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Toxic effects of cadmium on GABA and taurine content in different brain areas of adult male rats

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This work assesses the possible changes in gamma amino butyric acid (GABA) and taurine content in the hypothalamus, the median eminence and striatum after the exposure to various doses of cadmium. Cadmium chloride ($CdCl_2$) was administered in the drinking water at the doses of 5, 10, 25, 50 or 100 ppm to adult male rats for 1 month. In the anterior hypothalamus, taurine and GABA content decreased with the dose of 10 ppm of $CdCl_2$ only. Cadmium exposure decreased both GABA and taurine content in mediobasal hypothalamus except for the 50 ppm dose. In posterior hypothalamus GABA and taurine content was not affected by cadmium treatment. As far as the median eminence, 5 or 10 ppm of $CdCl_2$ increased taurine concentration, and at a dose of 5 ppm enhanced GABA content. A significant decrease of GABA and taurine concentration was seen in the striatum at any dose of cadmium used. The concentration of cadmium increased in the hypothalamus and in the striatum in animals receiving $CdCl_2$ in the drinking water at doses of 25, 50 or 100 ppm. The results indicate that cadmium globally decreased GABA and taurine content in the brain areas studied through effects that were not dose dependent.

Key words: Cadmium, Oral exposure, GABA, Taurine.

Cadmium is a heavy metal considered as an environmental poison. Its exposure induces several effects on both the neuroendocrine (1, 2, 6, 16, 17, 19) and the immune system (6, 23). Specifically, cadmium exposure is associated with changes in biogenic amines (1, 2, 16, 17, 19) or amino acid content (11, 18 20, 21, 25) within the brain.

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We previously demonstrated changes in the concentration of gamma amino butyric acid (GABA) and taurine, as well as of excitatory amino acids, in various areas of the hypothalamus and striatum of rats receiving cadmium in a dose of 50 ppm (18, 20, 21). That dose is high enough to be considered pharmacological, so that it is of interest to know if a lower dose of cadmium can induce similar changes on GABA and taurine content. Since GABA and taurine modulate pituitary hormone secretion (3, 13, 15), inhibitory amino acid changes could explain the modifications in plasma levels of pituitary hormones, e.g. prolactin, adrenocorticotropin hormone or growth hormone (22), seen after a low dose of cadmium, i.e. 5 ppm of $CdCl_2$ administered in the drinking water.

The objective of the present experiment was to analyze the effects of cadmium on anterior, mediobasal and posterior hypothalamic, median eminence and striatum GABA and taurine content in adult male rats. A dose response study for the effect of the metal was carried out.

Materials and Methods

Animals and experimental design.– Experiments were carried out in adult male Sprague-Dawley rats (250-300 g), kept under controlled conditions of light (light between 07.00 and 21.00 h daily) and temperature (22±2 °C). Food and water were available *ad libitum*. Six groups of 10 animals were used. Five groups were treated for one month with cadmium chloride ($CdCl_2$) at a dose of 5, 10, 25, 50 or 100 ppm of $CdCl_2$ in the drinking water. The control group was supplied with water from the State Company (36 µg Cd^{2+}/L). The lowest dose of cadmium administered to the animals in this work, is approximately 167 times higher than the Provisional Tolerable Weekly Intake (PTWI) of this heavy metal (35) that means an intake of 0.15 mg Cd^{2+}/Kg/day. Cadmium exposure did not modify water consumption (approximately 27 mL/day).

At the end of the treatment, animals were killed by decapitation. Care was taken to avoid any major stress before sacrifice and the decapitation procedure was completed within 5-7 seconds. Immediately after sacrifice the heads were introduced in liquid nitrogen, and kept at –80 °C until analyzed. A replicate experiment was performed to confirm the changes observed. The studies were conducted during the same season of the year (Spring) to avoid circannual variations (10).

The studies were conducted according with the principles and procedures outlined in the NIH guide for the Care and Use of the Laboratory Animals (26).

Tissue preparation.– After thawing, the median eminence, anterior, mediobasal and posterior hypothalamus and striatum blocks were immediately homogenized in cold 2 M acetic acid (1-4 °C), heated for 5 min in 100 °C water bath and centrifuged at 11,000 x g for 10 min at 4 °C. The supernatant was removed and kept frozen at –80 °C until amino acid determination. Before heating, an aliquot of the tissue homogenates were obtained and used to determine protein content by Bradford method.

