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Effects of Chronic LiCl and RbCl on Muricide Induced by Midbrain Raphe Lesions in Rats

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YAMAMOTO, T., H. ARAKI, Y. ABE AND S. UEKI. *Effects of chronic LiCl and RbCl on muricide induced by midbrain raphe lesions in rats.* PHARMACOL BIOCHEM BEHAV 22(4) 559–563, 1985.—Midbrain raphe lesions in rats (raphe rats) induce aggressive behavior including muricide. A single administration of LiCl (Li) 100 mg/kg to raphe rats produced only 25% of muricide inhibition. However, the inhibitory effect of muricide in raphe rats significantly increased from the 5th day following repeated administration of Li. Chronic Li also inhibited muricide in olfactory bulbectomized (OB) rats. The inhibition of muricide lasted until the next day to some extent. In this point, the effect of Li on muricide is similar to that of antidepressants, but not of neuroleptics. On the contrary, RbCl (Rb) showed a tendency to induce muricide. The single re-administration of Li 100 mg/kg on the withdrawal on the 7th day after repeated administration for 14 days showed a significant inhibition of muricide in raphe rats, unlike that in OB rats. Li also showed a partial prophylactic effect on muricide when Li 100 mg/kg was administered for 1 week before raphe lesions. These results suggest that raphe rats may serve not only as an experimental model of depression, but also as that of manic illness.

Lithium Rubidium Muricide Raphe lesions Olfactory bulbectomy Prophylactic effects

LITHIUM (Li) and rubidium (Rb), alkali metals, have been proposed to have therapeutic and prophylactic efficacy against manic-depressive psychosis. Li is effective chiefly for manic states [6,8], and Rb, mainly for depressive conditions [10,22].

In animal studies, Li exerts no marked effects on locomotor activity of animals in the normal state [7], while it effectively antagonizes the hyperactivity induced by amphetamine [3]. In contrast, Rb increases locomotor activity [7, 16, 22, 44]. Li inhibits release of noradrenaline (NA) [17,18], whereas Rb increases it [35]. Thus, Li and Rb exert effects opposite to each other in many ways.

It is well known that antidepressant drugs selectively inhibit muricide (mouse-killing behavior) of rats at doses below the neurotoxic level [14,41]. Although there is still no general agreement, Li is reported to be effective in suppressing aggression in humans and animals [9, 31, 32, 38]. So far, few detailed studies have been made on the effect of Li on muricide [5, 27, 29].

Destruction of the midbrain raphe nuclei of rat results in marked decrease in 5-HT content in the brain and induces hyperlocomotor activity and aggressive behaviors including muricide [13, 20, 40]. The muricide induced by destruction of the midbrain raphe nuclei is inhibited by antidepressants [41]. This behavior is more easily inhibited by antidepressants which inhibit serotonin (5-HT)-uptake more than NA-uptake.

In the present study, we attempted to demonstrate [1] the effect of chronic Li or Rb administration on muricide of rats with raphe lesions, and [2] the prophylactic effect of Li on muricide induction following raphe lesions.

Muricide can also be induced by other methods such as long-term isolation and olfactory bulbectomy [37]. Muricide differs in (1) mode of onset [40], (2) sensitivity to drugs [41] and (3) participation of the locus coeruleus [43] and amygdala [33] depending upon the method of induction. In the present study we have attempted to elucidate differences in the effect of Li on muricide induced by raphe lesions in comparison with muricide induced by olfactory bulbectomy and that of spontaneous killer rats.

METHOD

The animals used were male rats of the Wistar King A strain weighing 180 to 220 g at the time of surgery. They were supplied by The Kyushu University Institute of Laboratory Animals. After surgical manipulation, the rats were housed in individual wire-mesh cages (20×21×17 cm) throughout the experimental period and given food and water ad lib. Room temperature was maintained at 22±1°C on a 12 hr light-12 hr dark schedule with lighting off at 21:00.

The animals were anesthetized with sodium pentobarbital (40 mg/kg, IP) and placed on a stereotaxic instrument. For induction of the raphe lesion, a monopolar electrode (0.4 mm

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in diameter insulated stainless steel wire with bare tip) was inserted into the midbrain raphe nuclei according to the rat brain atlas of König and Klippel [19]. A direct current of 2 mA was applied for 15 sec to destroy both the medial raphe nucleus (frontal plane (F): 0.16, saggital plane (S): 0, horizontal plane (H): −2.5) and dorsal raphe nucleus (F: 0.16, S: 0, H: 1.0) (raphe rats). For olfactory bulbectomy, a hole of approximately 1 mm in diameter was opened in the skull and the olfactory bulbs were bilaterally removed by suctioning (OB rat). Animals were subjected to the muricide test for 15 min periods on the 2nd, 4th and 7th day after surgical operation.

