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Rat brain and serum lithium concentrations after acute injections of lithium carbonate and orotate

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Eight hours after intraperitoneal injections of 1·0, 2·0, and 4·0 m equiv Li kg^{-1}, the serum and brain lithium concentrations of rats were significantly greater after lithium orotate than after lithium carbonate. While little serum lithium remained at 24 h after injection of 2·0 m equiv kg^{-1} lithium carbonate, two-thirds of the 2 h serum lithium concentration was present 24 h after lithium orotate. Furthermore, the 24 h brain concentration of lithium after lithium orotate was approximately three times greater than that after lithium carbonate. These data suggest the possibility that lower doses of lithium orotate than lithium carbonate may achieve therapeutic brain lithium concentrations and relatively stable serum concentrations.

Lithium is now perhaps the most widely used drug in the treatment of mania and in the prophylaxis against the recurrence of depression in manic-depressive illness (Gershon & Shopsin, 1973).

Its toxicity has been observed at values very close to, and even overlapping with its therapeutic serum concentrations (Shopsin, Johnson & Gershon, 1970; Vacaflor, 1975), possibly because toxic or near toxic serum concentrations are reached shortly after each dose of lithium carbonate and then these rapidly decrease to therapeutic values. It is therefore necessary to monitor serum lithium or perhaps, intra-erythrocyte lithium during treatment (Frazer, Mendels & others, 1973; Mendels & Frazer, 1973).

There would therefore be advantages in a preparation of a lithium salt that maintains a relatively constant serum lithium concentration with as few doses as possible. Lithium orotate has been reported by Nieper (1973) to be effective at doses of 0·9 mmol lithium ion four to six times per week in cases of constitutional migraine, hemicrania, constant headache, and other disorders. In contrast to this, the correct dose of lithium carbonate for lithium prophylaxis has been recommended to be about 20 to 30 mmol lithium ion daily (Kerry, 1975).

The intraperitoneal injection of lithium in rats appears to be a good pharmacokinetic model for oral ingestion of lithium in man (Olesen, Schou & Thomsen, 1976). We have therefore examined the response in rats to lithium orotate or lithium carbonate (i.p.) for dose response and time course of lithium concentrations in serum and brain.

MATERIALS AND METHODS

Injections were prepared as follows: lithium carbonate (BDH Chemicals Ltd.) was dissolved in distilled water, the pH adjusted to 7·4 with HCl and the final concentration was 200 m equiv Li^+ $litre^{-1}$. Lithium orotate was prepared by dissolving 20 m equiv $LiOH \cdot H_2O$ (BDH Chemicals Ltd.) in distilled water and adding a stoichiometric amount of orotic acid (Sigma Chemical Co.). This mixture was heated with stirring until the orotic acid dissolved. From this upon cooling to 20°, a fine precipitate formed. The pH was adjusted to 7·4 with HCl, and the volume was adjusted to 100 ml. The resulting slurry was throughly and constantly stirred while portions were removed for injection with 25 gauge needles intraperitoneally into rats.

Male Sprague-Dawley rats, 201–337 g, housed individually and allowed free access to water and Purina lab chow, were decapitated, and blood from the cervical wound was collected and centrifuged to obtain serum which was stored frozen. Whole brains were removed immediately, dipped in a 0·02% solution of Acationox (Scientific Products), a non-ionic detergent, blotted thoroughly, frozen in aluminum foil on dry ice, and subsequently weighed. Afterwards, the brains were thawed and homogenized in a Waring Blender with microattachment in 9·3 ml 0·02% Acationox g^{-1} brain as described previously (King, Carl & others, 1969; Frazer & others, 1973) and centrifuged at 27 000 *g* for 40 min to remove cellular debris.

The serum was assayed for lithium at a 1:10 or a 1:20 dilution on a Varian Techtron 1200 atomic absorption spectrophotometer. Lithium serum standards were prepared by adding a known amount of lithium carbonate standard solution to serum from untreated rats and diluting 1:20 with distilled water. The supernatants from brain homogenates were assayed directly for lithium. Brain lithium standards were prepared by adding a known amount of lithium

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to brains from untreated rats before homogenization. Both serum and brain supernatants from untreated rats were included as blanks but did not absorb significantly above background. All deviations from the mean are expressed as s.e. of the mean.