Amino acid measurements.– GABA and taurine were separated and analyzed by using High Performance Liquid Chromatography (HPLC), with fluorescence detection after precolumn derivatization with O-phthaldialdehyde (OPA). An aliquot of the tissue supernatant contain-

ing homoserine as internal standard was neutralized with NaOH (4 M) and then was reacted at room temperature with OPA reagent (4mM OPA, 10% methanol, 2.56 mM 2-mercaptoethanol, in 1.6 M potassium borate buffer, pH 9.5) for 1 min; and at the end of this period, the reaction was stopped by adding acetic acid (0.5 v/v). Samples were immediately loaded through a Rheodyne (model 7125) injector system with a fifty-microliter loop sample. A C-18 reverse-column (4.6 mm ID x 150 mm, Nucleosi 5, 100 A) was used for separation. The amino acids were eluted with a mobile phase consisting of 0.1 M sodium acetate buffer (pH 5.5) containing 30% methanol, at a flow rate of 1 mL/min, resulting in a pressure of 140 Bars. The column was subsequently washed with the same buffer containing 70% methanol and re-equilibrated with the elution buffer before the next injection. The HPLC system consisted of a solvent delivery system coupled to a filter fluorometer (excitation 340 nm, emission 455 mm). This procedure allows for a clear separation and resolution of the amino acids measured in this study, as previously described (9).

GABA and taurine content in each tissue was calculated from the chromatographic peak areas by using standard curves and the internal standard. The linearity of the detector response for the amino acids studied was tested within the concentration range found in brain regions extracts.

Cadmium determination.– Cadmium concentration was determined in the whole hypothalamus plus median eminence and in the striatum of individual animals. Tissue cadmium concentrations were determined by graphite furnace atomic absorption spectrophotometry after microwave digestion (GFAAS) (24). The samples were mineralized in a Parr 4780 microwave acid digestion bomb and a Samsung M-745 microwave oven. Treating dried homogenized whole pituitary with 3.0 mL of ultra pure nitric acid and 1 mL of distilled water performed the mineralization step. The mineralization was complete after two digestions at 450 W for 2 min, 20 s each. For cadmium determination, an atomic absorption spectrophotometer (Perkin-Elmer, Varian Spectra 250 plus) with Zeeman background correction was used. Accuracy was assessed by calibration against aqueous standards. For the aqueous standard control, we checked that the absorbance measurement fit with the technical characteristics of the device, allowing a deviation of ±5%, i.e., RSD (relative standard deviation) was inferior to the 5% for the samples and for the patterns. Every ten samples a reslope was made. The lowest level of sensitivity was 1.6 ng/g. Samples of the whole experiment were analyzed in the same assay to avoid inter-assay variations; the intra-assay coefficient of variation was 4.5%.

Statistical analysis.– Amino acid concentrations were expressed as pg/µg protein. The results were tested for variance homogeneity through the Snedecor test. When the results did not follow a homogeneous variance, a Kruskal-Wallis test for comparison between groups was used. If the results follow a homogeneous variance, a one-way analysis of variance (ANOVA) followed by a Tukey test for multiple comparations were applied for comparisons between groups. The results were considered significant at $P \leq 0.05$. All values represent the mean ± S.E.M.

Results

Table I shows GABA and taurine content in anterior, mediobasal and posterior hypothalamus, median eminence and striatum of adult animals exposed to various doses of cadmium in the drinking water for 1 month.

Cadmium modified GABA and taurine concentration in the anterior hypothalamus ($F=10.02$, $P\leq0.001$ and $F=7.29$, $P\leq0.001$ respectively). GABA and taurine content were significantly reduced by low doses of cadmium (10 ppm) in the anterior hypothalamic region ($P\leq0.01$) as compared to the values observed in controls. No changes were observed at any of the other doses of metal employed.

Cadmium affected GABA and taurine concentration in the mediobasal hypothalamus ($F=35.01$, $P\leq0.001$ and $F=21.22$, $P\leq0.001$ respectively). Both GABA and taurine content were significantly reduced by cadmium exposure in mediobasal hypothalamus ($P\leq0.001$), except for the 50 ppm dose. In the posterior hypothalamus, cadmium exposure did not change GABA or taurine content at any of the concentrations used.

Cadmium exposure affected both taurine and GABA concentration in the median eminence ($F=10.48$; $P\leq0.01$ for taurine and $F=2.05$; $P\leq0.05$ for GABA). Taurine concentration increased with the lower doses of cadmium ($P\leq0.001$ *vs.* control group for 5ppm of cadmium or $P\leq0.01$ *vs.* control group for 10 ppm of cadmium). GABA content increased with the dose of 5 ppm of $CdCl_2$ only ($P\leq0.001$ *vs.* control group).

