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The distribution of lithium and its effects on the distribution and excretion of other ions in the rat

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Summary

1. In rats, lithium (ca 1 mEquiv/kg body weight) decreased brain sodium and magnesium, bone sodium and calcium and increased muscle calcium, plasma magnesium, urinary calcium and urine volume.
2. Lithium was particularly concentrated in bone.

Introduction

Lithium ions are apparently effective in the treatment of affective disorders (Baastrup, Poulsen, Schou, Thomsen & Amdisen, 1970; Coppen, Noguera, Bailey, Burns, Swani, Hare, Gardner & Maggs, 1971; Hanna, Jenner, Pearson, Sampson & Thompson, 1972) but the mode of action remains unknown (Pearson & Jenner, 1971).

Many studies have considered interactions between lithium and sodium or potassium ions (Coppen & Shaw, 1967; Hullin, Swinscoe, McDonald & Dransfield, 1968; Murphy, Goodwin & Bunney, 1969; Baer, Platman & Fieve, 1970). Birch (1970) however, emphasized the 'diagonal relationship' in the periodic table, between lithium and magnesium and calcium ions, reflected in terms of polarizing powers and ionic radii. This study is an initial attempt to explore the possible biological significance of such chemical similarities.

Since lithium is only clinically effective after several days of treatment, comparatively long-term studies have been performed in contrast to much of the earlier work.

Methods

Lithium citrate was administered to two groups of female Wistar rats, to one group by daily intraperitoneal injections of 2 ml of 0·1 mmol/ml aqueous solution and to the other by using drinking fluid with a concentration of 8 mmol/litre. Control groups received equimolar solutions of sodium by these routes. Two further control groups received water, one by daily injection (2 ml), the other as drinking fluid. Injected groups had free access to drinking water. All animals had free access to diet (Pilsbury diet 41B).

The six groups of seven rats each were selected by a weight ranking technique so that the group means varied from $144{\cdot}17 \pm 9{\cdot}45$ g to $145{\cdot}08 \pm 9{\cdot}83$ g. The standard deviation of the mean weight of all six groups fell between $\pm 9{\cdot}07$ g and 9·83 g.

The animals were in sound proof chambers which were temperature controlled and had a 12 h light, 12 h dark regime. 'Lights on' commenced at 09.30 in the

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first room and was staggered by 15 min for each of the two other rooms used. Each room was entered by the operator daily at the time of 'lights on' and at no other time.

The animals were allowed to acclimatize to the cages and the routine for a period of seven days (Grant, Hopkinson, Jennings & Jenner, 1971) after which they were given water alone by their allocated treatment route for a period of sixteen days. On day seventeen and for the following twenty-eight days treatment commenced, as outlined above.

At the end of the treatment period all animals were killed and tissue samples were taken of whole brain, left gastrocnemius muscle, left femur and plasma. In order to reduce the effect of the variability due to possible diurnal rhythms of tissue ion concentrations a killing order was designed so that the groups were successively sampled in order of their within-group weight rank.

Animals which had received lithium orally were allowed access to fluid until half an hour before killing. However, since rats are nocturnally active animals and the killing commenced at 08.00 hours and ended at 15.00 hours it is likely that most of the test dose received in the 24 h prior to killing would have been taken during the early 'lights out' period. Since the injected groups received their daily dose of the test substance at the beginning of the 24 h period in one dose it follows that these animals were sampled one from each group in turn during a period from 22·5 h to 29 h after the last dose. The animals were killed by exsanguination under nitrous oxide anaesthesia. The total time taken for anaesthesia and dissection of each animal was less than 9 minutes.

Blood was collected from the cut jugular vein via a nylon funnel into a polypropylene centrifuge tube containing strontium heparin (Evans). The plasma was immediately separated.

The whole of the above experiment was repeated using equimolar solutions of the chloride salts of lithium and sodium. The starting weights in this case were from $150{\cdot}14 \pm 8{\cdot}24$ g to $151{\cdot}07 \pm 9{\cdot}74$ g. The standard deviation range was from $\pm 8{\cdot}19$ g to $\pm 9{\cdot}74$ g.

Since one animal was removed from the citrate administration experiment because it had severe respiratory infection, 83 animals were sampled.

