SEROTONIN IS REDUCED IN THE FRONTAL CORTEX OF SARDINIAN ETHANOL-PREFERRING RATS

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Abstract — Ethanol-naive Sardinian alcohol-preferring (sP) and Sardinian alcohol-non-preferring (sNP) rats were tested to evaluate the levels of serotonin (5-HT) and 5-hydroxyindol-3-yl-acetic acid (5-HIAA) in the frontal cortex, hypothalamus, and nucleus accumbens, and the levels of dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) in the hypothalamus and nucleus accumbens. Compared with the sNP line, the sP rats had lower 5-HT and 5-HIAA concentrations in the frontal cortex, whereas no differences were found in the other brain areas tested, neither for neurotransmitters nor their metabolites. As the decreased 5-HT function is a feature shared by different alcohol-preferring strains, it could be linked to the genetic predisposition to voluntary ethanol consumption.

INTRODUCTION

The existence of a genetic predisposition towards alcohol abuse in humans is accepted and the selective breeding of laboratory animals for their preference to voluntary ethanol intake is considered a useful tool for studying the neurochemical basis of alcoholism. In our laboratories, Sardinian alcohol-preferring (sP) and Sardinian alcohol-non-preferring (sNP) rats have been selected from Wistar rats according to a breeding programme started in the early 1980s. The daily ethanol intake of an sP rat is over 4 g/kg, and the preference ratio for ethanol to water reaches 80–100%, while sNP rats avoid drinking ethanol solution (Colombo et al., 1995). Furthermore, sP rats display a higher degree of anxiety than sNP rats, when tested in the elevated plus-maze, which is reversed by voluntary ethanol intake (Colombo et al., 1995). The neurochemical characterization of these rat strains has until now been focused on the dopaminergic system. Fadda et al. (1990) have shown that the activation of DA metabolism, as indicated by the levels of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid, is higher in the caudate nucleus, medial prefrontal cortex, and olfactory tubercle of sP rats compared with sNP rats. Furthermore, between the two lines of rats, a lower density of DA D1 (De Montis et al., 1993) and D2 receptors (Stefanini et al., 1992) was found in the limbic system of ethanol-naive sP rats, suggesting that an impairment of dopaminergic transmission is linked to their inheritable attitude towards alcohol intake.

Recently sP rats have been shown to display a lower reactivity to serotonin (5-HT) system stimulation (Ciccocioppo et al., 1995). Thus, the behavioural responses to 5-HT agonists, to the 5-HT precursor 5-hydroxytryptophan, and to the 5-HT releasing agent sekindine, were significantly less pronounced in sP than in sNP or Wistar rats, suggesting a diminished 5-HT system function in sP rats. There is compelling evidence for the involvement of 5-HT in the control of ethanol intake, both in humans (LeMarquand et al., 1994a) and laboratory animals (LeMarquand et al., 1994b). Decreased 5-HT and 5-hydroxyindol-3-yl-acetic acid (5-HIAA) content was found in some brain areas of different alcohol-preferring rat lines compared with their corresponding alcohol non-preferring lines, namely P/NP rats (Murphy et
SEROPTONIN DEFICIENCY IN sP RATS

Between the better characterized strains, the alcohol-preferring P and alcohol-non-preferring NP rats of Indiana University, 5-HT immunoreactive fibre density was found to be lower in P rats (Zhou et al., 1994). Furthermore, 5-HT deficient Fawn-Hooded rats, selected for their bleeding disorder (Tschopp and Zucker, 1972), display a preference towards ethanol intake (Rezvani et al., 1990). In the light of these findings, the possibility was considered that the 5-HT system may also exhibit differences between sP and sNP rats. In the preceding paper (Bano et al., 1998), the serotonin status was studied in whole brains of alcohol-naive sP and sNP rats in conjunction with various aspects of tryptophan and related metabolism, whereas in the present investigation, we quantified 5-HT levels in selected brain areas of our Sardinian rats.

