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The effect of lithium salts on the urinary excretion of α-oxoglutarate in man

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Summary

1. Lithium ions in therapeutic doses cause an increase in the renal excretion of α-oxoglutarate and glutaric acid.
2. The excretion is probably due to reduced renal tubular reabsorption.
3. Neither citrate, lactate nor pyruvate excretion rises.

Introduction

The therapeutic efficacy of lithium ions in some manic depressive psychoses seems well established (Baastrup, Poulsen, Schou, Thomsen & Amdisen, 1970; Coppen, Noguera, Bailey, Burns, Swani, Hare, Gardner & Maggs, 1971; Hanna, Jenner, Pearson, Sampson & Thompson, 1972). The mode of action is, however, still obscure. Hence there is increasing interest in the pharmacology of lithium. A number of actions have been described and reviewed for example by Pearson & Jenner (1971). This paper adds one further observation; the increased renal excretion of α-oxoglutarate and glutarate.

Methods

The subjects were manic depressive patients (indicated by letters A, B, etc.) or controls (indicated by numbers 1, 2, 3, etc.). The diets were kept constant with a daily repeating menu, but were different for each individual. Urines were collected directly into the deep freeze, lithium itself does not affect the rate of loss of α-oxoglutarate but a complex combination of pH, temperature and other factors do.

Creatinine and urea were measured in a Technicon auto analyser by the recommended methods; lithium was estimated by atomic absorption spectroscopy. Urinary titratable acidity was estimated with an automatic titrator (Radiometer, Copenhagen). α-Oxoglutarate and pyruvate were estimated by a modification of the method of Bergmeyer & Bernt (1963). Urine (0·05 ml) was put into a 2 cm spectrophotometer microcell with 0·71 ml phosphate buffer (0·1 M pH 7·6) and 0·02 ml reduced nicotine adenine dinucleotide (NADH) solution (5 mg per ml). The optical density was measured at 340 mμ then 0·02 ml of glutamate dehydrogenase (beef liver, ammonium sulphate suspension diluted to 2 mg protein per ml, Sigma Ltd.) was added for the α-oxoglutarate determination and 0·02 ml of lactic dehydrogenase (rabbit muscle, ammonium sulphate suspension undiluted, Sigma Ltd.) for the determination of pyruvate. The optical density was measured after 4 min or when no further change occurred. The concentration of α-oxoglutarate and

pyruvate in urine was proportional to the optical density change and was determined from internal standards added to the urine. The optical density change with aqueous standards was higher than that of the same standards added to urine. Internal standards were added to each series of urines from which results are published to obtain more accurate absolute values. Blood and plasma α-oxoglutarate was determined by the method of Bergmeyer & Bernt (1963).

Citrate was estimated by the method of Beutler & Yek (1959). Lactate was estimated with lactate dehydrogenase. Urine (0·02 ml), was put into a 2 cm microcell together with 0·75 ml of glycine buffer (pH 9·2 containing hydrazine, Sigma Ltd.), and 0·02 ml NAD solution (10 mg/ml) and the optical density was read at 340 mμ ; 0·02 ml of lactate dehydrogenase was added and the optical density was read again after a further 30 min or when the reaction was complete. Concentrations were estimated from standards added to urine. The original observation, which suggested a correlation between lithium intake and urinary excretion of α-oxoglutarate, was made while using a gas chromatographic method for phenolic acid estimation (Sprinkle, Porter, Greer & Williams, 1969).

A more complete survey of urinary acids was then carried out by methods derived from those described by Dalgliesh, Horning, Horning, Knox & Yarger (1966), and Zaura & Metcoff (1969) with OV-17 or OV-101 columns, temperature programmed from 70–250° at 2°/min.

α-Oxoglutarate and glutaric acid were identified by means of a Perkin Elmer model 270 gas chromatograph-mass spectrometer.

