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Effects of buspirone on brain indoleamines and catecholamines in wild-type mice and *Lurcher* mutants

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Abstract

The effects of a chronic serotoninergic stimulation on brain monoamine levels and metabolism were studied in wild-type (+/+) mice and *Lurcher* (*Lc*/+) mutants. Endogenous serotonin, dopamine, noradrenaline and some of their major metabolites were measured in the frontal cortex, neostriatum, thalamus, brainstem, cerebellum and spinal cord. In +/+ mice, buspirone (1 mg/kg; i.p.) treatment during 40 days increased indoleamines, albeit with moderate changes in the ratios between tissue serotonin metabolites and endogenous serotonin, augmented noradrenaline contents in the spinal cord, and caused elevations of dopamine metabolites in most regions. In *Lc*/+ mutants, the effects of buspirone were attenuated, but higher L-tryptophan and indoleamine levels, suggest a storage of serotonin in a non-releasable compartment. In the hypoplastic *Lc*/+ cerebellum, indoleamine content was accrued, but with a decreased [serotonin metabolites]/[serotonin] ratio, indicating that the reorganized nerve terminals in *Lc*/+ mutants although they can synthesize and accumulate serotonin, may not utilize it efficiently in synaptic transmission.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); 5-HIAA (5-hydroxyindole-3-acetic acid); 5-Hydroxytryptophol; Dopamine; Noradrenaline; Ataxia, cerebellar

1. Introduction

The mutation *Lurcher* in mice causes the loss of cerebellar Purkinje and granule cells, as well as of inferior olive neurons (Caddy and Biscoe, 1979; Heckroth 1992, 1994; Heckroth and Eisenman, 1991). Recent findings have revealed a mutation in the δ2 glutamate receptor gene (Zuo et al., 1997) that is mainly expressed by Purkinje cells (Lomeli et al., 1993), as well as a Purkinje-cell intrinsic defect during the 2nd postnatal week (Soha and Herrup, 1995). The mutation causes death of the Purkinje neurons by apoptosis (Norman et al., 1995; Wullner et al., 1995); in heterozygous mutants, this cellular loss begins approximately on the 12th postnatal day, while in the homozygotes, neuronal degeneration starts as early as embryonic day 18, and they rarely survive beyond the 1st postnatal day (Cheng and Heinz, 1997; Resibois et al., 1997). The heterozygotes (*Lc*/+) reach adulthood, and at 3 months of age have lost their entire Purkinje cell population, over 90% of granule cells (Caddy and Biscoe, 1979), about 60% of inferior olive neurons (Heckroth and Eisenman, 1991) and 20–30% of neurons belonging to cerebellar deep nuclei (Heckroth, 1994). As expected, this neuropathology leads to overt symptoms of cerebellar ataxia and deficits in equilibrium tests (Lalonde et al., 1992; Le Marec et al., 1997). The *Lc*/+ mutant has been considered as an animal model of human cerebellar ataxia, particularly of olivopontocerebellar atrophy, because of the above-mentioned brainstem lesions. Quantitative autoradiography of serotonin transporters, or uptake sites, la-

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belled with [^{3}H]citalopram has shown that in most forebrain areas and cerebellar cortex, the innervation of *Lc/+* mutants is conserved, but with increased densities in cerebellar deep nuclei, as well as in some of the ascending efferent regions. In association with such increased densities of serotonin transporters, indicating an abnormal up-regulated innervation, endogenous levels of serotonin and of its main metabolite 5-hydroxyindole-3-acetic acid (5-HIAA) were found to be elevated in the cerebellum of the *Lc/+* mutant (Strazielle et al., 1996; Le Marec et al., 1999; Reader et al., 1999); however, these alterations could simply be a consequence of the structural reorganization ensuing the postnatal cerebellar atrophy.

Although patients with heredo-degenerative ataxia may suffer from multiple neurochemical disorders (Botez et al., 1998), one of the neurotransmitter systems that has often been implicated in cerebellar ataxia and related motor disorders is serotonin (Trouillas, 1993; Trouillas et al., 1980, 1988; Campanella et al., 1980; Plaitakis, 1993). Moreover, these observations have led to several attempts to treat spinocerebellar ataxia with serotoninergic therapies; however, the outcome of some of these clinical trials have been variable, depending on the nature of the lesions and the duration of the therapy (Trouillas et al., 1988, 1995; Lou et al., 1995; Wessel et al., 1995). Since cerebellar ataxic patients probably suffer from multiple neurochemical disorders, any future replacement therapies will have to be sustained by a greater knowledge of the interacting chemically identified neuronal systems (Peterson et al., 1988; Botez et al., 1991, 1996), as well as on the modifications that can be induced by compounds proposed for the management of such patients. For example, a combination of drugs can improve obstructive apnea, one of the lethal consequences of Friedreich's ataxia; this cocktail consisted of amantadine hydrochloride, thiamine and L-tryptophan (Botez et al., 1997, 1998) but in spite of its beneficial effects, the specific targets where these compounds act, either singly or in an interactive manner, still remain to be established.

The aim of this study was to determine the effects of the chronic administration of a serotoninergic agonist on the indoleamine and catecholamine systems in the *Lc/+* mutant. For this, wild-type mice (*+/+*) and *Lc/+* mutants were treated daily for 40 days with the 5-HT$_{1A}$ receptor agonist buspirone. At the end of the treatment, endogenous levels of serotonin, dopamine and noradrenaline, as well as some of the major metabolites, were measured in the frontal cortex, neostriatum, thalamus, brainstem, cerebellum and spinal cord. The steady-state tissue levels of these neurotransmitters and metabolites were then used as a biochemical index of the function of the serotoninergic and catecholaminergic innervations in this animal model of cerebellar ataxia, in particular, to assess the turnover of serotonin, that was calculated by the ratios between the levels of total indoleamine metabolites and serotonin contents.

