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BRIEF COMMUNICATION

Angiotensin II Mediates a Conditioned Taste Aversion in Water-Replete Rats

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GRUPP, L. A. AND S. Y.-M. CHOW. *Angiotensin II mediates a conditioned taste aversion in water-replete rats.* PHARMACOL BIOCHEM BEHAV 44(4) 985-988, 1993.—Many compounds that exert an influence on behavioral processes will, under the unique conditions of the conditioned taste aversion (CTA) procedure, cause animals to avoid consuming an otherwise preferred fluid. While angiotensin II (ANG II) is a peptide with a number of behavioral and physiological actions, previous research did not support its role as an agent capable of inducing a CTA. Those studies used fluid deprivation to induce fluid intake and only a single conditioning trial. Fluid deprivation can elevate endogenous ANG II levels that may have interfered with or masked the ability of ANG II injections to exert an effect as a CTA-inducing agent. The present study reassessed the ability of ANG II to induce a CTA using fully hydrated animals and a number of conditioning trials. ANG II was able to induce a significant taste aversion at a dose five times lower than that used in previous studies.

Angiotensin Conditioned taste aversion Rats Water deprivation

THE peptide angiotensin II (ANG II) has long been recognized to play an important role in the maintenance of fluid and electrolyte balance, as well as in the control of blood pressure. In addition to these classic functions, a growing body of research has demonstrated that this peptide can influence behavioral processes involving learning (1,6,11), memory (3,11), and the regulation of alcohol intake (7). Angiotensin can thus be cast among other peptides such as oxytocin, vasopressin, and adrenocorticotrophic hormone that have both behavioral and physiological functions.

One behavioral process in which peptides can be assessed is their ability to produce a conditioned taste aversion (CTA). In the CTA paradigm, the ingestion of a preferred solution with a distinctive taste is followed by an injection of a behaviorally active compound. Only a few pairings of the solution with the compound are required before animals begin to avoid consuming the solution. Sickness-inducing agents such as lithium chloride will produce a CTA, but psychoactive drugs such as morphine, amphetamine, and alcohol will also produce the aversion even though they are considered positive reinforcers, are addictive substances, and are avidly self-administered (5).

In an investigation of the potential of ANG II to mediate a CTA, Rabin et al. (9) tested fluid-deprived rats in the standard single-bottle procedure. They were unable to demonstrate an aversion with a rather high—1 mg/kg—dose of ANG II after a single conditioning trial. They did observe a modest aversion with the same dose when the more "sensitive" two-bottle procedure was employed, a finding confirmed by Bluthe et al. (3). Two procedural variables in the Rabin et al. study that may have prevented ANG II from producing a CTA were the single conditioning trial and the use of chronic fluid deprivation, a state known to stimulate ANG II in both animals (2,8) and man (10). Fluid deprivation for 24 h or more has been shown to elevate plasma renin activity (2,10), and preventing the synthesis of ANG II (2) or blocking the ANG II receptor (8) significantly reduces the amount of water consumed by 24-h water-deprived animals. Together, these findings illustrate a greatly intensified ANG II activity as a result of chronic water deprivation. The failure of ANG II to produce a CTA in the Rabin et al. study might not reflect a weak aversion-inducing agent but may have been due to a combination of only one conditioning trial and the chronic fluid deprivation, which, by elevating endogenous levels of ANG II, may have rendered the exogenously administered ANG II functionally weak. In the present study, we examine the ability of repeated

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injections of ANG II to induce a single-bottle CTA in animals that are not chronically fluid deprived.

SUBJECTS

Subjects were 16 naive, male Wistar rats weighing 200–275 g at the beginning of the experiment. Animals were housed individually in hanging wire cages and maintained on a 12 L : 12 D cycle with lights off at 7:00 a.m. All testing was done in the dark cycle, when animals are most active. Food and water were always available in the home cage.

METHODS

Conditioned taste aversion was assessed using the single-bottle test. Once per day, animals were removed from their home cages and placed for 20 min in individual drinking cages equipped with a graduated (0.1 ml) drinking tube. Every other

