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Rubidium (Rb) Treatment of Rats: Biological Effects and Implications for Psychiatry

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ABSTRACT

Experiments were carried out on rats in order to find out the implications for psychiatry of the basic biological effects of rubidium (Rb).

The forced swimming test (FST), used to evaluate the effects on rats of treatment with Rb, was conducted after 1 or 3 mEq/kg Rb was given chronically or subacutely. The weight of rats treated with 1 or 3 mEq/kg Rb, once daily, was observed daily for two weeks. Rb levels in the blood and brain of rats treated with Rb chronically or subacutely were determined by atomic absorption spectrophotometry. The rectal temperature was observed at 30, 60, 90, 120, 180, and 240 min after injections of 1 or 3 mEq/kg Rb.

The increase in the mean body weight of treated rats was almost the same as that of the control. The rectal temperature in rats treated with Rb showed a hyperthermic response. With both the 1 and 3 mEq/kg Rb treatments, Rb levels in the brains were significantly higher in the chronic experiments than in the subacute experiments. The experiments conducted to determine the effect of chronic and subacute treatment of Rb are hereafter termed 'chronic experiment' and 'subacute experiment' respectively. Almost the same significant difference was also observed in the Rb levels in the blood. In the FST, a decrease in the mean immobility time was not observed in either the subacute or the chronic experiments. Thus, antidepressant effects, as judged from the FST, were not observed although Rb did actually accumulate in both brain and blood.

Key words: *Rubidium, Rats, Biological effects*

Rubidium (Rb) is one of the group of 1A alkali metals, belonging to the same series as lithium (Li). Clinically, Li exhibits both antimanic and prophylactic effects on manic-depressive illness (MDI), and is widely used in the treatment of MDI. The usefulness of Li in the treatment of MDI spread our attention to other alkali metals. Among them, we focused on Rb, since Rb exhibits some neurophysiological, neurochemical, and behavioral effects which are contrary to those of Li. For example, Li decreases the behavioral activity of rats and Rb increases it[6]. In the first trial of Rb treatment in human subjects, Rb was administered orally, and a notable improvement in the patients' subjective sense of well-being was observed[2]. From this result, Rb appears to possess an antidepressive property, and has been used tentatively for the treatment of depression[3,5,10]. It is not yet certain whether or not Rb has uses in clinical psychiatry, especially in the therapy of MDI.

Experiments were conducted in order to gain basic knowledge about the biological effects of Rb relevant to the field of psychiatry.

MATERIAL AND METHODS

Animals

Male Wistar rats, weighing 150—180 g, were received at 5 weeks of age and reared for more than 1 week in our laboratory. Food and water were given *at libitum*. Room temperature was 23 ± 1°C and relative humidity was 55 ± 5%. Light was turned on at 7.00 a.m. and off at 7.00 p.m.

The forced swimming test (FST)

The FST, one of the models of depression, has been widely used to detect antidepressant activity[1]. In this study, the FST was used to evaluate the effects of Rb.

The rat was put into an acrylic resin cylinder (40 cm height, 18 cm inside diameter, depth 15 cm) containing warm water. The water temperature was 25 ± 1°C. As pretreatment, the rat was placed for 15 min in the water and then picked up and dried for 15 min in a heated chamber (air temperature: 32°C).

Clinically, the effects of most antidepressants are observed after 2 - 3 weeks of treatment. We therefore set the duration of the chronic experiment to 14 days. In the chronic experiments, 1 mEq/kg Rb,

3 mEq/kg Rb, or physiological saline (NaCl 0.9%) was given to the rats just after the pretreatment. Rubidium chloride, made by Wako Pure Chemical Industries, was dissolved in bacteria-free distilled water. One ml/kg was given intraperitoneally once daily for 14 consecutive days. On the last day, the chemical was given 1 hr before the FST. The immobility time in the FST was measured according to the procedure of Porsolt et al[11], i.e., rats placed in the cylinder were observed for 5 min and total immobility time was measured. Immobility time was defined as the time when the rat remained floating in the water, making only the necessary movement to keep its head above water. In the subacute experiments, the FST was conducted after 24 hr of pretreatment. The chemicals mentioned above were given to the rats three times, 24, 5, and 1 hr before the FST was conducted and the immobility time was measured.

