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MECHANISM OF LITHIUM CARBONATE INDUCED HYPERDIPSIA

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Pshychiatric patients and laboratory animals treated with lithium carbonate develop an important polydipsia and polyuria. Polydipsia could be a primary phoenomenon which induces a secondary polyuria or alternatively polyuria could be primary and polydipsia secondary. Experiments using nephrectomized male rats treated with lithium suggest that polyuria seems to be primary and polydipsia secondary. This is confirmed when water is restricted to lithium treated animals because their urine volumes are greater than the water ingested; our results also suggest that there is some kind of renal damage which could cause a primary polyuria. Kidneys morphollogical studies show non specific damages.

Mecanismo de la sed inducida por carbonato de litio. Los pacientes psiquiátricos y los animales de laboratorio tratados con carbonato de litio desarrollan una importante poliuria y polidipsia. La polidipsia puede ser un fenómeno primario que induce una poliuria secundaria o alternativamente, una poliuria primaria podría ser la causa de la polidipsia observada. Nuestros experimentos utilizando ratas macho nefrectomizadas tratadas con litio, sugieren que la poliuria sea primaria y la polidipsia secundaria. Estos resultados se confirman cuando se restringe el agua a los animales tratados con litio, quienes muestran unos volúmenes de orina superiores a los de agua ingerida, lo que sugiere que debe haber algún tipo de daño renal que justifique una poliuria primaria. Los estudios morfológicos renales muestran daños inespecíficos.

Lithium carbonate is almost exclusively used for the treatment of some psychiatric disorders (Marples, Christensen , Christensen , Ottosen and Nielsen, 1995). Many bipolar disorder patients become stabilized during the administration of lithium carbonate. Nevertheless, it produces several unwanted side effects, including polydipsia and polyuria (Penney and Hampton, 1990). At present, it is not clear the origin and mechanism of these alterations of water homeostasis. Two non excludent theories can be postulated to explain it. The polydipsia could be a primary phenomenon which in turn induces secondary polyuria (Penney and Hampton,1990; Cox and Singer, 1975); alternatively, polyuria could be primary and polydipsia a secondary phenomenon (Cox and Singer, 1975). The purpose of this work is to further investigate the origin of lithium induced thirst (LIT). For this, water and food intake, body weight, diuresis, plasma lit-

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hium levels and the effect of the nephrectomy were investigated in several groups of rats under different experimental conditions.

Materials and methods

Animals

Male Wistar rats weighing 200-350 g, obtained from the vivarium of the Faculty of Medicine of Oviedo were used throughout this study. Animals were normally housed individually in standard plastic and top wire cages. They were fed standard laboratory diet (Sandersmus) with continuous access to tap water, delivered from graduated tubes fitted with glass spouts. Lighting schedule was 12 hours on, 12 hours off (on at 9 am, off at 9 pm).

Procedure

The following substances were used in different experiments: Li_2CO_3, LiCl, Na_2CO_3; EDTA, Ether, Urethane, Karnowsky fluid, Cacodilate buffer, Ethanol.

For determination of plasma lithium levels, under ether anaesthesia (12 hours after the last Li_2CO_3 injection), a blood sample of about 1 ml was collected from the jugular vein. Blood was contained in plastic tubes with EDTA, and then centrifuged for plasma separation. Plasma samples were kept frozen (-20°C) until lithium content was measured using a Corning 455 flame photometry apparatus, callibrated using two commercial standard solutions of lithium and a reference potassium solution, according to the apparatus specifications.

Metabolic experiments were performed in individual metabolic cages (Tecniplast 1700). A three days period was allow to accustom the rats to new environment. Water and food intake and diuresis were recorded during another 3 days when actual experiments started.

Nephrectomy or sham operation was made via a single dorsal skin incission and two lateral muscular incisions under ether anaesthesia. To sham operated rats one dose of Li_2CO_3 was administered after surgery, in order to mantain plasma lithium levels. Nephrectomized rats did not receive lithium at that stage to avoid intoxication.

For histological studies, when the experiments were finished, the anaesthetized (urethane) animals were perfused using Karnowsky fluid. Kidneys were removed, introduced in Cacodilate buffer and stored at 4ºC until processed. Routine hematoxiline-eosine stain was made on 10µ slices for optical microscopy, and toluidine blue on 800Å slices for electronic microscopy.