2. Materials and methods

2.1. Animals and treatments

This study was carried out with female heterozygote *Lurcher* mutants (*Lc/+*) and female wild-type (*+/+*) controls from the B6CBACa/a strain, purchased from the Jackson Laboratory (Bar Harbor, ME), and housed in a temperature- and humidity-controlled room upon arrival. All animal use procedures were in strict accordance with the Canadian Council on Animal Care *Guide to the Care and Use of Experimental Animals*, and the protocol approved by the *Comité de déontologie pour l'expérimentation sur des animaux* from the Université de Montréal. A group of *+/+* mice ($n = 6$) and a group of *Lc/+* mutants ($n = 7$) received buspirone (1 mg/kg) for 40 days; the daily intraperitoneal injections were carried out 4–7 h after the beginning of their light period to avoid differential effects during the circadian cycle. As controls, another group of *+/+* mice ($n = 6$) and one of the *Lc/+* mutants ($n = 6$) received daily an equivalent volume (2 ml/kg) of saline solution (0.9% NaCl) for 40 days. The treatments began at about 3–4 months (80–120 days) of age, and at the end (120–160 days), the mice were all killed by decapitation 15–30 min after the last injection of saline or buspirone, their spinal cords and brains removed, frozen in *N*-methylbutane at −40°C, and then kept at −80°C.

2.2. Monoamine assays

The brains were gently thawed on a cold plate, and samples of defined regions were dissected from using stereotaxic coordinates (Franklin and Paxinos, 1997) under a binocular microscope (Reader and Grondin, 1987; Reader et al., 1999). The samples were rapidly weighed, placed in tubes containing 40–50 vol of cold monochloroacetic acid (0.1 M) with 2.15 mM NA_2EDTA, homogenized (Tissumizer™, Tekmar, Cincinnati, OH) and then centrifuged at 39,000 × *g* for 45 min at 4°C. The pellets were dissolved overnight for protein assays (Lowry et al., 1951), while the supernatants were filtered through 0.45-μm pores (GS, Millipore, Bedford, MA) and stored at −80°C until the actual analyses, usually carried out within a few days, by high-performance liquid chromatography (HPLC) with electrochemical detection (Sauvé and Reader, 1988; Reader, 1989; Reader et al., 1989, 1999). The mobile phase was made of 0.1 M monochloroacetic acid containing 2.15 mM of Na_2EDTA, 1.0 mM of sodium octyl sulfate (Sigma, St. Louis, MO), 10% methanol, and adjusted with 1 N OHNa to pH 3.3. The flow was set at 0.6 ml/min, and the temperature of the column kept at 35–38°C. The samples were injected directly into a 3-μm particle size 100.0 × 4.6 mm Adsorbosphere Catecholamine™ chromatographic column (Alltech, Deerfield, IL). The substances were oxidized with a glassy carbon

electrode at a potential of +680 mV relative to the Ag/AgCl reference electrode, and the electrochemical detector (EE & G M-400, Princeton Applied Research; Princeton, NJ) set at a gain of 10 nA full scale. The oxidation peaks generated were recorded, and integrated with a Hewlett-Packard 3392A instrument. Since there were no pre-column purification or recovery steps, only external standards were used that were made with authentic noradrenaline HCl, 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxy-L-tryptophan, 5-hydroxytryptophol, 5-HIAA, dopamine HCl, homovanillic acid (HVA), L-tryptophan, serotonin HCl and 3-methoxytyramine; these compounds were purchased from Research Biochemicals International (RBI, Natick, MA). The external standards solutions contained 0.5 or 1.0 ng of each of these authentic monoamines, and they were injected for every chromatographic run, i.e.: every four to six samples, in order to quantify peak areas and verify the retention times, which showed coefficients of variation never exceeding 5%. Finally, to avoid any differences due to storage time, samples from +/+ mice and from *Lc*/+ mutants were alternated.

2.3. Statistics

The data is reported as the means ± S.E.M., in nanograms per milligram of protein. Statistical comparisons were made by analysis of variance, followed by Fisher's *F*-distribution test (Barlow, 1983; Frank and Althoen, 1994), with a level of significance that was established at 0.05.

3. Results

3.1. Frontal cerebral cortex

The sample of frontal cortex was the most rostral and medial part of the prefrontal cerebral cortex, the primary and secondary motor cortices and primary sensory cortical areas; its dissection limits extended from the most rostral tip of the brain up to the anterior commissure. In the saline-treated +/+ mice (Table 1), the two most abundant monoamines here were serotonin and noradrenaline, but there were also moderate levels of dopamine. The

Table 1
Endogenous indoleamines and catecholamines in frontal cortex and neostriatum in wild-type (+/+) and *Lurcher* (*Lc*/+) mice following chronic administration of saline or buspirone
Values are means ± S.E.M. (in ng/mg protein). The statistical significance of the differences was determined by analysis of variance, followed by Fisher's *F*-distribution test.

