Ethanol desensitizes cannabinoid CB1 receptors modulating monoamine synthesis in the rat brain in vivo

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Abstract

The endocannabinoid system and the cannabinoid CB1 receptors are involved in the development of ethanol tolerance and dependence. This study aimed to investigate the in vivo sensitivity of a CB1 receptor agonist (WIN 55,212-2) modulating the synthesis of 3,4-dihydroxyphenylalanine/dopamine/noradrenaline (DOPA/DA/NA) and that of 5-hydroxy-tryptophan/serotonin (5-HTP/5-HT) in rat brain after ethanol treatment and withdrawal. In control rats, WIN 55,212-2 (4 mg/kg, i.p., for 1 h), through a mechanism sensible to the CB1 antagonist SR 141716A, increased the synthesis of DOPA/NA in a slice of brainstem containing the locus ceruleus (250%) and in the hippocampus (64%), and it reduced DOPA/DA synthesis in the striatum (47%). WIN 55,212-2 also decreased the synthesis of 5-HTP/5-HT in the locus ceruleus (43%), hippocampus (35%) and striatum (35%). In the locus ceruleus of ethanol-treated rats, the stimulatory effect of WIN 55,212-2 on DOPA/NA synthesis was abolished (acute treatment) or markedly attenuated (53–55%, chronic treatment and withdrawal), whereas in the hippocampus this effect was reduced only in chronic ethanol-withdrawn rats (33%). In the striatum of ethanol-treated rats (acute, chronic and withdrawal), the inhibitory effect of WIN 55,212-2 on DOPA/DA synthesis was completely blunted or markedly reduced. Similarly, the inhibitory effect of WIN 55,212-2 on 5-HTP/5-HT synthesis was reduced or abolished in the three brain regions after chronic ethanol and during withdrawal. These results indicate that treatment with ethanol in rats induces a functional desensitization of CB1 receptors modulating the synthesis of brain monoamines.

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Recent studies have provided clear evidence for the participation of the endocannabinoid system in the pharmacological and behavioral actions of alcohol, including preference and intake of ethanol, vulnerability and addiction [11,14]. Thus, mice lacking the CB1 receptor gene exhibited striking reduced voluntary ethanol consumption [12,18], consistent with other studies showing that treatment with selective CB1 receptor antagonists (SR 141716A and SR 147778) resulted in reductions of ethanol intake or in ethanol preference in normal and selected high drinking rat lines [1,5,9]. Moreover, chronic ethanol exposure in rodents has been shown to induce down-regulation (radioligand binding and function) of CB1 receptors [2,11,14]. On the other hand, previous investigations have demonstrated that cannabinoids, through the activation of CB1 receptors, also modulate monoamine synthesis [15] and release [6,13], and that ethanol intake is also reduced by drugs interfering with central monoamine systems [19,20]. Based on these observations, the aim of this study was to assess the in vivo sensitivity of a CB1 receptor agonist (WIN 55,212-2) modulating the activity of tyrosine hydroxylase (synthesis of 3,4-dihydroxyphenylalanine/dopamine/noradrenaline; DOPA/DA/NA), and tryptophan hydroxylase (synthesis of 5-hydroxytryptophan/serotonin; 5-HTP/5-HT) in rat brain after the acute and subchronic administration of ethanol and during ethanol withdrawal. Male Sprague-Dawley rats (220–240 g) were used and adequate measures were taken to minimize animal pain or discomfort. To quantitate the effects of ethanol on monoamine synthesis, groups of rats were treated with ethanol (20% ethanol/80% water) acutely (2 g/kg, i.p., for 2 h) and subchronically (2 g/kg, i.p., twice daily for 7 days), and then the effect of ethanol withdrawal was tested 24 h after the last injection of the repeated treatment (the observed behavioral signs were not quantitated) [8]. Control rats received equal volumes of saline vehicle. To assess the in vivo sensitivity of brain CB1 receptors, the acute