Raphe rats were divided into 2 groups: one consisted of animals which showed muricide at three consecutive tests: the other, those which showed no muricide at all. Only OB rats that showed muricide in all of three consecutive tests were used. For selection of spontaneous killer rats, rats were placed in the isolation cage for 1 hr and subjected to the muricide test for 15 min. Only rats that showed muricide in all of the same three tests after isolation housing were used as spontaneous killer rats.

Aggressive behavior was measured as described previously [40]. In addition to the muricide test for 3 min, the startle response to blowing with a fixed volume of air onto the back. Startle response was measured by scoring as follows: score 0, no reaction; score 1, slight; score 2, moderate; score 3, marked; score 4, extreme response.

LiCl (Li: E. Merck, Darmstadt) and RbCl (Rb: E. Merck, Darmstadt) were given subcutaneously to animals once a day for 14 days. After a 6-day withdrawal period, i.e., on the 7th day after the last dosing, Li was re-administered. The test for aggressive behavior was performed twice a day; before drug or saline administration (10:00 a.m.: pre-drug state) and 1 hr after administration (post-drug state). Water intake was measured during the pre-dosing period using "Touch-n-drink" (O'Hara and Co., Ltd) provided with two stainless steel balls at the end of the tube.

RESULTS

Effects of Li on Muricide

The effect of repeated administrations of Li 100 mg/kg on muricide in raphe rats are shown in Fig. 1 (N=12). The muricide in raphe rats was not affected markedly by chronic saline at either pre- or post-state (Fig. 1). A single administration of Li to raphe rats inhibited muricide in only 3 out of 12 rats (25%); however, the incidence of inhibition increased with repeated administration, being 66.6% on the 5th day. The inhibitory effect on muricide was maintained with chronic administration of Li (Fig. 1). Chronic administration produced an inhibition of muricide even at the non-drug state (23 hr after injection of preceding day; incidence of inhibition was 15.4% on the 3rd day and as high as 41.7% on the 9th day: Fig. 1). The re-administration of Li on the 7th day after the last administration resulted in an inhibition as high as 63.6%. The startle response, however, was scarcely affected by repeated administration of Li.

During the chronic administration of Li, rats were relatively sedated, but did not cause ataxia, tremor, or diarrhea. Chronic doses of Li increased water intake significantly; 39.7±3.4 ml/day (average ±SE) on the 3rd day in the Li-treated raphe rats compared to 24.2±4.7 ml in the saline-treated raphe rats ($p<0.002$; two tailed Mann-Whitney U-test). The significant increase of water intake persisted from the 3rd day, throughout the Li administration period.

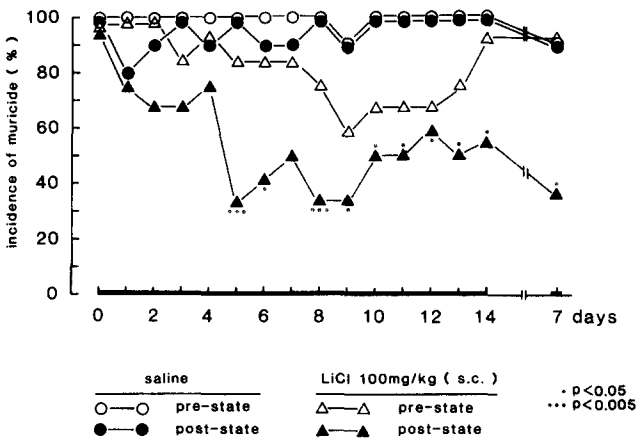

FIG. 1. Effects of chronic doses of LiCl on muricide in raphe rats.

The maximum effect was reached at the 8th day after repeated administration of Li; i.e., water intake increased to the maximum of 65.8±4.2 ml, compared to the control value of 26.6±3.4 ml.

A single administration of Li 200 mg/kg suppressed muricide in 5 of 6 raphe rats. Chronic administration, however, produced severe diarrhea and tremor, and 4 of 6 rats died by the 9th day.

In OB rats, a single administration of Li 100 mg/kg resulted in 37.5% muricide inhibition (N=12, Fig. 2). The percentage of inhibition increased with repeated administration of Li; muricide was completely inhibited on the 6th day, even at the pre-administration period after the 5th day muricide was inhibited in 50% of rats. The inhibitory effect of Li on muricide decreased slightly on the 6th day; however, the inhibition was 62.5% on the 14th day. On the 7th day following the cessation of administration, muricide inhibition was as low as 25% by Li re-administration.