Results

When the method of Sprinkle *et al.* (1969) was used for the estimation of phenolic acids in urine, an unidentified peak on the gas chromatograms was found to increase during treatment of manic depressive patients with lithium carbonate. The peak was identified as a tris-trimethylsilyl-derivative of α-oxoglutarate by mass spectrometry. The parent ion had m/e 362, with prominent fragments at m/e 347, 318, 291, 173, 157, 147, 93 and 73. An identical spectrum is obtained with authentic α-oxoglutarate, though multiple derivative formation was observed (Dalgliesh *et al.*, 1966). The complete acid profile technique used more forcing conditions for trimethylsilylation and α-oxoglutarate appeared to be destroyed. The presence of increased amounts of α-oxoglutarate in urine after lithium treatment was confirmed with the methoxime-trimethylsilyl derivative. This gave a parent ion at m/e 319 and prominent fragments at m/e 304, 287, 229, 202, 198, 186, 156, 147, 89 and 73. The corresponding derivative of authentic α-oxoglutarate gave an identical spectrum.

The urinary acid chromatograms showed approximately 80 peaks, most of which were reasonably constant from day to day on closely controlled diets, though some fluctuated in an apparently random manner. A further smaller peak was found to increase on lithium treatment in two subjects studied in this way. This peak was present in the same position in both trimethylsilyl and trimethylsilyl-methoxime profiles and was identified as bis-trimethylsilyl glutarate. The mass spectrum had parent ion m/e 276 (very small) and prominent fragments at m/e 261, 204, 158, 147, 129, 97 and 73, and was identical with that of the authentic compound.

When α-oxoglutarate had been identified, enzymatic estimations were employed and the increased urinary excretion of α-oxoglutarate on treatment with lithium was confirmed in nine individuals.

Figure 1 shows the urinary content of α-oxoglutarate per 24 h from five control subjects before, during and after receiving lithium. The clear increase in α-oxoglutarate excretion produced by lithium is obvious in each case. One subject (No. 1), who received the chloride, gave a similar response to those receiving the carbonate. The increased excretion continues as long as lithium is administered and there appears to be an increase over months or years. Subject B in Fig. 3 had received lithium for three years, and has a high rate of excretion. Subject A had received lithium for 3 months and also has a high rate of excretion. There were, however, no clear cut differences between patients and controls studied in comparable situations. Nevertheless in subject B in Fig. 3 a very low value is found when lithium carbonate was replaced by sodium bicarbonate. This we consider to be a rebound phenomenon.

In four of the five control subjects, the increase of α-oxoglutarate occurred rapidly. In three of these four subjects, where measurements were made at shorter time intervals, α-oxoglutarate excretion rose sharply after about three hours. The fifth subject, however, showed a delay of at least eight hours before the rise in output, and the increase observed, though obvious, was not as marked as in the other four. Those individuals who had the same dose on two or more successive days showed an increased output on subsequent days. Figure 2 shows the 24 h urinary output of α-oxoglutarate, lithium and titratable acidity in one of the subjects (No. 5), and is quite typical of results from the other persons studied. The pattern of α-oxoglutarate excretion closely follows urinary lithium. The titratable acidity, which showed a drop immediately following lithium treatment, returned to normal over the next two days of lithium treatment. This is followed by a compensatory rise on discontinuing the lithium. Similar changes in titratable acidity also occurred when lithium chloride was administered.

