

# Positron Emission Tomography Study of Brain Benzodiazepine Receptors in Friedreich's Ataxia

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**ABSTRACT:** Central type benzodiazepine receptors were studied in 9 patients with Friedreich's ataxia and 12 healthy subjects using positron emission tomography (PET) and [<sup>11</sup>C]Ro 15-1788, a specific antagonist of the central type benzodiazepine receptors, as radioligand. A standard PET procedure was used in 5 patients and 8 controls to obtain brain kinetics of the total binding of the radioligand. The remaining subjects were intravenously injected with a saturating dose of unlabeled Ro 15-1788, 30 minutes after the tracer injection, to determine the nondisplaceable binding of [<sup>11</sup>C]Ro 15-1788. A semi-quantitative method was used to quantify the [<sup>11</sup>C]Ro 15-1788 data. None of the quantification indices in the cerebellar hemispheres, or in the other brain areas investigated, was significantly modified in patients with Friedreich's ataxia. These findings suggest that brain benzodiazepine receptors are unaffected in Friedreich's ataxia.

**RÉSUMÉ:** Étude des récepteurs des benzodiazépines par tomographie par émission de positons dans l'ataxie de Friedreich Les récepteurs des benzodiazépines de type central ont été étudiés, par tomographie par émission de positons (PET), chez 9 patients atteints d'ataxie de Friedreich et chez 12 témoins, en utilisant le [<sup>11</sup>C]Ro 15-1788 comme radioligand. Une procédure PET standard, permettant d'obtenir la cinétique de la liaison totale du radioligand au niveau du cerveau a été utilisée chez 5 patients et chez 8 témoins. Les autres sujets ont reçu, 30 min après l'administration du traceur, une injection intraveineuse d'une dose saturante de Ro 15-1788 non radioactif, afin de déterminer la fraction non déplaçable du [<sup>11</sup>C]Ro 15-1788. Une méthode semi-quantitative est proposée pour la quantification des données. Quelle que soit la région cérébrale considérée (hémisphères cérébelleux, cortex occipital, cortex temporal ou substance blanche), aucun des index de quantification n'était significativement modifié chez les patients atteints d'ataxie de Friedreich. Ces résultats suggèrent que les récepteurs des benzodiazépines ne sont pas altérés dans l'ataxie de Friedreich.

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Friedreich's ataxia (FA) is an autosomal recessive disorder characterized by severe degeneration of the spino-cerebellar pathways, the dorso-lateral columns of the spinal cord, and the dentate nuclei, with few changes in the cerebellar cortex itself.<sup>1</sup> Four main reasons have prompted us to investigate whether the central type benzodiazepine receptors, known to be involved in the GABAergic inhibitory mechanisms,<sup>2</sup> are involved in this disease: 1) These receptors are found at high concentration in the cerebellum.<sup>3</sup> 2) Little is known about neurochemical changes in the brain of patients with FA although decreased cerebellar concentrations of GABA and glutamate have been found in two patients.<sup>4</sup> 3) Benzodiazepine receptors have already been studied in other kinds of ataxic experimental and human diseases. For example, severe alterations of cerebellar benzodiazepine receptors have been reported in different strains of ataxic mutant mice.<sup>5-7</sup> These changes are, however, associated with extensive pathological changes in the cerebellar cortex, which do not occur in FA. By contrast, a post-mortem analysis of some cases of olivo-ponto-cerebellar degeneration failed to detect marked

changes of these receptors<sup>8</sup> but the neuropathology of this progressive ataxic disease is also very different from FA. 4) In vivo examination of benzodiazepine receptors has become possible using positron emission tomography (PET) and [<sup>11</sup>C]Ro 15-1788 as a radioligand.<sup>9-13</sup> This could be an additional diagnostic element for the classification of cerebellar atrophies, which is still lacking in precision. Because of the well-known neuropathology of FA and its well-established clinical criteria that allow the determination of an homogeneous population of ataxic patients, we decided to study first the cerebellar benzodiazepine receptors in FA. This study must thus be considered as a first step towards further improvement in the classification of progressive cerebellar diseases.

## METHODS AND MATERIALS

Nine patients with FA (mean age  $\pm$  SD: 32  $\pm$  12 years), members of the "Association Française de l'Ataxie de Friedreich", gave their informed consent to participate in the study. All subjects

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satisfied the diagnostic criteria of FA (group 1a) as defined by Geoffroy et al.<sup>14</sup> However, for one subject the age of onset was late (21 years of age). Mean duration of the disease was  $18 \pm 11$  years. Six out of the 9 patients were wheelchair-bound. Twelve healthy subjects (mean age  $\pm$  SD:  $41 \pm 17$  years) were used as a control group. None of the patients or controls had taken benzodiazepines for at least one month prior to testing and none were previously regular users.