Measurement of Rb levels in the blood and brain

Just after the FST was finished, the rats were anesthetized with sodium pentobarbital (40 mg/kg), and blood was collected from hearts into plastic syringes containing disodium ethylene-diamine-tetraacetate (EDTA). EDTA was used to interfere with the coagulation of the blood. The rats were then killed by decapitation and the brains collected. The blood and brains were stored at −80°C until the assay.

Rb levels in the blood and brain were measured according to the method of Okada et al[9], with some modifications, i.e., samples were wet-ashed with HNO_3 using decomposition vessels (Uniseal Decomposition Vessels Co., Ltd. Israel) at 120°C for 5 hr. The ashed solution was diluted with distilled water and potassium was added until the concentration became 10,000 ppm, to achieve increased sensitivity.

A flame atomic absorption spectrophotometer, (Nippon-Jarrel Ash Co., Ltd. Japan Model AA-780) was used in the analysis. Samples were atomized in an air-acetylene flow (air 8 liters/min; acetylene 2 liters/min). An Rb-Ne hollow cathode lamp (WL-22824A, Westinghouse Co., Ltd. USA) was used at 20 mA using the 7800.2 Å Rb resonance line. The Rb level was determined with the standard curve method.

Measurement of body weight

When the chronic experiments were carried out, the body weight of the rats was measured daily at 10:00 a.m. using an automatic balance (Toyo-Riko Co., Ltd. Japan).

Observation of rectal temperature

The rats were acclimated to room temperature at 23 ± 1°C more than 1 hr before the experiment. The three test chemicals which were given in the FST were injected once into the rats intraperitoneally. The movement of the rats was lightly restrained, and a probe was inserted 7.5 cm into the rectum. Rectal temperature was observed before injection and 30, 60, 90, 120, 180, and 240 min after the injection. A rectal probe (PD-K061, Termo Co., Ltd. Japan) and a digital thermometer (CTM303, Termo Co., Ltd. Japan) were used to measure rectal temperature.

RESULTS

The forced swimming test

In the subacute experiments, the mean immobility times in the FST of 1 and 3 mEq/kg groups were 175 and 200 sec, respectively (Table 1): slightly longer than those of the control (170 sec). In the chronic experiments, the mean immobility times in the FST of 1 and 3 mEq/kg groups were 121 and 119 sec, respectively (Table 1): somewhat shorter than those of the control (132 sec).

Measurement of Rb levels in the blood and brain

In the subacute experiments, Rb levels in the blood were 0.71 and 2.1 mEq/kg in 1 and 3 mEq/kg groups, respectively. Levels in the brain were 0.51 and 1.4 mEq/kg in 1 and 3 mEq/kg groups, respectively (Table 2). There was statistically significant difference in the Rb level between blood and brain in the 1 mEq/kg group ($p<0.01$). The significant difference was observed between these two in the 3 mEq/kg group ($p<0.01$). On the other hand, in the chronic experiments, Rb levels in the blood were 4.1 and 6.5 mEq/kg in 1 and 3 mEq/kg groups, respectively. Levels in the brain were 3.6 and 6.6 mEq/kg in 1 and 3 mEq/kg groups, respectively (Table 2). There was a significant difference in the Rb level between blood and brain in 1

Table 1. Effect of subacute and chronic treatment of rubidium (Rb) on the forced swimming test

Treatment	No. of Animals	Body Weight (g) (Mean ± SE)	Immobility Time (sec) (Mean ± SE)
Subacute			
Control	(6)	257 ± 10	170 ± 17
Rb (1mEq/kg)	(6)	253 ± 10	175 ± 16
(3mEq/kg)	(6)	260 ± 8	200 ± 17
Chronic			
Control	(6)	290 ± 6	132 ± 11
Rb (1mEq/kg)	(6)	270 ± 11	121 ± 18
(3mEq/kg)	(7)	281 ± 6	119 ± 15

Statistical analysis was by Mann-Whitney U-test.