The tissue samples, but not the plasma samples, were digested by a modification of the technique described by Chang, Gover & Harrison (1966). After dissection the tissues were placed in preweighed Pyrex boiling tubes and reweighed. The samples were then dried at 105° C for 18 h, and the dry weight was recorded.

Eight ml of Analar nitric acid was added to each sample and the sample was left overnight in a dust-free area to allow readily oxidized portions to be destroyed. This reduced the initial frothing with heat which had previously been a problem. The sample was heated gently until all frothing subsided, then the mixture was boiled until a clear yellow solution resulted. Further nitric acid was added, if required, to ensure at least a four times excess before 1–2 ml of perchloric acid (Analar 70%) was added. The solution was boiled vigorously until dense white fumes of perchloric acid were evolved.

The solution obtained was diluted to a volume of 5 ml with distilled water and allowed to stand overnight to allow hydrolysis of perchlorate salts to occur. The solution was made up to 10 ml. The sample obtained was clear and colourless.

Analysis was carried out using a Hilger and Watts 'Atomspek' atom absorption spectrometer. The following elements were determined by atomic absorption techniques: lithium, sodium, potassium, magnesium, and calcium. All samples were diluted as required with a solution of 15 mM lanthanum nitrate and 4 mM caesium chloride made up in 0·1 mM hydrochloric acid (Sanui & Pace, 1966).

Plasma samples were estimated with no pretreatment but with a diluent supplemented with 6% n butanol and 0·02% n octanol, the former to reduce the viscosity and the latter to reduce foaming (Zettner & Seligson, 1964). These solutions were designed to reduce, as far as possible, interference from other cations.

On four rats maintained in metabolic cages, balance studies were performed. During the 16 day control period all rats received 2 ml of water i.p. daily. They then received 2 ml of a 100 mM solution of either lithium citrate, sodium citrate, lithium chloride, or sodium chloride, intraperitoneally daily for 28 days respectively. Free access was given to both food and water and the daily intake of each was recorded. Urine and faeces were collected daily.

The following estimations were carried out on each urine specimen: Li, Na, K, Mg, Ca, phosphate, chloride, creatinine and urea. All cations were estimated by atomic absorption spectroscopy after dilution with the lanthanum-caesium diluent previously described. Anions and metabolites were estimated on a Technicon 'Auto Analyser' by the standard methods described by the manufacturer.

Faeces specimens were digested using a modification of the procedure described for the tissues. The specimen was transferred to a 250 ml Erlenmeyer flask and 20 ml nitric acid was added. After standing overnight to allow frothing to take place, the sample was gently heated until frothing ceased. Heating was then increased until a moderately clear yellow solution was obtained. Further additions of nitric acid were sometimes required. After ensuring a fourfold excess of nitric acid, approximately 8 ml of perchloric acid (70%) was added and the solution was very gently heated to dryness. After cooling 25 ml of 0·1 mM hydrochloric acid was added and the mixture was allowed to stand for several hours with occasional shaking. The supernatant was decanted off.

After dilution with the lanthanum-caesium diluent, the five cations were determined by atomic absorption methods.

Statistical analysis

(a) Tissue distribution study

The changes in tissue electrolytes following lithium administration are not gross and interpretation is complicated by biological variations as well as by different intakes of lithium in individuals receiving the drug by the oral route. These groups drank more fluid and hence more lithium than had been predicted (Table 3). Furthermore, since the lithium intake was spread throughout the day, the interval between killing and the last drink varied. Hence it was decided to look at the correlation coefficients (Draper & Smith, 1966) between the tissue concentrations of lithium and the other metals in all animals irrespective of treatment.

Since sodium was administered to four of the twelve groups under analysis it was necessary to exclude effects of this treatment on the overall coefficients. Correlation coefficients were therefore determined between tissue sodium and other metals;

some of these correlations were significant. The significant correlations either indicate a response to administered sodium or a tendency for the particular ion's concentration to vary physiologically with that of sodium. In order to exclude the former, Analysis of Variance with Estimation of Individual Effects (Scheffé, 1959) was carried out. When tested against treatment by this method, rather than against tissue concentration by correlation coefficients, only the lithium treatment effect was confirmed and was of a similar order of significance to that indicated by the correlation coefficients between lithium concentration and tissue concentration. Sodium and some other ions varied together for physiological reasons not dependent on sodium administration.