The PET studies were performed using the LETI time-of-flight positron camera, which simultaneously provides a series of seven adjacent slices. The axial and the lateral resolutions were both 12 mm FWHM (full width at half maximum using medium-resolution shadow shields) with an undetected interslice space of about 3 mm. All subjects were studied at rest, with their eyes closed and ears unplugged. The head was carefully positioned in a head-holder, parallel to the orbitomeatal plane, by means of a crossed laser-beam system that was also used to prevent head movements during the studies.

Ro 15-1788, a specific antagonist of central type benzodiazepine receptors, was labeled with carbon 11 using a methylation process described previously<sup>15</sup> and used as a radioligand. Just before the tracer injection, a <sup>68</sup>Ge transmission scan was performed for the correction of auto-attenuation. A 65 minute PET acquisition was initiated immediately after the intravenous bolus injection of  $39 \pm 20$  nmoles (mean  $\pm$  SD) of [<sup>11</sup>C]Ro 15-1788 (dose:  $20 \pm 6$  mCi; specific radioactivity at the injection time:  $634 \pm 462$  mCi/micromole). Sequential sets of 29 to 31 images each were subsequently reconstructed with an automatic correction for <sup>11</sup>C decay and measured auto-attenuation. The initial 1 minute duration of the images was progressively expanded to 10 minutes to maintain their statistical quality. A total of 16 venous blood samples were collected during the study (1 per minute for the first 10 minutes and then 1 every 10 minutes thereafter) to obtain a whole-blood radioactivity concentration time-course. [<sup>11</sup>C]blood radioactivity was measured in an external counter cross-calibrated with the positron camera.

The standard PET procedure allows the sequential and regional determination of the total brain binding of [<sup>11</sup>C]Ro 15-1788. It was performed on 5 patients with FA and 8 controls. For the remaining 4 patients and 4 controls, displacement studies with high doses of unlabeled RO 15-1788 were performed in order to estimate the nondisplaceable binding (ND) of [<sup>11</sup>C]Ro 15-1788. This was done by injecting, 30 minutes after administration of the radioligand, unlabeled Ro 15-1788 ( $0.20 \pm 0.04$  mg/kg, i.e.  $660 \pm 132$  nmoles/kg), which is a sufficient dose to achieve full saturation of benzodiazepine receptors in controls.<sup>13</sup> ND was measured 20 minutes later ( $t = 50$  minutes post tracer injection). No pharmacological effects were reported by the subjects during these displacement studies.

#### Data Analysis

In each study, circular standardized regions of interest (area, 3 cm<sup>2</sup>) were placed according to Mazziotto's PET atlas,<sup>16</sup> on a set of early, essentially flow-dependent images ( $t = 2$  min.) in 4 brain structures: cerebellar hemispheres, temporal cortex, occipital cortex and white matter. These brain regions are easy to recognize on early images and are relevant to FA (cerebellar hemispheres) or typical examples of areas with high, intermediate and low, if any, density of benzodiazepine receptors (occipital cortex, temporal cortex and white matter respectively).

Radioactivity levels, expressed as a percentage of the injected dose per litre of tissue, were obtained for each sequential scan and used to determine regional time-activity curves for each subject. Three different indices were then determined in both patients and controls: 1) brain to blood radioactivity ratios were calculated at  $t = 20$  and 30 minutes (in all studies) and at  $t = 40$ , 50 and 60 minutes (in studies without displacement); 2) in displacement studies, a regional "displacement index" was calculated. It was defined as the ratio of the cerebral radioactivity measured just before the injection of the saturating dose of unlabeled Ro 15-1788 ( $t = 29$  minutes) and the radioactivity measured 20 minutes after this injection ( $t = 50$  minutes); 3) in studies without displacement, we calculated the specific to nondisplaceable binding ratio (B/ND) 50 minutes after the tracer injection, according to the following equation:

$$B/ND = (T - ND)/ND$$

where T corresponds to the total binding measured at  $t = 50$  minutes in each study without displacement and ND corresponds to the averaged value of the radioactivities measured at  $t = 50$  minutes in displacement studies.

Statistical analyses were made using Student's *t*-test.

