Pharmacology Biochemistry and Behavior, Vol. 1, pp. 207–210. ANKHO International Inc., 1973. Printed in the U.S.A.

Salivarectomy: Effect on Drinking Produced by Isoproterenol, Diazoxide and NaCl Loads[1]

JOHN L. FALK AND RICHARD W. BRYANT

Department of Psychology, Rutgers University, New Brunswick, New Jersey 08903

(Received 20 February 1973)

FALK, J. L. AND R. W. BRYANT. *Salivarectomy: effect on drinking produced by isoproterenol, diazoxide and NaCl loads.* PHARMAC. BIOCHEM. BEHAV. 1(2) 207–210, 1973.–Male rats were given sham, partial or complete salivarectomies and their drinking responses to various stimuli were evaluated. There was an attenuated response to subcutaneously administered isoproterenol and diazoxide in desalivate animals. The drinking response to intraperitoneally administered hypertonic NaCl was the same across all groups, although the response to intragastrically administered hypertonic NaCl was decreased in complete desalivate animals. These results, together with those from other studies, indicate that the desalivate animal has a drinking response deficit to various dipsogenic stimuli. The physiological basis of this deficit cannot yet be characterized.

Salivary glands Drinking Isoproterenol Diazoxide Water metabolism

THE beta-adrenergic agent isoproterenol produces both marked fluid intake in water-satiated rats [8] and hypertrophy of the salivary glands [11]. The possibility that these two effects of isoproterenol might be related is strengthened by noting two additional facts. First, rodent salivary glands contain large amounts of renin [14,15], a substance which has been critically implicated in fluid intake [5] and second, isoproterenol produces a variety of changes in rat salivary composition [16]. Thus, the salivary glands contain a substance (renin) significantly related to water intake and they function also as a target area for isoproterenol, a dipsogenic agent.

EXPERIMENT 1: ISOPROTERENOL-INDUCED DRINKING AND DESALIVATION

In order to determine if the salivary glands were implicated in the fluid intake produced by subcutaneous doses of isoproterenol, the isoproterenol dose-effect relation was determined in sham and desalivate animals. Salivarectomy, rather than simple occlusion of the salivary ducts, was performed in order to remove unequivocally this glandular source of renin.

Procedures

Animals. Nineteen, male Holtzman rats weighing 260–350 g prior to surgery were used. They were housed in individual, stainless steel cages in a temperature-controlled room with a light cycle of 12 hr on and 12 hr off.

Surgery. Animals were anesthetized with sodium pentobarbital (35 mg/kg) intraperitoneally (i.p.), and supplemented with chloral hydrate (250 mg/kg, i.p.) if required. Atropine sulfate (2 mg i.p.) was given 5–10 min after sodium pentobarbital administration.

A sham, partial, or complete salivarectomy was performed on each animal. Sham operations (N = 4) consisted of reflecting the skin, muscle and facia overlying the submaxillary-sublingual glandular complex and manipulating the fat and lymph nodes in this general region for a length of time approximately equal to that of a complete salivarectomy (30 min). In the partial desalivate operations (N = 7) two types of removals were used. Either the submaxillary and sublingual glands were removed with the parotid glands left intact, or the left parotid was removed along with the submaxillary and sublingual glands with the right parotid left intact. In complete desalivation, submaxillary, sublingual and parotid glands were removed bilaterally (N = 8). The minor sublingual glands were not removed. The general procedure used in removing the glands was that of Cheyne [1]. As well as removing the glands, glandular ducts and blood vessels were tied off. Parotid ducts were dissected carefully away from the branch of the seventh cranial nerve which runs within the same overlying sheath before tying. At the end of the experiment animals were sacrificed and areas of glandular removal were inspected for evidence of gland regeneration. None was found.

Drugs. Isoproterenol hydrochloride (Winthrop Laboratories) was dissolved in isotonic NaCl solution, and concentrations were adjusted so that all subcutaneous (s.c.) doses were less than 0.5 ml in volume. Isoproterenol solutions were prepared just prior to injection. Doses are specified in terms of the salt.