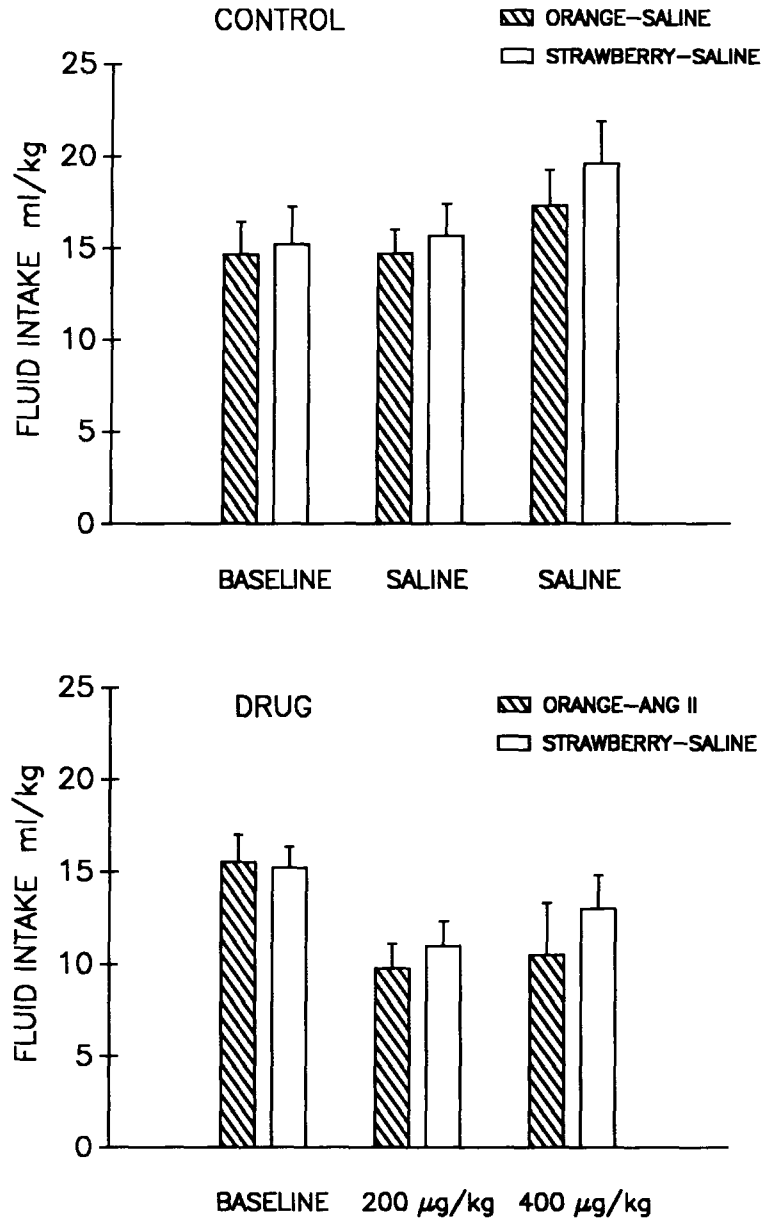

FIG. 1. Top: Control group intake of orange- and strawberry-flavored solutions across the three phases of the experiment. Each flavor was available on alternate days and both were followed by injections of the saline vehicle. Bottom: Drug group intake of flavored solutions. Orange was paired first with 200 μg/kg angiotensin II (ANG II) (seven pairings) and then with 400 μg/kg ANG II (seven pairings). Strawberry was always paired with saline injections. Each flavor was available on alternate days. Error bars indicate the SEM.

day, the tube contained an orange-flavored Kool-Aid solution while a strawberry-flavored Kool-Aid solution was presented on the intervening days. Both flavored solutions were made by adding 1.5 g of the crystals to 1 l water containing 40 g glucose. Animals were assigned to two groups (n = 8 per group) designated to receive a SC injection of either angiotensin II (ANG II group) or the saline vehicle (control group) immediately following consumption of the strawberry-flavored solution. A saline injection followed consumption of the strawberry-flavored solution. The two groups received seven pairings of 200 μg ANG II/kg bw followed by a phase of seven pairings with 400 μg ANG II/kg bw. Prior to taste aversion training, the two groups were run through a 20-day baseline phase where the two solutions were available on alternate days but no injections were given. Ile^5 ANG II (Sigma Chemical Co., St. Louis, MO) was dissolved in saline and prepared fresh daily.

RESULTS

Daily intake for each animal was averaged across each of the three phases and the group means are presented in Fig. 1. The top panel of this figure shows that the control group that received saline injections paired with both the orange- and strawberry-flavored solutions, tended to increase its consumption of both fluids across the final two phases of the experiment. The bottom panel of Fig. 1 shows that the group receiving 200 and 400 μg ANG II/kg bw decreased its consumption of both fluids, indicating that ANG II produced a CTA. A three-way analysis of variance (ANOVA) revealed a significant effect of group, $F(1, 14) = 4.8$, $p = 0.04$, and a group × phase interaction, $F(2, 28) = 3.45$, $p = 0.04$, confirming that animals receiving ANG II drank significantly less of the flavored solutions than the vehicle-injected control group. A significant effect of flavor, $F(1, 14) = 7.75$, $p = 0.01$, and flavor × phase interaction, $F(2, 28) = 4.26$, $p = 0.02$, shows that different amounts of orange and strawberry solutions were consumed but only at certain phases of the experiment. A nonsignificant group × flavor × phase interaction reflected a decrease in the intake of both the orange and strawberry solutions. Posthoc Duncan's analysis ($p \leq 0.05$) indicated that the increased intake of orange and strawberry in the control group was not significant compared to baseline even though there was an obvious tendency. However, in the drug group where intake of the orange solution was paired with ANG II both the 200- and 400-μg/kg doses produced significant reductions in intake, indicating the development of a taste aversion. The intake of strawberry solution also decreased but this was not significantly different from baseline.

