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BRIEF COMMUNICATION

Acquisition of Lithium Chloride- and Radiation-Induced Taste Aversions in Hypophysectomized Rats

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RABIN, B M , W A HUNT AND J LEE *Acquisition of lithium chloride- and radiation-induced taste aversions in hypophysectomized rats* PHARMACOL BIOCHEM BEHAV **18**(3) 463–465, 1983 —The effects of hypophysectomy on the acquisition of conditioned taste aversions following injection of lithium chloride and following exposure to ionizing radiation were studied using a two-bottle preference test Hypophysectomy did not disrupt the acquisition of a taste aversion following either treatment The results are interpreted as (a) suggesting that pituitary/adrenal hormones do not mediate the acquisition of a conditioned taste aversion following injections of lithium chloride or following exposure to ionizing radiation in a two-bottle preference test, and (b) consistent with other research suggesting that the involvement of pituitary/adrenal hormones in taste aversion learning may be related to the conflict induced by using a one-bottle test and not to the learning itself

Conditioned taste aversion Hypophysectomy Ionizing radiation Lithium chloride Pituitary/adrenal hormones

A CONDITIONED taste aversion (CTA) is produced when a novel taste solution is paired with irradiation (100 Rad) or with injection of a toxic solution such as lithium chloride (LiCl), such that an organism will avoid further ingestion of that solution at a later time [3] Lesions of the area postrema, the chemoreceptive trigger zone for emesis [1], disrupt the acquisition of a CTA following injection of LiCl [13] and following exposure to ionizing radiation [12], thereby suggesting that there may be mechanisms in common underlying the acquisition of an aversion induced by either treatment Hunt *et al* [7], working with parabiotic rats, have shown the transfer of a CTA to the shielded member of the parabiotic pair following irradiation of the unshielded member of the pair This finding indicating the involvement of a blood-borne factor in mediating the acquisition of a radiation-induced CTA is consistent with the role of the area postrema in CTA acquisition and with the hypothesis that similar mechanisms may be involved in the acquisition of both radiation- and lithium chloride-induced taste aversions

The nature of such a humoral factor is at present unknown Possible candidates might involve hormones of the pituitary or adrenal glands Exposing rats to 650 Rad doses of X-irradiation causes an increase in levels of plasma and adrenal corticosteroids which can be prevented by hypophysectomy [4] Concordant with this finding, Cairnie and Leach [2] using deprived rats in a one-bottle experimental design have reported that treating rats with dexamethasone produces a significant attenuation of a radiation-induced CTA Adrenal corticosteroids have also been implicated in LiCl-induced aversions Using one-bottle experimental designs, Hennessy *et al* [5,6] have shown that treatment with LiCl produced an increase in plasma corticosteroids that could be suppressed by pretreatment with dexamethasone Similarly, dexamethasone pretreatment could attenuate the acquisition of a LiCl-induced CTA Therefore, it might seem reasonable to propose that a radiation- or drug-induced release of pituitary/adrenal hormones can serve to mediate the acquisition of a CTA produced by both of these treatments However, working with non-deprived rats in a one-bottle test, Kimeldorf *et al* [9] have reported that hypophysectomy does not disrupt the acquisition of a radiation-induced CTA Similarly, more recent research on LiCl-induced taste aversions using behavioral manipulation of pituitary/adrenal hormones [16] seems to suggest that the involvement of these hormones is more related to the conflict produced by a one-bottle experimental design than to the acquisition of the aversion itself In these experiments, Smotherman *et al* [16] have shown that it is possible to produce a dissociation between the corticosteroid and behavioral effects of LiCl injections by manipulating the number of LiCl preexposures to which the animals are subjected. Under these conditions, increasing the number of preexposures decreased the effectiveness of LiCl-induced taste aversion learning without producing a corresponding decrease in plasma corticosteroid levels.

These results suggest that an intact pituitary/adrenal system may not be necessary for the acquisition of either drug- or radiation-induced taste aversions. However, there are some difficulties in interpreting the results of previous studies. First, the pharmacological manipulation of pituitary/adrenal hormones involves the use of non-physiological doses and does not provide direct evidence of endogenous hormone release in the acquisition of an LiCl- or radiation-induced CTA. Second, since previous research has indicated that the taste aversion paradigm is sensitive to variations in procedure [11,14], the results of studies of LiCl-induced and radiation-induced taste aversions may not be directly comparable because of the different experimental procedures used to evaluate the role of pituitary/adrenal hormones in this behavior. Consequently it is important to reevaluate the question of whether or not pituitary/adrenal hormones can serve to mediate taste aversion learning using a design in which the experimental procedures are the same for both LiCl and radiation treatments. Third, the use of deprived animals in a one-bottle experimental design may produce a conflict which can be affected by the manipulation of pituitary/adrenal hormones independently of a possible role of such hormones in mediating the acquisition of a CTA itself. The present experiment utilized hypophysectomized rats and a two-bottle preference test to allow the direct assessment of the role of endogenous hypophyseal hormones in the acquisition of both LiCl- and radiation-induced conditioned taste aversions and to eliminate the possibility that the conflict produced by the use of one-bottle designs could influence the results.