Correlation matrices were set up for each tissue, the tissue concentrations of the ions and the percentage water content for each sample being entered irrespective of treatment. A maximum sample population of 83 was therefore obtained. In the case of bone, the sample size was 78 since part of one batch of digests was lost. Not more than two samples are missing from any one treatment group.

(b) Metabolic cage experiments

Correlation coefficients were also applied to these data. This time, however, the matrices were set up individually for each of the four animals and 24 h output of ions in both urine and faeces were entered together with food intake, faeces weight, water intake and urine output day by day for each animal. In addition derived data for lithium intake, sodium intake, total excretion of each ion, total balance of each ion and water balance were entered. The matrices represented 44 days (16 days control and 28 days treatment) and 576 correlation coefficients were obtained for each animal over this period.

The coefficients of greatest relevance to the present discussion were those between lithium and sodium intakes and other parameters and these are presented in the results section.

It should be noted that the above methods involved obtaining correlation coefficients between lithium injections which were only either 0 or 0·2 mEquiv/day and other parameters. However, this situation is one for which correlation coefficient methods are most powerful provided there is an approximately linear relationship between the two parameters (Kendal & Stuart. 1963).

Results

(a) Tissue studies

The overall effects of lithium on the tissue concentrations of cations are set out in Table 1. This table represents a simplification of the detailed results available and it is included to indicate the normal tissue concentrations obtained in control animals and the order of magnitude of the changes demonstrated. Results from lithium treated groups in the table are means from all animals given lithium regardless of the mode of administration or the lithium salt used. Similarly, control values in the table consist of both sodium and water treated animals regardless of actual type of treatment. Because of the variability of the data, this type of presentation masks the significant changes of tissue concentrations which are occurring.

Table 2 gives *P* values derived from parts of the correlation coefficients matrices obtained for the tissue concentrations. Correlation coefficients were obtained

between tissue *concentrations* of the elements and tissue lithium and sodium *concentration*. The Analysis of Variance with Estimation of Individual Effects (Scheffé, 1959) tested the effects of *treatment* with either lithium or sodium and the tissue *concentrations*. The tests indicate similar statistical probabilities for the changes due to lithium treatment and by comparison with lithium concentration. In contrast the correlation coefficients indicate several correlations with tissue sodium concentrations, none of which were confirmed by the analysis of variance. This in-

TABLE 1. *Concentrations of ions in tissues of lithium treated and control rats (mEq/kg wet weight of tissue)*

		Lithium treated		Control	
		Mean	±S.D.	Mean	±S.D.
Brain	Na	45·85	1·76	47·53	1·77
	K	60·16	11·15	59·08	7·37
	Mg	12·69	0·35	12·95	0·45
	Ca	3·29	1·01	3·58	1·24
	% H_2O	77·73	0·25	77·69	0·41
n=		28		55	
Muscle	Na	23·77	1·34	23·39	1·37
	K	69·98	10·07	69·67	10·64
	Mg	23·28	0·69	23·22	0·60
	Ca	1·09	0·13	0·99	0·16
	% H_2O	75·24	0·53	75·36	0·43
n=		28		55	
Bone	Na	180·00	10·37	185·00	8·71
	K	41·24	4·09	42·43	7·59
	Mg	239·60	13·86	243·30	18·92
	Ca	8,535·00	163·60	8891·00	495·30
	% H_2O	32·00	1·68	31·96	1·58
n=		26		52	
Plasma	Mg	1·60	0·19	1·39	0·27
	Ca	4·68	0·48	4·60	0·57
n=		28		55	

The table shows average values for tissue concentrations of ions of animals treated by lithium salts by injection or in drinking fluid, and average values from control animals including those receiving sodium salts.

TABLE 2. *Maximum values of* P *for correlation coefficients between either lithium or sodium concentration and other ion concentrations and for analysis of variance between either lithium or sodium treatment and tissue concentration of other ions*

	$P<$	Correlation coefficients		Analysis of variance	
	Ionic	Lithium	Na	Lithium	Na
Tissue	concentration	concentration	concentration	treatment	treatment
Brain	Na	0·001(−)		0·0025(−)	*
n=83	K	*	*	*	*
	Mg	0·001(−)	0·001	0·01(−)	*
	Ca	*	0·01	*	*
Muscle	Na	*		*	*
n=83	K	*	0·05	*	*
	Mg	*	*	*	*
	Ca	0·01	*	0·005	*
Bone	Na	0·05(−)		0·05(−)	*
n=78	K	*	*	*	*
	Mg	*	*	*	*
	Ca	0·01(−)	0·001	0·005(−)	*

* Indicates $P>0·05$. Negative correlations and changes are indicated by (−).

dicates that though tissue sodium concentration was correlated in some instances with the concentration of other metals, this was not a result of administered sodium, but rather of the natural metabolic interrelationships of the metals in the tissues.

