

BEHAVIORAL CHANGES IN THE COURSE OF CHRONIC RENAL INSUFFICIENCY IN RATS

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In addition to the changes in various biochemical parameters chronic renal insufficiency (CRI) leads to progressive behavioral disturbances both in humans and rats. To further characterize these changes, the present study aimed to investigate locomotor, exploratory and emotional activity of rats with experimental CRI. Our experiments with the open field test have shown a marked decrease in locomotor, exploratory and emotional activity of the animals suffering from CRI. These changes were parallel with an increased water intake, reduced food intake and body weight. Thus, behavioral disturbances accompanying CRI in rats are similar to those occurring in human patients. The experimental model of CRI described by us seems to be a good tool for pharmacological studies.

Key words: *chronic renal insufficiency, behavior, open field test, food intake, water intake, rat*

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Abbreviations: CA – catecholamines, CNS – central nervous system, CRI – chronic renal insufficiency, 5-HIAA – 5-hydroxyindoleacetic acid, 5-HT – serotonin, TRY – tryptophan

INTRODUCTION

Chronic renal failure results in profound biochemical disturbances affecting numerous organs and regulatory systems, including central nervous system (CNS) functioning. Patients with uremic encephalopathy manifest disorientation, somnolence, asterixis and comma symptoms [13]. Speech disorders, memory failure, myoclonic jerks, flapping tremor, restless legs, paresthesia and difficulties in walking are other serious problems in chronic renal insufficiency (CRI) [31]. Also anorexia, nausea, vomiting [22], sleep disturbances [14], sexual dysfunction and depression [15, 16] are common symptoms in the uremic patients. Besides central dysfunctions, some of these symptoms are due to peripheral neuropathy, autonomic insufficiency or peripheral vascular disease [25].

The mechanisms involved in the pathologies within CNS seen in renal insufficiency are not fully understood. One limitation is the lack of biochemically and behaviorally characterized animal model of the disease, which could considerably facilitate the research efforts directed to prevent or counteract the CNS-related pathologies. In our laboratory, we have adopted a simple experimental model of CRI in rats, in which the biochemical changes closely resemble these observed in humans [4, 5, 18, 27]. The current study was undertaken to clarify if behavioral disturbances in this model of CRI in rats can imitate changes occurring in the chronic renal failure in man.

MATERIALS and METHODS

Animals

Male Wistar rats weighting 260–480 g were housed in the cages as appropriate with *ad libitum* access to chow and water. A 12:12 h light-dark cycle was maintained, and the temperature and humidity were controlled.

Surgical induction of chronic renal insufficiency

CRI was induced by a partial resection of the renal tissue according to Ormrod and Miller [24]. Briefly, rats were anesthetized with pentobarbital (40 mg/kg, *ip*). In sham-operated rats (control group) only the surgical extraction of the renal capsule was performed. The moderate CRI (CRI 1) was induced by the removal of the left kidney, while the right kidney was decorticated in 60%. The rats with severe CRI were subjected to the same surgical procedure as in the case of CRI 1 group and after 2 weeks the additional 20% of the right kidney cortex was removed. The group of animals with severe CRI was divided in two subgroups: CRI 2 and CRI 3.

Behavioral tests

Before the induction of CRI all animals were tested on open field test. These group served as control (100%) to which all animals were referred. The behavioral tests were performed a month after the surgical procedures. In addition, in CRI 3 group, the tests were carried out 2 months after the last surgical procedure.

Open field test

The animals were placed in a white, wooden, open field box (100 × 100 × 47 cm) with the floor divided into 25 equally sized squares measuring 20 × 20 cm each. Four wooden bars (20 cm high) were located at the intersections of the lines in the central area of the floor. During the experimental session, the testing room was illuminated with dim white light. The animals' behavior was characterized by a total number of crossings (locomotor activity), rearings (exploratory activity), groomings and the count of fecal pellets excreted during a 10-minute session (emotional activity) [20].

Parameters of renal insufficiency

The blood for the biochemical analyses was taken one day after the behavioral tests. Rats were anesthetized with pentobarbital (40 mg/kg, *ip*). The blood was collected by a heart puncture and transferred to a tube containing 3.13 % sodium citrate (citrate/blood = 1:9 v/v). Platelet poor plasma was obtained by a centrifugation of the blood at 3000 rpm for 10 min at 4°C. Urea and creatinine levels were measured by the use of commercial kits (Cormay, UK).