Mice	+/+		*Lc*/+		*F*
Treatment	Saline	Buspirone	Saline	Buspirone	
Frontal cortex					
L-Tryptophan	51.021 ± 4.968	87.572 ± 4.995[b]	60.208 ± 6.094	73.407 ± 8.541	$F_{3,21} = 5.676$
5-Hydroxytryptophan	0.088 ± 0.013	0.165 ± 0.017	0.124 ± 0.048	0.098 ± 0.017	$F_{3,16} = 1.366$
Serotonin	3.492 ± 0.144	5.442 ± 0.344[a]	4.613 ± 0.446	5.659 ± 0.687	$F_{3,21} = 4.224$
5-HIAA	2.454 ± 0.100	3.863 ± 0.421[a]	3.382 ± 0.498	3.711 ± 0.431	$F_{3,21} = 2.452$
5-Hydroxytryptophol	0.127 ± 0.015	0.234 ± 0.030[b]	0.121 ± 0.013	0.150 ± 0.020[g]	$F_{3,20} = 5.894$
Noradrenaline	3.184 ± 0.361	3.859 ± 0.180	4.296 ± 0.380[d]	4.430 ± 0.222	$F_{3,21} = 3.659$
Dopamine	0.798 ± 0.115	0.814 ± 0.094	1.019 ± 0.175	1.154 ± 0.152	$F_{3,18} = 1.416$
DOPAC	0.248 ± 0.045	0.547 ± 0.070[b]	0.276 ± 0.047	0.399 ± 0.046	$F_{3,20} = 6.400$
HVA	0.882 ± 0.102	1.902 ± 0.232[b]	1.039 ± 0.236	1.229 ± 0.139[g]	$F_{3,21} = 5.673$
Neostriatum					
L-Tryptophan	48.475 ± 5.405	99.155 ± 13.290[b]	104.840 ± 12.244[e]	72.032 ± 4.598[f]	$F_{3,19} = 6.385$
5-Hydroxytryptophan	0.155 ± 0.054	0.056 ± 0.000	0.125 ± 0.026	0.152 ± 0.012	$F_{3,7} = 0.767$
Serotonin	2.953 ± 0.109	4.394 ± 0.490	5.730 ± 0.675[e]	5.424 ± 0.630	$F_{3,20} = 4.559$
5-HIAA	2.239 ± 0.164	4.558 ± 0.757[a]	4.462 ± 0.792[d]	4.791 ± 0.506	$F_{3,20} = 3.199$
5-Hydroxytryptophol	0.232 ± 0.025	0.188 ± 0.030	0.350 ± 0.117	0.190 ± 0.010	$F_{3,12} = 0.825$
Noradrenaline	0.281 ± 0.026	0.479 ± 0.075[a]	0.455 ± 0.075	0.528 ± 0.068	$F_{3,19} = 3.090$
Dopamine	97.290 ± 6.845	86.712 ± 2.512	86.843 ± 4.099	100.258 ± 8.328	$F_{3,21} = 1.318$
DOPAC	12.200 ± 0.960	18.286 ± 2.106[a]	12.439 ± 1.169	13.870 ± 0.911[g]	$F_{3,19} = 4.170$
HVA	7.076 ± 0.448	22.434 ± 3.496[c]	9.777 ± 1.161	13.611 ± 1.474[h]	$F_{3,20} = 10.804$
3-Methoxytyramine	4.764 ± 0.331	10.825 ± 0.525[c]	6.015 ± 0.346	7.691 ± 0.685[f,h]	$F_{3,19} = 22.539$

[a] $P < 0.05$, between saline- and buspirone-treated +/+ mice.
[b] $P < 0.01$, between saline- and buspirone-treated +/+ mice.
[c] $P < 0.001$; between saline- and buspirone-treated +/+ mice.
[d] $P < 0.05$, between saline-treated +/+ mice and *Lc*/+ mutants.
[e] $P < 0.01$; between saline-treated +/+ mice and *Lc*/+ mutants.
[f] $P < 0.05$;between saline- and buspirone-treated *Lc*/+ mutants.
[g] $P < 0.05$, between buspirone-treated +/+ mice and *Lc*/+ mutants.
[h] $P < 0.01$; between buspirone-treated +/+ mice and *Lc*/+ mutants.

immediate serotonin precursor, namely, 5-hydroxy-tryptophan, was measured in trace amounts, and of the two indoleamine metabolites, 5-HIAA was the most abundant, while 5-hydroxytryptophol represented only about 5% of total metabolite contents. Also, HVA and DOPAC were found in low amounts, indicating that the dopamine measured in the frontal cortex was not only a precursor contained in noradrenergic fibers, but an authentic and releasable neurotransmitter. After buspirone treatment of the +/+ mice, there were increases in tissue levels of L-tryptophan (+72%), of serotonin (+56%), of the two indoleamine metabolites 5-HIAA (+57%) and 5-hydroxytryptophol (+84%), as well as of the dopamine metabolites DOPAC (+120%) and HVA (+116%), but with an unchanged dopamine contents. Cortical noradrenaline contents in saline-treated *Lc*/+ mutants, was about 35% greater than in saline-treated +/+ mice. Although serotonin levels were slightly higher in the *Lc*/+ than in the +/+ mice, this difference was not significant for saline-treated animals (Table 1). After buspirone treatment of *Lc*/+ mutants, there were only increases in 5-hydroxytryptophol and HVA levels caused by buspirone in the *Lc*/+ mutants, but were significantly lower than in the +/+ mice.

3.2. Neostriatum

The neostriatum (caudate–putamen) was made up mainly of its rostral division, extending up to the anterior commissure. As expected, dopamine was the most abundant catecholamine; there were also high levels of DOPAC, HVA and 3-methoxytyramine (Table 1), but only very low levels of noradrenaline in this structure. The indoleamines serotonin and 5-HIAA were measured in moderate amounts, and 5-hydroxytryptophol amounted to about 9% of total serotonin metabolites. Buspirone treatment of +/+ mice increased L-tryptophan (+104%), 5-HIAA (+104%), noradrenaline (+70%), DOPAC (+50%), HVA (+217%) and 3-methoxytyramine (+127%) levels. In the saline-treated *Lc*/+ mutants, the neostriatal monoamine content was essentially the same as in +/+ mice (Table 1), except for higher tissue levels in L-tryptophan (+116%), serotonin (+94%) and 5-HIAA (+99%). Treatment of the *Lc*/+ mutants with buspirone significantly increased only 3-methoxytyramine (+28%) contents; the slight elevations in DOPAC and HVA levels compared to saline-treated *Lc*/+ mutants were of lesser magnitude than the changes caused by buspirone in +/+ mice.

Table 2
Endogenous indoleamines and catecholamines in thalamus and brainstem in wild-type (+/+) and *Lurcher* (*Lc*/+) mice following chronic administration of saline or buspirone
Values are means ± S.E.M. (in ng/mg protein). The statistical significance of the differences was determined by analysis of variance, followed by Fisher's *F*-distribution test.