From Table 2 it may be seen that both brain sodium and magnesium were decreased following lithium treatment. Lithium also increased muscle calcium, decreased bone calcium and probably decreased bone sodium. A highly significant correlation between Li treatment and plasma Mg was also noted ($P<0{\cdot}01$). In bone, tissue water was negatively correlated with sodium, magnesium and calcium ($P<0{\cdot}001$ in all cases).

The difference in effects produced by the two modes of administration of lithium are seen in Table 3. The plasma lithium is considerably lower in the injected groups and probably reflects the longer period between administration and killing as well as the lower dose.

TABLE 3. *Mean lithium concentrations of tissues within treatment groups*

Mode of administration	Salt	Mean Li dose mEq/animal	mEq/l. Plasma Li	mEq/kg wet weight Brain Li	Muscle Li	Bone Li
Drinking fluid *ad lib*	Li citrate	0·278	0·182	0·191	0·322	1·30
	$n=7$	(0·049)	(0·054)	(0·036)	(0·144)	(0·105)
	Li chloride	0·250	0·136	0·175	0·220	1·15
	$n=7$	(0·048)	(0·027)	(0·033)	(0·039)	(0·145)
	Control	0·000	0·000	0·008	0·018	0·158
	$n=28$			(0·006)	(0·009)	(0·031)
Daily injection i.p.	Li citrate	0·200	0·023	0·162	0·104	0·912
	$n=7$		(0·004)	(0·035)	(0·021)	(0·071)
	Li chloride	0·200	0·020	0·107	0·058	0·829
	$n=7$		(0·016)	(0·034)	(0·034)	(0·131)
	Control	0·000	0·000	0·008	0·027	0·157
	$n=27$			(0·008)	(0·009)	(0·044)

Standard deviations are given in parentheses.

TABLE 4. *Significance of correlation coefficients between daily intake by injection of lithium or sodium and daily measures of other variables*

	Lithium treated		Na treated	
	Citrate	Chloride	Citrate	Chloride
Variable	$P<$	$P<$	$P<$	$P<$
Urine vol.	0·001	0·001	*	*
Water intake	0·001	0·05	*	*
Food intake	0·001(−)	*	*	*
Faeces wt.	0·001(−)	*	*	*
Urine Li	0·001	0·001		
Urine Na	0·001(−)	0·01(−)	0·05	*
Urine K	0·001(−)	0·05(−)	*	*
Urine Mg	0·05	0·05(−)	*	*
Urine Ca	0·001	0·001	0·01(−)	*
Faeces Li	0·001	0·001		
Faeces Na	0·01(−)	*	*	*
Faeces K	*	*	*	0·05(−)
Faeces Mg	*	0·05(−)	*	0·01(−)
Faeces Ca	*	*	*	0·05(−)

* Indicates $P>0{\cdot}05$. (−) Indicates negative correlation.

(b) Metabolic balance studies

In Table 4 some of the correlations found between administration of either lithium or sodium and other parameters in the balance studies are presented. Sodium did not clearly correlate with the various factors studied whereas lithium was consistently correlated with a number of parameters.

The frequently reported polyuria and polydipsia following lithium is clearly shown by the appropriate positive correlations in the lithium treated animals. Major changes were found in urine electrolyte excretions. Thus, excretion of calcium was markedly enhanced whereas that of sodium and potassium was reduced.

No significant finding emerged from faecal electrolyte estimation, total balance of electrolytes nor phosphate, chloride, creatinine and urea in urine.

Discussion

The results show that in rats lithium ions interfere with the distribution and handling of magnesium, calcium, sodium and potassium.

Hullin *et al.* (1968) have noted ' episodes of lithium release ' occurring over a considerable period of time in urine of a patient whose lithium medication had been discontinued. The present results indicate that much of this lithium may have originated in bone which has a much higher retention of lithium than the other tissues studied.