Statistical analysis

Statistical significance of the results was assessed by two tail Student's «t» tests (paired or unpaired as appropriate). The values of $p<0.05$ were deemed significant. Numeric values are expressed as the mean±SEM.

Experiments

Dipsogenic effect of lithium carbonate: Dose-response relation

Doses from 0.01 to 4 mmol/kg bw/day of Li_2CO_3 were tested for its dipsogenic effect. The dose-response relation was assessed on seven different groups of 4-10 rats (Total n = 44). Li_2CO_3 was diluted for injecting in distiled water and administered through two i.p. boluses separated by 12 hours (9 am and 9 pm). The total daily volume of Li_2CO_3 solution injected ranged from 0.1 ml/kg bw for the lowest dose of Li_2CO_3 to 40 ml/kg bw for the highest. A control group (n= 10 rats), received only vehicle injections (10 ml/kg bw of distiled water divided in two equal boluses administered separated by 12 hours).

During three days the spontaneous water intake was recorded; then Li_2CO_3 treatment began and daily water intake was recorded during another four days. Plasma lithium content was measured according to the method above described.

Dipsogenic effect of lithium carbonate: lithium vs carbonate

In order to test the contribution of both lithium cathion and carbonate anion to the LIT phenomenon, the following experiments were performed:

a) A dose of 1 mmol/kg bw of Na_2CO_3 was tested for its dipsogenic effect on a group of 12 rats following a procedure similar to the above mentioned in exp #1. Another group of 8 rats received an equimolar dose of Li_2CO_3. A third group of 8 rats received only vehicle (distiled water). In all cases the volume i.p. injected was 10 ml/kg bw divided in two equal boluses separated by 12 hours.

b) Doses from 1 to 8 mmol/kg bw/day of LiCl were tested for its dipsogenic effect. The dose-response relation was assessed on 4 different groups of 7-10 rats (Total n= 34). LiCl was diluted for injecting in distiled water and administered through two i.p. boluses separated by 12 hours (9 am and 9 pm). The total daily volume of LiCl solution injected ranged from 2.5 ml/kg bw for the lowest dose of Li_2CO_3 to 20 ml/kg bw for the highest.

Metabolic balance of rats during Li treatment

Eleven rats housed in individual metabolic were i.p. injected 1 mmol/kg bw/day of Li_2CO_3 during 4 days. Another group of 6 rats was submitted to the same protocol but only vehicle (distiled water, 10 ml/kg bw/day) was i.p. administered.

Effect of nephrectomy on LIT

This experiment was performed to assess the possible nephrogenic origin of LIT. For this, 46 animals were distributed in 5 groups:

a) Fourteen male rats of 200-285 g bw, receiving 1 mmol/kg bw of Li_2CO_3 following the usual procedure, were nephrectomized at the end of a 4 days treatment. Water intake was recorded during that time and for 24 hours after nephrectomy.

b) Fourteen males of 200-275 g bw, receiving 1 mmol/kg bw of Li_2CO_3 following the usual procedure were pseudonephrectomized at the end of a 4 days treatment. Water intakes were recorded as in the previous group.

c) Five males of 210-280 g bw, were treated as in group «a» but nephrectomy was performed on day 10th.

d) Five males of 230-290 g bw, were treated as group «b» but pseudnephrectomy was made on day 10.

e) Eight males of 235-290 g bw which did not receive treatment were bilaterally nephrectomized. Water ingestion was recorded as usual for one more day.

Gastric water load: Crossed administration

The experiment is designed to confirm that LIT is a secondary phenomenon to polyuria. Six male rats of 300 - 400 g bw were treated with Li_2CO_3 1 mmol/kg bw using the standard protocol. Another group of 6 animals of similar bw received the same treatment but administering distiled water instead of Li_2CO_3. On day 5th each group of animals received via gastric gavage the averaged amount of water drunk by the other group on day 4th. The animals were housed in metabolic cages without water available and urine volume excreted was recorded.

Kidney morphological study.

To investigate the possible lithium induced renal damage, kidneys from the animals of experiments #3 and 5 were microscopically studied as described above.