Mice	+/+		*Lc*/+		*F*
Treatment	Saline	Buspirone	Saline	Buspirone	
Thalamus					
L-Tryptophan	55.213 ± 5.869	108.786 ± 17.861[a]	72.827 ± 11.122	80.520 ± 11.070	$F_{3,20} = 3.319$
5-Hydroxytryptophan	0.080 ± 0.010	0.167 ± 0.020[b]	0.097 ± 0.022	0.086 ± 0.010[f]	$F_{3,17} = 6.193$
Serotonin	4.650 ± 0.130	5.225 ± 0.763	8.265 ± 1.047[d]	7.243 ± 0.601	$F_{3,20} = 5.589$
5-HIAA	5.321 ± 0.231	10.314 ± 1.830[a]	12.849 ± 2.156[d]	11.728 ± 1.663	$F_{3,20} = 4.078$
5-Hydroxytryptophol	0.162 ± 0.015	0.374 ± 0.075[b]	0.206 ± 0.042	0.172 ± 0.017[f]	$F_{3,21} = 5.203$
Noradrenaline	4.505 ± 0.247	5.050 ± 0.408	5.385 ± 0.587	4.996 ± 0.330	$F_{3,21} = 0.763$
Dopamine	1.628 ± 0.245	2.305 ± 0.737	2.743 ± 0.474	2.138 ± 0.410	$F_{3,18} = 1.134$
DOPAC	0.508 ± 0.070	1.454 ± 0.651[a]	0.619 ± 0.104	0.598 ± 0.099	$F_{3,19} = 2.101$
HVA	1.642 ± 0.189	3.259 ± 0.587[a]	2.089 ± 0.364	2.145 ± 0.379	$F_{3,20} = 2.796$
Brainstem					
L-Tryptophan	53.339 ± 2.103	121.029 ± 16.751[b]	79.614 ± 9.968	75.322 ± 7.863[e]	$F_{3,19} = 6.404$
5-Hydroxytryptophan	0.090 ± 0.016	0.266 ± 0.047[b]	0.098 ± 0.023	0.086 ± 0.013[g]	$F_{3,21} = 10.115$
Serotonin	6.839 ± 0.343	9.268 ± 0.709	10.217 ± 1.415[c]	10.807 ± 1.387	$F_{3,20} = 2.689$
5-HIAA	12.513 ± 1.294	15.077 ± 1.938	14.021 ± 1.433	14.708 ± 1.555	$F_{3,20} = 0.519$
5-Hydroxytryptophol	0.229 ± 0.039	0.552 ± 0.030[b]	0.317 ± 0.078	0.243 ± 0.037[f]	$F_{3,20} = 7.909$
Noradrenaline	4.986 ± 0.360	6.539 ± 0.303	6.392 ± 0.872	5.718 ± 0.288	$F_{3,21} = 1.944$
Dopamine	1.148 ± 0.199	1.766 ± 0.118	1.523 ± 0.268	1.643 ± 0.218	$F_{3,21} = 1.579$
DOPAC	0.373 ± 0.071	1.106 ± 0.227[b]	0.401 ± 0.080	0.525 ± 0.063[f]	$F_{3,21} = 7.226$
HVA	0.834 ± 0.144	1.835 ± 0.137[b]	0.991 ± 0.194	1.113 ± 0.179[e]	$F_{3,20} = 7.184$

[a] $P < 0.05$, between saline- and buspirone-treated +/+ mice.
[b] $P < 0.01$; between saline- and buspirone-treated +/+ mice.
[c] $P < 0.05$, between saline-treated +/+ mice and *Lc*/+ mutants.
[d] $P < 0.01$; between saline-treated +/+ mice and *Lc*/+ mutants.
[e] $P < 0.05$; between buspirone-treated +/+ mice and *Lc*/+ mutants.
[f] $P < 0.01$, between buspirone-treated +/+ mice and *Lc*/+ mutants.
[g] $P < 0.001$; between buspirone-treated +/+ mice and *Lc*/+ mutants.

3.3. Thalamus

In the thalamus, comprising both its anterior and posterior divisions, of saline-treated +/+ mice, the main monoamines serotonin and noradrenaline were present in slightly higher levels than in the frontal cortex, and the indoleamine metabolites somewhat paralleled this distribution, with 5-hydroxytryptophol representing about 3% of the total (Table 2). The dopamine contents and the levels of its metabolites DOPAC and HVA were about 1.5–2-fold higher than in the frontal cortex. After buspirone treatment, in the +/+ mice, there were changes in tissue L-tryptophan (+97%), as well as of 5-hydroxytryptophan (+109%), 5-HIAA (+94%) and 5-hydroxytryptophol (+131%); the latter metabolite increases suggesting an accrued turnover. The changes in noradrenaline and dopamine were not significant, but DOPAC and HVA levels were augmented by 186% and 98%, respectively, when compared to the saline-treated +/+ mice. In saline-treated *Lc*/+ mutants, serotonin and 5-HIAA contents were higher than in the saline-treated +/+ by 78% and 141%, respectively (Table 2). There were no monoamine changes in the thalamus of *Lc*/+ mutants after buspirone treatment; in fact, 5-hydroxytryptophan and 5-hydroxytryptophol contents were lower by respectively 48.5% and 54.0% than in the buspirone-treated +/+ mice.

3.4. Brainstem

In the brainstem, made of the pons, medulla and mesencephalon, serotonin and 5-HIAA levels were higher than in all the other regions (Table 2), reflecting the presence of the serotoninergic neurons that are localized in the raphe nuclei pertaining to these structures. The main indoleamine metabolite was again 5-HIAA, while 5-hydroxytryptophol represented $< 2\%$ of total indoleamine metabolites. Also, noradrenaline was measured in high amounts, and there were significant levels of dopamine together with DOPAC and HVA. After buspirone treatment, there were increases in tissue L-tryptophan (+127%), 5-hydroxytryptophan (+195%), 5-hydroxytryptophol (+141%), DOPAC (+196%) and HVA (+120%)

Table 3
Endogenous indoleamines and catecholamines in cerebellum and spinal cord in wild-type (+/+) and *Lurcher* (*Lc*/+) mice following chronic administration of saline or buspirone
Values are means ± S.E.M. (in ng/mg protein). The statistical significance of the differences was determined by analysis of variance, followed by Fisher's *F*-distribution test.

