RNA, water consumption and toxicity¹

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Two studies were done. The first dealt with the effect of RNA on water consumption under deprivation schedule, and it was found that RNA animals consume less water under those conditions than do saline control animals. It was also observed that RNA-treated animals in this experiment became weaker. The second experiment dealt with the effects of RNA on health under conditions of food deprivation. A highly significant interaction between RNA injection and food deprivation was found. All food deprived animals treated with RNA died. None in the other three groups seemed in any way affected. These results are discussed in terms of possible effects of RNA on digestion and evaluated in relation to studies where RNA is to be administered to humans.

The first purpose of this research was to find the effect of injections of yeast Ribonucleic Acid (RNA) on water consumption under conditions of water deprivation in order to duplicate conditions of other experiments (Solyom, Beaulieu, & Enesco, 1966; Solyom, Enesco, & Beaulieu, in press), where water was used as a reinforcement. Corson & Enesco (1966) reported that less water was consumed under ad lib conditions by RNA injected animals while the studies involving water deprivation showed a larger quantity of water consumed during each drinking period. In these latter studies, water was used as reinforcement for a bar pressing response and led to general conclusions concerning learning ability. The results of Corson & Enesco (1966), however, suggest that the motivation for water per se may be influenced by RNA injections. This study is an attempt to clarify this issue. The effect of a single ECS on water consumption was also investigated in relation to a previous experiment (Beaulieu, 1966) which suggested that ECS might interact with RNA to increase rate of water consumption under conditions of water deprivation. The second purpose of the research was to find out if food deprivation would interact with RNA in influencing the S's physiological state. In order to maximize any such effects, the amount of RNA injected per kilogram body weight was double that used in most studies dealing with RNA and learning.

EXPERIMENT I

Subjects

Eighteen male hooded rats were the Ss of this study, nine in each group. The yeast RNA was obtained from Pabst Laboratories; 10% neutral solution and prepared by the addition of 16 M NaOH to pH7. An amount of 320 mg/kg (body weight) of RNA was injected intra-peritoneally every day for approximately 3 months before the beginning of this study as well as throughout the study. An equivalent volume of saline (1 cc) was daily injected into the control Ss.

Apparatus

The animals were tested in a 9 in. x 14 in. wooden box with one Plexiglas wall and a grid floor with a water spout protruding from one wall. The spout was fitted by means of a rubber sleeve to a standard 10 ml laboratory pipette calibrated to 0.1 ml, with its tip cut off to permit easy water flow. The water was held in the pipette by means of a thin rubber stopper at the top end.

Procedure

Each animal was given access to water in the box for 5 min., at the end of which total amount of water consumed was read directly from the calibrated pipette. This was done once each day and Ss had no other access to water. On day 15, immediately following their daily drinking session, all animals were given an electroconvulsive shock of 0.5 sec. at 50 mA via alligator clips attached to each ear. Full convulsions were induced in all animals.

Results and Discussion

Mean water consumption is shown in Fig. 1 and indicates that the saline animals consistently drink more than the RNA animals. Analysis of variance reveals this difference to be significant at the .01 level ($F=13.82$, $df=1/16$, $p<.01$). Administration of ECS did not appear to produce any changes in drinking behavior. If there are changes, they are certainly not of the magnitude encountered in the previous study (Beaulieu, 1966).

Quantity of water consumed does not appear in this study to be very stable, fluctuating as much as 40% between sessions. This alone may account for

Fig. 1. Mean water consumption (in mls) for saline and RNA-treated animals, in blocks of 3 days.

the apparent increase in water consumption after an ECS following I trial traumatic avoidance learning in the previous study. In any event, it seems that no serious interaction takes place between RNA and ECS in terms of water consumption.

It is, however, quite clear that RNA-treated animals drink significantly less than saline animals which is in accord with the previously mentioned findings of Corson & Enesco (1966). This result is interesting in view of the fact that in previous studies using water reinforcement it was found that RNA-injected animals pressed more for water reinforcement than saline animals, though deprivation was more severe in the present study. It seems possible that if motivation had been equal in the two groups, the difference in the rate of bar pressing would have been even larger.

It was also incidentally observed that the RNA animals seemed weaker than the saline animals under these conditions of water deprivation. It seems likely that this is due to the interaction of water deprivation with the effects of RNA injections, rather than to the injections of RNA alone, since Corson & Enesco (1966) found no difference in tests of strength or endurance between saline and RNA injected groups which were not water deprived.

EXPERIMENT II

Subjects

Fourty male hooded rats were the Ss of this study, 10 in each of the four groups of a factorial design having RNA versus saline orthogonal to deprivation versus no deprivation. Assignment to groups was random. Injection procedures were the same as for Experiment I, except that the animals were injected daily in the late afternoon for approximately two weeks before onset of deprivation, and daily during the two weeks of deprivation. The Ss were kept in individual cages at all times.

Procedure

The non-deprived animals had free access to food and water at all times. The deprived animals had access to water at all times but were given only one or two pellets of Purina Chow a day, in an attempt to reduce their body weight to 75% of their ad lib body weight within a week, and to maintain it at this level. This schedule was terminated when all animals in the group injected with RNA and deprived of food had died.

Results

In the last three days of the two week schedule of food deprivation, all deprived animals treated with RNA died. The symptoms at the time of death consisted of sudden onset of weakness (the animal being unable to remain on his four legs), as well as a complete disinterest in food. No animal in the three remaining groups showed any sign of ill health, regardless of whether they received RNA or whether they were deprived. The interpretation of these results is that a normally safe dosage of RNA can be lethal when combined with a normally safe food deprivation schedule.

DISCUSSION

Evidently RNA is a potentially lethal substance when combined with poor dietary conditions. These results should be considered before RNA is administered to humans, especially to aged individuals who frequently have poor eating habits. These results suggest that it would be useful to investigate the effects of RNA on digestion, as it was found that the food deprived RNA-treated animals lost weight at a significantly slower rate than control animals. Other results implicating a difference in digestion are those of Corson & Enesco (1966) who found that RNA-injected animals under ad lib feeding and drinking conditions gained weight at a slower rate than saline-injected controls. These problems warrant further investigation in view of the possible dangers of RNA therapies. It is also possible that research on these aspects of the effect of injections of yeast RNA could clarify some of the confusion concerning the effect on learning and memory functions.

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