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BRIEF COMMUNICATION

The Effect of Nitrites on Isolation-Induced Aggression in Mice

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GRUENER, N. *The effect of nitrites on isolation-induced aggression in mice.* PHARMAC. BIOCHEM. BEHAV. 2(2) 267–269, 1974. – Chronic administration of sodium nitrite (1 g/l) in drinking water of pregnant mice and their offspring caused a significant increase in the isolation-induced aggression of the male young. The cessation of administration of sodium nitrite reduced the aggressive behavior of the experimental group to the control level.

Nitrites Isolation Aggression

THE INTEREST in the effect of environmental agents on behavior is on the increase as a growing segment of the population is exposed to such agents. Nitrates and nitrites are frequently ingested in drinking water, natural food such as vegetables, and in processed foods when food additives have been utilized. One of the complications following excessive nitrate and nitrite intake is methemoglobinemia. This disease is characterized by decrease in the oxygen transport capacity of the blood. Nitrite which is the direct toxic agent can oxidize hemoglobin to methemoglobin which is biologically inactive. Nitrates can be reduced to nitrites mainly by microbiological activity in the food or in digestive systems. Infants in the first months of life are especially sysceptible to methemoglobinemia from these salts [3].

The question of the health implications at the subclinical level of exposure to nitrates and nitrites is of considerable importance, because detection of symptoms at such levels is difficult and thus preventive action is not likely to be taken.

We have already shown [2] that $C_{57}B1$ mice, that have been given sodium nitrite in their drinking water, showed an increase in methemoglobin level. The average was 1.2% (% of total hemoglobin as methemoglobin) at a nitrite concentration of 1 g/l (control mean level 0.6%) and 10.1% of methemoglobin at 2 g/l of nitrites. Levels of 10% or higher of methemoglobin are considered clinically significant. In the same report we have shown that nitrites reduced the motor activity of the mice but only at the highest concentration (2 g/l).

In the following communication, we show that nitrites cause a significant increase in the aggression of mice.

METHOD

Ten $C_{57}B1$ female mice were mated with males of the same strain. Each couple was placed in a separate cage. Five couples were given a sodium nitrite solution (1 g/l in tap water) for their drinking water and the other five couples drank tap water. At birth, the adult males were removed from the cages and the newborn mice were kept with their mothers for the next 21 days. The mothers who drank $NaNO_2$ during gestation continued to receive the same solution through the nursing period.

The young were weighed twice a week through the nursing period. At weaning (after 21 days) 12 young males from each group were randomly chosen and each was placed in a separate cage and isolated for 8 weeks. The nitrite group continued to get the nitrites at the same level (1 g/l) throughout the isolation period, and for the first 5 weeks of the behavioral tests.

At the end of the isolation period the mice were tested for isolation induced aggression. The rating scale of scoring aggressiveness used here was essentially the same as described by Banerjee [1]. Each confrontation between 2 mice was allowed to take place for 10 min in a square fighting cage, and the behavior was rated according to a predetermined aggression scale with maximum score of 20.

Each animal was exposed to another animal once a week, for 6 weeks; 4 of the 6 sessions were intra-group confrontation and 2 were extra-group confrontations. There was no set order for these confrontations because the order might influence the results of the confrontations.

After 6 weeks the experimental animals were taken off

the nitrite solution and were given tap water to drink. After a break of 2 weeks the tests were resumed and the same first 4 sessions were repeated. During the latter period all the animals drank tap water.

RESULTS AND DISCUSSION

The results of the confrontation sessions are presented in Tables 1 and 2. The cumulative score for each group was averaged over all the sessions. Table 1 shows that there was an increase in the aggressive behavior of the treated group particularly where this group met the controls. At these sessions, the controls had a significantly low score. Returning the experimental group to regular tap water was accompanied by a decrease in aggression to the level of the control group which did not change during this time. The simultaneous increase in the mean score of the water group when exposed to the previous nitrite group shows that the score for the individual in each session is not independent of its opponent.

Several reports have recently described the various effects of nitrites and nitrates on the central nervous-system [4, 5, 6]. However, no suggestion has been made regarding the site(s) or mode of action of these ions in the tissue. This study hints that at least the behavioral effect may be reversible and that it disappears after a short interval. Elucidation of the mechanisms and significance of this aggression phenomenon might help in the evaluation of the risks of exposure to nitrates and nitrites.

TABLE 1

THE EFFECT OF SODIUM NITRITE ON AGGRESSION IN MICE

Group	Number of Confrontations	Mean Aggression Score	S.D.	p*
1. H_2O – intra	44	9.01	3.14	–
2. H_2O – extra	22	5.68	3.55	0.01
3. $NaNO_2$ (1 g/l) – intra	38	10.88	4.68	0.05
4. $NaNO_2$ (1 g/l) – extra	22	12.95	3.66	0.01

*The differences in the mean aggression score between Group 1 and Groups 2, 3 and 4, are analyzed statistically according to the t-test.

Intra – sessions in which mice were confronted with mice from the same experimental group.

Extra – sessions in which mice exposed to others belong to the other experimental group.

TABLE 2

AGGRESSION OF MICE AFTER RETURNING TO TAP WATER

Group	Number of Confrontations	Mean Aggression Score	S.D.	p*
1. H_2O – intra	32	8.97	5.00	–
2. H_2O – extra	15	8.77	5.94	N.S.
3. $NaNO_2$ (1 g/l) – intra	28	9.43	5.31	N.S.
4. $NaNO_2$ (1 g/l) – extra	15	9.50	5.20	N.S.

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