Mice	+/+		*Lc*/+		*F*
Treatment	Saline	Buspirone	Saline	Buspirone	
Cerebellum					
L-Tryptophan	53.931 ± 2.339	97.424 ± 8.974[b]	59.095 ± 6.391	67.900 ± 4.199[i]	$F_{3,20} = 9.718$
5-Hydroxytryptophan	0.063 ± 0.009	0.089 ± 0.012	0.037 ± 0.006	0.048 ± 0.004[i]	$F_{3,12} = 8.336$
Serotonin	1.169 ± 0.131	1.451 ± 0.127	2.532 ± 0.281[d]	4.280 ± 0.726[e,i]	$F_{3,21} = 10.789$
5-HIAA	1.496 ± 0.149	2.435 ± 0.230[a]	2.470 ± 0.162[d]	3.209 ± 0.310[e,h]	$F_{3,20} = 9.979$
5-Hydroxytryptophol	0.086 ± 0.011	0.203 ± 0.017[b]	0.158 ± 0.016[d]	0.202 ± 0.013[e]	$F_{3,19} = 10.881$
Noradrenaline	3.308 ± 0.276	3.061 ± 0.186	4.797 ± 0.338[d]	6.334 ± 0.520[e,j]	$F_{3,21} = 17.384$
Dopamine	0.252 ± 0.086	0.237 ± 0.051	0.280 ± 0.032	0.605 ± 0.079[f,i]	$F_{3,18} = 8.836$
DOPAC	0.071 ± 0.014	0.243 ± 0.026[c]	0.093 ± 0.013	0.167 ± 0.033[e,h]	$F_{3,21} = 10.012$
HVA	0.354 ± 0.125	0.426 ± 0.035	0.390 ± 0.089	0.419 ± 0.090	$F_{3,20} = 0.131$
Spinal cord					
L-Tryptophan	48.145 ± 3.489	58.838 ± 3.418	64.622 ± 6.325	93.196 ± 8.417[f,i]	$F_{3,21} = 10.545$
5-Hydroxytryptophan	0.042 ± 0.003	0.134 ± 0.011[c]	0.064 ± 0.011	0.093 ± 0.008[i]	$F_{3,17} = 16.969$
Serotonin	3.738 ± 0.109	5.968 ± 0.476[a]	5.760 ± 0.819[d]	4.679 ± 0.566	$F_{3,21} = 3.326$
5-HIAA	4.812 ± 0.783	6.384 ± 0.742	6.993 ± 0.529[d]	10.179 ± 0.851[e,i]	$F_{3,21} = 9.551$
5-Hydroxytryptophol	0.147 ± 0.016	0.598 ± 0.093[c]	0.216 ± 0.034	0.465 ± 0.073[e]	$F_{3,20} = 11.585$
Noradrenaline	2.308 ± 0.136	3.417 ± 0.228[b]	2.550 ± 0.195	2.807 ± 0.231	$F_{3,21} = 5.212$
Dopamine	0.333 ± 0.026	0.418 ± 0.050	0.528 ± 0.060	1.487 ± 0.258[g,j]	$F_{3,20} = 15.687$
DOPAC	0.100 ± 0.007	0.212 ± 0.030[a]	0.158 ± 0.024	0.230 ± 0.031	$F_{3,21} = 5.264$
HVA	0.188 ± 0.049	0.328 ± 0.070	0.243 ± 0.070	0.300 ± 0.061	$F_{3,19} = 0.886$

[a] $P < 0.05$, between saline- and buspirone-treated +/+ mice.
[b] $P < 0.01$, between saline- and buspirone-treated +/+ mice.
[c] $P < 0.001$; between saline- and buspirone-treated +/+ mice.
[d] $P < 0.05$; between saline-treated +/+ mice and *Lc*/+ mutants.
[e] $P < 0.05$, between saline- and buspirone-treated *Lc*/+ mutants.
[f] $P < 0.01$, between saline- and buspirone-treated *Lc*/+ mutants.
[g] $P < 0.001$; between saline- and buspirone-treated *Lc*/+ mutants.
[h] $P < 0.05$, between buspirone-treated +/+ mice and *Lc*/+ mutants.
[i] $P < 0.01$, between buspirone-treated +/+ mice and *Lc*/+ mutants.
[j] $P < 0.001$; between buspirone-treated +/+ mice and *Lc*/+ mutants.

in +/+ mice. In the *Lc*/+ mutants, monoamine levels were similar as in +/+ mice (Table 2), but for higher serotonin contents (+48%). After buspirone, monoamines in *Lc*/+ mutants were unchanged when compared to saline-treated *Lc*/+ mice, and lower than in the buspirone-treated +/+ mice in the case of L-tryptophan (−38%), 5-hydroxytryptophan (−68%), 5-hydroxytryptophol (−56%), DOPAC (−52%) and HVA (−39%).

3.5. Cerebellum

The cerebellum was carefully separated from the brainstem; the samples included the cortex of both vermis and hemispheres, as well as the deep cerebellar nuclei. In +/+ mice (Table 3), there were low levels of serotonin, a moderate noradrenaline content and traces of dopamine. The metabolites of serotonin were also measured in very low amounts; the principal one was 5-HIAA, while 5-hydroxytryptophol represented about 5% of total indoleamine metabolites. The treatment of the +/+ mice with buspirone lead to changes in tissue levels of L-tryptophan (+81%), 5-HIAA (+63%), 5-hydroxytryptophol (+136%) and DOPAC (+242%). In the *Lc*/+ mutants, serotonin, 5-HIAA, 5-hydroxytryptophol and noradrenaline contents were higher than in +/+ mice by 117%, 65%, 84% and 45%, respectively; otherwise, all remaining monoamines had levels in the range of values measured for the +/+ mice. After buspirone treatment of the *Lc*/+ mutants, there were increases in cerebellar contents of serotonin (+69%), 5-HIAA (+30%), 5-hydroxytryptophol (+28%), noradrenaline (+32%), dopamine (+116%) and DOPAC (+80%) when compared to the saline-treated mutants.

