Research report

Effects of agmatine on ethanol withdrawal syndrome in rats

I. Tayfun Uzbay *, Özgür Yeşilyurt, Turgay Çelik, Hakan Ergün, Aşkin İşmer

Department of Medical Pharmacology, Psychopharmacology Research Unit, Faculty of Medicine, Gülhane Military Medical Academy, Etlik 06018, Ankara, Turkey

Received 5 July 1999; received in revised form 10 August 1999; accepted 10 August 1999

Abstract

Effects of agmatine, which is an endogenous polyamine metabolite formed by decarboxylation of L-arginine, have been investigated on the ethanol withdrawal syndrome in rats. Adult male Wistar rats were used in the study. Ethanol (7.2% v/v) was given to the rats by a liquid diet for 21 days. Agmatine (20, 40, 80 and 160 mg/kg) and saline were injected to rats intraperitoneally 30 min before ethanol withdrawal testing. After 30th min, 2nd and 6th h of ethanol withdrawal, rats were observed for 5 min, and withdrawal signs which included locomotor hyperactivity, agitation, stereotyped behavior, wet dog shakes and tremor were recorded or rated. A second series of injections was given at 6 h after the first one, and subjects were then tested for audiogenic seizures. Agmatine caused dose-dependent and significant inhibitory effects on stereotyped behaviors, wet dog shakes and tremors during the observation period. It did not cause any significant change in motor coordination of naive (not ethanol-dependent) rats. Our results suggest that agmatine attenuates withdrawal syndrome in ethanol-dependent rats; thus, this drug may be beneficial in the treatment of ethanol dependence. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Agmatine; Ethanol withdrawal syndrome; Ethanol dependence; Rat

1. Introduction

Ethanol abuse and dependence remain among the greatest substance abuse problems worldwide. Ethanol withdrawal syndrome precipitated by discontinuing chronic ethanol intake is the most important evidence indicating the presence of physical ethanol dependence [22]. Although the signs of ethanol withdrawal in humans [36] and rats [20,40,44] have been well described, the mechanisms underlying physical dependence to ethanol are poorly understood.

Agmatine is a cationic amine formed by decarboxylation of arginine by the enzyme arginine decarboxylase [33]. It is a biologically active substance [19], but the mode and sites of action have not been fully defined. Agmatine binds with high affinity to both imidazoline and α2-adrenergic receptors of all subclasses [18,25,28,30] and is widely distributed in rat tissue, such as serum, viscera and brain including astrocytes [23,27,29]. It has also been suggested that agmatine blocks nitric oxide synthase (NOS) in rats [3,9]. Current evidence is consistent with a hypothesis that agmatine meets many criteria for a neurotransmitter in brain. It is synthesized, stored, and released in brain; is contained in neurons and axon terminals; interacts with cell specific receptors; and elicits biological actions within the central nervous system [30,31].

Results of the some recent studies indicate modulatory effects of agmatine on opioid analgesia in rats [13] and mice [14]. The role of increased glutamergic transmission in development of the opioid [16,37,38] and ethanol [15,32] physical dependence has been well known and agmatine selectively inhibits the NMDA subclass of glutamate receptor channels in rat hippocampal neurons [46]. Furthermore, in our previous study, we observed that agmatine prevents all the signs of naloxone-precipitated abstinence syndrome in morphine dependent rats in a dose-dependent manner...
and hypothesized that it may be a potent neuropharmacologically active agent on mechanisms involved in development of morphine physical dependence [2]. However, the effects of agmatine on development of tolerance or physical dependence to ethanol has not been investigated yet. It may also have some beneficial effects on ethanol withdrawal.

In the present study, we aimed to investigate the possible effect of agmatine on withdrawal syndrome in ethanol-dependent rats.

2. Methods

2.1. Animals and laboratory

All procedures in this study are in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health (USA). Adult male Wistar rats (205–286 g weight at the beginning of the experiments) were subjects. They were housed in a quiet and temperature and humidity-controlled room (22 ± 3°C and 65 ± 5%, respectively) in which a 12-h light/dark cycle was maintained (08:00–20:00 h light). Exposure to ethanol and all behavioral experiments involved in ethanol withdrawal syndrome were carried out in the separate and isolated laboratories, which have the same environmental conditions with the colony room.