DISCUSSION

The present results demonstrate that peripheral injections of ANG II can produce a significant conditioned taste aversion in rats using the single-bottle procedure. These findings are in contrast to Rabin et al. (9), who used the single-bottle procedure, a single conditioning trial, and a dose five times larger than the present one (i.e., 1 mg/kg vs. 200 μg/kg in the present study) and failed to find a significant CTA. Only when the more sensitive two-bottle procedure was used was a modest CTA observed.

Although the present study and the report of Rabin et al. do differ in a number of procedural elements, one major element of difference that may have contributed to the diverse outcomes is the state of hydration of rats—our animals were fully hydrated while those in the Rabin et al. study were committed to a chronic 23.5-h fluid deprivation schedule. It is well documented that ANG II levels are elevated as a result of chronic water deprivation (2,8,10) because the decrease in arterial blood pressure and kidney blood flow that follow chronic fluid deprivation represent stimuli to the juxtaglomerular cells of the kidney, causing renin release and ultimately the formation of ANG II. Thus, injection of ANG II in the Rabin et al. study was almost certainly occurring against a background of elevated endogenous ANG II. The failure to observe an ANG II-induced CTA in that study may have happened because the unconditioned effects of exogenously administered ANG II were not easily perceived against an already elevated background level of ANG II activity. These results suggest that ANG II is not a weak ANG II CTA-inducing agent in rats given that we were able to observe a significant taste aversion in fully hydrated rats that were preexposed to the conditioned stimulus using the same single-bottle procedure and a dose of ANG II that was five times lower than that of Rabin et al. (i.e., 200 μg/kg vs. 1,000 μg/kg). Further, animals in the present study were preexposed to the orange-flavored conditioned stimulus prior to any pairings with ANG II. Because preexposure is a condition that is known to retard the development of a CTA [see (4) for a review], the development of the CTA in these animals suggests that ANG II is a potent unconditioned stimulus. Angiotensin II is a behaviorally active compound capable of influencing memory, thirst, and alcohol intake. The present findings demonstrate that a significant ANG II-induced CTA can be produced in fully hydrated rats with a series of conditioning trials. The behavioral effects mediated by ANG II are observable in the CTA paradigm.

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REFERENCES

1. Baranowska, D.; Braszko, J. J.; Wisniewski, K. Effect of angiotensin II and vasopressin on acquisition and extinction of conditioned avoidance in rats. Psychopharmacology (Berl.) 81:247–251; 1983.
2. Barney, C. C.; Threatte, R. M.; Fregly, M. J. Water deprivation-induced drinking in rats: Role of angiotensin II. Am. J. Physiol. 244:R244–R248; 1983.
3. Bluthe, R. M.; Dantzer, R.; LeMoal, M. Peripheral injections of vasopressin control behavior by way of interoceptive signals for hypertension. Behav. Brain Res. 18:31–39; 1985.
4. Braveman, N. S. What studies on preexposure to pharmacological agents tell us about the nature of the aversion-inducing agent. In: Barker, L. M.; Best, M. R.; Domjan, M., eds. Learning mechanisms in food selection. Waco, TX: Baylor University Press; 1977:511–553.
5. Gamzu, E. The multifaceted nature of taste-aversion-inducing agents: Is there a single common factor? In: Barker, L. M.; Best, M. R.; Domjan, M., eds. Learning mechanisms in food selection. Waco, TX: Baylor University Press; 1977:477–509.
6. Georgiev, V. P.; Yonkov, D. I.; Kambourova, T. S. Interactions between angiotensin II and baclofen in shuttle-box and passive avoidance performance. Neuropeptides 12:155–158; 1988.

7. Grupp, L. A.; Perlanski, E.; Stewart, R. B. Regulation of alcohol consumption by the renin-angiotensin system: A review of recent findings and a possible mechanism of action. Neurosci. Biobehav. Rev. 15:265–275; 1991.
8. Malvin, R. L.; Mouw, D.; Vander, A. J. Angiotensin: physiological role in water-deprivation-induced thirst in rats. Science 197: 171–173; 1977.
9. Rabin, B. M.; Hunt, W. A.; Bakarich, A. C.; Chedester, A. L.; Lee, J. Angiotensin II-induced taste aversion learning in cats and rats and the role of the area postrema. Physiol. Behav. 36:1173–1178; 1985.
10. Rolls, B. J.; Wood, R. J.; Rolls, E. T.; Lind, H.; Lind, R. W.; Ledingham, J. G. G. Thirst following water deprivation in humans. Am. J. Physiol. 239:R479–482; 1980.
11. Wisniewski, K.; Braszko, J. J. The significance of central monoamine systems in the angiotensin II improvement of learning. Clin. Exp. Hypertens. (A) 6:2127–2131; 1984.