Pharmacology Biochemistry and Behavior, Vol. 1, pp. 739–741. ANKHO International Inc., 1973. Printed in the U.S.A.

BRIEF COMMUNICATION

Alcohol Ingestion Following Intracerebral Angiotensin Administration and Water Deprivation

DAVID A. JOHNSON AND ROBERT P. ANDERSON

Physiological Psychology Laboratory, Department of Psychology
San Francisco State University, San Francisco, California, 94132

(Received 3 July 1973)

JOHNSON, D. A. AND R. P. ANDERSON. *Alcohol ingestion following intracerebral angiotensin adminstration and water deprivation.* PHARMAC. BIOCHEM. BEHAV. 1(6) 739–741, 1973.–Rats which drank a significant amount of water when angiotensin was injected into the septum were allowed to choose between 4, 8 and 12% ethanol following angiotensin administration and following 24 hr water deprivation. Ethanol intake was similar under both conditions. The results indicate that drinking following central angiotensin adminstration has some of the motivational properties of normal thirst.

Angiotensin Septum Thirst Ethanol consumption

COPIUS drinking in the rat can be elicited by central adminstration of carbachol or angiotensin [4,8]. Research on the basis of this behavior has focused on two related questions: (1) whether these compounds act on the same neural substrates [12] and (2) whether these agents produce drinking behavior by activating a motivational system which is involved in water deprivation [5, 6, 10].

The neural substrates activated by carbachol or angiotensin stimulation are probably proximal though not identical. White *et al.* [12] reported that 75% of the drinking sites they found responded to both carbachol and angiotensin, while the other 25% responded to only one of these agents. Furthermore, optimally effective doses of angiotensin had on the average a shorter drinking latency than optimal carbachol doses: 134 sec as opposed to 309 sec [12]. Also, centrally or peripherally administered anticholinergic agents at doses which suppress carbachol drinking had little or no effect on angiotensin elicited drinking [2,12].

Some evidence supports the view that carbachol has motivational properties similar to those of water deprivation [8], but most work suggests that they are different. Specifically, Franklin and Quartermain have shown that carbachol injected rats bar pressed for water less than water deprived rats; though, they all drank the same amount when water was freely available [5]. Moreover, the intake of alcohol [3], sucrose [6], and quinine [5] obtained following carbachol injection differed markedly from the intake after water deprivation. Centrally applied angiotensin, on the other hand, has many of the same motivational properties as normal thirst. Rats injected with angiotensin bar pressed for water as much as rats 24 hr water deprived [10]. Additionally, the intake of saline [12] and quinine [10] were the same following both angiotensin adminstration and water deprivation.

While 23 hr water deprived rats will drink 4, 8, and some 12% alcohol solution, Cicero and Myers [3] have shown that carbachol injected rats drank little if any alcohol. The purpose of this study is to investigate further the difference between the motivational properties and possible neural substrates activated in angiotensin and carbachol elicited drinking by comparing the choice behavior toward different concentrations of alcohol following angiotensin administration and water deprivation.

METHOD

Eleven male and female Long-Evans rats weighing between 310–500 g at the beginning of the experiment were used in this study. The animals were individually housed in hanging cages and, except for test periods, were allowed free access to food and water. All animals were stereotaxically implanted under Nembutal anesthesia (50 mg/kg) with cannulae aimed to terminate just above the septum (De Groot coordinates: AP 7.8, L 0.5, and D + 4.0). The animals were allowed at least one week to recover from surgery. Cannulae were similar to those of Russell *et al.* [11]. The cannula system consisted of the guide cannula, constructed of a 23 ga. hypodermic needle and a series of five injector cannulae (30 ga.) which, in increments of 0.5 mm, ranged from 1.0–3.0 mm longer than the guide cannula.

Injections of 1 μl containing 0.1 μg of angiotensin (Hypertensin, CIBA) dissolved in 0.9% NaCl were made using the injector canulla connected to a 100 μl Hamilton

microsyringe via a polyethylene tube (PE 10). The microsyringe was mounted to a Kopf No. 1208 Micro-Injection Unit which allowed for a more accurate control of the volume injected.

Testing Procedure

Each rat was tested for sensitivity to angiotensin in a preliminary screening session. A criterion of 4.0 ml of water consumed within 30 min after the injection was used to determine the drinkers. The animals were first tested with an injector cannula which projected 1.0 mm beyond the tip of the guide cannula. Those rats which did not meet the criterion were allowed free access to water for 24 hr and then reinjected with the 1.5 mm injector cannula. This procedure was continued with the 2.0, 2.5, and 3.0 mm injector cannulae until the criterion was met. Once the criterion was met the size of the injector cannula was noted and was not changed for that animal for the remainder of the experiment. Eighteen animals were initially implanted and tested but only eleven met all the criterion.

Two groups were formed and each was tested under two conditions with ethanol: (1) 24 hr water deprivation and (2) angiotensin stimulated. Group 1 (N = 6) was tested with angiotensin followed by water deprivation. The order for Group 2 (N = 5) was simply reversed. A final screening session with angiotensin was preformed to determine that the rats drank to the established criterion. Finally the animals were tested to determine the amount of water drunk following 24 hr fluid deprivation. All test sessions and screening sessions were separated by a 48 hr interval. The screening sessions took place in the animal's home cage, whereas the test sessions took place in a cylindrical Plexiglas cage (12 in. dia.) in which the three alcohol solutions were available from three no drip spouts, connected to inverted 50 ml burettes, equally spaced about the perimeter of the cage. This method was used to minimize the effects of position habit.