#### RESULTS

Total binding of [<sup>11</sup>C]Ro 15-1788 was higher in patients with FA than in controls in all brain regions studied. This increase was statistically significant ( $p < 0.01$ ) in cerebellar cortex, occipital cortex and white matter (51%, 43% and 43% increase, respectively, at  $t = 50$  minutes), but not in temporal cortex (18% increase at  $t = 50$  minutes). As shown in Figure 1, this difference was observed at every time period after the tracer injection. [<sup>11</sup>C]Blood radioactivity levels were also higher in FA than in controls (Figure 1), a 55% increase ( $p < 0.01$ ) being observed at time  $t = 50$  minutes. The brain/blood ratios were similar in both groups, except in the temporal cortex where this ratio was slightly lower in patients than in controls (Figure 2). The results for the patient with late onset disease did not differ from those for the others.

Injection of a saturating dose of unlabeled Ro 15-1788 resulted in an abrupt decrease in the brain radioactivity in both groups. In less than 15 minutes, the brain radioactivity fell to levels similar to those reported in full saturation coinjection studies.<sup>13</sup> This means that at any time from 45 minutes onwards post-tracer injection, regional values of the radioactivity reflected ND. The displacement index was similar in patients with FA and in controls in all brain regions studied (mean  $\pm$  SD:  $10.4 \pm 1.3$ ,  $13.7 \pm 2.2$ ,  $10.1 \pm 4$  and  $6.6 \pm 2$  in the 4 patients vs.  $8.5 \pm 2.4$ ,  $13 \pm 1.9$ ,  $9.4 \pm 2.5$  and  $5.4 \pm 1.6$  in the 4 controls, in cerebellar hemispheres, occipital cortex, temporal cortex and white matter respectively). Furthermore, the B/ND ratios determined at  $t = 50$  minutes were similar in controls and in patients in the four brain areas considered (Figure 3).

#### DISCUSSION

##### Methodological Considerations

[<sup>11</sup>C]Ro 15-1788 is a suitable ligand to study the central type benzodiazepine receptor in normal and pathological subjects

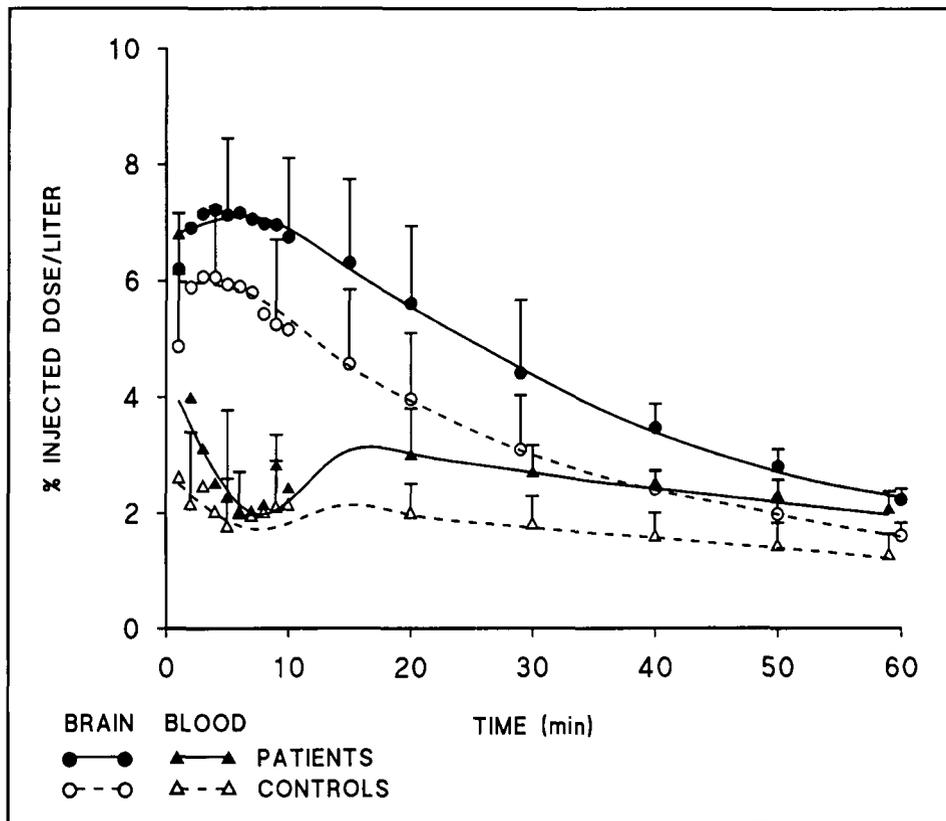


Figure 1 — Cerebellar and blood kinetics of [ $^{11}\text{C}$ ]Ro 15-1788. Mean  $\pm$  SD values of cerebellar and [ $^{11}\text{C}$ ]blood radioactivity were obtained in 9 patients and 12 controls ( $t \leq 30$  minutes) or 5 patients and 8 controls ( $t > 40$  minutes). Cerebellar and blood kinetics were both significantly increased in patients with FA, at every time post-tracer injection.

