Evidence for a modulatory effect of sulbutiamine on glutamatergic and dopaminergic cortical transmissions in the rat brain

Fabrice Troveroa,*, Marco Gobbib, Jeanne Weil-Fuggazac, Marie-Jo Bessona, Denis Brocheted, Sylvain Pirotec

aKey-Obs S.A., Centre d’Innovation, 16, rue Leonard de Vinci, 45074 Orleans, Cedex 2, France
bIstituto di Ricerche Farmacologiche Mario Negri, Via Eritrea, 62, 20157 Milan, Italy
cLaboratoire de Neurochimie-Neuroanatomie URA 1488, 9 Quai Saint Bernard, 75005 Paris, France
dPsypharm S.A., B.P. 0102, 53001 Laval, France
eA.N.P.P., 25 rue de la Plaine 75020 Paris, France

Received 26 June 2000; received in revised form 2 August 2000; accepted 4 August 2000

Abstract

Chronic treatment of rats by sulbutiamine induced no change in density of N-methyl-d-aspartate (NMDA) and (+)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors in the cingular cortex, but a significant decrease of the kainate binding sites, as measured by quantitative autoradiography. In the same treated animals, an increase of D1 dopaminergic (DA) binding sites was measured both in the prefrontal and the cingular cortex, while no modification of the D2 binding sites was detected. Furthermore, an acute sulbutiamine administration induced a decrease of kainate binding sites but no change of the density of D1 and D2 DA receptors. Acute sulbutiamine injection led to a decrease of the DA levels in the prefrontal cortex and 3,4-dihydroxyphenylacetic acid levels in both the cingular and the prefrontal cortex. These observations are discussed in terms of a modulatory effect of sulbutiamine on both dopaminergic and glutamatergic cortical transmissions. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Sulbutiamine; Glutamate receptors; Dopaminergic receptors; Prefrontal cortex; Cingular cortex; Autoradiography

Sulbutiamine is a hydrophobic molecule that easily crosses the blood–brain barrier and gives rise to thiamine and thiamine phosphate esters in the brain [2,3]. It does not have psychostimulant properties and is currently used for treatment of somatic and psychic inhibition. For example, it improves the functioning of episodic memory in schizophrenic patients [11] and hastens the resorption of psycho-behavioural inhibition occurring during major depressive disorder [7]. Moreover, it improves performances in behavioural models of inhibition (induced by an aversive situation) such as the ‘learned helplessness’ and the ‘forced swimming test’ (Lacroix and Bourin, personal communications). Given the role of dopaminergic and glutamatergic transmissions in the anterior cortical regions, (e.g. prefrontal and cingular cortices) where decisions and strategies are organized, the present study has been conducted in order to determine the effects of sulbutiamine on these systems. For this aim, the effects of acute and chronic sulbutiamine treatments on glutamatergic and dopaminergic binding sites in the rat brain were analyzed by autoradiographic analysis in prefrontal and cingular cortices and compared to a subcortical structure, the nucleus accumbens.

Male Sprague–Dawley rats weighing 200–250 g (Charles River France, Saint-Aubin les Elbeuf) have been used. For chronic treatments, rats have been treated intraperitoneally (i.p.) with sulbutiamine (12.5 mg/kg) or vehicle (Arabic gum, 15% w/v) once a day for 14 days, and killed 2 h or 5 days after the last injection. For acute treatments, rats have been treated intraperitoneally with sulbutiamine (12.5 mg/kg) or vehicle (arabic gum, 15% w/v), and have been sacrificed after various times following the injection (15 and 30 min, 1, 4, 12 and 24 h) for autoradiographic analysis of binding sites. The brain was rapidly dissected out and was frozen in isopentane (−40°C) Sections (20 μm thick) were cut with a cryostat at −20°C at different levels of the brain. They were mounted onto gelatine-coated glass slides and stored at −20°C until the day of incubation with ligand. Autoradiographic experiments of D1 dopaminergic (D1-DA), N-methyl-d-aspartate (NMDA), (+)-α-amino-3-hydroxy-5-methylisox-
azole-4-propionic acid (AMPA) and kainate were performed according to the procedures previously described [1,9,14]. For D2 dopaminergic binding sites, sections were incubated for 60 min at 20°C in a 50 mM Tris–HCl buffer (pH 7.4) containing (125I)-iodosulpride (0.2 nM) (from NEN Dupont, France). Non-specific binding was determined by adding 10⁻⁶ M unlabelled sulpiride. Sections were washed five times in ice-cold 50 mM Tris–HCl buffer (pH 7.4) and dried under a stream of cold air. Finally, the sections were exposed to ³H-ultrofilm (LKB) for 10 days. After developing, binding quantification was obtained by densitometric analysis of autoradiographic films. Autoradiograms were transformed into digitised diagrams (512 x 512 pixels) with an image analyzer giving 256 grey levels per pixel. Each value of grey level was transformed into optical density (OD) by the computer. Autoradiographic film densities were converted to apparent tissue ligand concentrations on the basis of film densities overlying the radioactive standards and the specific activity of the radioligand. Differences between treatments were evaluated using one-factor analysis of variance for repeated measures (ANOVA), followed by the Tuckey–Kramer t-test. For estimation of monoamine levels, rats were sacrificed 2 h following the injection of sulbutiamine (12.5 mg/kg) and the brain was rapidly dissected out and was frozen at −80°C, until the dissection. On frontal brain sections (400 μm thick) realized with a microtome, prefrontal cortex, anterior cingular cortex and nucleus accumbens were dissected and kept at −80°C until analysis. Tissue samples were put into 100 μl of perchloric acid 0.1 M containing ethylene diamine tetra-acetic acid (EDTA) 0.1 mM and 0.01% cysteine and sonicated. Following centrifugation (35 000 × g, 40 min at 4°C), supernatants were kept at −80°C until dosages. They were purified on alumine microcolumns and were injected into a high-pressure liquid chromatography C18 column equilibrated with a