Experimental design. Following the operative procedure, all desalivate rats were maintained on wet Purina Laboratory chow (powdered) until recovery proceeded to a point

[1]Supported by Grant AM 14180 from the National Institutes of Health, and Grant MH 18409 from the National Institute of Alcohol Abuse and Alcoholism.

where the dry chow could be consumed readily. Tap water was available from calibrated animal drinking tubes (Richter tubes) at all times. Sham-operated animals were maintained on the dry, powdered food from the immediate postoperative period.

The animals were adapted to a simple food and water schedule in which water was always available (no deprivation) and food was removed only during a 4-hr water intake test period. Thus, at 12 noon rats were weighed, Richter tubes were filled, any scheduled drug injection was administered, and food was removed. Water intake for the following 4 hr was recorded (12 noon - 4 p.m.) and then food was replaced. This 4-hr water intake measure constituted the major dependent variable. The food and water intakes from 4 p.m. - 12 noon were measured also, but did not change as a function of drug administration and therefore are not reported.

Isotonic saline (control doses) or isoproterenol (0.05, 0.10 or 0.33 mg/kg) was injected s.c. at 12 noon. Each isoproterenol dose day was followed by a saline dose day (control) except on weekends when no injections were given, although the feeding-drinking and weighing procedures were maintained. Each dose level of isoproterenol was given to each animal from two to five times (usually three times) in a nonsystematic order.

Upon completion of this phase of the experiment, the food schedule was changed so that animals were deprived of food for 2 hr prior to injection as well as during the 4-hr water intake test period. Only one isoproterenol dose level was administered (one to four times for each animal) under this condition.

Results

The control saline injections resulted in an insignificant amount of 4-hr water intake (0.1 - 0.5 ml). For sham-operated animals, isoproterenol yielded a dose-related increase ($F = 7.89$, $p<0.01$) in water intake (see Fig. 1).

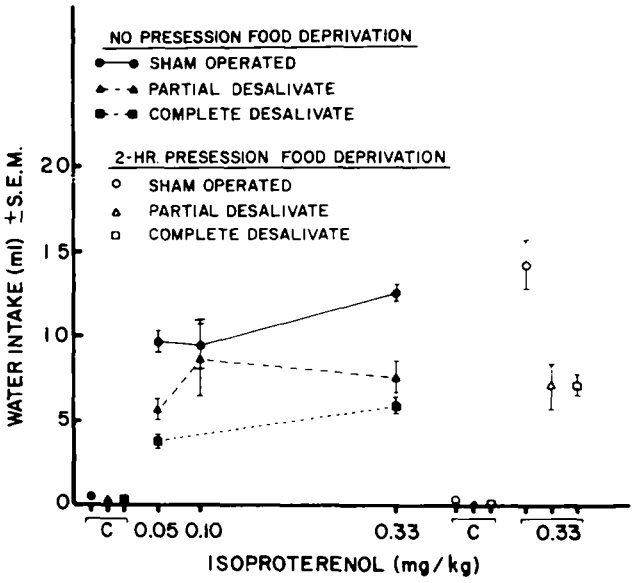

FIG. 1. Water intake (4-hr) in water-satiated rats as a function of subcutaneous isoproterenol doses or control (C) dose of isotonic saline in sham, partial, or completely salivarectomized animals.

Similar dose-related results occurred for the complete desalivate animals ($F = 11.91$, $p<0.005$). However, the dose-related increase was not significant for the partial desalivate rats. While isoproterenol produced increased water intakes in all groups of animals, the degree of increase was less in desalivate than in sham animals. At both dosage points, the complete desalivate group intakes were significantly less ($p<0.001$) than the sham group values, and less ($p<0.02$) at the 0.05 mg/kg dose point than the point for the partial desalivate group (t-tests for uncorrelated means, 2-tailed). The partial desalivate group intakes differed from the sham group values at the 0.05 mg/kg ($p<0.001$) and 0.33 mg/kg ($p<0.01$) dose points, but not at the 0.10 mg/kg point. Clearly, the dipsogenic response to isoproterenol injection was attenuated in desalivate animals, with the partial desalivate values falling between those of the sham and complete desalivate groups.