MATERIALS AND METHODS

Table 1. Serotonin (5-HT) and 5-hydroxyindol-3-yl-acetic acid (5-HIAA) concentrations in different cerebral areas of Sardinian alcohol-preferring (sP) and -non-preferring (sNP) rats

<table>
<thead>
<tr>
<th>Cerebral area</th>
<th>5-HT (pmol/mg wet wt of tissue)</th>
<th>5-HIAA (pmol/mg wet wt of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothalamus</td>
<td>sP: 3.56 ± 0.09, sNP: 3.45 ± 0.13</td>
<td>2.35 ± 0.14</td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>sP: 2.78 ± 0.08**, sNP: 3.40 ± 0.08</td>
<td>1.26 ± 0.08*</td>
</tr>
<tr>
<td>Nucleus accumbens</td>
<td>sP: 4.28 ± 0.28, sNP: 4.54 ± 0.40</td>
<td>3.04 ± 0.12, 3.18 ± 0.20</td>
</tr>
</tbody>
</table>

Values are the means ± SEM of 13 animals, processed in three different experiments. The significance of the differences between sP and sNP values is expressed as follows: *P < 0.05; **P < 0.01 (Student’s t-test).

Male sP and sNP rats from the 39th generation of our breeding programme were used. Selection criteria of the lines are described elsewhere (Colombo et al., 1995). Animals were 6 months old at the time of the experiments, and were housed four per cage in a temperature-controlled (24°C) environment, with a 12-h light–dark cycle (lights on at 08:00). Food (standard rat chow, MIL Morini, San Polo d’Enza, RE, Italy) and water were accessible ad libitum, and all rats were naive with regard to ethanol experience. After death by decapitation, the brain was rapidly removed and rinsed in chilled saline. The pia mater was eliminated and the hypothalamus, frontal cortex, and nucleus accumbens were dissected out on ice and immediately frozen on solid CO₂, then stored at −70°C until processed. Tissues were sonicated on ice in cold 0.1 N-HClO₄ (1:30 w/v), centrifuged at 1000 g in a refrigerated Beckman Super-speed centrifuge, and the supernatant stored at −70°C for no more than 24 h or immediately injected into an HPLC apparatus, consisting of two Gilson pumps, two 7125 Rheodyne injectors, a Hewlett Packard series 1100 column thermostat equipped with two Beckman columns (Ultrasphere ODS, 5 μm, 4.6 × 150 mm) and an ESA Coulomex II detector with two independent analytical cells. Data were recorded by a Waters 746 Data Module integrator, and quantified following standard calibration. For DA and DOPAC evaluation, the mobile phase consisted of 0.5 M sodium acetate, 0.2 mM EDTA, 0.43 mM sodium octyl sulphate, 14% methanol, pH 4.8 with acetic acid, delivered at 1.1 ml/min; the column temperature was set at 22°C, the Coulomex analytical cell first electrode was set at +350 mV and the second at −180 mV. For 5-HT and 5-HIAA separation, the mobile phase was 0.5 M sodium acetate, 0.2 mM EDTA, 10% methanol, pH 4.2 with acetic acid, delivered at 1.0 ml/min; the column temperature was set at 38°C, the Coulomex analytical cell first electrode was set at +50 mV and the second at +350 mV. All reagents were HPLC grade, standard solutions were prepared with 5-HT–HCl, 5-HIAA, DA–HCl, and DOPAC purchased from RBI.

Statistical analysis was performed by Student’s t-test, with a probability level (P) of <0.05 for significant differences.