Fig. 1. Representative autoradiograms of rat brain coronal sections obtained following incubations with specific ligands. (A) D1 dopaminergic binding sites. Sections were incubated in presence of 1 nM (³H)-SCH 23390 and 10 nM spiroperidol to avoid labelling of 5-hydroxytryptamine trifluoroacetate (HT)₂ serotonergic binding sites. Sections were exposed to ³H-ultrofilm (LKB) for 15 days. (B) D2 dopaminergic binding sites. Sections were incubated in presence of 0.2 nM (125I)-iodosulpride. Sections were exposed to ³H-ultrofilm (LKB) for 10 days. (C) AMPA glutamatergic binding sites. Sections were incubated in presence of 34 nM (³H)-glutamate. Sections were exposed to ³H-ultrofilm (LKB) for 2 weeks. (D) Kainate glutamatergic binding sites. Sections were incubated in presence of 60 nM (³H)-kainic acid. Sections were exposed to ³H-ultrofilm (LKB) for 6 weeks. (E) NMDA glutamatergic binding sites. Sections were incubated with 200 nM (³H)-glutamate and of 5 μM AMPA and 1 μM kainic acid. Sections were exposed to ³H-ultrofilm (LKB) for 6 weeks.

Fig. 2. Effect of a chronic treatment by sulbutiamine on the density of glutamatergic and dopaminergic binding sites in various cerebral structures. The values are expressed in fmol/ mg of tissue and are the means ± SEM calculated from data obtained with six animals per group. (a) Significantly different from the control-5 days group P < 0.05. (b) Significantly different from the control-2 h group P < 0.05.
solvent consisting of sodium phosphate buffer (0.1 M), 1-octanesulphonic acid (2.75 mM), triethylamine (0.25 mM), EDTA (0.1 mM), NaH₂PO₄ (0.1 M) and methanol (15%), adjusted with phosphoric acid to pH 2.9. The injection rate was 1 ml/min and DA and 3,4-dihydroxyphenylacetic acid (DOPAC) levels were quantified by electrochemistry using a carbon electrode (Chromato®eld Eldec 102) set at 0.65 V.

Fig. 1 shows representative autoradiograms obtained from rat brain sections following incubations with various ligands at an anteriority level showing the nucleus accumbens and the cingular cortex. The densitometric quantitative analysis of the binding in the different groups is reported on Fig. 2. For comparison, densitometry has been systematically performed on the same structures, except for ³H-glutamate, whose binding is only enriched in indicated regions. The chronic treatment induced a significant increase in the density of D1 binding sites in both the prefrontal and the anterior cingular cortex (+26 and +34%, respectively), only 2 h following the interruption of the treatment. No change in the density of D2 DA receptor was observed. Furthermore, 5 days following the chronic treatment, a significant decrease in the density of the ³H-kainate binding was observed in the cingular cortex and the nucleus accumbens (−36 and −28%, respectively). A significant and similar decrease has also been observed in the striatum and the hippocampus (data not shown). The chronic treatment induced no significant change in the density of other glutamatergic binding sites (NMDA and AMPA subtypes).