In the 20-hr period during which the animals had access to both food and water, desalivate rats, especially the complete desalivates, drank more water than the sham group animals [cf. 3, 13]. The possibility might exist under such conditions that the decreased fluid intake response to isoproterenol in desalivate animals could result from an initial state of overhydration at the injection time. Since the overdrinking in desalivate rats is associated with the intake of dry food, withholding food for a period of time prior to the water intake test should prevent this food-intake-related overhydration. Accordingly, the 0.33 mg/kg isoproterenol dose was repeated for all groups under the additional condition of food deprivation for 2 hr prior to injection. Figure 1 (right side) shows that the previous values for this dosage point were replicated rather closely. Therefore, the attenuated drinking response of desalivate animals to isoproterenol injection cannot be attributed to a state of initial overhydration.

A total of 10 animals from the present study died as a result of the 0.33 mg/kg dose level of isoproterenol after having been exposed to the other dose levels, as well as one or more doses at this greatest dose value. In related work, some of which did not involve surgery, we have found that the lower dose values also kill about one-quarter of the animals.

EXPERIMENT 2: DIAZOXIDE-INDUCED DRINKING AND DESALIVATION

Since the full expression of isoproterenol-induced drinking was partially dependent upon the integrity of the salivary glands, it was decided to check the generality of this finding by using diazoxide, a more efficacious dipsogenic agent [4], in an experiment similar to the previous one.

Procedures

Animals. Fourteen, male Holtzman rats weighing 260–350 g prior to surgery were used. Eight of these had been used in the previous experiment. The additional animals were surgically prepared as previously described. Housing conditions were also maintained as in the previous experiment.

Drugs. Diazoxide (3-methyl-7-chloro-1, 2, 4-benzothiadiazine-1, 1-dioxide), obtained as a gift from Schering Corp., Bloomfield, N. J. (Hyperstat), was dissolved in a vehicle consisting of one part 1 N NaOH to three parts isotonic NaCl solution. Diazoxide solutions were prepared

immediately prior to injection.

Experimental design. As in the second part of the previous experiment, food was removed from the cages 2 hr prior to injection and the start of the water intake test period. However, the water intake test period was shortened to 3 hr as this period was known from other of our studies to encompass the major drinking response to the dose of diazoxide selected (80 mg/kg, s.c.). As before, food was not available during the fluid intake test period.

Each animal was injected with the diazoxide dose four times, with 3–9 days between injections. Vehicle doses were given a total of three times at volumes the same as those comprising the diazoxide injections and were randomly interposed between diazoxide injections. Three to nine days also elapsed between vehicle injections.

Results

Figure 2 shows that diazoxide injection produced a dipsogenic response in all groups of animals, while the mean water intake following vehicle injections was virtually zero. However, the intake response of the sham desalivate animals was significantly greater than the complete (t = 5.26, df = 33, $p<0.001$) and the partial (t = 2.44, df = 21, $p<0.05$) desalivate rats. The difference between the complete and partial desalivate groups was not significant.

Unlike the case of isoproterenol in the previous experiment, diazoxide did not result in deaths.

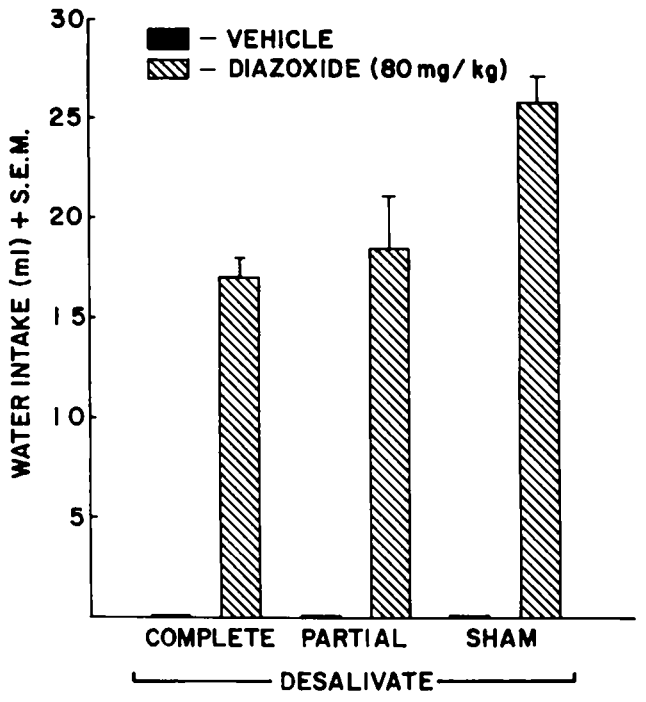

FIG. 2. Water intake (3-hr) in water-satiated rats given subcutaneous doses of diazoxide or vehicle. Animals were either sham, partial, or completely salivarectomized.