Results

Dipsogenic effect of lithium carbonate: Dose-response relation

The lower doses of Li_2CO_3 tested (0.01 and 0.1 mmol/kg bw/day) did not increase water intake in a period of four days when compared with the average of the last three days before treatment. The 1.0 mmol/kg bw/day of Li_2CO_3, had a steady enhancing effect on water intake whose differences in comparison with the basal intake became significant by the second ($t(7)= 3,56$; $p< 0.001$), third ($t(7)= 6,0$; $p<0.001$) and fourth days ($t(7)= 6,6$; $p<0.001$). On the contrary, 2.0 m mol/kg bw /day of Li_2CO_3 dose had a depressant effect on water intake during the first ($t(6)= 13,4$; $p<0.001$) and second days of treatment ($t(6)= 6,1$; $p<0.001$). Intake increases by the third and fourth days although it do not reaches statistical significance when compared with basal intake. The highest dose tested, 4.0 m mol/kg bw /day of Li_2CO_3, showed a marked deleterious effect on the animals, diminishing their water intake already during the first day of treatment. On the second day the animals were sacrificed due to their bad condition (Fig 1).

Plasma lithium concentration after treatment with 1 mmol/kg bw/day of Li_2CO_3 was 0.24±0.01 mEq/l.

Dipsogenic effect of lithium carbonate: lithium vs carbonate

The water intake during 4 days of treatment with 1 mmol/kg bw/day of Na_2CO_3 did not change when compared to averaged 3 days basal values (Fig 2). Neither changes were observed in water intake when rats received 1 mmol/kg bw of LiCl even after four days of treatment (basal intake 130.8±3.6 vs 135.9±6.3 on day 4th n= 8; N.S.); 2 mmol/kg (basal 134.0±7.0 vs 141.0±7.2, n= 7, N.S.); 4 mmol/kg (basal 193.2±3.8 vs 225.0±15.6, n= 9, N.S.). On the contrary, when rats received 8 mmol/kg of LiCl a significant dipsogenic effect was evident after 3 days (190.2±7.9 vs 272.7±22.3; n=10, $p<0.01$), and 4 days of treatment (440.4±26.7, n= 10, $p<0.001$).

Metabolic balance of rats during Li treatment

Results of this experiment are summarized on Table 1. Lithium treatment (1 mmol/kg) increased water intake of rats vs control on days 2 ($p<0.01$), 3 and 4 ($p<0.001$). This tre-

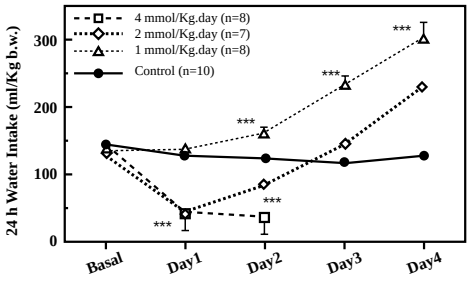

*Fig. 1. Dipsogenic effect of lithium carbonate: Dose-response relation. Basal intakes are the average of three previous days. (*** p<0.001 vs basal values).*

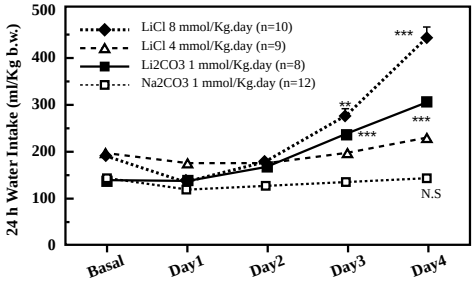

*Fig. 2. Water intake after different doses of Li_2CO_3, Na_2CO_3 and LiCl. (** p<0.01; *** p<0.001 vs basal values).*

Table 1
Metabolic Balance of rats during Li2CO3 (1 mMol/day. Kg) treatment

	Treatment	Basal #	Day 1	Day 2	Day 3	Day 4
Water Intake	Control	80.7±3.3	75.1±9.1	65.8±7.7	74.8±5.7	71.9±7.3
(ml/Kg. 24 h)	Li2CO3	95.4±6.9	96.5±7.3	113.2±10.4**	168.5±19.2***	228.7±22.9***
Diuresis	Control	30.4±3.9	34.2±4.4	34.1±2.6	38.2±3.4	36.1±1.1
(ml/Kg. 24 h)	Li2CO3	37.5±4.5	39.7±3.8	64.1±7.8	113.0±17.0***	164.7±18***
Food intake	Control	67.0±4.2	66.9±4.1	61.8±3.3	65.4±3.6	60.1±2.3
(g/Kg. 24 h)	Li2CO3	66.0±3.4	62.9±3.2	63.0±2.7	56.0±3.3	54.4±1.8

Water intake, diuresis and food intake of control and lithium carbonate treated rats. (** p<0.01 vs control and *** p<0.001 vs control).

atment also increased diuresis on days 2 (p<0.01), 3 and 4 (p<0.001) when compared with the control group. During the experimental period there were no differences on food intake between lithium treated and control groups. Both experimented a significant increase of body weight along the experiment.