RESULTS

Table 1 shows 5-HT and 5-HIAA levels in the hypothalamus, frontal cortex, and nucleus accumbens of sP and sNP rats. In sP rats compared with sNP rats, there was an 18% decrease in 5-HT and 5-HIAA concentrations in the frontal cortex, whereas no differences were observed in the
Table 2. Dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) concentrations in different cerebral areas of Sardinian alcohol-preferring (sP) and -non-preferring (sNP) rats

<table>
<thead>
<tr>
<th>Cerebral area</th>
<th>DA (pmol/mg wet wt of tissue)</th>
<th>DOPAC (pmol/mg wet wt of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothalamus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sP</td>
<td>2.54 ± 0.31</td>
<td>0.71 ± 0.02</td>
</tr>
<tr>
<td>sNP</td>
<td>2.43 ± 0.29</td>
<td>0.64 ± 0.01</td>
</tr>
<tr>
<td>Nucleus accumbens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sP</td>
<td>32.92 ± 2.52</td>
<td>7.70 ± 0.69</td>
</tr>
<tr>
<td>sNP</td>
<td>34.06 ± 2.06</td>
<td>8.58 ± 0.69</td>
</tr>
</tbody>
</table>

Values are the means ± SEM of 13 animals, processed in three different experiments, for the nucleus accumbens and of five animals for the hypothalamus. No statistically significant differences were found between sP and sNP values (Student’s t-test).

DISCUSSION

The present results indicate that the levels of 5-HT and its major metabolite 5-HIAA are lower by about 20% in the frontal cortex of sP rats compared with the sNP line. These differences are a genetic trait, being present in alcohol-naive animals. No differences in 5-HT concentrations between sP and sNP rats were observed in the hypothalamus and nucleus accumbens. In contrast with our rat lines, the reduction in 5-HT in other alcohol-preferring and non-preferring rat lines, such as P and NP (Murphy et al., 1982), N and Nih (Murphy et al., 1986), HAD and LAD (Gongwer et al., 1989), was found to be present not only in the frontal cortex, but also in other brain areas, such as the striatum, septal nuclei, hippocampus, and also hypothalamus and nucleus accumbens. An immunocytochemical study (Zhou et al., 1994) has shown that the lower 5-HT content in the alcohol-preferring P line is due to a reduction in the number of 5-HT thin fibres with small varicosities originating from the dorsal raphe, whereas no reduction in the thick neurons from the median raphe was present in these animals. It was then suggested that changes in the number of 5-HT neurons may be restricted to a specific neuronal subpopulation.

Since 5-HT deficiency in the frontal cortex seems to be a common feature in alcohol-preferring rats from different lines, it might be suggested that reduction in serotonergic transmission in this area, rather than in the other brain regions, plays a more relevant role in alcohol preference. In accordance with this hypothesis, changes in 5-HT receptors have been found in P rats, compared with NP rats, predominantly in the frontal cortex (McBride et al., 1993, 1994, 1997; Wong et al., 1993). Moreover, a microdialysis study from our laboratory (Portas et al., 1994) has shown that acute alcohol administration increases 5-HT release in the frontal cortex of sP rats more effectively than in sNP ones, suggesting that alcohol consumed by sP animals acts as a means to compensate for 5-HT deficiency. Another difference between our rat lines and others genetically selected for alcohol preference or avoidance concerns dopaminergic neurons. Whereas alcohol-preferring P rats exhibit lower DA concentration in the nucleus accumbens as well as in other forebrain areas when compared to NP rats (Murphy et al., 1982), no differences in DA and its metabolite DOPAC have been observed between sP and sNP rats. However, since we have previously shown that sP rats have fewer D₁ (De Montis et al., 1993) and D₂ (Stefanini et al., 1992) receptors in the limbic system than sNP rats, a reduction in dopaminergic transmission may still be sustained by a postsynaptic mechanism. There is neurochemical (Di Chiara and Imperato, 1985) and electrophysiological (Gessa et al., 1985) evidence that ethanol can activate the mesolimbic DA system. This may compensate for the deficiency in DA release or the reduction in DA receptors. Overall, the data suggest the involvement of both mesolimbic DA and fronto-cortical 5-HT in controlling alcohol preference and intake. It has been suggested that an alcohol-induced increase in 5-HT outflow involves the activation of the mesolimbic DA system (Carboni et al., 1989). However, the interactions between serotonergic and dopaminergic
transmissions are quite complex and more research is needed to clarify this problem.

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