Table 1
Rating scale for some behavior signs induced by ethanol withdrawal in rats

<table>
<thead>
<tr>
<th>Signs</th>
<th>Scoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stereotyped behavior</td>
<td>0: no stereotyped behavior</td>
</tr>
<tr>
<td></td>
<td>1: rats showing only one stereotyped behavior</td>
</tr>
<tr>
<td></td>
<td>2: two stereotyped behavior</td>
</tr>
<tr>
<td></td>
<td>3: three stereotyped behavior</td>
</tr>
<tr>
<td></td>
<td>4: four stereotyped behavior</td>
</tr>
<tr>
<td></td>
<td>5: all of stereotyped behavior</td>
</tr>
<tr>
<td>Agitation</td>
<td>0: no irritability or aggressive behavior</td>
</tr>
<tr>
<td></td>
<td>1: rats showing mild or moderate irritability</td>
</tr>
<tr>
<td></td>
<td>2: very irritable</td>
</tr>
<tr>
<td></td>
<td>3: handling vocalization and moderately aggressive</td>
</tr>
<tr>
<td></td>
<td>4: handling vocalization and very aggressive</td>
</tr>
<tr>
<td></td>
<td>5: spontaneous vocalization and very aggressive</td>
</tr>
<tr>
<td>Audiogenic seizures</td>
<td>0: no seizure</td>
</tr>
<tr>
<td></td>
<td>1: rats showing only wild running</td>
</tr>
<tr>
<td></td>
<td>2: rats showing tonic seizure</td>
</tr>
<tr>
<td></td>
<td>3: rats showing tonic-clonic seizure</td>
</tr>
<tr>
<td></td>
<td>4: rats showing tonic-clonic seizure &gt; 90 s</td>
</tr>
<tr>
<td></td>
<td>5: mortality following the seizure</td>
</tr>
</tbody>
</table>

2.2. Oral ethanol self administration

For chronic ethanol exposure, the rats were housed individually and ethanol was given in the modified liquid diet as previously described [40]. At the beginning of the study, rats were given the modified liquid diet without ethanol for 7 days. Then liquid diet with 2.4% ethanol was administered for 3 days. The ethanol concentration was increased to 4.8% for the following 4 days and finally to 7.2% for 21 days. Liquid diet was freshly prepared daily and presented at the same time of the day (10:00 h). The weight of the rats was recorded every day, and daily ethanol intake was measured and expressed as g per kg per day. Control rats (n = 8) were pair fed an isocaloric liquid diet containing sucrose as a caloric substitute to ethanol.

2.3. Drug used in the study

Agmatine sulfate were purchased from Sigma Chemical (USA) and dissolved in saline. Agmatine or saline were injected to rats intraperitoneally at a volume of 1 ml/200 g body weight. Drug solutions were prepared freshly in the morning of each experiment.

2.4. Evaluation of ethanol withdrawal syndrome

At the end of the exposure to 7.2% ethanol-containing liquid diet, ethanol was withdrawn from the diet by replacing the diet with one that did not contain ethanol at 10:00 h. Ethanol-dependent rats were then assigned into five groups (n = 8 for each group). Agmatine (20, 40, 80 and 160 mg/kg) and saline were injected 30 min before ethanol withdrawal testing.

The rats were then observed for 5 at 30 min, then 2 and 6 h of the withdrawal period. At each observation time, rats were assessed simultaneously for the following behavioral conditions: agitation, wet dog shakes, stereotyped behavior and tremor. Horizontal and vertical locomotor activities of the rats were also recorded (Opto Varimex Minor, Columbus, OH, USA) and expressed as mean ± SEM. Wet dog shakes and tremors were assessed as incidence. Wet dog shakes behavior was considered positive if they occurred at least three times during the observation period. Tremor was determined after lifting rats vertically by the tail; positive was assigned to rats showing clearly distinct forelimb tremor when they were rotated 180° around axis of the tail. Grooming, sniffing, head weaving, gnawing and chewing were observed as major stereotyped behaviors during the ethanol withdrawal in the study. Stereotypic behaviors and agitation were scored using a rating scale as previously described [44] (Table 1).

