The effects of intracerebroventricular injection of ascorbic acid on feeding behavior in male rats

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Abstract

Introduction:
Several factors such as glutamate and dopamine affect appetite, food and water consumption. Recent research has shown that the release of these two neurotransmitters from their relevant neurons is involved in the central ascorbic acid (AA) release, and the released ascorbic acid can modulate their effects. In the present study, we evaluated the effects of intracerebroventricular injection of ascorbic acid on food consumption in male rats at light phase.

Materials and Methods:
For this purpose, 50 NMRI rats weighing about 250-300 g were selected randomly and divided into 5 equal groups including control, sham (ascorbic acid solvent), and 25, 50 and 100 µg/10 µl ascorbic acid. First, the animals were annulated (within the right ventricle of the brain). Seven days after the recovery period, all the drugs were injected into the right brain ventricle, after 12 h of food deprivation. Then, the animals were put in the metabolic cages equipped with computer software and the number of container reference and food intake was measured. The amounts of food consumption every 60 minutes and for about 4 hours after injection were recorded.

Results:
The findings showed that intracerebroventricular administration of ascorbic acid decreased food intake at all three doses, which were important in dosage of 25 µg (P< 0.05). Also, the number of container reference was reduced in ascorbic acid groups compared to the control group. All three doses of ascorbic acid also reduced food intake. This reduction was significant only in the 100 µg ascorbic acid group (P<0.05).

Conclusion:
Findings show intracerebroventricular injection of ascorbic acid affects the animals’ feeding and its effect occurs possibly through interference with the brain chemical mediators, particularly dopamine and glutamate. The cellular involved mechanism must be identified with molecular research.

Keywords: Ascorbic acid, Feeding Behavior, Rat

Introduction:
Nutrition control includes a complex set of physiological mechanisms at different regulating levels that involves different areas of brain and some loci outside the central nervous system. Several factors such as hormones, neural mediators and modulators, pathways, nuclei, autonomic system and metabolic needs have a role in this phenomenon. Despite extensive research in the past decades, there is still a lot to learn about nutritional mechanism.
Most of the information in this regard about mammals has come from studies on rats (1). One of the most effective systems on nutrition is dopamine system. It has been shown that prescribing drugs which stimulate this system decreases appetite and food intake in male rats (2). In the cancers characterized by anorexia, dopamine receptors gene expression increases in nutrition control areas and dopamine receptors of D1 and D2 are more important in this regard (3). It has been also reported that prolonged dopamine cycle, increased release of this neurotransmitter from dopaminergic terminals of brain and increased activity of dopaminergic neurons in limbic system and in striatum cause increased locomotor activity, euphoria and stereotyped behaviors in animals (4, 5).

Glutamate system is another system which influences nutrition. It has been demonstrated that prescription of drugs stimulating this system affects appetite and food consumption in mammals. For instance, glutamic acid injection into hypothalamus in the sheep increases food consumption 30 min after injection, which seems to be generated either directly or through changing into GABA neurotransmitter (6).

Ascorbic acid is an antioxidant vitamin which is abundantly found in brain of mammals and plays an important role in modulating these two nervous systems and modifying behaviors of animals (7). Ascorbic acid is released from glutaminergic neurons and modulates activities of both glutaminergic and dopaminergic systems so that its low concentration stimulates these two systems and its high concentration inhibits them (8).

Also, ascorbic acid slows dopamine oxidation and as a result hinders its rapid removal from the synapses (9). Considering the importance of these two glutaminergic and dopaminergic systems in regulating nutrition and the effect of ascorbic acid on them, the present study aimed to investigate the effect of central injection of ascorbic acid on the rate of food intake in male rats during light phase.

