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Attenuation of Pilocarpine-Induced Drinking By Chronic Treatment with Estrogens[1] (40844)

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Rats treated chronically with estrogens have an attenuated drinking response to acute administration of isoproterenol (1–3), renin (3), angiotensin I (4), and angiotensin II (4). In addition, drinking in response to administration of hypertonic saline is attenuated (3), as it is in the estrogen-treated rabbit (5), although drinking in response to a 24-hr period of dehydration is not (3).

The objective of the present study was to assess the drinking response of estrogen-treated rats to pilocarpine, a parasympathomimetic agent known to elicit drinking in the rat (6).

Methods. Experiment 1. Effect of graded doses of pilocarpine on water intake, urine output, and urinary excretion of electrolytes. Eighty-seven female rats of the Blue Spruce Farms (Sprague-Dawley) strain weighing 244 to 337 g were used. They were maintained three per cage in a windowless, thermoregulated room (26 ± 1°C) which was illuminated from 0600 to 1800 hr. All animals received Purina Laboratory Chow and tap water *ad libitum* prior to the study but were allowed no food during the study. Twenty-nine of the rats were untreated and received 1 ml of distilled water/kg ip at the beginning of the experiment. The three remaining groups received 1 (21 rats), 2 (29 rats), and 6 mg (8 rats) of pilocarpine hydrochloride ip (Amen Drug and Chemical Co., Inc., New York, N.Y.) The experiment began at 0900 hr. After injection, each rat was placed in an individual, stainless-steel metabolism cage and given a preweighed water bottle consisting of an infant nursing bottle with a cast aluminum spout as described by Lazarow (7). Water intake and urine output were measured hourly for 3 hr thereafter. The cumulative 3-hr urine sample from each rat was pooled and the urinary output of sodium and potassium measured by flame photometry.

A one-way analysis of variance was used to analyze the data resulting from this study (8). Comparison between individual groups was made by Student's *t* test using the pooled variance from the analysis of variance (9). Significance was set at the 95% confidence limit.

Experiment 2. Effect of chronic treatment with estrogens on the drinking response to pilocarpine. Study 1. Eighteen female rats of the Blue Spruce Farms (Sprague–Dawley) strain were used. Six rats served as controls; six others received 21 μg estradiol benzoate/kg/day. All treated rats received estrogen for 29 weeks prior to this study.

Estradiol benzoate was administered via Silastic tubes. Silastic tubing (No. 602-235, 0.25 mm wall thickness, 10 mm long) containing, crystalline estradiol benzoate was implanted sc between the shoulder blades of six rats while twice the length of the same tubing containing estradiol benzoate was implanted sc in a second group of six rats. The third group of six rats (control group) was implanted sc with a 10-mm length of empty Silastic tubing. At the end of the experiment each tube was removed from its subcutaneous site, cleaned of adhering tissue and placed in a vacuum desiccator for 48 hr. It was then weighed on an analytical balance. Drug dose was based on meanweight loss of the tube and mean body weight during the period of implantation. Dimethylpolysiloxane (Silastic) tubing has been shown to allow diffusion of certain crystalline steroids into various media at a constant rate over relatively long periods of

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time (10, 11). Previous experience in this laboratory has indicated that this method of steroid administration provides a reliable means of achieving reasonably constant drug release for periods up to 6 months (1–4).

At 0900 hr on the day of the study, all rats were administered 3 mg pilocarpine hydrochloride/kg ip. They were then placed in individual metabolism cages and water intakes and urine outputs measured hourly for 3 hr as described in Experiment 1. Urinary sodium and potassium concentrations were also measured as described in Experiment 1.

Study 2. This study was carried out in a fashion similar to that of Study 1 excepting that estradiol-17β was used instead of estradiol benzoate. The estrogen was administered via the same type of Silastic tubes as in Study 1 for 24 weeks prior to the study at doses of 6.5 and 16.5 μg/kg/day. At 0900 hr on the day of the study, all rats were administered 3 mg of pilocarpine hydrochloride/kg ip. They were then placed in individual cages and water intakes, urine output, and urinary sodium and potassium concentrations measured as described above.

Statistical analysis of all data was carried out as described above.

Results. Experiment 1. Administration of graded doses of pilocarpine induced graded increases in water intake (Fig. 1A). The threshold dose of pilocarpine appeared to be between 1 and 3 mg/kg. The latter dose induced nearly maximal water intake. The major effect of pilocarpine on water intake occurred during the first hour of measurement with the subsequent two hours adding relatively smaller increments to the 1-hr measurement.

Urine output increased strikingly with graded doses of pilocarpine (Fig. 1B). The threshold dose was below 1 mg/kg. Thus, urine output appeared to respond at a dose lower than that required to elicit a drinking response. Urine output also differed from water intake in that the incremental increase during the second and third hours contributed significantly to the total urine output. The urine volume excreted during the second hour was approximately one half that excreted during the first hour.

Both urinary sodium and potassium excretion rates increased significantly ($P < 0.01$) above that of controls when 1 mg pilocarpine/kg was administered (Fig. 1C). Thus, the threshold dose which increased urinary sodium and potassium excretion rates significantly above that of controls was below 1 mg/kg. This also suggests that urinary sodium and potassium excretion rates are more sensitive to peripheral administration of pilocarpine than is water intake.