with PET.<sup>10-13, 17-18</sup> Its cerebral penetration and binding to benzodiazepine receptors are extremely fast. For example, as soon as the 10th minute after the intravenous injection of the tracer, 80% of the occipital radioactivity is specifically bound to the benzodiazepine receptors in normal subjects.<sup>11</sup> It has also been shown that the in vivo nonspecific brain binding of Ro 15-1788 is negligible.<sup>12,19</sup> Moreover, the metabolites of the tracer do not interfere with [ $^{11}\text{C}$ ]brain concentration measurements since the metabolism is essentially peripheral, leading to water soluble blood metabolites that do not cross the blood-brain barrier.<sup>12,20</sup> Finally, the lack of pharmacological effects of this compound allows saturation studies to be performed.

We have recently shown that these characteristics allow a quantification of brain benzodiazepine receptors, based on an equilibrium method.<sup>13</sup> Briefly, in this method, total binding and ND were measured in separate PET procedures using two different groups of subjects. ND was determined after coinjection of the tracer with unlabeled Ro 15-1788, injected at doses higher than or equal to 330 nmoles/kg, which led to a full saturation of the receptors in vivo. The specific binding (B) was calculated by subtracting this ND value from the total binding. B/ND ratio reached a constant value at  $t \geq 20$  min., indicating an equilibrium state. This value provided an estimation of the  $B_{\text{max}}/K_d$  ratio (number of receptors/affinity of the ligand for its receptors), which is known as the binding potential.<sup>21</sup> Indeed, from the Michaelis-Menten equation ( $B/F = B_{\text{max}} [K_d + F]$ ), we can consider that  $B/ND \approx B_{\text{max}}/K_d$  since 1) ND accounts essentially

for the free ligand (F) in the brain, in vivo non specific binding being negligible and 2) receptor occupancy by the tracer is low ( $<5\%$ ) in standard PET procedures<sup>10-12</sup> indicating that F is negligible relative to  $K_d$ . Because this method is based on the ratio of two brain radioactivity measurements (B and ND), it is unaffected by the peripheral metabolism of the tracer. However, the major limitation of this method is that total binding and ND were determined in different groups of subjects. To obtain these two parameters in a single PET study, and therefore in the same subject, we replaced the coinjections with displacements (injection of unlabeled Ro 15-1788 30 minutes after the tracer injection). At time  $t = 50$  minutes, we found regional brain radioactivity values in our displacement studies similar to those found in coinjection studies previously reported.<sup>13</sup> These values may, thus, be considered as nondisplaceable binding. Thus, in the present study, two different indices of quantification could be determined: the B/ND ratio and a regional displacement index. The B/ND ratio was determined at time  $t = 50$  minutes, as in reference 13, from standard and saturation PET studies. As previously specified, this B/ND ratio reflects the binding potential ( $B_{\text{max}}/K_d$ ). The regional displacement index was calculated in each displacement study as the ratio between the total binding of [ $^{11}\text{C}$ ]Ro 15-1788 measured at  $t = 29$  minutes and ND measured at  $t = 50$  minutes. Because we found a moderate decrease in ND between  $t = 30$  minutes and  $t = 50$  minutes,<sup>13</sup> this index slightly differs from  $B_{\text{max}}/K_d$ . However, since it was calculated in single PET studies, it remains unaffected by inter-individual variations