In the striatum, a significant effect of treatment on both GABA and taurine was observed ($F=6.41$, $P\leq0.001$ and $F=6.36$, $P\leq0.001$ respectively). After cadmium

Table I. *GABA and taurine content (ng/μg protein) in the anterior, mediobasal and posterior hypothalamus, median eminence and striatum in adult male rats treated for one month with cadmium-free water (control) or with different doses of cadmium chloride (5-100 ppm) in the drinking water.*
The values are expressed as mean ± S.E.M. (n=10 in each group). *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001 *vs.* control group.

Amino acid (ng/μg protein)	Anterior hypothalamus	Mediobasal hypothalamus	Posterior hypothalamus	Median eminence	Striatum
Taurine					
Control	40.26±5.73	64.09±13.33	29.44±6.63	64.57±11.66	51.65±4.82
5 ppm $CdCl_2$	37.90±2.33	25.46±1.85***	30.79±3.24	138.53±7.87***	27.39±3.77***
10 ppm $CdCl_2$	12.48±1.97**	23.59±3.01***	26.63±4.19	128.12±11.96**	36.13±4.40*
25 ppm $CdCl_2$	39.47±5.68	11.62±0.35***	18.97±3.58	119.02±7.42	31.99±4.43*
50 ppm $CdCl_2$	25.73±3.69	41.25±9.03	40.13±5.81	125.17±22.34	27.88±5.05**
100 ppm $CdCl_2$	48.94±6.15	23.33±3.86***	40.67±2.82	118.36±22.90	28.84±2.66**
GABA					
Control	67.79± 6.84	100.4±13.64	47.57±6.28	71.44±7.52	133.64±10.26
5 ppm $CdCl_2$	78.00±9.55	60.37±5.96***	65.03±10.87	220.04±15.28***	80.62±12.13*
10 ppm $CdCl_2$	12.21±2.04**	43.51±11.04***	42.17±3.31	113.88±19.92	95.58±10.08*
25 ppm $CdCl_2$	94.46±9.32	36.53±1.57***	76.17±0.60	151.10±24.49	90.41±11.87*
50 ppm $CdCl_2$	45.90±8.21	75.72±13.74	80.95±12.49	145.55±24.50	45.58±11.76***
100 ppm $CdCl_2$	83.13±15.69	51.74±4.53***	74.94±14.20	115.09±20.05	69.64±7.33**

exposure, GABA and taurine concentration decreased with every dose of metal employed.

As shown in Fig. 1, cadmium content in the whole hypothalamus and in the striatum increased with every cadmium dose used, but reached statistical significance only in those animals treated with 25-100 ppm of $CdCl_2$ ($P \leq 0.01$).

Discussion

Both GABA and taurine are involved in the modulation of a great variety of physiological functions, from endocrine regulation to immunity or behavior (3, 7, 32). GABA is the main inhibitory neurotransmitter in the central nervous system (28), and is associated with the development of neurodegenerative diseases (35). Taurine is also a central nervous system neurotransmitter (28), associated with protective functions (30) and since recently, it is considered a toxicological biomarker (31). The foregoing results suggest that cadmium differentially affects GABA and taurine content within the brain, according to the dose and the specific area of the brain analyzed. Generally the effects of the metal were not dose dependent.

Cadmium, at the doses used, did not modify the content of GABA and taurine in the posterior hypothalamus, a region involved in the regulation of both autonomic nervous system and immune system activities (4). The absence of significant changes of 50 ppm of $CdCl_2$ on both GABA and taurine concentration in the median eminence and the hypothalamus in adult male rats treated for 1 month, agrees with previous data from the laboratory using the same dose of the metal in a similar experimental design (20) or administered in an alternate sc. schedule (12). The increase in taurine content in the median eminence with the lowest cadmium dose may explain, at least in part, the increase in plasma prolactin levels previously demonstrated using similar doses of the metal (22). However, the changes in GABA content did not explain the associated changes in plasma prolactin levels,