**Materials and Methods:**

In this experimental study, 5 male Anne Marie rats weighting 250-300 g were used. They were kept at 23 °C with adequate ventilation and 12 h light/dark cycle. They had free access to food and water. The animals were randomly divided to five groups with equal numbers: these groups included a control, a sham-operated (cannula + ascorbic acid solvent), 25 mcg / volume of 10 Ml ascorbic acid, 50 mcg / volume of 10 Ml ascorbic acid, 50 mcg / volume of 10 Ml ascorbic acid and 100 mcg / volume of 10 Ml ascorbic acid.

The rats were anesthetized by intraperitoneal administration of ketamine-xylazine mixture. Then, using stereotaxis, a sterile guide cannula (No. 23) was placed in the right ventricle of their brains. To find it, a Paxinos stereotaxic atlas with coordinates of AP=-0.92, DV=3.5 and ML=1.6 was used. The cannulas were inserted into the skull and fixed using dental cement. After that, the rats were rested for one week and then the experiment started. Drugs were administrated using Hamilton syringe through a polyethylene flexible pipe and a stainless steel injection cannula (No. 27) which was 1.5 mm below the guide cannula at the time of injection.

In order to get accustomed to the environment, the rats were transferred to the experiment site 12 h before the experiment. The animals were not fed during this time. Before starting the experiments, the samples were weighted by an accurate scale and the drugs were intracerebroventricularly injected. The rats were kept for four hours in the metabolic cages with containers of weighed water and food which were equipped with computer software. The number of their access to food container and rate of food consumption were recorded every hour. In
the end, the rats were anesthetized and beheaded and a cut of their brain was provided to determine the exact site of injection. If the drug was injected outside the ventricle, the sample was excluded from the study.

The obtained data from different experimental treatments were compared and analyzed using one-way ANOVA and then Tukey test. P value of less than 0.05 was considered significant.

**Results:**
The effect of intracerebroventricular administration of ascorbic acid on the number of access to food container and rate of food consumption in male rats was studied in the present paper. The results showed no significant difference between control and sham-operated groups. Comparison of the effect of ascorbic acid on the access to food container during the four-hour experiment revealed that all three doses of ascorbic acid decreased the number of access to food container, but this difference was not significant compared with the control and sham-operated groups (Fig. 1). Additionally, all three doses of ascorbic acid decreased food consumption, showing a significant difference only in 25 mcg ascorbic acid group (p<0.05) (Fig. 2). Also, all three doses of ascorbic acid decreased the number of access to food container at each hour compared with the control and sham-operated groups, with a significant difference at the first hour in the 25 mcg ascorbic acid group (p<0.05) and at the second hour in the 100 mcg ascorbic acid group (p<0.01) (Fig. 3). These three doses of ascorbic acid also decreased food consumption. This decrease was significant only at the second hour in the 100 mcg ascorbic acid group (p<0.05) (Fig. 4).

![Fig. 1: Comparison of access to food container in different groups during 4 h of experiment](image)

The effects of intracerebroventricular injection of ascorbic acid on the number of access to food container and rate of food consumption in male rats was studied in the present paper. The results showed no significant difference between control and sham-operated groups. Comparison of the effect of ascorbic acid on the access to food container during the four-hour experiment revealed that all three doses of ascorbic acid decreased the number of access to food container, but this difference was not significant compared with the control and sham-operated groups (Fig. 1). Additionally, all three doses of ascorbic acid decreased food consumption, showing a significant difference only in 25 mcg ascorbic acid group (p<0.05) (Fig. 2). Also, all three doses of ascorbic acid decreased the number of access to food container at each hour compared with the control and sham-operated groups, with a significant difference at the first hour in the 25 mcg ascorbic acid group (p<0.05) and at the second hour in the 100 mcg ascorbic acid group (p<0.01) (Fig. 3). These three doses of ascorbic acid also decreased food consumption. This decrease was significant only at the second hour in the 100 mcg ascorbic acid group (p<0.05) (Fig. 4).
The effects of intracerebroventricular injection of...  