Each group received a second injection of its original drug given at 6 h after the first injection. After 6 h of withdrawal testing, rats were exposed to an audiogenic
Fig. 1. Changes in horizontal (A) and vertical (B) locomotor activities during the first 6 h of ethanol withdrawal (n = 8 for each group; h = hour; Hor. = horizontal; Ver. = vertical; *P < 0.05 significantly different from control).

### Table 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Incidence (%)</th>
<th>Intensity (mean score)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>62.5</td>
<td>2.25</td>
</tr>
<tr>
<td>Agmatine (20 mg/kg)</td>
<td>37.5</td>
<td>1.12</td>
</tr>
<tr>
<td>Agmatine (40 mg/kg)</td>
<td>37.5</td>
<td>1.00</td>
</tr>
<tr>
<td>Agmatine (80 mg/kg)</td>
<td>25.0</td>
<td>0.75</td>
</tr>
<tr>
<td>Agmatine (160 mg/kg)</td>
<td>25.0</td>
<td>0.50</td>
</tr>
</tbody>
</table>

* n = 8 for each group.

stimulus (100 dB) for 60 s in a separate and soundproof place in the laboratory. The intensity of the audiogenic seizures was scored according to Morisset et al. [21] with a slight modification (Table 1).

All experiments were carried out during the light period. All ratings were done by a naive observer who was unaware which treatment the rats received.

#### 2.5. Measurement of accelerod performance in naive control rats

Agmatine (20, 40, 80 and 160 mg/kg) and saline were administered in five groups of naive (not ethanol-dependent) Wistar rats. A total of 30 min after the injections, rats were put into the accelerod performance test apparatus (Rotamex V-EE/85, Columbus, OH, USA) and accelerod performance of the rats was measured as described in previous studies [6,41,42]. Training for accelerod tests was performed during the week when ethanol-free diet was given. Rotational velocity of the rod was linearly increased from 0 to 79 rpm within 4 min. The time (seconds) at which the animal fell off the accelerating rods was automatically measured by the built-in timer of the apparatus. The results of the accelerod performance tests were expressed as mean ± SEM.

#### 2.6. Statistical analysis

Changes in horizontal and vertical locomotor activities of ethanol-dependent rats as compared with ethanol non-dependent control rats were analyzed by unpaired (between groups) Student’s t-test. Analysis of variance (one-way ANOVA) followed by Dunnett’s test were used in evaluation of the effects of agmatine on the locomotor activities and accelerod performance. The intensities of the agitation, stereotyped behavior and audiogenic seizures in different groups were compared by Mann–Whitney U-test. Comparison of the incidences of the audiogenic seizures, wet dog shakes behavior and tremors were done by Chi-square test. The level of significance was set at P < 0.05 level.

### 3. Results

#### 3.1. Ethanol consumption

Daily ethanol consumption of the rats ranged from 12.67 ± 2.01 to 14.76 ± 2.48 during the exposure to ethanol (7.2%). No significant difference between the ethanol ingesting groups was observed.

#### 3.2. Behavioral changes during ethanol withdrawal

Significant horizontal and vertical locomotor hyperactivity were observed in the ethanol-dependent group from the 30th min of the withdrawal-testing period as compared with the ethanol non-dependent saline group (Fig. 1A and B). Other behavioral signs of ethanol withdrawal syndrome such as stereotyped behavior, wet dog shakes, tremor and agitation appeared at 30 min and lasted during the whole observation period (Fig. 3A–D dark bars). Audiogenic seizures occurred at 6 h
of ethanol withdrawal with an incidence of 62.5% in control group (Table 2). No ethanol withdrawal signs were observed in the ethanol non-dependent rats.

3.3. Effects of agmatine on ethanol withdrawal syndrome

Agmatine did not cause any significant change on the horizontal and vertical hyperactivity induced by ethanol withdrawal at the 30th min, 2nd and 6th h of the observation period \[F_s(4, 35) < 1.23; P_s > 0.05\] (Fig. 2A and B).