3.6. Spinal cord

In the spinal cord, always removed as a whole structure from the medullary canal, the serotonin and noradrenaline levels were higher than in the cerebellum; in the range of values measured in the cerebral cortex (Table 3). However, and contrary to cerebral cortex, the 5-HIAA levels were higher than those of serotonin, indicating a normally higher turnover here than in the cerebral cortex. The levels of 5-hydroxytryptophol amounted to about 3% of total indoleamines. Also, dopamine was present, albeit in very low amounts, and there were only traces of DOPAC and HVA. The buspirone treatment of the +/+ mice increased 5-hydroxytryptophan (+219%), serotonin (+60%), 5-hydroxytryptophol (+307%), noradrenaline (+48%) and DOPAC (+120%) contents. In saline-treated *Lc*/+ mutants (Table 3), tissue serotonin and 5-HIAA levels were higher than in the saline-treated +/+ mice, by 54.1% and 45.3%, respectively. The buspirone treatment of *Lc*/+ mutants lead to increases in L-tryptophan (+44%), 5-HIAA (+46%), 5-hydroxytryptophol (+115%) and dopamine (+182%) contents, when compared to the levels measured in saline-treated *Lc*/+ mutants. There were no differences in serotonin, noradrenaline, DOPAC and HVA contents when saline-treated and buspirone-treated *Lc*/+ mice were compared. On the other hand, in buspirone-treated *Lc*/+ mutants, spinal tissue levels of L-tryptophan

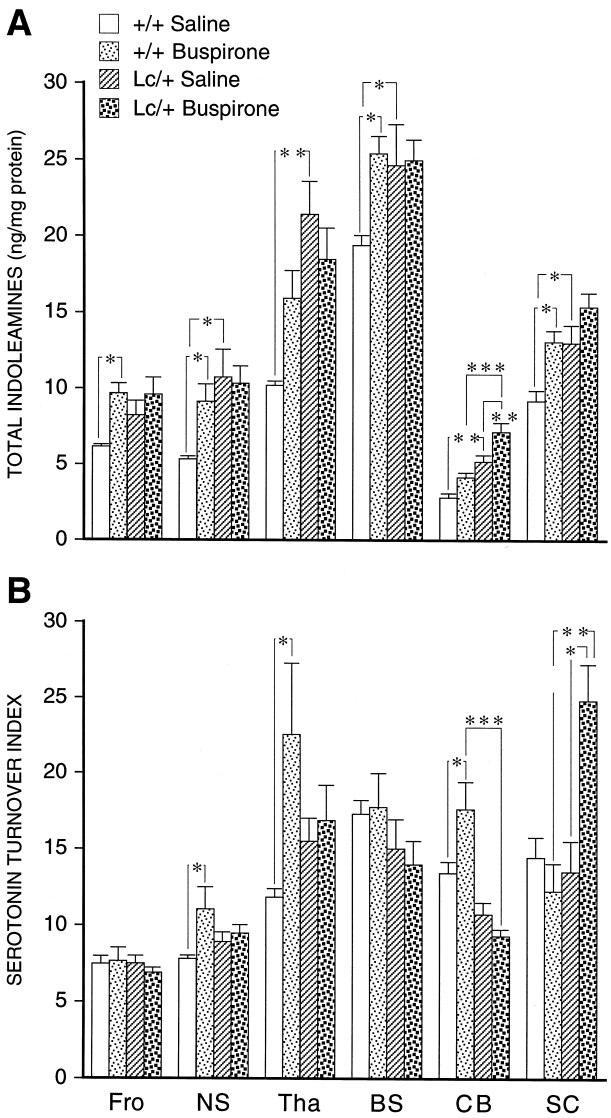

Fig. 1. The total indoleamines contents and the serotonin turnover index were calculated from the values given in Tables 1–3 for wild type (+/+) mice and *Lurcher* (*Lc*/+) mutants, that had been treated with either saline or with buspirone. The regions are Fro = frontal cortex; NS = neostriatum; Tha = thalamus; BS = brainstem; CB = cerebellum; SC = spinal cord. (A) The values of the total indoleamines are the means ± S.E.M. in nanograms per milligram of protein of the amounts in the tissues of [5-hydroxytryptophan] + [serotonin] + [5-HIAA] + [5-hydroxytryptophol], and calculated separately for every animal. (B) The serotonin turnover indexes are the means ± S.E.M. of the ratios between indoleamine metabolites ([5-HIAA] + [5-hydroxytryptophol]) and endogenous serotonin, calculated separately for every animal. The statistical significance of the differences, which was determined by analysis of variance followed by Fisher's *F*-distribution test, is indicated for the comparisons shown here as $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

5-HIAA and dopamine were higher by 58%, 59% and 256%, respectively, while 5-hydroxytryptophan content was lower (−31%), when compared to the buspirone-treated +/+ mice.

3.7. Total indoleamines and serotonin turnover

As illustrated in Fig. 1, buspirone treatment of the +/+ mice elevated total indoleamine contents in the frontal cortex (+57%), neostriatum (+72%), brainstem (+31%) and spinal cord (+42%), and the serotonin turnover index in the neostriatum (+42%), thalamus (+90%) and cerebellum (+31%). The *Lc*/+ mutants have already higher total indoleamines, and the buspirone treatment further increased these levels only in the cerebellum. In addition, buspirone treatment of *Lc*/+ mutants did not affect serotonin turnover index, except for an increase (+84%) in the spinal cord.

4. Discussion

In the present study, indoleamines and catecholamines were measured in well-defined anatomical regions of the *Lc*/+ mouse, a mutant characterized by a severe cerebellar atrophy, with manifest gait and movement disorders (Lalonde et al., 1992; Le Marec et al., 1997), and that resembles some forms of human heredo-degenerative cerebellar ataxia. The protocol was designed to determine if there was any abnormal biochemical reactivity of the central serotoninergic systems after chronic administration of the 5-HT_{1A} receptor agonist buspirone over a 40-day period. In this way, we tried to mimic the temporal course of some of the serotoninergic therapies (Plaitakis, 1993; Trouillas, 1993, Botez et al., 1997), that more recently have resorted to the administration of buspirone (Lou et al., 1995; Trouillas et al., 1996, 1997).