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The acute treatment of rats with ethanol (2 g/kg, i.p., for 2 h) increased the synthesis of DOPA/NA in the locus ceruleus (158%, \( p < 0.001 \), but not in the hippocampus, and this treatment also failed to alter DOPA/DA synthesis in the corpus striatum (Table 1). Subchronic ethanol administration (2 g/kg, i.p., twice daily for 7 days) and ethanol withdrawal after the repeated treatment (24 h) did not modify significantly the accumulation of DOPA in any brain region (Table 1). On the other hand, acute and subchronic ethanol treatments did not alter the synthesis of 5-HTP/5-HT in the locus ceruleus, hippocampus or corpus striatum (Table 1). In ethanol-withdrawn (24 h) rats, and as expected [8], 5-HTP/5-HT synthesis was found increased in the locus ceruleus (60%, \( p < 0.01 \)) and hippocampus (28%, \( p < 0.01 \)), but not in the corpus striatum (Table 1). These results were in general agreement with the known complex interactions between ethanol and the monoaminergic transmitter systems in the brain [16].

In control rats, the acute administration of the CB1 receptor agonist WIN 55,212-2 (4 mg/kg, i.p., for 1 h) [15] increased the synthesis of DOPA/NA in the locus ceruleus (250%, \( p < 0.001 \)) and the hippocampus (64%, \( p < 0.005 \)) (Fig. 1), and it reduced DOPA/DA synthesis in the corpus striatum (47%, \( p < 0.001 \)) (Fig. 2). Acute treatment with WIN 55,212-2 also decreased the synthesis of 5-HTP/5-HT in the locus ceruleus (43%, \( p < 0.001 \)), hippocampus (35%, \( p < 0.001 \)) and corpus striatum (35%, \( p < 0.01 \)) (Fig. 3). These stimulatory (DOPA/NA) and inhibitory (DOPA/DA and 5-HTP/5-HT) effects of WIN 55,212-2 on the syntheses of brain monoamines were blocked by SR 141716A, a selective CB1 receptor antagonist (data not shown) [15]. In the locus ceruleus of ethanol-treated rats, the stimulatory effect of WIN 55,212-2 on DOPA/NA synthesis was abolished (acute treatment) or markedly attenuated (53–55%, \( p < 0.001 \)).

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Locus ceruleus</th>
<th>Hippocampus</th>
<th>Striatum</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOPA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>93 ± 9</td>
<td>40 ± 4</td>
<td>1564 ± 78</td>
</tr>
<tr>
<td>Acute ethanol</td>
<td>240 ± 20</td>
<td>32 ± 6</td>
<td>1425 ± 64</td>
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<tr>
<td>Subchronic ethanol</td>
<td>111 ± 13</td>
<td>59 ± 2</td>
<td>1442 ± 77</td>
</tr>
<tr>
<td>Ethanol withdrawal</td>
<td>127 ± 8</td>
<td>33 ± 4</td>
<td>1333 ± 60</td>
</tr>
<tr>
<td>5-HTP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>494 ± 14</td>
<td>172 ± 8</td>
<td>253 ± 16</td>
</tr>
<tr>
<td>Acute ethanol</td>
<td>616 ± 106</td>
<td>133 ± 6</td>
<td>201 ± 20</td>
</tr>
<tr>
<td>Subchronic ethanol</td>
<td>576 ± 112</td>
<td>190 ± 13</td>
<td>210 ± 10</td>
</tr>
<tr>
<td>Ethanol withdrawal</td>
<td>794 ± 38</td>
<td>221 ± 37</td>
<td>205 ± 18</td>
</tr>
</tbody>
</table>

* Bars represent means ± S.E.M. derived from four to eight experiments as a percentage of the respective control (light bars). †† *p < 0.001; †† † *p < 0.01; †** *p < 0.05; †* *p < 0.05 when compared with the acute stimulatory effect of WIN in the saline group (ANOVA followed by Bonferroni’s test).