RESULTS

Dose response

Thirty-two rats were divided into six groups of five animals each, plus two controls used for preparation of standards. Each group received an intraperitoneal injection of either lithium carbonate or lithium orotate at one of three doses of lithium: 1·0, 2·0, and 4·0 m equiv kg^{-1}. As the largest dose entailed injecting a volume of 2 ml per 100 g body weight, the injection was made slowly. Rats were killed 8 h after injection and serum and brains taken.

Serum and brain lithium concentrations correlate significantly after injection of the orotate ($r = 0.984$, $P < 0.001$) or lithium carbonate ($r = 0.970$, $P < 0.001$). As shown in Fig. 1, serum and brain concentrations correlate significantly with dose for both drugs (range of r's, 0·926 – 0·980, $P < 0.001$).

At each of the three doses, the mean serum and brain lithium concentrations were greater after orotate than after carbonate. The regression coefficient of serum lithium concentrations after the orotate, 0·401, is significantly greater than that for the carbonate, 0·209 ($P < 0.001$) (Fig. 1) (Edwards, 1967). Similarly, the regression coefficient of brain lithium concentrations after orotate, 0·259, is significantly greater than that for carbonate, 0·200 ($P < 0.05$, one-tailed test). In two of the animals, brain lithium concentrations after 2·0 m equiv kg^{-1} of orotate were extremely high (1·75, 1·78 m equiv kg^{-1}) and were not included in any statistical analysis.

Time course

The y-intercepts of the four regression lines in Fig. 1 are negative which suggests non-linearity at low doses of both orotate and carbonate. A dose of 2·0 m equiv kg^{-1} was used to study the time course of lithium serum and brain concentrations since it is evident from Fig. 1 that a linear relation exists between dose and serum or brain lithium concentrations over the dose range 1·0–4·0 m equiv kg^{-1}.

To determine how differences in serum and brain lithium concentrations observed at 8 h changed with time, some rats were killed at 2, 8 or 24 h after injection. Six groups of five animals each, plus two controls, each received an intraperitoneal injection of 2·0 m equiv Li^+ kg^{-1} of either lithium carbonate or orotate. Little serum lithium remains 24 h after

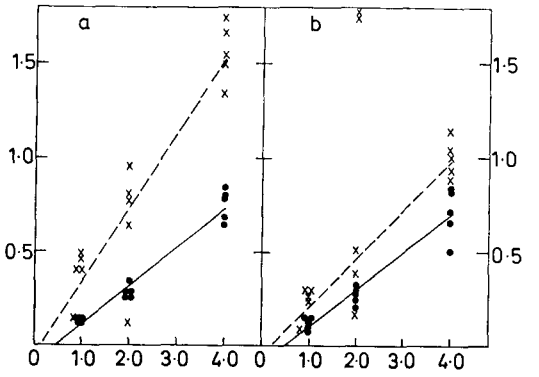

FIG. 1. Serum (a) and brain (b) lithium concentrations of rats injected with various doses of the orotate or carbonate. Animals were killed 8 h after injection. Each point represents a lithium concentration from one rat after either lithium orotate (×) or lithium carbonate (●) injection. Regression equations were calculated from these points excluding the 1·75 and 1·78 m equiv kg^{-1} lithium brain values (see text). These equations are a: Li concn = 0·401 (dose LiOr) —0·07. Li concn = 0·209 (dose Li_2CO_3) —0·10. b: Li concn = 0·259 (dose LiOr)— 0·06. Li concn = 0·200 (dose Li_2CO_3) —0·10. The dotted lines are the regression lines obtained for the lithium orotate data and the solid lines for the lithium carbonate data. Ordinates a: Serum concentration (m equiv $litre^{-1}$); b: Brain concentration (m equiv kg^{-1}). Abscissa: Dose (m equiv Li^+ kg^{-1}).

injection of the carbonate, while two-thirds of the 2 h serum lithium concentration is present 24 h after the orotate (Fig. 2). The 24 h serum lithium value after the orotate (1·11 ± 0·09 m equiv $litre^{-1}$) differs significantly from that after the carbonate (0·10 ± 0·02 m equiv $litre^{-1}$, $P < 0.001$).