Effect of nephrectomy on LIT

As shown in Fig. 3, nephrectomy, either at 4th or 10th day of treatment abolished LIT. Water ingestion of nephrectomized animals decreased immediately afterwards being even lower than in rats receiving no treatment.

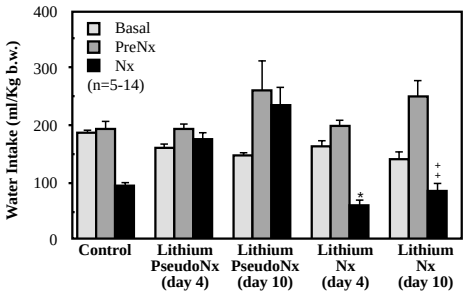

Fig. 3. Effect of nephrectomy on lithium induced water intake. For clarity significances of only most important results on water intake are represented. (p<0.05 vs control-Nx and vs lithium day 10-Nx; ¥ p< 0.05 vs lithium day 4-Nx and not significant vs control Nx).*

Gastric water load: Crossed administration

When ad lib water availability was abolished and water was administered only through gastric gavage, Li_2CO_3 (1 mmol/kg bw) treated animals excreted urine volumes greater than those water infused, (Urine: 122.0±30.2 vs water infused: 82.7 ml/kg bw). On the contrary, control rats excreted urine volumes adequate to the water amount infused (Urine: 234.9±37.9 vs water infused: 272.4 ml/kg bw) (Fig.4).

Kidneys morphological study

As can be seen on Fig 5 kidneys of Li_2CO_3 treated animals showed areas of

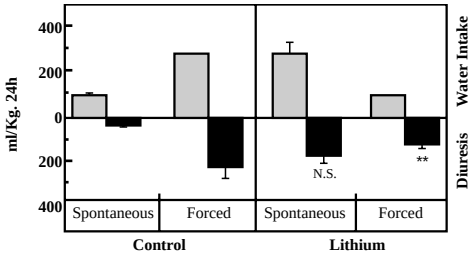

*Fig. 4. Effect on diuresis of a crossed water load. Lithium treated rats received a water load equal to the averaged amount drunk by control rats the prior day. Control rats received the same averaged volume of water drunk the prior day by lithium treated rats. (N.S. = Not significant vs diuresis of control forced group; ** p<0.01 vs of diuresis of control spontaneous group).*

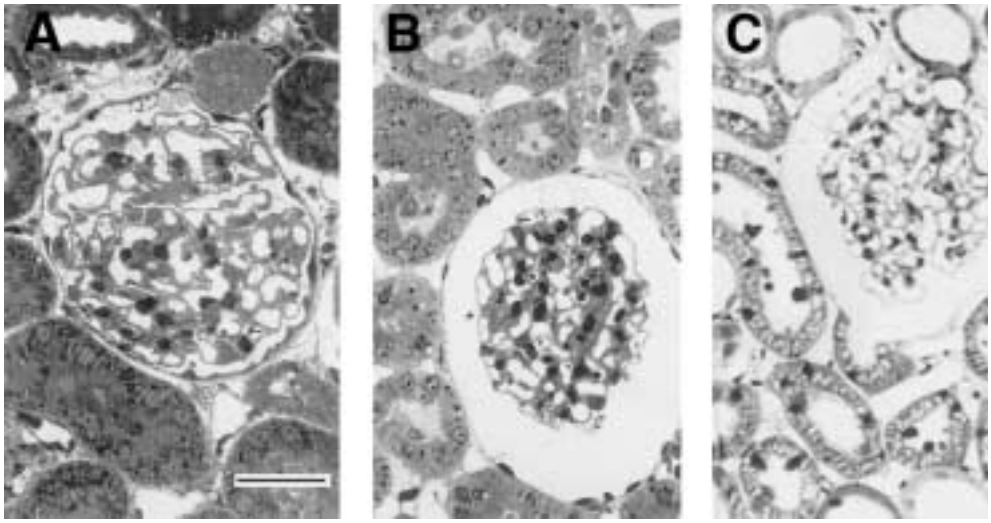

Fig.5. Standard-stained sections of kidneys showing glomerulus from Control (A), Li2CO3 treated (B) and forced hidrated rats (C). The horizontal bar represents 20 µm aprox.