**Fig. 2:** Comparison of food consumption in different groups during 4 h of experiment  
\[ n=10, \text{ mean}\pm\text{SEM}, \,* P< 0.05 \]

**Fig. 3:** The number of access to food container in different groups in terms of hours after injection  
\[ n=10, \text{ Mean}\pm\text{SEM}, \,* P< 0.05, \,** P< 0.001 \]
Discussion:
Based on the findings of the present study, intracerebroventricular injection of ascorbic acid in all three doses decreased both the number of access to food container and the rate of food consumption in male rats compared with the control and sham-operated groups. However, these changes were not dose-dependent and dose of 25 mcg ascorbic acid had a more reducing effect. The results also showed that central injection of ascorbic acid had a significant impact on nutritional behavior of rats. No report has been published on the effect of ascorbic acid on nutrition so far to be compared with the results of the present study. Cellular mechanism of the effect of ascorbic acid on brain and nutritional behavior of animals has not been clearly known yet. Ascorbic acid is an essential vitamin for body which is abundantly found in brain of mammals and plays an important role in modifying behaviors of animals. Despite the high concentration of ascorbic acid in the brain, only few of its functions have been identified so far (6). Ascorbic acid is released from glutaminergic neurons and modulates activities of both glutaminergic and dopaminergic systems (4, 8). According to the existing findings, one of the main mechanisms of the effect of ascorbic acid is probably its influence on activity of the brain’s dopamine system. There are many reasons confirming that ascorbic acid prolongs the life cycle of dopamine in many parts of brain. Ascorbic acid also slows dopamine oxidation and hinders its rapid removal from the synapses (9). Evidence has suggested that dopaminergic system is considered an important factor in appetite regulation and dopamine receptors of D1 and D2 are more important in the control of nutrition. Prescription of agonists of these two receptors reduces food intake in hungry rats (10, 11). Dopamine effects on nutritional behavior of animals can be imposed either directly or by reducing the secretion of prolactin and peptide Y, both of which are nutritional stimulators (12). It has been reported that impairment in striatum dopamine signaling system causes changes in food intake and appetite of rats (13, 14). Therefore, it can be said that intracerebroventricular injection of ascorbic acid in the present study could probably strengthen the inhibitory effect of...
Glutamate affects excitatory and inhibitory motor neurons involved in the nutrition control (15). It has been proven that stimulation of NMDA glutamate receptors of post-synaptic neurons produces nitric oxide and this neurotransmitter which is easily spread to the surrounding would have several impacts on the nervous system. Nitric oxide can cause changes in the activation of dopamine system. For example, nitric oxide is capable of reducing dopamine re-absorption at synapses through inhibiting re-absorbing proteins (16), increasing the amount of dopamine release in different parts of brain (17) and enhancing the dopaminergic system (18, 19). Hence, it seems that stimulation of the brain's glutaminergic system by ascorbic acid could activate the production cycle of nitric oxide while influencing dopamine system in terms of nutrition. In addition, brain's glutaminergic system itself can directly intervene in the reduction of food intake. For instance, it has been reported that the inhibitory effect of cholecystokinin on nutrition and satiety is mainly performed through activation of NMDA glutamate receptors, especially in the nuclei of isolated pathway of hindbrain. Also, it has been observed that inhibition of glutamate activity of ventral region of the accumbans nucleus can act as a nutritional stimulator (20, 21). On the other hand, since ascorbic acid was administrated centrally into the lateral ventricle of brain in the present study, it was consequently able to affect all parts of brain. Hence, direct impacts of ascorbic acid on nutritional control centers, metabolism of neurons or other brain neurotransmitters can be ignored. Probably, a part of the effects of ascorbic acid is mediated through interfering with other neural mediators of brain (12). Further studies are needed to confirm or reject this hypothesis.

**Conclusion:** The results of the present study showed that intracerebroventricular injection of ascorbic acid could lead to a significant reduction in nutrition times and rate of food consumption.

**References:**