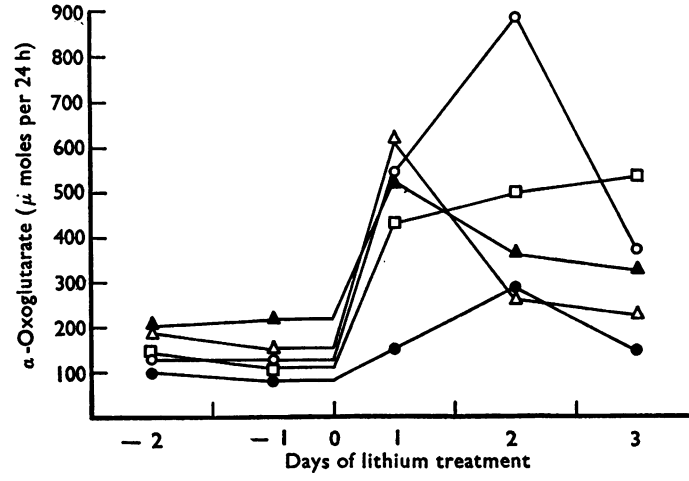

FIG. 1. Excretion of α-oxoglutarate in 5 subjects given lithium. ▲, (Subject 1) lithium chloride, as a single dose 1·15 g. ○, (Subject 2) lithium carbonate, 1 g, at time 0 and two doses of 0·5 g on day 2. ●, (Subject 3) lithium carbonate 1 g at time 0, and two doses of 0·5 g on day 2. △, (Subject 4) lithium carbonate 1 g at time 0 and two doses of 0·25 g on day 2. □, (Subject 5) lithium carbonate, 1 g, at time 0 and on days 2, 3 and 4.

Blood and plasma levels of α-oxoglutarate were studied before and during lithium administration. There was no discernible change.

α-Oxoglutarate clearance (Smith, 1956) was always below the creatinine clearance both before and during lithium studies. Hence, in these studies net tubular reabsorption of α-oxoglutarate is apparently reversibly inhibited by lithium.

An increased urinary excretion of the tricarboxylic acid cycle intermediates can occur in states of alkalosis (Ostberg, 1931 ; Crawford, Milne & Scribner, 1959), therefore the comparative contribution of the effect of the carbonate and lithium ion was studied. Figure 3 shows two studies on controlled diets, one patient received lithium carbonate followed by equivalent amounts of lithium as lithium chloride, another received lithium carbonate followed by equivalent amounts of carbonate ions as sodium bicarbonate. From the figure, it is clear that the carbonate ion does not significantly contribute to the α-oxoglutarate response.

In alkalotic states, citrate in particular is excreted in increased amounts as well as and in excess of the increase in α-oxoglutarate excretion (Evans, MacIntyre,

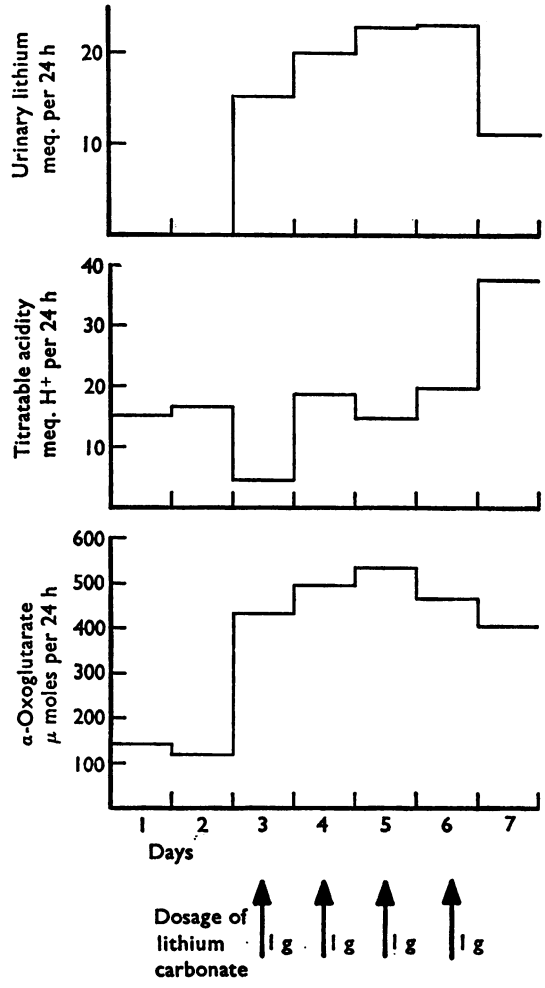

FIG. 2. Urinary excretion of α-oxoglutarate, lithium and titratable acidity in subject No. 5 given 1 g of lithium carbonate daily.

Macpherson & Milne, 1956). Studies of citrate, lactate and pyruvate showed no evidence that any increase occurred with the rise in α-oxoglutarate in any subject receiving lithium.