The acute administration of sulbutiamine did not induce any change in the binding density of ³H-SCH 23390 and of ¹²⁵I-iodosulpride (Fig. 3). Fifteen minutes following sulbutiamine administration, a significant decrease of the density of kainate binding sites was observed in the cingular cortex. Such a decrease was also observed in the prefrontal cortex one hour after the sulbutiamine administration. In both these structures, the decrease was still persistent 24 h after the injection. Since no change was observed 2 h following the chronic treatment despite the presence of sulbutiamine metabolites in the brain [3], the occupation of kainate binding sites by sulbutiamine and/or by its metabolites can be excluded to explain the binding decrease. No variation in

Fig. 3. Effect of an acute administration of sulbutiamine (12.5 mg/kg, i.p.) on the density of D1 and D2 dopaminergic and kainate binding sites in the cingular and prefrontal cortex. For each time following sulbutiamine injection, the values are expressed in percent of the control value (±SEM calculated from data obtained with six animals per group). *Control* column is the mean ± SEM of control values obtained at various times. * Significantly different from the control group P < 0.01.
the density of D1, D2 and kainate binding sites has been observed in the nucleus accumbens (data not shown).

Table 1 shows that following an acute injection of sulbutiamine, DOPAC levels were significantly reduced (−30%) in the prefrontal cortex. In the cingular cortex, DA and DOPAC levels were decreased (−34 and −26%, respectively), as compared to control animals. Finally, no change in dopaminergic transmission was observed in the nucleus accumbens. The decreased levels of DOPAC suggest a reduced release of DA in the prefrontal cortex. The increased density of D1 DA receptors 2 h following a chronic treatment (Fig. 2), may result from this chronic modification and its induced compensatory mechanisms. These mechanisms disappear with the interruption of the sulbutiamine treatment, no more modification of D1 binding sites being observed five days later (Fig. 2). A single injection of sulbutiamine should not be sufficient to change the D1 receptor density (Fig. 3). These observations suggest that changes of receptor sensitivity take place following a chronic change in the cortical dopaminergic transmission induced by sulbutiamine.

Thus, the changes in density of kainate receptor in the cortex lead to suggest that sulbutiamine and/or its metabolites may modulate the cortical glutamatergic transmission. In fact, the rapid decrease observed immediately following a single injection suggests a direct effect on the cortical glutamatergic transmission, for instance a modulation of the intra-synaptic glutamate concentration. Cellular mechanisms responsible for the effects of sulbutiamine are unknown. Thiamine triphosphate, a derivative significantly enhanced in rat brain following an injection of sulbutiamine, could have modulatory effect on neuronal membrane permeability [2,3]. Such a modulation induces ionic changes, and further modulate the binding on kainate receptors. Changes on glutamatergic transmission could be at the origin of the modulation of dopaminergic cortical transmission. Indeed, numerous behavioural, anatomical and electrophysiological studies report the modulation of dopaminergic cortical transmission by glutamate, notably through kainate receptors [4,8,10,12]. On the contrary, chronic blockade of dopaminergic transmission by antipsychotic drugs does not change the density of cortical kainate binding sites, even though the expression of their mRNA is affected [6,13]. In the nucleus accumbens kainate receptors are down regulated without any significant change in dopaminergic transmission.

The interactions between dopaminergic and glutamatergic transmissions within the prefrontal cortex could play a pivotal role in the therapeutic action of sulbutiamine. Those results strongly support recent findings that demonstrate improvement in behavioural, cognitive, attentional and functional disorders in schizophrenic, alcoholic and depressed patients [5,7,11]. Other experiments, including release and/or uptake studies, have to be conducted in order to further investigate the respective role of the cortical glutamatergic and dopaminergic cortical transmissions in the effect of sulbutiamine.

Table 1 shows that following an acute injection of sulbutiamine (12.5 mg/kg) on levels of dopamine and its metabolites in the prefrontal cortex, the cingular cortex and in the nucleus accumbens.*

<table>
<thead>
<tr>
<th></th>
<th>Prefrontal cortex</th>
<th>Cingular cortex</th>
<th>Nucleus accumbens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Sulbutiamine</td>
<td>Control</td>
</tr>
<tr>
<td>Dopamine</td>
<td>2.06 ± 0.21</td>
<td>1.65 ± 0.08</td>
<td>3.64 ± 0.39</td>
</tr>
<tr>
<td>HVA</td>
<td>3.18 ± 0.27</td>
<td>2.97 ± 0.15</td>
<td>2.88 ± 0.25</td>
</tr>
<tr>
<td>DOPAC</td>
<td>1.05 ± 0.09</td>
<td>0.75 ± 0.03*</td>
<td>1.41 ± 0.1</td>
</tr>
</tbody>
</table>

* Data are the mean ± SEM of ten determinations per group and are expressed in pmoles/mg of protein. * Significantly different from the control group P < 0.01.