Table 2. Concentration of Rubidium (Rb) in rat blood and brain at the termination of the forced swimming test in subacute and chronic treatment of Rb

Treatment	No. of Animals	Blood (mEq/kg.wet tissue) (Mean ± SE)	Brain (mEq/kg.wet tissue) (Mean ± SE)
Subacute			
Rb (1mEq/kg)	(6)	0.71 ± 0.03^{**a}	0.51 ± 0.01
(3mEq/kg)	(6)	2.1 ± 0.05^{**a}	1.4 ± 0.06
Chronic			
Rb (1mEq/kg)	(6)	4.1 ± 0.09^{**a} ^{**b}	3.6 ± 0.06^{**b}
(3mEq/kg)	(7)	6.5 ± 0.5 ^{**b}	6.6 ± 0.3 ^{**b}

^{**a}: $p<0.01$, compared to the brain; ^{**b}: $p<0.01$, compared to the subacute experiments.
Statistical analysis was by Student's t-test.

Table 3. Correlation coefficient between rubidium (Rb) levels in blood and brain

Treatment	No. of Samples	Correlation Coefficient
Subacute Rb (1mEq/kg)	(6)	0.11
(3mEq/kg)	(5)	0.37
Chronic Rb (1mEq/kg)	(5)	0.60
(3mEq/kg)	(7)	0.84*

*: $p<0.05$

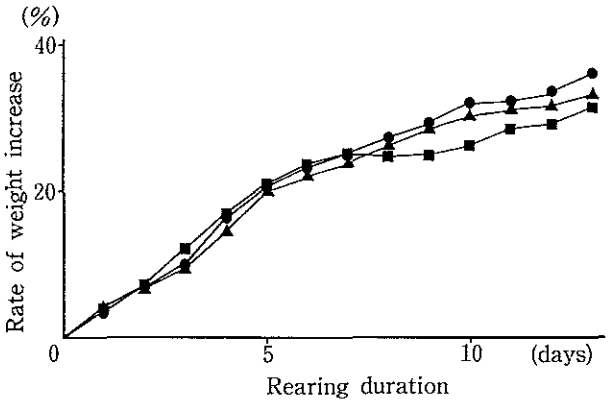

Fig. 1. Changes in the weight of rats in the chronic experiment.
Statistical analysis was by Mann-Whitney U-test.
●: control (n=6), ■: 1 mEq/kg Rb (n=6), ▲: 3 mEq/kg Rb (n=7)

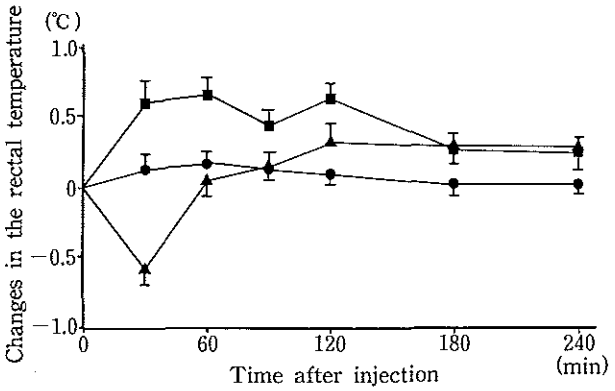

Fig. 2. Changes in the rectal temperature.
●: control (n=16), ■: 1 mEq/kg Rb (n=8),
▲: 3 mEq/kg Rb (n=8)

mEq/kg group ($p<0.01$), but not in 3 mEq/kg group.

The Rb levels in the chronic experiment were significantly higher than those in the subacute experiment in both 1 and 3 mEq/kg groups (Table 2, $p<0.01$).

The correlation between the Rb levels in the blood and brain was analyzed (Table 3). In the chronic experiment of 3 mEq/kg group, a significant correlation was observed ($r=0.85$).

Measurement of body weight

The rate of weight increase in the control, 1 and 3 mEq/kg groups were 36%, 32%, and 33%, respectively (Fig. 1). No significant difference was observed in these three groups.

