EFFECTS OF XYLAZINE ON GLUTAMATE AND GABA CONTENTS IN THE HIPPOCAMPUS AND THALAMENCEPHAL IN THE RAT

BAI-SHUANG YIN1,2, HONG-BING WANG3, DU-QIANG GONG3, GUO-JIANG LI1, AND LI GAO1

1College of Veterinary Medicine, Northeast Agricultural University, Harbin 150030, China Department of Veterinary Medicine, 2Jilin Agriculture Science and Technology College, Jilin 132101, China gaoli43450@163.com

Received: December 11, 2010     Accepted: May 6, 2011

Abstract

The purpose of the study was to define if anaesthetic action of xylazine could conceivably result from the potentiation of inhibitory neurotransmitters or the inhibition of excitatory neurotransmitter systems in the brain. Rats were injected with xylazine at a dose of 50 mg/kg b.w., and then the hippocampus and thalamencephal were removed at 0.1, 0.25, 0.5, 1, 1.5, 2, 4, and 6 h after the injection. Glutamate (Glu) and γ-aminobutyric-acid (GABA) were measured in the brain tissue by reversed-phase high-performance liquid chromatography. The results revealed that the hippocampus Glu level decreased significantly 0.1 h after the injection of xylazine, the thalamencephal GABA increased significantly 0.1 h after the injection, while the changes in hippocampus GABA and thalamencephal Glu were not significant. However, all of these changes returned to the normal level after 2 and 4 h, respectively. The results indicated the relative effects of xylazine on Glu and GABA levels in the hippocampus and thalamencephal.

Key words: rat, xylazine, glutamate, γ-aminobutyric-acid, brain.

Xylazine exhibits analgesic, muscle relaxant, and slight sedation properties. Furthermore, xylazine also can evoke some other effects: restrain of ventricle conduction, decrease in heart rate and venous pressure (14), restrain of the respiratory system, decrease in body temperature (13), decrease in plasma concentration of T3, and significant increase in plasma concentration of insulin and glucagon (11). Xylazine has been used as a component of a variety of anesthetics in domestic and wild animals in the developing countries (16, 18). It was demonstrated that anaesthesia could change the concentration of amino acid neurotransmitters such as glutamate (Glu) and γ-aminobutyric-acid (GABA) (4, 10, 15). Changes in the concentration of both compounds may induce many kinds of nervous system diseases (9).

Despite an extensive use of xylazine in veterinary practice, there is very little information about the effects of xylazine on amino acid neurotransmitters. Thus this investigation focuses on the effects of xylazine on Glu and GABA concentrations.

Material and Methods

Animals. Male and female Sprague-Dawley rats weighing 200±20 g were used. The animals were housed at a controlled temperature (20±2°C) and maintained under light-dark cycles, each consisting of 12 h of light and 12 h of darkness (lights on from 06:00 to 18:00 h), with feed and water available ad libitum. The animals were divided into two groups: the control group and xylazine treated group (five rats in each group). The experimental procedures were performed in accordance with the Local Ethics Committee for Animal Experiments.

Experimental procedures. A brain tissue was removed before the treatment (0 h, control) in control group. The rats were injected intraperitoneally with 50 mg/kg of xylazine (Bayer Ltd, USA), and decapitated 0.1, 0.25, 0.5, 1, 1.5, 2, 4, and 6 h after the injection. The hippocampus and thalamencephal of control and experimental rats were dissected on ice and frozen immediately. Each sample was homogenised in 1 ml of ice-cold deionised methanol (V/V=50:50) and centrifuged at 3,000 rpm for 30 min at 4°C. Forty
microlitres of supernatant was removed to another test tube and with added 40 µl of acetonitrile was centrifuged at 15,000 rpm for 10 min at 4°C. Then 49 µl of 0.5 mol/L NaHCO₃ and 20 µl of 0.5% dinitro-fluorobenzene were added to the supernatant, mixed, and heated for 50 min in 65°C water bath.

**Chromatography.** Detection by RP-HPLC was performed using a C₁₈ column (4.6 mm×200 mm, 5 µm) and chromatographic system (Waters 2695, USA) at room temperature. The eluent containing 0.05 mol/L sodium acetate (pH 6.9) and deionised acetonitrile (V/V=50:50) was pumped through the column at 1 ml/min. The detection was made at 350 nm.

**Statistical analysis.** The results were analysed by variance analysis. Experimental data were expressed as the mean±standard deviation (X±SD). SPSS 13.0 was used for statistical analysis of the data. The differences between control values and those obtained after treatment were considered significant at P<0.05.