Water and food intake

On the day of the behavioral tests the 24-hour water and food intake was measured.

Body weight

On the last day of the experiment the body weight of rats was measured.

Statistical analysis

The results of the experiment were evaluated by a one-way analysis of variance (ANOVA) followed by the Tukey-Kramer Multiple Comparisons Test. Student's *t*-test was used to compare the urea and creatinine concentration in the blood. The data were deemed statistically significant when $p < 0.05$.

Ethics

The study was approved by the Ethics Committee of the Medical Academy in Białystok as being in compliance with the guidelines for care and use of animals in physiological sciences recommended by the national and international law and Guidelines for the Use of Animals in Biomedical Research (Thromb. Haemost., 1987, 58, 1078–1084).

RESULTS

Parameters of renal insufficiency

Renal cortex extraction led to an increase in blood urea and creatinine concentration. Urea and creatinine levels were significantly elevated in all groups of animals in comparison with the control group. The biochemical changes were more pronounced in the group with severe CRI and increased with time (Tab. 1).

Table 1. The blood urea and creatinine concentration of the control and rats with CRI

| | CON 1 | CRI 1 | CON 2 | CRI 2 | CON 3 | CRI 3 |
|-----------------|------------|--------------------------|------------|--------------------------|------------|-----------------------------|
| Urea [mM] | 3.3 ± 0.3 | 9.3 ± 1.0 $p < 0.05$ | 3.1 ± 0.3 | 19.6 ± 2.6 $p < 0.01$ | 3.5 ± 0.4 | 85.9 ± 7.4 $p < 0.001$ |
| Creatinine [M] | 23.3 ± 2.5 | 43.1 ± 3.6 $p < 0.05$ | 24.5 ± 3.3 | 86.3 ± 9.5 $p < 0.01$ | 27.2 ± 2.3 | 313.6 ± 20.1 $p < 0.001$ |

CON 1, 2, 3 – control group for moderate, severe 1 and severe 2 CRI; CRI 1 – moderate chronic renal insufficiency, CRI 2 – rats with severe chronic renal insufficiency tested 1 month after surgery and CRI 3 – rats with severe chronic renal insufficiency tested 2 months after surgery (experimental details are described in Materials and Methods). Values are presented as means ± SEM, significance of the difference in comparison with the control group

Open field test

A significant decrease in the number of crossings (Fig. 1) and groomings (Fig. 2) were observed in rats with moderate CRI and severe CRI 1 month after the CRI induction but there were no changes in the number of rearings (Fig. 3) and defecations (Fig. 4). In the rats with severe CRI, in which the tests were performed 2 months after the last surgical procedure, in addition to the reduction in crossings and groomings number, a decrease in rearings and excreted fecal pellets count was also observed.

Water and food intake

Water (Fig. 5) and food (Fig. 6) intake was not altered in rats with moderate CRI. In the rats suffer-

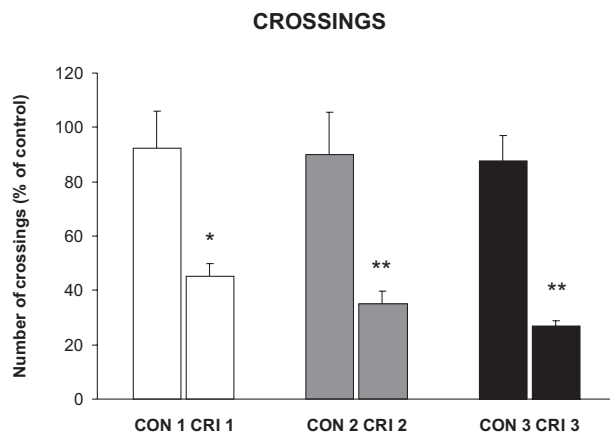


Fig. 1. The effect of CRI on the number of crossings in the open field. CON 1, 2, 3 – control groups for moderate, severe 1 and severe 2 CRI; CRI 1 – moderate chronic renal insufficiency, CRI 2 – group with severe chronic renal insufficiency tested one month after surgery and CRI 3 – group with severe chronic renal insufficiency tested 2 months after surgery (experimental details are described in Materials and Methods). Values are presented as means ± SEM, significance of the difference in comparison with the control group: * $p < 0.05$, ** $p < 0.01$

