diazepam, as a positive control, significantly increased MCS latency and suppressed GTCS. Pretreatment with flumazenil reversed the anticonvulsant effects of diazepam. Moreover, pretreatment with flumazenil inhibited the significant anticonvulsant effects of Pasipay. Flumazenil reversed the effects of Pasipay for the increment of MCS and GTCS latency as well as the protection percentage against MCS and GTCS. Upon pretreatment with flumazenil, mortality was also increased, and it might therefore be assumed that Pasipay exerted its anticonvulsant effects through GABA_A-benzodiazepine receptor complex. Thus, its active components may be bind to the benzodiazepine receptors. These results are in agreement with our previous study. It was found that peripheral administration of Pasipay had anticonvulsant effects in the PTZ model and flumazenil could reverse its anticonvulsant effects. Besides, the role of GABAergic system on the anxiolytic effects induced by *Passiflora actina* extracts was recently reported and the effects were reversed by flumazenil. In contrast, Soulimani et al. reported that the effects of *P. incarnata* were not mediated through an action on the GABA receptors. On the other hand, it seemed that peripheral administration of Pasipay was more effective than central administration. However, other mechanisms had discussed about the anticonvulsant effects of peripheral administration. Naloxone only antagonized the effects of Pasipay on
decreasing the duration of clonic seizures in the PTZ model compared to the control group. Nevertheless, it did not show any significant reversal of Papisay effects. It seemed that some part of its anticonvulsant effects related to activation of opioid system, which was attenuated by naloxone in peripheral administration of Papisay.

In our study, it is possible that anticonvulsant effects of Papisay may be due to its flavonoids which include rutin or vitexin found in the extract. On the contrary, there was a controversial study which reported that pure vitexin and isovitexin of P. incarnata had no activity in central nervous system tests. However, rutin increased thiopental-induced sleeping time in mice, and it also reduced the exploratory parameters and locomotor activity. There are some studies reported on the neuroprotective effect of Papisay extract and the possible anticonvulsant effects of the extract may be due to rutin (3, 4, 5, 7-pentahydroxyflavone-3-rhamnoglucoside), which is a flavonoid of the flavonol type. Recently, some anticonvulsant effects have been shown in the PTZ-model.

In summary, central administration of Papisay possesses the agonistic activities on the GABAAergic system, and this effects can be reversed by a benzodiazepine receptor antagonist. Further studies need to make clear that these flavonoids have anticonvulsant effects via binding to the benzodiazepine-site of GABA receptors, and allosterically modulating the chloride flux through the ion channel complex.

REFERENCES