Observation of rectal temperature

The temperature after injection of 1 mEq/kg Rb was higher than that before the injection throughout the experiment (Table 4, Fig. 2). The maximal elevation of the rectal temperature was 0.66°C at 60 min after the injection. Significantly higher temperature was observed at 30, 60, and 120 min, compared with the control ($p<0.05$). After treatment

Table 4. Changes in the rectal temperature

Treatment	No. of Animals	Time after injection 30 min (°C;Mean±SE)	60 min (°C;Mean±SE)	90 min (°C;Mean±SE)	120 min (°C;Mean±SE)	180 min (°C;Mean±SE)	240 min (°C;Mean±SE)
Control	(16)	0.13 ± 0.10	0.17 ± 0.08	0.14 ± 0.09	0.09 ± 0.09	0.02 ± 0.09	0.02 ± 0.07
Rb (1mEq/kg)	(8)	0.60 ± 0.15*	0.66 ± 0.10**	0.44 ± 0.11	0.62 ± 0.11**	0.26 ± 0.10	0.24 ± 0.11
(3mEq/kg)	(8)	−0.59 ± 0.12**	0.05 ± 0.12	0.15 ± 0.10	0.33 ± 0.12	0.31 ± 0.06*	0.30 ± 0.06**

*: $p<0.05$, **: $p<0.01$, compared with control. Statistical analysis was by Mann-Whitney U-test.

with 3 mEq/kg Rb, the temperature gradually lowered, and the temperature 30 min after the injection was lower than that before the injection by 0.59°C. The temperature then gradually rose, and by 180 and 240 min after injection, was significantly higher than that of the control ($p < 0.05$).

DISCUSSION

There was little difference between body weight increase in the Rb treated groups and in the control. Jenner et al[7] reported that Rb treatment was not related to any specific weight-reducing effect in humans for over 2 years. The findings obtained from the present experiment are in good agreement with the observation of Jenner et al[7].

Regarding rectal temperature, 1 mEq/kg Rb caused a hyperthermic response throughout the experiment, while 3 mEq/kg Rb caused a hypothermic response at the beginning but caused a relative hyperthermic response later. When a high dose of Rb was administered, the rats appeared to be stressed; hypothermic response occurred simultaneously. Following this, the rat gradually recovered from the stress and the rectal temperature rose with the absorption of Rb. Thus, it can be inferred that Rb essentially causes a hyperthermic response in rats.

When treating patients, the Li level in serum is often measured in order to determine the appropriate dose of Li. However, Del Vecchio et al[4] reported that Rb in whole blood is clinically of greater significance than Rb in plasma. Therefore, we decided to examine the Rb level in whole blood.

In 1 and 3 mEq/kg groups in the subacute and 1 mEq/kg group in the chronic experiments, the Rb level in blood was higher than in brain. The results suggest that Rb, injected intraperitoneally, pass into the blood, but did not fully pass into the brain, since the blood and brain were collected shortly after injection of Rb.

The Rb levels in the blood and brain in the chronic experiments were higher than those in the subacute experiments. This finding suggests that Rb accumulated in the brain and blood. Taking into account the observation of Paschalis et al[10], it can be inferred that Rb in the blood accumulates in the red blood cells.

The biological half-life of ^{86}Rb administered to rats intraperitoneally is 7.5 days, and that administered orally to humans is 80.2 days[12]. On the other hand, the half-life of lithium administered to rats subcutaneously is 6.1 hr[14], and that administered orally to humans is 28.9 hr[8]. Thus, Rb has a very long biological half-life compared to other antipsychotic drugs.

In the chronic experiment of 3 mEq/kg Rb, significant correlation in the Rb levels between blood and brain was observed. Spirtes et al[13] reported almost the same result in monkeys. It is useful, therefor, to examine the Rb level in whole blood to estimate the Rb level in the brain in the chronic treatment of Rb.

In the FST, no essential differences in the immobility time in the subacute and chronic experiments were noted. The results suggest that, despite the relatively long half-life of Rb and its accumulation in the brain and blood, Rb dose not exhibit antidepressive effects, judged from the FST.

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