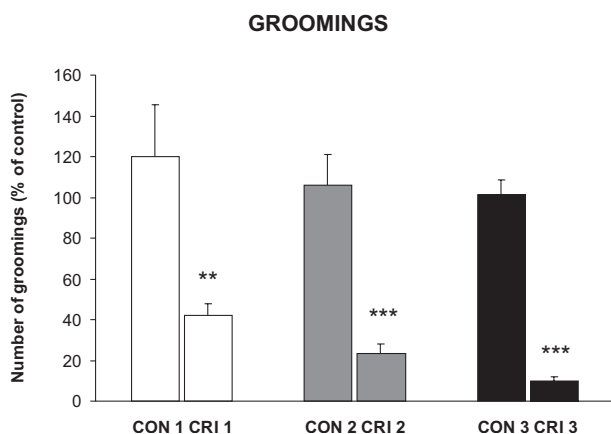


Fig. 2. The effect of CRI on the number of groomings in the open field. CON 1, 2, 3 – control groups for moderate, severe 1 and severe 2 CRI; CRI 1 – moderate chronic renal insufficiency, CRI 2 – rats with severe chronic renal insufficiency tested 1 month after surgery and CRI 3 – rats with severe chronic renal insufficiency tested 2 months after surgery (experimental details are described in Materials and Methods). Values are presented as means \pm SEM, significance of the difference in comparison with the control group: ** $p < 0.01$, *** $p < 0.001$

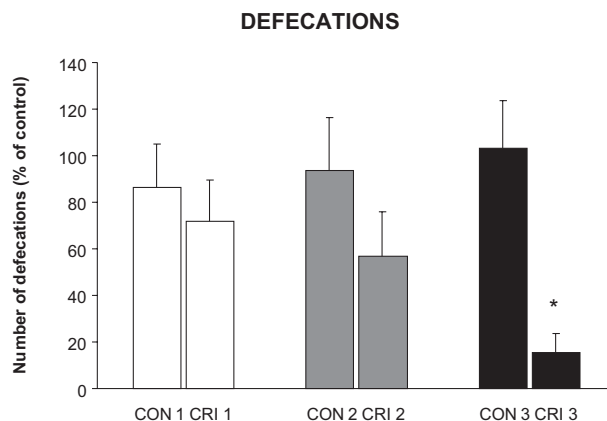


Fig. 4. The effect of CRI on the number of defecation in the open field. CON 1, 2, 3 – control groups for moderate, severe 1 and severe 2 CRI; CRI 1 – moderate chronic renal insufficiency, CRI 2 – rats with severe chronic renal insufficiency tested 1 month post surgery and CRI 3 – rats with severe chronic renal insufficiency tested 2 months post surgery (experimental details are described in Materials and Methods). Values are presented as means \pm SEM, significance of the difference in comparison with the control group: * $p < 0.05$

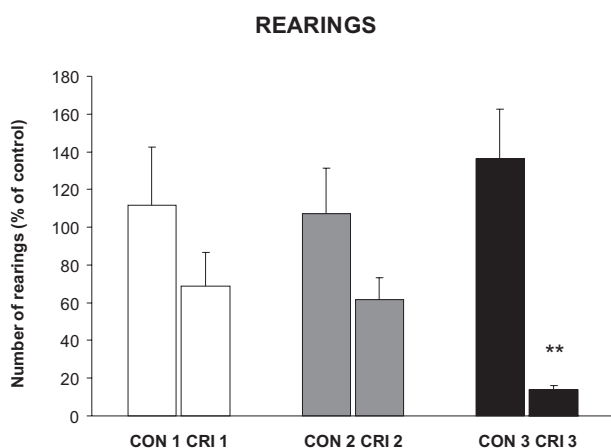


Fig. 3. The effect of CRI on the number of rearings in the open field. CON 1, 2, 3 – control groups for moderate, severe 1 and severe 2 CRI; CRI 1 – moderate chronic renal insufficiency, CRI 2 – group with severe chronic renal insufficiency tested 1 month after surgery and CRI 3 – group with severe chronic renal insufficiency tested 2 months after surgery (experimental details are described in Materials and Methods). Values are presented as means \pm SEM, significance of the difference in comparison with the control group: ** $p < 0.01$