EXPERIMENT 3: NACL-INDUCED DRINKING AND DESALIVATION

In Experiment 2, desalivate animals once again proved less responsive to a dipsogenic agent than sham-operated rats. In order to determine if this decrement would occur to a more traditional, osmotic stimulus to drinking, the present experiment was performed.

Procedures

Animals. Those used in Experiment 2 were retained for the present experiment.

Experimental design. The same feeding-drinking schedule used in Experiment 2 was continued for this experiment.

In the first phase, a 3% body weight intragastric load of 0.5 M NaCl solution was administered at the start of the 3-hr drinking test period. Animals were lightly anesthetized with ether. A Number 8, French rubber catheter was passed down the esophagus and into the stomach. The hypertonic NaCl was allowed to flow out of a burette into the stomach by gravity. Each animal was stomach loaded twice, with at least 3 days between successive loads. Two sham loads were administered as well, with at least 2 days between these treatments. A sham load consited of all the above manipulations except that no stomach load was administered. Stomach and sham loads were given in a random order.

In the second phase of the experiment, hypertonic NaCl solution was administered intraperitoneally. A 1% body weight load of 1.0 M NaCl solution was injected intraperitoneally at the start of the drinking test period. Each animal was injected twice with 3 days between the injections.

Results

Figure 3 shows that with sham loading the 3-hr drinking remained at the inconsequential baseline level. The intragastric NaCl load produced significantly less drinking in complete desalivate rats than in sham operated animals (t = 2.46, df = 22, $p<0.05$). The other t-tests performed on the intragastric data were not significant.

There were no significant differences among the comparisons of drinking responses produced by intraperitoneal NaCl injection in desalivate and sham desalivate animals.

DISCUSSION

The decreased drinking response of salivarectomized rats to stimulation by s.c. isoproterenol and diazoxide is of interest since the dipsogenic properties of isoproterenol [7,9] and diazoxide (unpublished study) have been shown to depend upon the presence of the kidneys. While nephrectomy completely eliminates the dipsogenic effect of these drugs, salivarectomy attenuates it in the presence of kidneys. Since salivary gland renin plays a doubtful role in the physiological response of renin secretion ([10], p. 58; [12]), the lack of a drinking response to isoproterenol or diazoxide in the nephrectomized animal remains difficult to interpret until plasma renin activities are measured under these conditions. It is not known whether these agents can elicit renin secretion from the salivary glands, as they do from the kidneys, nor if any such elicitation is increased or decreased by nephrectomy.

The present experiment agrees with previous research showing that the drinking response of desalivate rats to

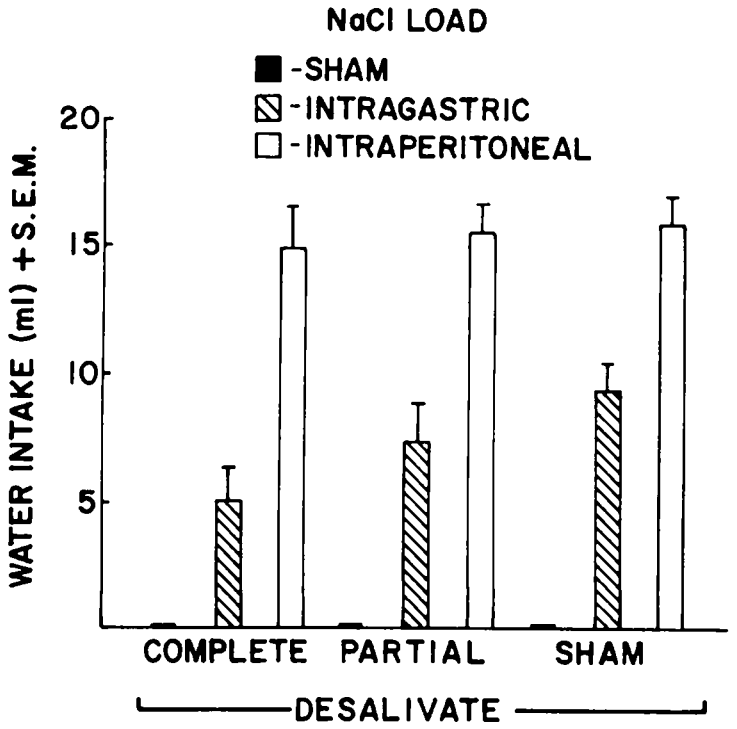

FIG. 3. Water intake (3-hr) in water-satiated rats given intragastric (3% body weight of 0.5 M NaCl) or intraperitoneal (1% body weight of 1.0 M NaCl) hypertonic loads. Animals were either sham, partial, or completely salivarectomized.