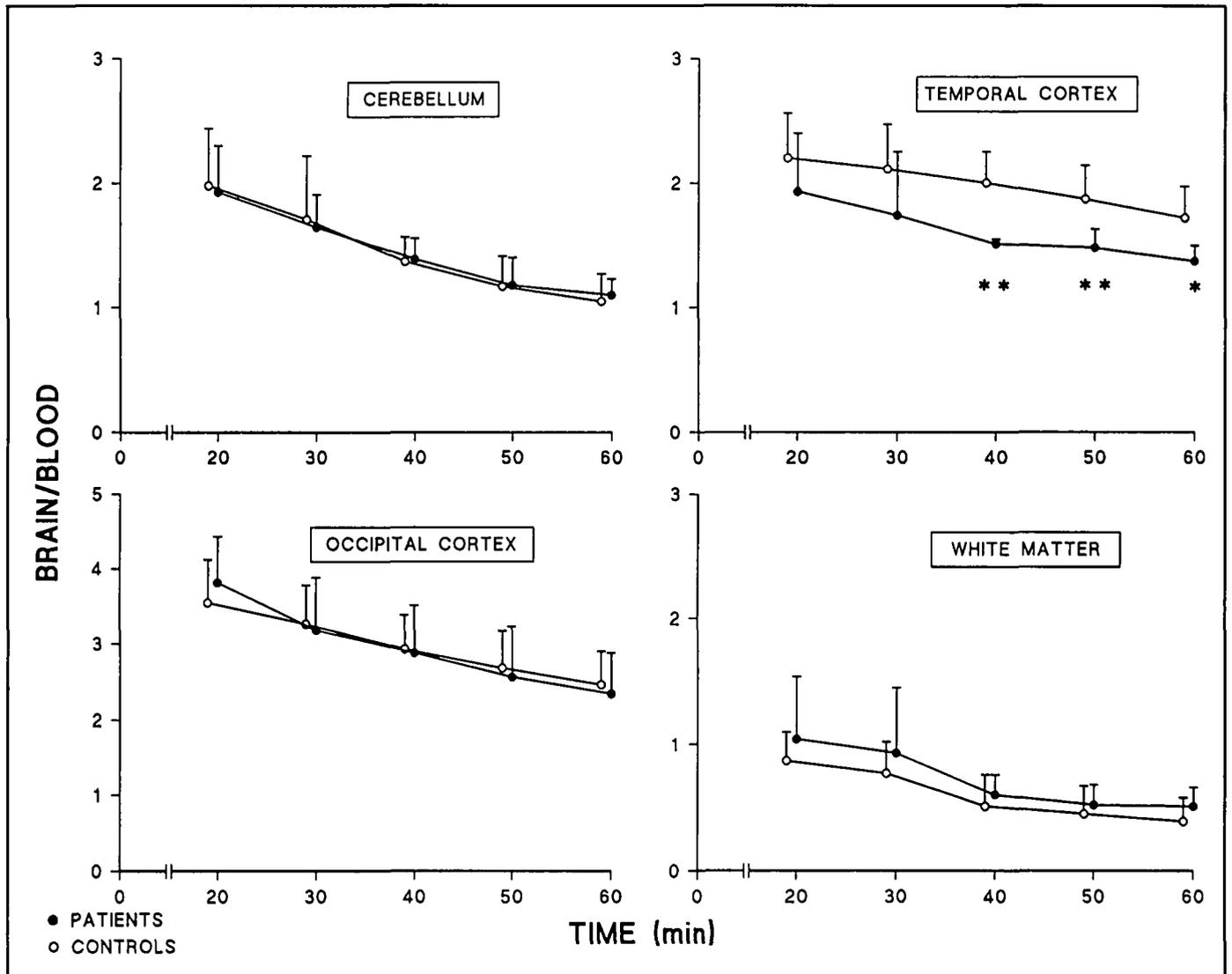


Figure 2 — Regional  $[^{11}\text{C}]$ Ro 15-1788 brain/blood radioactivity ratio. The brain/blood ratio of  $[^{11}\text{C}]$  radioactivity was calculated for each subject at 20, 30, 40, 50 and 60 minutes after the injection of the radioligand. At 20 and 30 minutes, data (mean  $\pm$  SD) were obtained in all 9 patients and 12 controls, whereas at times 40, 50 and 60 minutes, mean values derived only from studies of the 5 patients and 8 controls who had not received a displacing dose of Ro 15-1788. No statistically significant difference was observed between groups in the different areas studied, except in temporal cortex (\*:  $p < 0.02$ ; \*\*:  $p < 0.01$ ).

of the radioligand binding. Both indices may therefore be considered as complementary methods of quantification.