Agmatine reduced, dose-dependently and significantly, the intensity of stereotyped behavior and incidence of wet dog shakes and tremors appearing during the ethanol withdrawal. The effect of agmatine on the signs were more prominent at the 30th min and 6th h of the observation period in its higher doses (40, 80 and 160 mg/kg) (Fig. 3A–C). It also reduced significantly the severity of agitation at the 2nd h of the ethanol withdrawal in doses of 80 and 160 mg/kg (Fig. 3D). It reduced both incidence and intensity of the audiogenic seizures appearing at the 6th h of ethanol withdrawal, dose-dependently (Table 2) but the inhibitory effect of agmatine did not reach a statistically significant level.

3.4. Effects of agmatine on accelerod performance in ethanol non-dependent (naive) rats

Agmatine did not cause any significant change on motor coordination of the naive (no ethanol-dependent) rats [\(F(4, 25) = 2.238; P = 0.093\)] (Fig. 4).

4. Discussion

The present study demonstrates that agmatine, an arginine metabolite, has some inhibitory effects on the withdrawal syndrome in ethanol-dependent rats. Consistent with our previous findings Refs. \[43–45\] the present data demonstrated that daily ethanol consumption ranged from 12.67 to 14.76 g/kg for 21 consecutive days produced physical dependence in rats. Majchrówicz \[20\] also showed that dependence and signs of ethanol withdrawal could be produced in rats with 4-day intragastric administration of 9–15 g/kg of ethanol per day. Because we did not observe any significant change on the locomotor activities of the dependent rats during the observation period and on the motor coordination in naive group, the beneficial effects of agmatine on ethanol withdrawal syndrome are not related to other non specific effects such as sedation or muscle relaxation.

These inhibitory effects of agmatine on ethanol withdrawal syndrome may be explain by three mechanisms: First, agmatine binds to \(\alpha_2\)-adrenoceptors \[18,\] and drugs such as clonidine which bind to these receptors also have prominent inhibitory effects on the ethanol withdrawal syndrome in rats \[24\] and humans \[4,5\]. These data suggest that perhaps agmatine may inhibit ethanol withdrawal by a clonidine-like effect. However, unlike clonidine, agmatine has not been shown to have agonist activity at \(\alpha_2\) receptors \[26\]. Alternatively, it has been reported that agmatine acts as an agonist at imidazoline receptors \[18,28,30,31\]. This action could be responsible for its beneficial effects on ethanol withdrawal syndrome, because this property is shared with clonidine, another potent inhibitor of the ethanol withdrawal syndrome.

A second explanation of these beneficial effects of agmatine on ethanol withdrawal syndrome may be a central inhibition of NOS by agmatine. Some recent studies have been shown that NOS inhibitors cause a prominent attenuation signs of ethanol withdrawal syndrome in rats \[1,17,44\]. Recently, the results of some studies indicated that agmatine inhibits NOS in isolated rat aorta \[3\] and in brain \[9\].

Another interesting possibility explaining the inhibitory effects of agmatine on ethanol withdrawal may
be an interaction with central excitatory amino acidergic mechanisms via nitric oxide (NO). Activation of excitatory amino acid receptors, particularly the NMDA subtype, causes an influx of calcium into neurons leading to calmodulin-dependent activation of NOS [11]. Thus, activation of NMDA receptors may be accompanied by formation of NO [10]. The role and importance of NMDA receptors and excitatory amino acid stimulation in the development of ethanol physical dependence is well known [7,12,32,35,39] and NMDA receptor blockers inhibit the ethanol withdrawal syndrome in rats [8,34]. NMDA receptor activation relies upon NO as a significant neuronal messenger, then NOS inhibition in the glutamate system may also be responsible for the inhibitory effect of agmatine on ethanol withdrawal signs. On the other hand, agmatine selectively blocks NMDA receptor channels in rats [46] and its beneficial effects on ethanol withdrawal may also be directly related to a direct interaction with NMDA receptors.

In conclusion, agmatine seems to be a new and a potent pharmacologically active agent on mechanisms involved in development of ethanol physical dependence in rats, and it may have therapeutic potential in the treatment of ethanol-type dependence.
Acknowledgements

This study was granted by the Scientific and Technical Research Council of Turkey (Project No. TUBITAK, SBAG-AYD-235). Authors would like to thank to Hakan Kayir for his technical assistance during the study.

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