Water intake in OB rats was significantly increased by chronic Li from the 5th day (54.8±5.7 ml: $p<0.002$). This effect significantly persisted till the 9th day (49.0±2.7) but disappeared after the 10th day (42.8±2.9). The maximum increase in water intake was 56.0±3.5 ml at the 6th day after repeated administration ($p<0.002$).

In spontaneous killer rats (N=14), a single administration of Li 100 mg/kg produced 57.1% muricide inhibition, and at the 2nd day, 14.3%; the 4th day, 71.4%; the 7th day, 28.6%; the 10th day, 64.3%; the 14th day, 35.7%. Thus, Li-induced inhibition of muricide in spontaneous killer rats did not necessarily increase with repeated administration and is not always reproducible in contrast to that of raphe rats and OB rats. The startle response of spontaneous killer rats was not significantly affected by Li throughout the experimental period.

Prophylactic Effect of Li on Muricide Induction

In the rat (N=8) which had been prophylactically administered Li 100 mg/kg/day for a week, the incidence of muricide following raphe lesions was lower than that in the rats (N=8) treated with saline in the same way. The incidence of muricide in rats (N=8) pretreated with Li was 25% at the 2nd and 4th day after raphe lesions and 37.5% at the 7th day, as contrasted to the value of 50, 62.5 and 75%, respectively, in rats given saline in the same way. In the rats

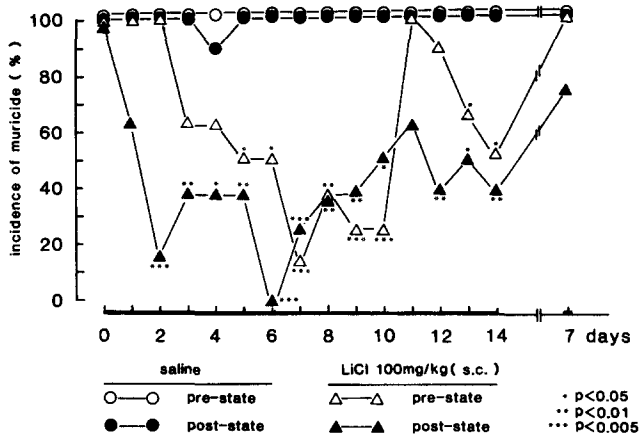

FIG. 2. Effects of chronic doses of LiCl on muricide in OB rats.

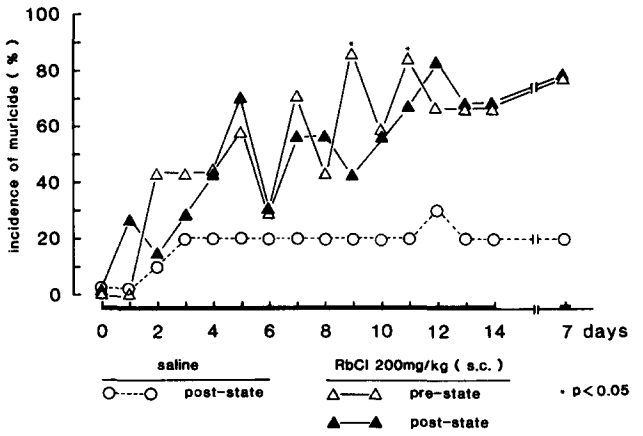

FIG. 3. The occurrence of muricide following chronic administration of RbCl in intact rats.

(N=9) pretreated with Li 100 mg/kg/day for 2 weeks, the muricide incidence was 44.4% at the 2nd day after raphe lesions and 66.7% at the 4th and 7th days, but no significant difference in incidence of muricide was observed between the Li-or saline-pretreated rats. The score of the startle response in rats pretreated with Li for 1 week was low, though not significant, being 2.3±0.2 at the 2nd day. Similar low scores were recorded at the 4th day after lesions. The startle response score of the rat pretreated with Li for 2 weeks was significantly low, i.e., 1.8±0.3 ($p<0.02$) at the 2nd day after lesions compared to 2.9±0.3 in the saline-pretreated rats.

Effect of Rb on Muricide

Muricide in raphe rats (N=5) was not affected during the chronic administration of Rb 200 mg/kg. Scores of the startle response were increased as Rb was repeated. On the last day of administration, the score of startle response in the Rb-treated rats showed significantly high score (2.8±0.2, $p<0.02$) when compared with that of saline-treated rats (2.0±0.1). On the contrary, Rb administration to rats (N=4) which had shown no muricide even after raphe lesions induced muricide in 2 out of 4 rats after repeated administrations for 4 days, though a single administration failed to cause muricide. On the other hand, a single Rb administration induced muricide in the intact rats (N=8) showing no muricide (Fig. 3). The incidence of muricide increased with repeated administration, being 83.3% after 12-day administration. Muricide was seen even 7 days after withdrawal following repeated administration for 14 days. The startle response in the Rb-treated rats did not differ in the score from those in the saline-treated rats.