The differences in tissue concentration of lithium of rats 'injected ' or given lithium in the ' drinking fluid ' is in agreement with the data of Schou (1958) on the ability of brain and muscle to take up and release lithium. Schou indicated that lithium passed fairly quickly into muscle but very slowly into brain and left the tissues at equally different rates. In the present work, the injected groups of animals had a greater time between last lithium and sampling than did animals drinking fluid containing lithium. The greater the difference between the two groups therefore for any tissue the greater the rate at which lithium leaves that tissue. Muscle lithium in the ' injected ' groups was between one-third and one-quarter of muscle lithium from ' drinking fluid ' groups. Despite the difference in dose received, the respective differences in brain tissue are much smaller. Movement into and out of brain is therefore slower.

The very high concentration of lithium in bone and the relatively small difference between the ' injected ' and ' drinking fluid ' groups indicate a relatively fixed state of lithium in bone. This could be explained by incorporation of the element into bone structure. It can be seen from Table 3 that the bone lithium concentration is approximately proportional to the mean lithium dose.

The magnitude of the changes in bone calcium following lithium treatment does not suggest a 1 : 1 exchange of calcium and lithium. The changes may therefore be due to metabolic effects of lithium on calcium homeostasis.

Recently other workers have shown effects of lithium on calcium metabolism. Gotfredsen & Rafaelsen (1970) have shown that both calcium and magnesium excretion are increased in rats during 25 days of lithium treatment though one must interpret the calcium finding with some caution since the control group considerably decreased its output of calcium over the same period, a result which we find rather unusual and contrary to that of the present experiment and of preliminary

adaptation experiments (Birch, 1971). In untreated female, growing rats we found a gradual increase in excretion of both calcium and magnesium with time roughly commensurate with body size up to the age of about 70 days (200 g body weight).

Mellerup, Plenge, Ziegler & Rafaelsen (1970) have shown an increase in plasma calcium a short time after administration of lithium and a decrease in calcium uptake into bone for up to 2 h following the dose.

Creek, Lund, Thomas & Pollard (1971) have shown that addition of lithium carbonate to the diet of laying hens causes increase in the proportion of soft shelled eggs proportional to the dose of lithium administered. Even at the lowest dose (282 mg/kg diet, intake of diet unspecified) all eggs layed after two days were soft shelled. In addition it was found that plasma calcium decreased roughly in proportion to the dietary dose.

The decrease in magnesium concentration of brain demonstrated in the present study differs from changes described by King, Carl, Archer & Castellanet (1969). These workers found an increase in brain magnesium following three days treatment with lithium to mice. It might be that a time dependent factor is involved in the effect of lithium on magnesium metabolism as a whole since not only the brain concentration but also plasma concentration following lithium has been the subject of contradictory reports (Nielsen, 1964a, b ; Bunney, Goodwin, Davis & Fawcett, 1968 ; Frizel, Coppen & Marks, 1969 ; Aronoff, Evens & Durell, 1971). Much of the difference might be explained if plasma magnesium were initially decreased following lithium therapy but later became elevated (Nielsen, 1964b). Plasma magnesium was highly significantly ($P<0{\cdot}001$) correlated with plasma lithium in the present study.

The decrease in brain sodium following lithium confirms the earlier studies of Davenport (1950) and Baer, Kassir & Fieve (1970). However, the results of the metabolic study in rats in which lithium treatment was negatively correlated with urinary sodium and potassium conflict with those of Baer *et al.* (1970). It is possible, however, that the results presented are due to the reduced intake of those elements following lithium administration. Baer and his co-workers were at pains to ensure that sodium intake was adequate and therefore supplemented the diet.

The literature regarding the effects of lithium on sodium and potassium has been frequently reviewed (Baer *et al.*, 1970 ; Schou, 1968 ; Aronoff *et al.*, 1971), and is not exhaustively discussed here particularly as so much less effort has been devoted to the possible significance of changes of the divalent ions produced by lithium. Further the present findings support the view that lithium might achieve its pharmacological effect by means of an action on calcium and magnesium homeostasis.

The relevance of these studies to the specific inhibition of vasopressin by lithium ions (Harris & Jenner, 1972), for which calcium and magnesium levels are critical or similarly its inhibition of net tubular reabsorption of α-oxoglutarate (Bond, Jenner, Lee, Lenton, Pollitt & Sampson, 1972) which is also likely to depend on these ions or its many other actions, remains to be explored.

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