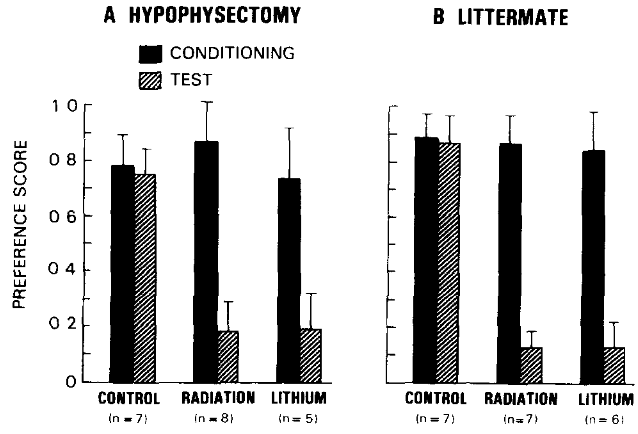

FIG. 1. Sucrose preference scores of animals given control procedures or exposed to 100 rad radiation or 3 mEq/kg IP, injections of LiCl. The number in parentheses indicates the number of rats in each group. Variance bars indicate the standard deviation. A. Hypophysectomized rats. B. Untreated littermate rats.

METHOD

The subjects were 27 hypophysectomized (hypox) and 26 littermate rats received from Charles Rivers Labs. The rats were adapted to the lab for 2 weeks. All rats were maintained on food ad lib and a 5% sucrose solution. During this period, three of the hypox and one of the littermate rats died.

For taste aversion training the rats were adapted to a water deprivation schedule for four days during which tap water was presented for 30 min during the early part of a 12:12 light:dark cycle. Five hr after the first bottle, all rats were given a second bottle for 30 min which contained their usual 5% sucrose solution. This procedure was adopted to reduce possible stress on the hypox rats resulting from the deprivation procedure and to continue to provide them with their maintenance sucrose solution. All testing was done during the first drinking period. On the conditioning day, all rats were presented with two calibrated drinking tubes, one containing tap water and the other containing a 10% sucrose solution which had been flavored with vanilla extract (1% v/v) to produce a distinctive and novel taste solution as the conditioned stimulus. Intake of each solution was recorded. Immediately following this drinking period, the hypox and littermate rats were each divided into three equal treatment groups. The first group of rats (8 hypox and 8 littermate) was exposed to 100 Rad gamma radiation using a cobalt-60 source at 40 Rad/min. Dose measurements were determined with a 3.3 cc Victoreen ionization chamber and with thermoluminescent detectors (LiF TLD 100s). The second group of rats (7 hypox and 7 littermate) was given an intraperitoneal injection of 3 mEq/kg LiCl. The remaining rats (7 hypox and 7 littermate) served as controls, 4 were put in restraining boxes for sham irradiation procedures and 3 were given an intraperitoneal injection of isotonic saline. After treatment, all rats were returned to their home cages for 24 hr. On the morning of the test day, all rats were again given a choice between tap water and the vanilla-flavored sucrose solution and the intake of each solution was recorded. In order to make certain that all rats were exposed to the conditioned stimulus, 9 rats which did not show a preference for the vanilla-flavored sucrose on the conditioning day or which did not show significant fluid intake on either conditioning or test day were excluded from the experiment.

Relative intake of sucrose solution and tap water is presented as preference score, defined as sugar intake divided by total fluid intake. For data analysis, the arcsin transformation [17] was used to normalize the distribution of scores. Comparisons of preference scores across treatment conditions on conditioning and test days were made using one-way analyses of variance. For comparisons within individual treatment conditions, 2-way analyses of variance were performed to obtain the appropriate error terms for a series of planned comparisons [8].

RESULTS AND DISCUSSION

The sucrose preference scores are summarized in Fig. 1. Despite the fact that the absolute fluid intake of the hypox rats on the conditioning day (mean=12.50, SD=5.26) was less than that of the controls (mean=23.15, SD=3.83), as indicated in Fig. 1 there were no differences in the relative intake of sucrose and water on either the conditioning or test day. All rats, both hypox and littermate, showed a similar preference for the vanilla-flavored sucrose solution on the conditioning day, $F(5,34)=1.662$, $p>0.10$. Because neither of the control procedures, sham-irradiation or saline injection, had an effect on test day sucrose preference, the scores of both groups have been combined. In contrast to the rats given the control procedures, a significant reduction in sucrose preference was observed following exposure to radiation and to LiCl injection in both the hypox, $F(1,17)=57.56$, $p<0.01$, and littermate, $F(1,17)=224.90$, $p<0.01$, rats. This finding not only indicated that a CTA had developed but also that the addition of the vanilla extract to the higher concentration of sucrose solution provided a sufficiently novel taste to serve as a distinctive conditioned stimulus for the establishment of a CTA. There were no significant differences between the hypox and littermate rats from either the irradiation or injection procedures on sucrose preference, $F(1,22)=0.019$, $p>0.10$.