Buspirone is a non-benzodiazepine anxiolytic agent (Wu et al., 1972) with a seemingly complex mechanism of action, that calls upon the primary activation of 5-HT_{1A} receptors (Peroutka, 1985; Jann 1988; Eison et al., 1991). For example, buspirone has been shown to increase the head twitch behavior induced by 5-hydroxytryptophan administration in the presence of pargyline, and it was proposed to exert this through postsynaptic 5-HT_{1A} receptors (Kitamura et al., 1994). In addition, it has been documented that buspirone effects are multiple; indeed, besides acting on postsynaptic 5-HT_{1A} receptors, it could also stimulate presynaptic 5-HT_{1A} sites regulating release and/or metabolism, influence 5-HT_2 receptors and even block autoreceptors of the dopamine D_2 receptor subtype (Jann, 1988; Tunnicliff, 1991). In electrophysiological studies, buspirone increased the firing frequency of dopaminergic neurons of the ventral tegmental area, and it was found to block the effects of dopamine when applied by iontophoresis on these dopaminergic neurons. However, in these same studies, buspirone was found in addition to activate noradrenergic neurons of the locus coeruleus via a probable blockade of α_2-adrenoceptors and to inhibit the firing rate of cells in the nucleus raphe dorsalis, presumably serotoninergic, through an undetermined mechanism (Scuvée-Moreau et al., 1987), but that could involve 5-HT_{1A} autoreceptors. That buspirone manifests multiple effects acting on several receptor subtypes is in addition compounded by the differences in outcome following acute or chronic drug administration. Indeed, a single administration of buspirone was found to decrease dopamine levels, but to elevate DOPAC and 3-methoxytyramine in the neostriatum, most probably due to an enhanced release. Such an increase in the ratio between metabolites and dopamine can be interpreted as an augmented metabolism, and was already verified by voltammetry in neostriatum and nucleus accumbens (Algeri et al., 1988). Also, in healthy subjects, an acute oral administration of 20 mg of buspirone increased plasmatic levels of noradrenaline, dopamine and free plasmatic serotonin, but with no changes in adrenaline, tryptophan or platelet serotonin (Lechin et al., 1998); these findings were considered to be caused by hyperactivity of sympathetic and parasympathetic nerves rather than by an increased release from the adrenal medulla. However, chronic treatments may lead to very different levels of neurotransmitters and metabolites, or even to quite varying metabolic states, as the monoaminergic systems adapt over time to the long-term drug challenge. For example, buspirone given for a month reverses haloperidol-induced catalepsy (Queiroz and Frussa-Filho, 1997), and a buspirone treatment lasting only 2 weeks caused a decrease in neostriatal dopamine metabolism (McMillen, 1985). More recently, it has been shown with the technique of α-[^{14}C]methyl-L-tryptophan incorporation, that a single dose of buspirone decreases serotonin synthesis throughout the brain, but a chronic treatment for 2 weeks does not modify it, leading the authors to propose as underlying mechanism the desensitization of 5-HT_{1A} autoreceptors on the serotoninergic neurons (Okazawa et al., 1999). Based on the latter study, the chronic effects of buspirone can be regarded to differ from those caused by a single (acute) dose. There were no attempts here to examine the effects of a single dose of buspirone, since the aim of the present study was to mimic in the ataxic *Lc*/+ mouse the therapy proposed for humans. Also, the effects of other related serotonin$_{1A}$ compounds, such as the more selective agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin, were not compared to buspirone, but could warrant further investigations.

In the present study, buspirone was injected intraperitoneally at a daily dose of 1 mg/kg, and this is a relatively low dose but close to that used for patients suffering from cerebellar ataxia, and that showed beneficial effects after 2–4 months of treatment (Lou et al., 1995; Trouillas et al.,1996, 1997). Also, since the present buspirone treatment lasted for 40 days, it can be assumed that the changes

in endogenous monoamines followed a chronic adaptation, and may reflect more than the effect on a single neurotransmitter the balance or 'net effect' on interacting systems. In the +/+ mice, buspirone significantly increased serotonin only in the frontal cortex and spinal cord; even though the total indoleamine contents were higher than in saline-treated +/+ mice, the ratios between the metabolites and serotonin were unmodified, resulting in a conserved turnover index (Fig. 1).

The monoamine contents in saline-treated *Lc*/+ mutants and saline-treated +/+ mice were overall similar, but serotonin, 5-HIAA and 5-hydroxytryptophol, as well as noradrenaline levels, were higher in the *Lc*/+ cerebellum. In addition, the *Lc*/+ mutants had a higher noradrenaline content in the frontal cortex, as well as greater serotonin and 5-HIAA levels in the thalamus and in the spinal cord. In the neostriatum, thalamus, cerebellum and spinal cord, total indoleamines were significantly higher in the *Lc*/+ mutants than in the +/+ mice; however, there were no differences in the serotonin turnover index (Fig. 1) in any single region of the saline-treated *Lc*/+ mutants when compared to the saline-treated +/+ mice, and this is in agreement with the suggestion that in this mutant, indoleamines are accumulated but not released, even after a 'biochemical challenge' with an amino acid precursor. Indeed, it was shown for *Lc*/+ mice that after a chronic (40 days) treatment with L-tryptophan, endogenous serotonin contents increased in the neostriatum, brainstem and cerebellum, and the 5-HIAA levels were higher in the frontal cortex, neostriatum, thalamus, brainstem, cerebellum and spinal cord (Reader et al., 1999). These changes could be compatible with a higher retention, or incorporation, of the amino acid precursor in the nerve endings at the terminal fields of innervation, together with an accrued synthesis of indoleamines. Since the serotonin turnover index in the *Lc*/+ mutants treated with L-tryptophan increased only in the thalamus and spinal cord, but with higher total indoleamine contents in the thalamus, brainstem, cerebellum and spinal cord, it was suggested that the *Lc*/+ mutants can retain higher concentrations of the amino acid precursor L-tryptophan and increase total indoleamines, probably stored in a non-releasable compartment.