### Results

Thalamencephal concentrations of Glu and GABA in control animals and rats injected with xylazine are shown in Table 1. As can be seen from the Table, Glu concentrations did not change significantly after drug injection, compared with control group. GABA concentrations changed markedly after the injection compared with controls (P<0.01, P<0.05). The concentrations achieved the maximum value at 0.25 h after injection and the minimum value was detected at 4 h after injection.

In the hippocampus, Glu concentrations reached the minimum value at 0.25 h after injection, compared with controls; while the maximum value of Glu concentrations was detected in control rats (Table 2). This difference was significant (P<0.01 or P<0.05). GABA concentrations were not significantly changed after drug injection as compared with control group (P > 0.05).

<table>
<thead>
<tr>
<th>Time after injection (h)</th>
<th>GLU (µg/mL)</th>
<th>GABA (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>23.91 ± 1.09</td>
<td>13.69 ± 0.46</td>
</tr>
<tr>
<td>0.1</td>
<td>21.62 ± 1.27</td>
<td>13.94 ± 0.38</td>
</tr>
<tr>
<td>0.25</td>
<td>13.41 ± 0.83</td>
<td>14.02 ± 0.45</td>
</tr>
<tr>
<td>0.5</td>
<td>13.74 ± 0.67</td>
<td>14.13 ± 0.29</td>
</tr>
<tr>
<td>1</td>
<td>15.57 ± 0.55</td>
<td>14.17 ± 0.27</td>
</tr>
<tr>
<td>1.5</td>
<td>18.55 ± 1.23</td>
<td>14.33 ± 0.37</td>
</tr>
<tr>
<td>2</td>
<td>22.55 ± 1.08</td>
<td>14.43 ± 0.34</td>
</tr>
<tr>
<td>4</td>
<td>23.08 ± 1.31</td>
<td>14.38 ± 0.42</td>
</tr>
<tr>
<td>6</td>
<td>23.55 ± 1.08</td>
<td>14.31 ± 0.56</td>
</tr>
</tbody>
</table>

▲ P<0.05, ▲▲ P<0.01 compared with control group.
Discussion

The knowledge about the neurotransmitters involved in inhibition and excitation of the motor system is still incomplete. Within the mammalian central nervous system (CNS), Glu is the most abundant excitatory amino acid neurotransmitter. Apart from its physiological role in neuronal excitation, Glu is also known to cause structural and functional damage including cellular swelling and neuronal death (5). GABA is one of the major inhibitory neurotransmitter for fast inhibitory synaptic transmission and regulates many physiological and psychological processes (2). Hypoxia may increase GABA levels by inhibiting GABA metabolism by GABA transaminase due to induced low pH (12). Indirect evidence of GABA as a contributor to intracortical excitability has been gathered (19).

Past research has shown that the effect of anaesthesia on Glu and GABA varies. For example, it was demonstrated that the sevoflurane inhibits the release of Glu in an encephalic region (1). The higher doses of ketamine decrease release of Glu (15). It has been reported that enflurane reduces uptake of GABA and causes GABA accumulation in the synapse (7). Isoflurane, propofol, ethylether, and pentothal inhibit degradation of GABA and increase GABA concentration (9). Our results showed that the level of hippocampus Glu 0.1 h after xylazine administration was lower than that of the control group, but returned to a normal level 2 h after the start of anaesthesia. Thalamencephal GABA concentration was higher than that of the control group, and then returned to a normal level at 4 h after the start of anaesthesia.

It has been shown that isoflurane inhibits the release of Glu, without influencing uptake mechanisms (15). Propofol has been reported to reduce uptake of Glu without limiting neuronal glutamate release (3, 6). In addition, a wide range of anaesthetic agents inhibit excitatory synaptic transmission, an effect that could be mediated by blockade of Ca$$^{2+}$$ entry through receptor operated and/or voltage-sensitive Ca$$^{2+}$$ channels (VSCCs) (8). Intravenous anaesthetic agents significantly inhibit K$$^{-}$$-evoked glutamate release from rat cerebrocortical slices, due to depression of P/Q-type VSCCs and activation of GAB$$\alpha$$ receptors (10). In this study, xylazine inhibited Glu and facilitated GABA release mechanisms.

We found that at the beginning of injection, the hippocampus Glu level first decreased and then increased. On the other hand, thalamencephal GABA level first increased then decreased, as the action of xylazine had been reduced. These findings are very important to understand the role of xylazine for defending nerve injury in the combined anaesthesia.

Acknowledgments: This study was funded by two grants financed by the Northeast Agricultural University doctoral research start-up funding and Heilongjiang province post-doctoral research start-up funding (LBH–Q07016).

References