### Benzodiazepine Receptors in FA

In the present study, a significant increase in the total binding of  $[^{11}\text{C}]$ Ro 15-1788 was observed in the brains of patients with FA. Several arguments suggest that this abnormality resulted from differences in blood pharmacokinetics rather than from alterations of the benzodiazepine receptors: 1) the radioligand binding was increased significantly, not only in the cerebellar cortex, but also in the other areas; 2)  $[^{11}\text{C}]$ blood concentrations were higher in patients than in controls and the brain/blood ratios were similar in both groups in all brain regions except temporal cortex; 3) B/ND ratios and displacement indices were in the normal range in all brain areas.

Whether the abnormal  $[^{11}\text{C}]$ blood pharmacokinetics in FA resulted from a slower metabolism of the Ro 15-1788 itself or from an increase in labeled blood metabolites was not directly determined in this study since measurements of blood metabolites were not performed. However, the first hypothesis is the most likely since the increase in  $[^{11}\text{C}]$ blood radioactivity correlated with an increase in  $[^{11}\text{C}]$ brain radioactivity while the blood metabolites of this tracer do not cross the blood-brain barrier.<sup>12,20</sup> Thus, the hepatic metabolism of Ro 15-1788 may be slower in patients with FA than in controls.

The main finding of this study is that the B/ND ratios in patients with FA were normal, which indicates that regional Bmax/Kd ratios were unmodified. Although parallel variations of Bmax and Kd cannot be ruled out, these results strongly suggest that brain benzodiazepine receptors remain unaffected in

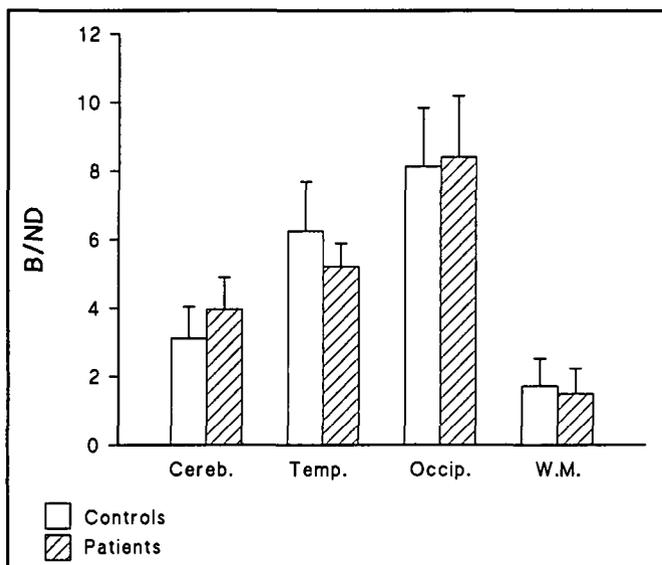


Figure 3 — Specifically-bound/non-displaceable radioactivity ratios (B/ND) in patients and in controls. These ratios were estimated at time  $t = 50$  minutes post-tracer injection using studies with and without displacement as described in the methods and materials section. No statistically significant differences were found between patients with FA and controls.

patients with FA. In agreement with our findings, a normal density of GABA and benzodiazepine receptors has been reported recently in a post-mortem study of a single patient with FA.<sup>22</sup> Taken into account that benzodiazepines modulate GABAergic transmission by acting at the GABA/BZ/chloride channel complex receptor on target cells of the GABAergic neurons,<sup>2</sup> the lack of benzodiazepine receptor abnormality suggests that GABAergic inhibitory mechanisms may not be affected in FA.

The localization of benzodiazepine receptors in cerebellar cortex is important for the interpretation of our results. Many mutant mice studies<sup>5-7</sup> have shown that benzodiazepine receptors are partially located on Purkinje cells, but conflicting results have been reported in other mutant mice studies<sup>23</sup> and in a study of olivopontocerebellar atrophy where no changes in benzodiazepine receptors have been found despite an apparent reduction of Purkinje cells.<sup>8</sup> More recently, immunohistochemical methods of cellular location of benzodiazepine receptors have become available.<sup>24-25</sup> In both studies, monoclonal antibodies labeled Purkinje dendrites shafts and interneurons in the molecular layer, and glomeruli and granule cells in the granular layer. Purkinje cells bodies were also strongly labeled in one study.<sup>25</sup> The lack of significant reduction in the number of benzodiazepine receptors in FA may, thus, reflect that pathological changes of the cerebellar cortex described in FA — a patchy loss of Purkinje cells<sup>1</sup> — are relatively mild.

Finally, these results, which were obtained in a well characterized population of ataxic patients, should be very useful in future PET studies investigating the benzodiazepine receptors in less defined forms of cerebellar atrophy, which may lead to improved classification of these diseases.

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