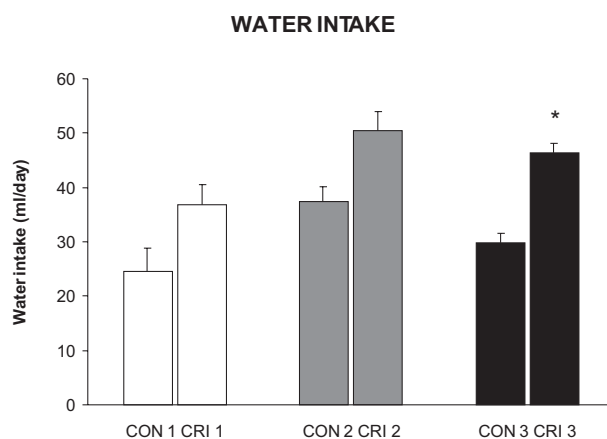


Fig. 5. The water intake of rats with CRI. CON 1, 2, 3 – control groups for moderate, severe 1 and severe 2 CRI; CRI 1 – moderate chronic renal insufficiency, CRI 2 – group with severe chronic renal insufficiency examined 1 month post surgery and CRI 3 – group with severe chronic renal insufficiency examined 2 months post surgery (experimental details are described in Materials and Methods). Values are presented as means \pm SEM, significance of the difference in comparison with the control group: * $p < 0.05$

ing from severe CRI, no change in water intake was observed 1 month after the surgery, but 2 months after the CRI induction, it was increased. A marked decrease in food intake was observed in the severe CRI independently of a duration of the renal insufficiency.

Body weight

The rats with moderate CRI have shown no difference in body weight in comparison to the control animals. The weight of the rats suffering from CRI 1 and 2 months after the surgery was signifi-

cantly lower than in a corresponding control groups (Fig. 7).

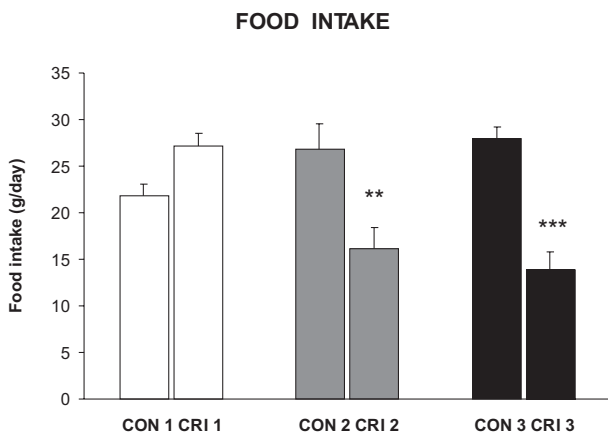


Fig. 6. The food intake in rats with CRI. CON 1, 2, 3 – control groups for moderate, severe 1 and severe 2 CRI; CRI 1 – moderate chronic renal insufficiency, CRI 2 – rats with severe chronic renal insufficiency examined 1 month after surgery and CRI 3 – rats with severe chronic renal insufficiency examined 2 months after surgery (experimental details are described in Materials and Methods). Values are presented as means \pm SEM, significance of the difference in comparison with the control group: ** $p < 0.01$, *** $p < 0.001$

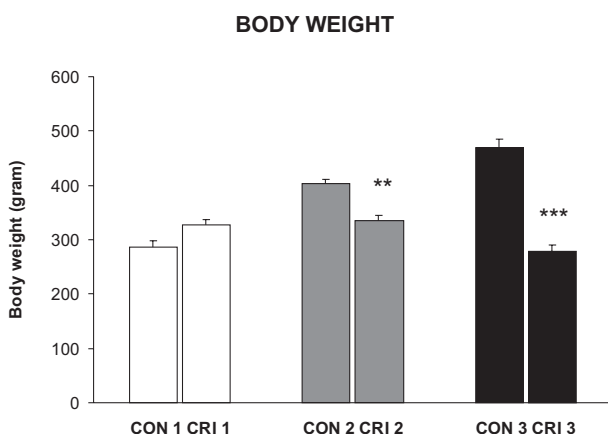


Fig. 7. The body weight of rats with CRI. CON 1, 2, 3 – control groups for moderate, severe 1 and severe 2 CRI; CRI 1 – moderate chronic renal insufficiency, CRI 2 – group with severe chronic renal insufficiency tested 1 month after surgery and CRI 3 – group with severe chronic renal insufficiency tested 2 months after surgery (experimental details are described in Materials and Methods). Values are presented as means \pm SEM, significance of the difference in comparison with the control group: ** $p < 0.01$, *** $p < 0.001$