Two controls took 0, 2, 4, 8 and 16 g of sodium bicarbonate on successive days while receiving a controlled food and fluid intake. Figure 4 shows their excretion of citrate, α-oxoglutarate and titratable acidity. It can be seen that despite a much greater drop in titratable acidity than is seen in Fig. 2 which is typical for a person receiving 1 g/24 h of lithium carbonate (i.e. <10 mequiv. hydrogen ions/24 h) α-oxoglutarate excretion does not rise significantly, although there is some evidence of a rise in citrate excretion. Evans *et al.* (1956) showed larger changes of citrate excretion but with larger doses of bicarbonate. Further the drop in titratable acidity in urine from subjects receiving one gramme of lithium carbonate or chloride per day occurs only on the first day. Hence the increased α-oxoglutarate excretion due to lithium is not a simple analogue of that due to alkalosis.

Anumonye, Reading, Knight & Ashcroft (1968) found a marked uricosuric effect of lithium and, because of this, suggested possible effects on the transport of organic acids. During the periods studied in these experiments, there was no correlation between uric acid excretion and α-oxoglutarate excretion, and no clear increase of uric acid excretion. However, Anumonye *et al.* (1968) observed the changes over longer periods of time and hence their findings are not necessarily in conflict with our results.

Discussion

The results presented demonstrate clearly that intake of lithium ions leads to an increased rate of excretion of α-oxoglutarate. This seems most likely to be due to a reversible inhibition of a renal tubular transport process. The fact that

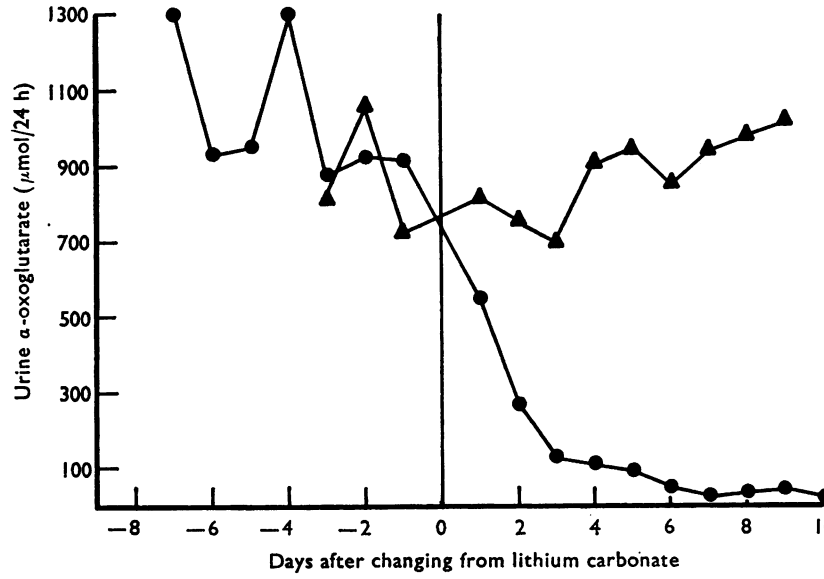

FIG. 3. Urinary excretion of α-oxoglutarate in two patients; A, changing from lithium carbonate to lithium chloride, and B, from lithium carbonate to sodium bicarbonate. A=▲, B=●.

glutaric acid excretion also rises seems consistent with this view as it may well share the same transport mechanism, but it is probably remote as an intermediary metabolite from α-oxoglutarate.

Net tubular transport of α-oxoglutarate is not a simple transport phenomenon as the kidney can avidly metabolize injected α-oxoglutarate and can produce it. Tubular reabsorption is active, i.e. against the electrochemical gradient at least in the dog (Cohen & Wittman, 1963 ; Balagura & Pitts, 1964), and this can occur actively from blood or tubular fluid. Net tubular secretion, which can also occur, is, however, passive and Pitts (1968) favours the view that there are two pumps, one on the luminal membrane, the other on the peritubular membrane ; both pump α-oxoglutarate actively into the cells but it can diffuse back through either membrane. Hence there are a number of possible sites for the action of lithium.