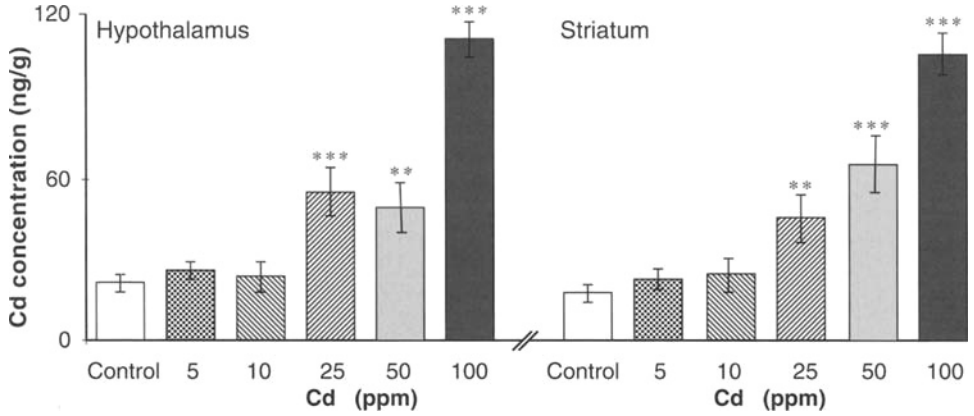

Fig. 1. *Cadmium concentration in the whole hypothalamus and in the striatum in adult male rats treated for one month with cadmium chloride (5-100 ppm) in the drinking water.*
The values are expressed as mean ± S.E.M. (n=10 in each group). **P ≤ 0.01, ***P ≤ 0.001 *vs.* control group.

thus indicating a disruptive effect of cadmium on the inhibitory mechanism that regulate prolactin secretion from the pituitary (5, 8, 22).

The changes in GABA and taurine content at the anterior hypothalamus with low doses of the metal together with the decrease in their content in the mediobasal hypothalamus suggest the existence of a disruption of the regulatory mechanism of prolactin exerted by GABA (3, 22, 24, 27, 33) as was previously demonstrated for dopamine (14, 16, 17, 19). All these data together suggest that cadmium exposure disrupts the regulatory mechanisms of pituitary hormone secretion exerted by GABA or taurine.

The striatum is connected with the hypothalamus (15) and so far modulates the activity of the hypothalamus. It is of interest to note that cadmium similarly affected both neurotransmitters in the striatum. However these changes do not explain the modification in biogenic amines and amino acids in hypothalamus, described in this and in previous works from the laboratory (17, 19-21) thus indicating that the connections between the striatum and the hypothalamus is altered by cadmium exposure. On the other hand, the striatum is connected with other brain areas involved in the regulation of behavior (like hippocampus), so that several behavioral test may be altered in animals exposed to this heavy metal as was previously suggested (29). The results resemble those found in aged rats (12), thus suggesting that metal accumulation at the brain may be involved in the acceleration of the aging process.

The changes observed in both GABA and taurine content in the brain regions studied cannot be explained by the alterations in the body weigh gain, as this parameter only decreased with the higher doses of the metal, but not with the doses of 5 or 10 ppm of $CdCl_2$. In addition, the changes are not due to cadmium accumulation in the analyzed tissues, since the concentration of this metal only increased in the animals treated with 25, 50 or 100 ppm of $CdCl_2$.

In conclusion, these data suggest the existence of differential cadmium effects on GABA and taurine, depending on the brain area analyzed. Surprisingly, the effects were not dose dependent. Further experiments are needed to fully understand the differential effects of the metal in the brain areas studied, and their correlation with other physiological function like pituitary hormone secretion.

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A. LAFUENTE, A. GONZÁLEZ-CARRACEDO, T. CABALEIRO, A. ROMERO y A. I. ESQUIFINO. *Efectos tóxicos del cadmio sobre el contenido de GABA y taurina en diferentes regiones cerebrales de rata macho adulta.* J. Physiol. Biochem., **61** (3), 439-446, 2005.

En este trabajo se evalúan las posibles alteraciones en el contenido de ácido gamma amino butírico (GABA) y taurina, inducidas por varias dosis de cadmio en el hipotálamo, la eminencia media y el estriado. Para ello, se administró cloruro de cadmio ($CdCl_2$) durante 30 días a ratas macho adultas en el agua de bebida a las dosis de 5, 10, 25, 50 ó 100 ppm. En el hipotálamo anterior, la exposición al cadmio no modificó el contenido de taurina y GABA, aunque la concentración de estos aminoácidos descendió con las dosis de 10 ppm. Tanto el contenido de GABA como el de taurina disminuyeron significativamente en el hipotálamo mediobasal tras la exposición al cadmio, excepto con la dosis de 50 ppm. Sin embargo, en el hipotálamo posterior, la exposición a este

metal no alteró los niveles de GABA y taurina. En la eminencia media, las dosis de 5 y 10 ppm aumentaron la concentración de taurina, mientras que el contenido de GABA sólo aumentó con la dosis de 5 ppm. Tras la exposición al cadmio, se observó un descenso en el contenido de GABA y taurina en el estriado con todas las dosis utilizadas. La concentración de cadmio aumentó en el hipotálamo y en el estriado con las dosis de 25, 50 y 100 ppm de $CdCl_2$. Globalmente, según estos resultados, la exposición al cadmio puede conllevar un descenso de GABA y taurina en las regiones cerebrales estudiadas, aunque dichos descenso no parece ser dependiente de las dosis del metal.

Palabras clave: Cadmio, Exposición oral, GABA, Taurina.

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