DISCUSSION

In our model of the progressive renal failure, we observed the changes similar to those observed

in the patients suffering from CRI, i.e. an increase in plasma creatinine and urea concentration proportional to the extent of a damage to renal tissue and the period of observation. We have also noticed the reduction in body weight, food intake and increase in water intake. Our experiments have also shown that behavioral changes in CRI rats manifest as the decrease in locomotor, exploratory and emotional activity. Similar symptoms of chronic renal failure have been observed in humans [13, 22, 31]. A mechanism of these disturbances is complex and not fully understood. Although patients with uremia may have symptoms attributable to anemia, myopathy, and bone disease or to other intercurrent diseases, most of the behavioral disturbances seem to be of central origin [9–11]. This suggestion is supported by our previous experiments, in which no changes were observed in coordination measured on rota-rod between healthy and CRI rats (unpublished data).

In people with CRI, a decrease in motor functions was observed [10]. It has also been reported that the patients with uremic encephalopathy exhibit a disturbance of the central nervous system neurotransmitters. Lam et al. [19] and Kinniburgh and Boyd [17] have reported an increased level of octopamine, a false neurotransmitter in the human brain. Ali et al. [2] demonstrated a significant depletion in the norepinephrine, epinephrine and dopamine levels in the brain of rats with acute and chronic uremia. On the contrary, the concentration of catecholamines (CA) in plasma was enhanced. Defects in catecholaminergic neurotransmission, being an evidence of dysfunction in the autonomic nervous system, may contribute to the development of neuropathy [2]. We cannot exclude that one of the reasons of locomotor, exploratory and emotional activity decrease in CRI rats is an inhibition of catecholaminergic transmissions in the brain.

Pharmacological studies have suggested an involvement of 5-HT_{1B}, 5-HT_{1A} and 5-HT_{2C} receptors in the control of locomotor activity [8, 30]. Thus, we should take under consideration the role of serotonergic neurotransmission in behavioral changes observed in CRI. Some symptoms of serotonergic types of neuronal dysfunctions, such as insomnia, hypothermia and altered mentation, have been noted in patients with uremia [28]. Therefore, some authors have intensively investigated tryptophan (TRY) and serotonin (5-HT) concentration in the CNS in CRI rats. In the brain, however, the

concentration of TRY and 5-HT remains unaltered but the 5-HT turnover rate is significantly accelerated although the magnitude of this increase is relatively small [28]. An increased brain 5-HT turnover in CRI with the accumulation of its metabolite 5-hydroxyindoleacetic acid (5-HIAA) may be involved also in the modulation of locomotor, exploratory and emotional activity.

The influence of a diet on plasma TRY level, brain concentrations of TRY, 5-HT and 5-HIAA in chronic uremic rats was investigated by Siassi et al. [29]. They have shown that the abovementioned parameters depend on protein intake.

The biochemical studies showed the changes in brain 5-HT and TRY concentrations, which play a key role in the regulation of eating behavior both in experimental animals and in humans [26]. Thus, the reduction of food intake observed in our experiments could be explained by the enhanced 5-HT synthesis in the brain [26]. Nevertheless, besides CA and 5-HT, other factors like cholecystokinin, glukagon and opiates can also be involved in the observed effect of uremia [7, 21].

We have also shown that the body weight was significantly diminished in CRI animals as compared with sham-operated animals which was associated with a decreased food intake. Our observations are in line with the experiments of Mehls et al. [23], who demonstrated that decreased intake of food was the major determinant factor of body weight in experimental renal failure. The most probable explanation of the decreased body weight is a loss of appetite due to a rise in toxic factors [3]. Enhanced water intake in severe CRI may be explained by an increase in plasma urea concentration that caused an increased osmolality. High osmolality have been shown to stimulate osmoreceptors in the hypothalamus, leading to polydipsia, and in turn to increased total body water and extracellular water content [1, 12]. Barrow and Lenconte [6] have shown that there is no influence of body weight on the open field activity. Thus, we can exclude the possibility that the decreased activity of rats suffering from CRI is associated with a loss of body weight. The literature data have demonstrated that food intake is regulated, among others, by CA and 5-HT [7].

In conclusion, this study shows that disorders in behavior of rats with experimental CRI are similar to those occurring in the patients suffering from chronic renal disease. Many of these disturbances

might have been caused by the changes in the biochemical parameters in the brain. The experimental model of CRI described by us seems to be a good tool for pharmacological studies.

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