The results presented above clearly show that removal of the pituitary has no effect on the acquisition of a CTA following injection of LiCl or following exposure to ionizing radiation in an experimental design using a two-bottle preference test. Because there were no differences in relative preference between the hypox and littermate rats, it is unlikely that the reduction in fluid intake produced by hypophysectomy affected these results.

The present results with LiCl are consistent with the results of previous CTA research using pharmacological [5,6] and behavioral [15,16] techniques to manipulate corticosteroid levels. This prior research has indicated that the effects of manipulation of pituitary/adrenal hormone levels may be related more to the conflict produced by the use of a single bottle CTA design than to the learning itself. In the present experiment, the use of a two-bottle test does not produce a conflict because the rat has an alternative to drinking the vanilla-flavored sucrose solution which has been associated with the unconditioned stimulus, and hypophysectomy has no effect on CTA acquisition. These data, obtained using a different method to modulate pituitary/adrenal hormone levels, therefore confirm and strengthen prior results.

The observation that hypophysectomy did not disrupt or modulate the acquisition of a radiation-induced CTA is also consistent with the previous results obtained by Kimeldorf *et al.* [9] using non-deprived rats in a one-bottle test, but is not concordant with the recent report that manipulation of corticosteroids by injection of dexamethasone in deprived rats given a one-bottle test can attenuate a radiation-induced CTA [2]. Since the studies that reduce potential conflict, either by using non-deprived rats or by using a two-bottle test, report no effect of manipulation of pituitary/adrenal hormones on the acquisition of a CTA, there is the strong implication that a radiation-induced release of these hormones does not function to mediate CTA learning following exposure to ionizing radiation.

Also, because this experiment allows a direct comparison of the role of pituitary/adrenal hormones in the acquisition of both LiCl- and radiation-induced taste aversions, these data form a critical part of an emerging pattern of results indicating the presence of a common mechanism underlying the acquisition of both radiation- and lithium chloride-induced taste aversions [10, 11, 12].

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REFERENCES

1. Borison, H. L. Area postrema. Chemoreceptor trigger zone for vomiting—is that all? *Life Sci.* **14:** 1807–1817, 1974.
2. Cairnie, A. B. and K. E. Leach. Dexamethasone: A potent blocker for radiation-induced taste aversion in rats. *Pharmacol. Biochem. Behav.* **17:** 305–311, 1982.
3. Garcia, J., W. G. Hankins and K. W. Rusiniak. Behavioral regulation of the milieu interne in man and rat. *Science* **185:** 824–831, 1974.
4. Hameed, J. M. A. and T. J. Haley. Plasma and adrenal gland corticosterone levels after X-ray exposure in rats. *Radiat. Res.* **23:** 620–629, 1964.
5. Hennessy, J. W., W. P. Smotherman and S. Levine. Conditioned taste aversion and the pituitary-adrenal system. *Behav. Biol.* **16:** 413–424, 1976.
6. Hennessy, J. W., W. P. Smotherman and S. Levine. Investigations into the nature of the dexamethasone and ACTH effects upon learned taste aversion. *Physiol. Behav.* **24:** 645–649, 1980.
7. Hunt, E. L., H. W. Carroll and D. J. Kimeldorf. Humoral mediation of radiation-induced motivation in parabiont rats. *Science* **150:** 1747–1748, 1965.
8. Keppel, G. *Design and Analysis: A Researcher's Handbook.* Englewood Cliffs: Prentice-Hall, 1973.
9. Kimeldorf, D. J., J. Garcia and D. O. Rubadeau. Radiation-induced conditioned avoidance behavior in rats, mice, and cats. *Radiat. Res.* **12:** 710–718, 1966.
10. Rabin, B. M., W. A. Hunt and J. Lee. Studies on the role of central histamine in the acquisition of a radiation-induced conditioned taste aversion. *Radiat. Res.* **90:** 609–620, 1982.
11. Rabin, B. M., W. A. Hunt and J. Lee. State-dependent interactions in the antihistamine-induced disruption of a radiation-induced taste aversion. *Radiat. Res.* **90:** 621–627, 1982.
12. Rabin, B. M., W. A. Hunt and J. Lee. Attenuation of radiation- and drug-induced conditioned taste aversions following area postrema lesions in the rat. *Radiat. Res.* 1983, in press.
13. Ritter, S., J. L. McGlone and K. W. Kelly. Absence of lithium-induced taste aversion after area postrema lesion. *Brain Res.* **201:** 501–506, 1980.
14. Sessions, G. R. Histamine and radiation-induced taste aversion conditioning. *Science* **190:** 402–403, 1975.
15. Sessions, G. R. and J. L. Meyerhoff. Dissociation of pituitary-adrenal activation and behavioral stress response following taste aversion conditioning. *Soc. Neurosci. Abstr.* **7:** 870, 1981.
16. Smotherman, W. P., A. Margolis and S. Levine. Flavor preexposures in a conditioned taste aversion situation: A dissociation of behavioral and endocrine effects in rats. *J. Comp. Physiol. Psychol.* **94:** 25–35, 1980.
17. Winer, B. J. *Statistical Principles in Experimental Design.* New York: McGraw-Hill, 1962.