normal structure mixed with areas in which glomerular filtration space is augmented. Electronic microscopy confirms that finding showing an increased size of the Bowman´s space. Loosed epithelial cells can be seen in the tubes lummen. On over-hydrated animals (experiment nº 5) very similar structural alterations were found in their kidneys.

Discussion

Polyuria - polydipsia syndrome is present in 60% of psychiatric patients treated with lithium and persistent in about 20-25% of them (Godin and Fillastre, 1989). In this cases acute lesions of distal and collector tubes have been reported as well as cronic lesions of the interstitium (fibrosis) and tubes (enlargement) but it is not proved that these are caused by lithium (Bendz, Aurell, Balldin, Mathé and Sjödin, 1994). According to Albrecht, Kampf and Müller-Oerlinghausen (1980) about 25% of the patients treated with lithium suffer a slow evolving nephropaty, while Coppen, Bishop, Bailey, Cattell and Price (1980) deny that lithium is the responsible of the renal disfunction. It has been reported renal disfunction consecutive to lithium treatment (Butch and Walling,1980) but also after neuroleptic treatment (Bendz et al, 1994). All this literature suggests that the role of kidneys in LIT phenomenon is not clear. Two non excluding hypothesis can be formulated to explain thirst induction by lithium therapy: LIT is a primary phenomenon or, alternatively, is secondary. Lithium could be the responsible of a primary thirst by different mechanisms (Penney and Hampton, 1990), among them, a renin increase and a greater generation of angiotensin II. There are also some evidences of the LIT dependence on the integrity of dopaminergic system and nigroestriatal pathway (Mailman, 1983 and Mailman, Krigman, Mueller, Mushak and Breese, 1978).

In our experiments the effect of lithium carbonate on water intake increase is in absolutely accordance with the extensive literature (Smith and Amdisen, 1983; Christensen, 1983; King, Aylard and Hullin, 1985; Conte et al 1982). The toxicity of the Li_2CO_3 has al-

so been demonstrated, although a middle range dose (1 mmol/kg/day) was well tolerated by rats. The bulk of experiments made in our study involve a dose of Li_2CO_3 of 1 mmol/kg /day which produced an averaged level of plasma lithium of 0.24 mmol/l well lower than necessary to induce in humans a therapeutic effect, normally in the range of 0.8-1.2 mmol/l (Silverstone and Romans, 1996; Flórez, 1997). Nevertheless, it should be considered the fact that whereas lithium carbonate is routinely administered p.o. for human treatment, in our experiments it was given i.p.

The other hypothesis is that LIT is secondary to polyuria. This seems to be demonstrated by our results since bilateral nephrectomy totally abolishes LIT. This results are confirmed when water is restricted on the animals treated with lithium who excrete urine volumes greater than the amount of water that they received via gastric gavage. This experiments suggest that LIT is secondary to polyuria at least in the beginning of the treatment.

Structural alterations of the kidneys are similar in Li_2CO_3 treated rats than in forced overhidrated rats. In the last group, alterations were even more intense than in lithium treated animals, perhaps because polyuria and polydipsia were more acutely established than in lithium treated rats. The quite often image of detached epithelial cells in the lumen of the tubes that could be seen in normal rats is considered as the expression of the turnover of the epithelium. This image was more frequent in lithium treated rats and also on overhydrated rats. As morphological alterations in the kidneys of control and lithium treated rats were very similar, it could not be inferred that kidneys alterations are directly responsible of LIT phenomenon. Nevertheless nephrectomy and cross water load experiments speak in favour of a principal role of the kidney in LIT and recent experiments (Hensen, Haenelt and Gross, 1996) demonstrated that biochemical renal mechanisms are also involved in LIT, because lithium induced a decrease in vasopresin receptor density.

Acknowledgements

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