Furthermore, in the orotate-treated animals, the 24 h brain lithium concentration increased above that at 8 h whereas there is no difference in the carbonate-treated animals. The 24 h brain lithium concentration after orotate (1·30 ± 0·02 m equiv kg^{-1}) is ~3 times greater than that after carbonate (0·41 ± 0·07 m equiv kg^{-1}, $P < 0.001$).

DISCUSSION

The results indicate that lithium concentrations in rat serum are higher after administration of the orotate than after the carbonate. There are several possible explanations for this. Since the orotate was injected as a slurry whereas the carbonate was injected as a clear solution, the solid orotate may act as a slow release form. Amdisen (1975) has presented data on sustained release preparations of lithium and suggested that sustained release can be achieved 'by involving the active drug principle in complex or salt formation. Some drugs with a limited solubility can

give a certain slow release effect without the use of special pharmaceutical formulations'.

Another explanation to account for the observed difference in serum lithium concentrations is that orotate causes metabolic changes which affect serum lithium values. For example, orotic acid has been shown to change the concentration of nucleotides *in vivo* (Marchetti, Puddu & Caldarera, 1962) which, in turn, have been shown to bind lithium *in vitro* (Birch & Goulding, 1975). Alternatively, it is conceivable that orotate may have an influence on factors reducing the glomerular filtration rate which could account for the higher serum lithium values.

In contrast to the serum lithium findings presented in this paper, Smith (1976) reported that intraperitoneal injection of 0·5 m equiv kg^{-1} lithium orotate or lithium carbonate, presumably as clear solutions, did not result in any significant difference in serum lithium concentrations 4·5 h after injection. There are several differences between Smith's and our procedure, notably, Smith's use of clear solutions and a lower dose of drug. Our results suggest that below 1·0 m equiv kg^{-1}, there is non-linearity between dose and serum or brain lithium concentrations. Although Smith found no difference in serum concentrations, he did find that chronic administration of the orotate protected partially against lithium-induced polydypsia and polyuria in rats.

The elevated brain concentrations after the orotate may be a consequence of the lithium serum concentrations falling off much more slowly than after the carbonate (Fig. 2). Lithium equilibrates slowly between blood and brain in contrast with the process between blood and other tissues (Schou, 1958). Presumably, if the serum lithium concentrations are maintained for longer this would facilitate equilibrium. Nieper (1973) offered an alternative explanation, suggesting that orotic acid forms a complex with lithium which can enter the brain preferentially through a specific carrier mechanism for orotate.

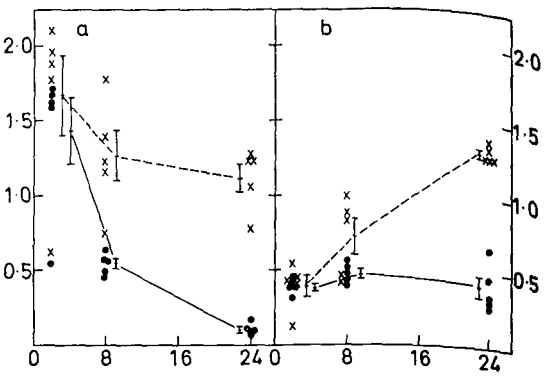

FIG. 2. Serum (a) and brain (b) lithium concentrations at various times after injection of lithium orotate or carbonate. Each point represents a lithium value from one rat. Each animal was injected with 2 m equiv Li^+ kg^{-1} ±, mean ± standard error of the mean. × - - - -×, lithium orotate. ●——●, lithium carbonate. Ordinates as for Fig. 1. Abscissa: Time (h) after injection.

Regardless of the mechanism, our results suggest that low doses of the orotate may achieve therapeutic brain lithium concentrations while avoiding transient toxic lithium concentrations in the serum.

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