parenterally administered hypertonic NaCl was comparable to control animals, whether the salt was administered s.c. [6] or i.p. [13]. The attenuated drinking of desalivates following intragastric hypertonic NaCl loads has not been reported previously, although a decreased response to water deprivation [13] and hypovolemia [6] has been noted.

The decreased drinking response of the desalivate rat to certain dipsogenic physiological and pharmacological stimuli cannot at this time be assigned a ready interpretation. An altered oral sensory environment, or some endocrine output from these glands may participate in the normal response to various dipsogenic stimuli.

REFERENCES

1. Cheyne, V. D. A description of the salivary glands of the rat and a procedure for their extirpation. *J. dent. Res.* **18**: 457–468, 1939.
2. de Jong, W., W. Lovenberg and A. Sjoerdsma. Renin-like activity in submaxillary gland in several strains of rats including the spontaneously hypertensive rat. *Biochem. Pharmac.* **21**: 2123–2129, 1972.
3. Epstein, A. N., D. Spector, A. Samman and C. Goldblum. Exaggerated prandial drinking in the rat without salivary glands. *Nature* **201**: 1342–1343, 1964.
4. Falk, J. L. and M. Tang. Severe polydipsia and antidiuresis produced by diazoxide. *Physiol. Behav.* **9**: 259–260, 1972.
5. Fitzsimmons, J. T. Thirst. *Physiol. Rev.* **52**: 468–561, 1972.
6. Gutman, Y., P. Livneh and J. Pietrokovski. Role of salivary glands in response to thirst stimuli. *Israel J. med. Sci.* **6**: 573–575, 1970.
7. Houpt, K. A. and A. N. Epstein. The complete dependence of beta-adrenergic drinking on the renal dipsogen. *Physiol. Behav.* **7**: 897–902, 1971.
8. Lehr, D., J. Mallow and M. Krukowski. Copious drinking and simultaneous inhibition of urine flow elicited by beta-adrenergic stimulation and contrary effect of alpha-adrenergic stimulation. *J. Pharmac. exp. Ther.* **158**: 150–163, 1967.
9. Meyer, D. K., B. Peskar and G. Hertting. Hemmung des durch blutdrucksenkende Pharmaka bei Ratten ausgelösten Trinkens durch Nephrektomie. *Experientia* **27**: 65–66, 1971.
10. Page, I. H. and J. W. McCubbin. *Renal Hypertension.* Chicago: Year Book Medical Publ, 1968.
11. Selye, H., R. Veilleux and M. Cantin. Excessive stimulation of salivary gland growth by isoproterenol. *Science* **133**: 44–45, 1961.
12. Takeda, T., J. DeBusk and A. Grollman. Physiologic role of reninlike constituent of submaxillary gland of the mouse. *Am. J. Physiol.* **216**: 1194–1198, 1969.
13. Vance, W. B. Observations on the role of salivary secretions in the regulation of food and fluid intake in the white rat. *Psychol. Monogr.* **79**: 1–22 (whole No. 598), 1965.
14. Werle, E., R. Vogel and L. F. Göldel. Über ein blutdrucksteigerndes Prinzip in Extrakten aus der Glandula submaxillaris der weissen Maus. *Arch. exp. Path. Pharmak.* **230**: 236–244, 1957.
15. Werle, E., I. Trautschold and A. Schmal. Über ein Iso-Enzym des Renins und über die Isolierung eines biologisch aktiven Spaltproduktes seines Substrates. *Z. Physiol. Chem.* **332**: 79–87, 1963.
16. Zengo, A. N., I. D. Mandel, A. Solomon and P. Block. Chemistry of rat saliva following isoproterenol administration. *J. oral Ther. Pharmac.* **4**: 359–363, 1968.