Water Deprived Condition

Twenty-four hr before each session the rats were deprived of all fluids. Prior to placement into the cylindrical cage each rat was given a mock injection in which the stylette plug was removed but no solution was injected. Once in the cylindrical cage the rat was allowed to select from three alcohol solutions: 4, 8, and 12%. At the end of 30 min the amount of each solution consumed was recorded. The animal was returned to his home cage and provided with free access to water.

Angiotensin Condition

Each animal was injected with 0.1 μg of angiotensin, then placed in the cylindrical cage and allowed to select from the 4, 8, and 12% alcohol solutions. At the end of 30 min the amount of each solution consumed was recorded and the animal was returned to his home cage and given free access to water.

Histology

After completion of the experiment, each rat was injected with an overdose of Nembutal and perfused with 10% Formalin. The brains were removed, frozen, and cut into 50 60 μ sections. Every third section was stained with thionine and cannulae sites were identified with the aid of the Pelligrino and Cushman atlas [9].

RESULTS

Since there was no difference between Group 1 and Group 2 in the initial screening sessions and in the alcohol test sessions, their data were combined. The results of these experiments are shown in the Table. Although the rats in the 24 hr water deprivation condition drank slightly more alcohol than under the angiotensin condition, their alcohol intake was essentially the same. There was no significant reduction of water intake between the two angiotensin screening sessions.

Histological examinations showed that the cannula tips were resting in the medial septum (7 rats), the lateral septum (3 rats), and the lateral pre-optic area (1 rat). No systematic differences in drinking behavior were found between these three areas.

TABLE 1

MEAN INTAKE (± SD) OF WATER AND OF ALCOHOL SOLUTION AFTER WATER DEPRIVATION AND ANGIOTENSIN

	Thirst Stimulus	
Condition	Angiotensin (ml)	Deprivation (ml)
Initial Water Test	9.0 ± 4.5	
Alcohol (%)		
4	2.6 ± 3.5	2.6 ± 3.4
8	0.4 ± 0.6	1.0 ± 1.0
12	0.1 ± 0.1	0.3 ± 0.2
Final Water Test	7.2 ± 2.9	11.5 ± 5.4

DISCUSSION

In contrast to carbachol elicited drinking, angiotensin stimulated rats drank a substantial amount of alcohol. Indeed, the choice behavior toward different concentrations of alcohol following angiotensin adminstration was comparable to that of 24 hr water deprivation. Thus, additional support is provided for the suggestion that angiotensin elicited drinking has the same motivational properties as normal thirst. However, preference behavior may not be the same for all motivational levels [1]. Further work examining a wide range of angiotensin doses, deprivation levels, and taste stimuli is therefore needed before a firm conclusion can be drawn about the similarity between angiotensin and deprivation induced thirst.

The basic procedures used in this study were the same as those used by Cicero and Myers [3] to determine the alcohol intake following carbachol adminstration except that in this study the animals were not run in thier home cages and none of the alcohol solutions were removed during the testing sessions. Because of the similarity between the

procedures and because Cicero and Myers found that carbachol injected rats drank little if any alcohol, angiotensin appears to induce a different drive state from carbachol. This is consistent with the view that angiotensin activates different neural mechanisms from carbachol.

REFERENCES

1. Beck, R. C. and V. T. Ellis. Sucrose reinforcement thresholds for hungry, thirsty and non-deprived rats. *Psychon. Sci.* **4**: 199 200, 1966.
2. Blass, E. M. and H. W. Chapman. An evaluation of the contribution of cholinergic mechanism to thirst. *Physiol. Behav.* **7**: 679–686, 1971.
3. Cicero, T. J. and R. D. Myers. Preference-aversion functions for alcohol after cholinergic stimulation of the brain and fluid deprivation. *Physiol. Behav.* **4**: 559 562, 1969.
4. Epstein, A. N., J. T. Fitzsimons and B. J. Rolls. Drinking induced by injection of angiotensin into the brain of the rat. *J. Physiol.* **210**: 457 474, 1970.
5. Franklin, K. B. J. and D. Quartermain. Compariosn of the motivational properties of deprivation-induced drinking with drinking elicited by central carbachol stimulation. *J. comp. physiol. Psychol.* **71**: 390–395, 1970.
6. Gandelman, R., J. Panksepp and J. A. Trowill. Preference behavior differences between water deprivation-induced and carbachol-induced drinkers. *Cummuns Behav. Biol. Part A* **1**: 341 346, 1968.
7. Giardina, A. R. and A. E. Fisher. Effect of atropine on drinking induced by carbachol, angiotensin and isoproterenol. *Physiol. Behav.* **7**: 653 655, 1971.
8. Grossman, S. P. Direct adrenergic and cholinergic stimulation of hypothalamic mechanisms. *Am. J. Physiol.* **202**: 872–882, 1962.
9. Russell, M. J., M. S. Godsey and K. W. Lydell. Omnitrode: A simple cannula for chemical and electrical stimulation. *Physiol. Behav.* **8**: 773–775, 1972.
10. Rolls, B. J., B. P. Jones, and D. J. Fallows. A comparison of the motivational properties of thirst induced by intracranial angiotensin and by water deprivation. *Physiol. Behav.* **9**: 777–782, 1973.
11. Pellegrino, L. J. and A. J. Cushman. *A Stereotaxic Atlas of the Rat Brain.* New York: Appleton-Century-Crofts, 1967.
12. White, G. S., R. A. Levitt and S. Boyer. Drinking elicited by CNS injection of angiotensin in the rat. *Psychon. Sci.* **26**: 283–284, 1972.