DISCUSSION

There are many clinical reports supporting prophylactic or therapeutic efficacy of Li in manic illness [2,8], monopolar depression [11] and schizophrenia [1,4]. In animal experiments, foot-shock induced aggression in mice is inhibited by Li [31], though at a relatively high dose. Sheard [29] has reported that Li at relatively high dose (5 mEq/kg) blocked parachlorophenylalanine (PCPA)-induced muricide. However, Rush *et al.* have reported that chronic Li fails to block muricide induced by food limitation in individually housed Sprague-Dawley rats [27]. In the present study, though a single dosing of Li 100 mg/kg failed to affect muricide induced by raphe lesions, repeated injections of Li inhibited muricide accompanied by no behavioral toxicity. This discrepancy may depend on the induction method of muricide or the strain of rats.

In the biochemical studies, single administration of Li did not cause detectable changes in the brain content of tryptophan and 5-HIAA. However, subacute treatment with Li increased those concentrations in the brain [15,25]. Sheard *et al.*, using probenecide, also demonstrated that Li accelerated the turnover rate of 5-HT in the brain [30]. It is interesting that inhibition of muricide by Li is parallel to the increased 5-HT turnover rate in the brain by Li. Our previous results showed that muricide in raphe rats is effectively inhibited by drugs which potentiate the activity of 5-HT neurons more than that of NA neurons, its muricide is presumed to be due to the reduction of brain 5-HT [40]. From these points, it seems therefore likely that the inhibitory effect of Li on muricide in raphe rats is exerted by activation of 5-HT neuron. Furthermore, Broderick *et al.* [5] recently reported that muricide in Long-Evans rats was markedly blocked by acute injection of Li when Li and L-tryptophan were administered in combination in their smallest effective doses. The biochemical effect of Li combined with L-tryptophan showed the synergistic increase in 5-HT turnover rate in the rat. These results also support our hypothesis. There are, however, some reports in which Li does not affect 5-HT metabolism in the brain [28]. These data concerning brain 5-HT are conflicting, possibly due to the difference in experimental conditions. According to Nakagawara [24], Li does not affect 5-HT metabolisms in intact brains, whereas it produces a marked increase in 5-HT and 5-HIAA in brain of PCPA-treated rats. It is therefore likely that Li exerts similar effect in raphe-lesioned rats as well as that of PCPA-treated rats.

Thus, chronic Li also inhibited muricide in OB rats and spontaneous killer rats. OB rats and spontaneous killer rats also showed a decrease in 5-HT turnover rate in the brain [21]. There is no significant difference in the manner of muricide inhibition of Li between OB rats and raphe rats. However, it is of interest that the inhibitory effect of Li on muricide of raphe rats, unlike that in OB rats, was evident even at 7 days after cessation of Li-administration. The mechanism of this effect remains unsolved.

On the other hand, neuroleptics and antidepressants also inhibit muricide. However, this effect of neuroleptics is gradually reduced with repeated administration, while that of antidepressants is not [34]. In this respect, the mode of Li action on muricide in raphe rat is different from that of neuroleptics but similar to that of antidepressants. It was reported that Li treatment significantly increased 5-HIAA level in the cerebrospinal fluid in manic patients, who showed low concentrations of 5-HIAA [12, 23, 39]. Raphe rats with decreased 5-HT and 5-HIAA brain content show a high arousal level not only in behavior [41], but also in EEG [42]. At this time, Li showed muricide inhibition in raphe rats. The above facts suggest that raphe rats may not only be an experimental model of depression [41], but also of manic illness. Rb, which is reported clinically to have an antidepressant effect [22, 26, 35], produced no inhibition of muricide in raphe rats. On the contrary, Rb showed a tendency to induce muricide in rats, which did not show muricide following raphe lesions, as well as in intact non-killer rats. The present report is the first to show that Rb induces muricide, though Rb has been reported to induce or potentiate irritability or attack response in rats [9, 10, 36]. This indicates that the drug which has clinical antidepressant effect does not necessarily exert an inhibitory activity on muricide. In any way, it may be mentioned that the mode of clinical effect of Rb seems to be different from that of typical antidepressants. Li has a prophylactic effect against manic illness in clinical practice [2,11]. For demonstration of the prophylactic effectiveness of Li in experimental animals, the pretreatment with Li, as administered for 7 consecutive days at a dose of 100 mg/kg before raphe lesions, tended to inhibit the appearance of muricide, though the degree of inhibition was not statistically significant. The increasing of the dose to 200 mg/kg produced no corresponding potentiation of muricide inhibition, suggesting that Li exerts its prophylactic effect only at an optimal dose; but it is not dose-dependent.

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