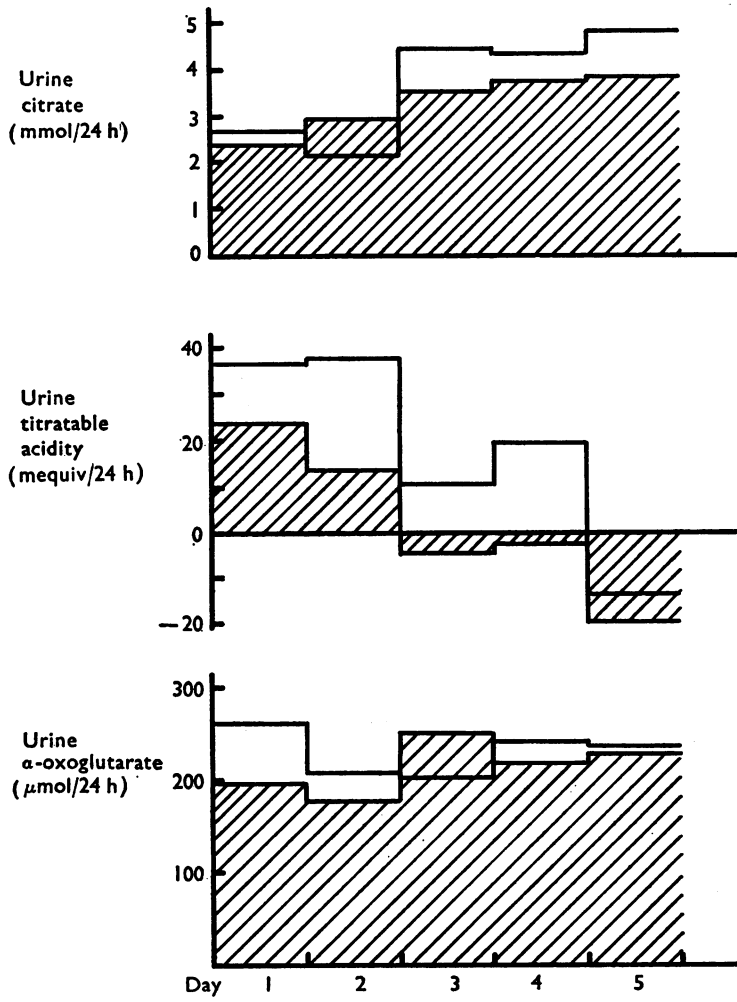

FIG. 4. Excretion in urine of citrate, α-oxoglutarate ,and titratable acidity by control subjects 1 and 6 who each ingested on consecutive days, 0, 2, 4, 8 and 16 g of sodium bicarbonate, in addition to a controlled and constant food and fluid intake. The considerable fall in titratable acidity far in excess of that in Fig. 2 due to lithium carbonate is not associated with a rise of α-oxoglutarate, but there is some evidence of a slight tendency for citrate excretion to increase (subject No. 1 open histograms, No. 6 cross hatched).

It is natural to try to think of analogous processes in brain. However, although brain actively metabolizes α-oxoglutarate (Krebs & Cohen, 1939), uptake from blood is distinctly less into brain than into liver and kidney (Selleck & Cohen, 1965). Compartmentalization of citric acid cycle metabolism in brain is affected by lithium (Berl & Clarke, 1972), perhaps a transport process similar to that occurring in the kidney is relevant.

Selleck & Cohen (1965) give evidence that α-oxoglutarate and the *p*-aminohippuric acid transport share one pathway, and Balagura-Baruch & Stone (1969) suggest there is a metabolic influence of Krebs cycle intermediates on *p*-aminohippurate transport, as they cause inhibition at all levels of *p*-aminohippuric acid tubular loads, above and below the tubular maximum. However, Selleck & Cohen (1965) feel that citrate also shares this pathway. So far, we are unaware of studies of lithium on *p*-aminohippuric acid transport though they would perhaps help to clarify some of these issues. The results do, however, suggest that lithium might help in separating out the mechanisms affecting citrate from those affecting α-oxoglutarate excretion.

The effect of lithium injection on α-oxoglutarate excretion may also be relevant to other renal actions of lithium, for example the inhibition of the action of vasopressin (Harris & Jenner, 1972).

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