**Fig. 1. Acute effects of WIN 55,212-2 (WIN, 4 mg/kg, for 1 h) on DOPA accumulation in the locus ceruleus (a slice of brainstem) and hippocampus of saline-treated rats (saline group), acute ethanol-treated rats (2 g/kg, i.p., for 2 h) and subchronic ethanol (2 g/kg, i.p., for 7 days) followed by withdrawal (24 h).** Each value is the mean ± S.E.M. of four to eight experiments per group. One-way ANOVA detected significant differences between treatments for DOPA synthesis in a piece of brainstem containing the locus ceruleus \( F(3,13) = 15.28, p < 0.001 \), but not in the hippocampus and striatum. ANOVA also detected significant changes for 5-HTP synthesis in the locus ceruleus \( F(3,13) = 3.57, p = 0.04 \) and hippocampus \( F(3,13) = 5.01, p = 0.02 \), but not in the striatum.† † *p < 0.01 when compared with the corresponding saline group (ANOVA followed by Bonferroni’s test).† † † *p < 0.001 when compared with the acute stimulatory effect of WIN in the saline group (ANOVA followed by Bonferroni’s test).
Fig. 2. Acute effects of WIN 55,212-2 (WIN, 4 mg/kg, for 1 h) on DOPA accumulation in the corpus striatum of saline-treated rats (saline group), acute ethanol-treated rats (2 g/kg, i.p., for 2 h), subchronic ethanol-treated rats (2 g/kg, i.p., for 7 days), and subchronic ethanol-withdrawn (24 h) rats (withdr group). Rats received vehicle (light bars) or WIN (dark bars) 30 min before NSD 1015 and were sacrificed after another 30 min. Bars represent means ± S.E.M. derived from four to eight experiments as a percentage of the respective control (light bars).* \( p < 0.001 \) when compared with the corresponding control (two-tailed Student’s \( t \)-test).† \( p < 0.05 \); †† \( p < 0.01 \) when compared with the acute inhibitory effect of WIN in the saline group (ANOVA followed by Bonferroni’s test).

subchronic treatment and withdrawal state) (Fig. 1), whereas in the hippocampus this effect was found significantly reduced only in subchronic ethanol-withdrawn rats (33%, \( p < 0.05 \)) (Fig. 1).

In the corpus striatum of ethanol-treated rats (acute, subchronic and withdrawal state), the inhibitory effect of WIN 55,212-2 on DOPA/DA synthesis was completely blunted or markedly reduced (Fig. 2). Similarly, the inhibitory effect of WIN 55,212-2 on 5-HTP/5-HT synthesis was reduced or abolished in the locus ceruleus, hippocampus and corpus striatum after acute and/or subchronic ethanol and/or ethanol withdrawal after the repeated treatment (Fig. 3).

These results indicated that ethanol treatments in rats (and more specifically the state of withdrawal after subchronic ethanol) were associated with decreases in CB1 agonist (WIN 55,212-2)-mediated functional responses, which suggests a desensitization of these cannabinoid receptors modulating the syntheses of DOPA/NA, DOPA/DA and 5-HTP/5-HT in the brain. The molecular mechanism involved in this in vivo desensitization of CB1 receptors could be related to a decrease in the number of receptors induced by ethanol, which would result in reduced agonist (WIN 55,212-2) sensitivity as demonstrated for other neurotransmitter agonists regulating monoamine synthesis in the brain [7]. In fact, chronic treatment with ethanol has been shown to induce down-regulation of CB1 receptor number [2] and also to attenuate cannabinoid-activated signal transduction [3,14]. Moreover, ethanol exposure in rats also decreased CB1 receptor mRNA levels in several brain regions, including the hippocampus and striatum [5,17]. This decrease of CB1 receptor function could also be related, in part, to a change in the availability of endogenous cannabinoids. Thus, chronic ethanol treatment, but not the acute exposure, has been shown to increase the content of anandamide and 2-arachidonylglycerol [3,10], probably due to inhibition of the endocannabinoid transport system [4]. This increase in the extracellular content of the endogenous agonists could contribute to the observed process of CB1 receptor desensitization.
In summary, treatment with ethanol in rats induces a functional desensitization of cannabinoid CB₁ receptors (activated by the agonist WIN 55,212-2) modulating the syntheses of DOPANA (stimulatory effect), DOPA/DA (inhibitory effect) and 5-HTP/5-HT (inhibitory effect) in the brain. These results further support a role of CB₁ receptors in the various molecular mechanisms associated with alcohol addiction [14].

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