Of particular interest was the cerebellum of the *Lc*/+ mutants, already characterized by higher endogenous serotonin, 5-HIAA, 5-hydroxytryptophol and noradrenaline levels (Strazielle et al., 1996; Reader et al., 1999). Since in the *Lc*/+ mice, the cerebellum has considerably atrophied, the indoleamine levels corrected by the average total cerebellar surface area reflect a preservation of serotonin contents, as reported for other cerebellar mutants (Ohsugi et al., 1986; Ghetti et al., 1988). Moreover, the serotonin turnover index of *Lc*/+ mutants was not higher than for the +/+ mice, but if 5-HIAA levels and turnover ratios were calculated for the total cerebellar surface area, they indicated a lower serotonin metabolic activity, as if serotonin accumulates presynaptically in this structure, but thereafter is not used in synaptic transmission (Strazielle et al., 1996). In the related mutant *Purkinje cell degeneration*, or *pcd* mouse, the 5-HT turnover is normal at 3–6 months of age, but is lower at 7–15 months of age; this is probably caused by maturation and aging (Ghetti et al., 1988). Moreover, increases in [^{3}H]citalopram binding in the cerebellar cortex and deep nuclei of *Lc*/+ mutants (Le Marec et al., 1999; Strazielle et al., 1996) indicate that the serotonin innervation is preserved, but within an hypoplastic cerebellum, while electron microscopical observations in *pcd* mutants have failed to reveal retrograde degeneration of nerve terminals (Triarhou and Ghetti, 1986). Taking into account the loss of tissue surface in the *Lc*/+ mutants, the relative [^{3}H]citalopram binding densities were determined to be normal in the cerebellar cortex, but higher in the deep cerebellar nuclei, indicating a reorganization of the serotonin innervation in the deep nuclei (Le Marec et al., 1999; Strazielle et al., 1996). The present results show that in the cerebellum, there were also no significant differences in the serotonin turnover indexes (Fig. 1) between saline-treated +/+ mice and saline-treated *Lc*/+ mutants; however, this index was increased (+31%; $P < 0.05$) by buspirone in the +/+ mice, but not in the mutants, and the [serotonin metabolites]/[serotonin] ratios were lower in the buspirone-treated *Lc*/+ mutants than in the buspirone-treated +/+ mice. In contrast, the total indoleamine contents, that is 85% higher in saline-treated *Lc*/+ mutants compared to +/+ mice ($P < 0.05$), increased further by buspirone treatment in the *Lc*/+ mutants (+38%; $P < 0.001$).

In the *Lc*/+ mutants, there were also effects of buspirone on catecholamines, namely, increases of noradrenaline in the cerebellum, of dopamine in the cerebellum and spinal cord, of HVA in the frontal cortex and neostriatum, of DOPAC in the neostriatum, brainstem and cerebellum, and of 3-methoxytyramine in the neostriatum. Interestingly, in the +/+ mice, the effects of buspirone on catecholamines seem more widespread; indeed, it leads to increases in DOPAC levels in all the regions examined here, to augmented HVA in the frontal cortex, neostriatum, thalamus and brainstem and to increased 3-methoxytyramine in the neostriatum. These effects of buspirone may be related to its putative action on autoreceptors of the dopamine D_2 subtype (Jann 1988; Tunnicliff, 1991) and are in line with decreases in neostriatal dopamine metabolism found after 2 weeks of buspirone treatment (McMillen, 1985). It may also be suggested that some of the effects of buspirone on catecholamines could be mediated by interactions between the central serotonin, dopamine and noradrenaline systems, either at the brainstem nuclei of origin of these projections, or at the terminal fields of innervation. It has already been reported that chronic treatment with L-tryptophan in *Lc*/+ mutants increases noradrenaline in the cerebellum and neostriatum, dopamine in the spinal cord, DOPAC in the cerebellum,

HVA in the frontal cortex, neostriatum and cerebellum as well as 3-methoxytyramine in the neostriatum (Reader et al., 1999). These effects of the precursor could be due to interactions between serotonin and catecholamine systems in the *Lc/+* mutants, since these effects of L-tryptophan were not documented in the *+/+* mice, except for an increase in noradrenaline in the frontal cortex. At that time, a possible interpretation was that *Lc/+* mutants can synthesize serotonin, but it is not released, or not available at regulatory targets. The catecholamine neurons could be normally inhibited by serotonin, and this lack of tonic inhibition normally exerted on dopamine and noradrenaline synthesis now leads to increases in these neurotransmitters. Whatever the cause, the impact of buspirone on catecholamines, particularly on dopamine and its metabolites, could lead to beneficial effects. Indeed, although structural and/or functional abnormalities of the serotonin system may play a role in human heredo-degenerative ataxia (Kish et al., 1992; Plaitakis, 1993; Trouillas, 1993; Botez et al., 1998), other neurotransmitters may also have to be accounted for in ataxic disorders. For example, low HVA levels have been found in both Friedreich's ataxia and in olivopontocerebellar atrophy (Campanella et al., 1980; Botez et al., 1991), leading to the proposal that dopaminergic drugs, such as amantadine, may be clinically useful. Behavioral studies in the *Lc/+* mutant, as well as both open and double blind studies in patients suffering from Friedreich's ataxia and olivopontocerebellar atrophy, have shown that amantadine leads to a mild improvement of motor coordination in this animal model (Lalonde et al., 1993), as well as to ameliorations of both reaction time and movement time in the human patients (Peterson et al., 1988; Botez et al., 1991, 1996). Moreover, the dopaminergic innervation in *Lc/+* mutants is seemingly spared as shown by normal tissue contents in dopamine and metabolites (Reader et al., 1998), as well as by autoradiographic surveys of dopamine transporters (Strazielle et al., 1998), and could thus be a target for drugs such as amantadine and buspirone that are seemingly unrelated, but which may share common dopaminergic mechanisms. On the other hand, the effects obtained with amantadine could be due to its antagonist properties on NMDA receptors, since improvements in performance observed following treatment are not related to initial cerebrospinal fluid levels of HVA (Botez et al., 1991). Indeed, sensorimotor coordination improvements have also been reported in *Lc/+* mice that were treated with ketamine (Lalonde et al., 1993), a NMDA receptor antagonist.

In conclusion, we examined the effects on monoaminergic systems of a chronic treatment with buspirone in the *Lc/+* mouse, a neurological mutant that resembles some forms of human heredo-degenerative ataxia. In saline-treated *Lc/+* mutants, the higher indoleamine levels in the cerebellum, thalamus and spinal cord, with a conserved turnover index, indicate that they can accumulate but may not be released. After buspirone treatment, the changes in indoleamine levels in the cerebellum of *Lc/+* mice suggest an accrued synthesis with a higher retention and/or and accumulation, perhaps in a pool that is not readily utilized, together with a stunted metabolism, judged by a decreased turnover index. As a consequence of this diminished availability of serotonin at brainstem targets, catecholamine neurons lack a tonic inhibition that regulates dopamine and noradrenaline synthesis, although a direct effect of buspirone on catecholamines, particularly on the central dopaminergic systems, has to be taken into account.

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