In view of the fact that water intake reached near maximal levels when a dose of 3 mg pilocarpine/kg was administered, this dose was chosen for use in the studies of Experiment 2. It was also chosen because 6 mg pilocarpine/kg appears to be near the maximal dose tolerated by rats without significant side effects.

Experiment 2. Study 1. Administration of 3 mg pilocarpine/kg ip to rats treated chronically with estradiol benzoate was accompanied by drinks that were approximately 50% less than that of controls during the first hour of the study (Table I). Water intakes of both estrogen-treated groups were reduced significantly below that of controls. During the second and third hours of the study only the water intake of the group receiving the higher dose of estrogen was reduced significantly below that of the controls. In contrast, treatment with estradiol benzoate had no significant effect on either urine output or on urine electrolyte excretion rate (Table I).

Study 2. Administration of 3 mg pilocarpine/kg ip to rats treated chronically with estradiol-17β was accompanied by a significantly reduced water intake compared to the control group during all 3 hr of the study. However, as in Study 1, treatment with estradiol-17β had no significant effect on either urine output or urine electrolyte excretion rate (Table I).

Discussion. Drinking accompanying administration of pilocarpine has been shown to depend in part on the dose administered and on the time after administration of this compound that measurements were made

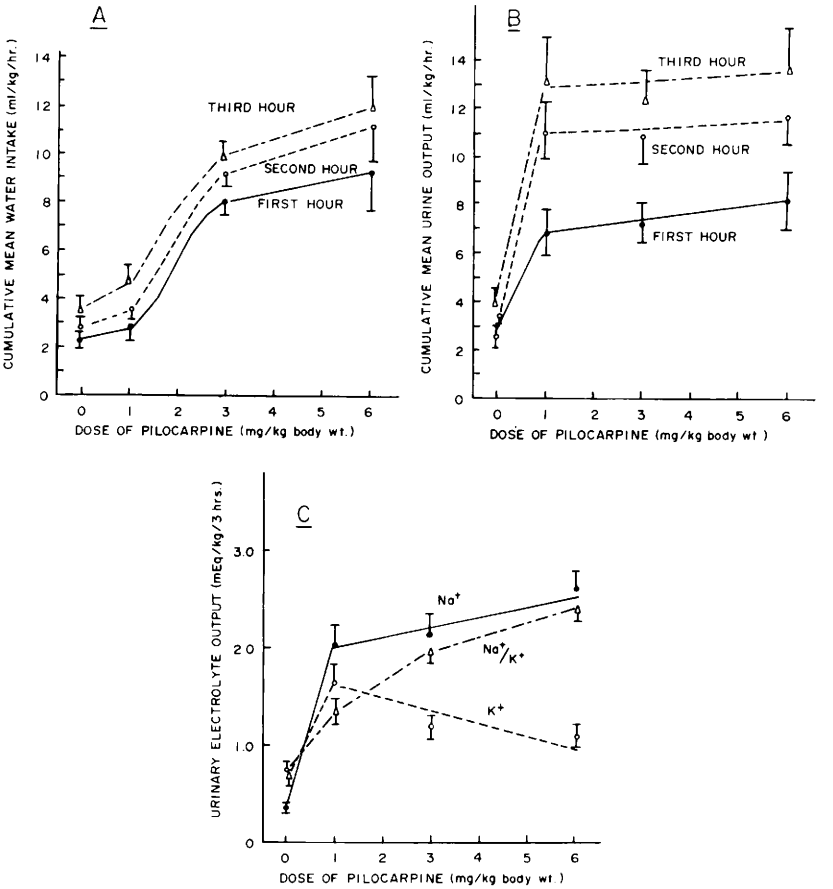

FIG. 1. Effect of graded ip doses of pilocarpine on water intake (A), urine output (B), and urinary sodium and potassium excretion rates (C) in rats at each hour during the 3-hr study. One standard error is set off at each mean.

(6). Maximal drinking response to graded ip doses of pilocarpine was observed for 3.75 mg/kg. Doses higher than this (7.5, 15, and 30 mg/kg) progressively reduced the drinking response during the first 90 min of the study. By 180 min after treatment a dose–response relationship appeared to have become established (6).

In the present study a dose–response relationship relating water intake to dose of pilocarpine administered (1 to 6 mg/kg) was observed (Fig. 1). The observation that the threshold dose required to increase the output of urine and electrolytes (sodium and potassium) is lower than that required to increase water intake is of interest. Reasons for this difference remain speculative. Of further interest is the mechanism by which urine sodium and potassium excretion rates are increased. Although urinary sodium excretion rate increased to a greater extent than urinary potassium excretion rate, the latter was elevated by all doses of pilocarpine administered (Fig. 1C). Whether the effect of pilocarpine on urinary electrolyte excretion is associated with an inhibitory effect on either the secretion of, or response to, aldosterone cannot be stated from these studies. Further, changes in renal hemodynamics and glomerular filtration rate might also account for the results. Additional study will be needed to determine the mechanism responsible. Similar results were observed by Saad et al (12)

TABLE I. EFFECT OF PILOCARPINE (3 mg/kg body wt, ip) ON WATER INTAKE, URINE OUTPUT, AND URINE ELECTROLYTE EXCRETION BY RATS TREATED CHRONICALLY WITH AN ESTROGENIC AGENT

Treatment group	No. of rats	Mean body weight (g)	Cumulative water intake (ml/kg body wt) during:			Cumulative urine output (ml/kg body wt) during:			Urinary Na output (meq/3 hr)	Urinary K output (meq/3 hr)	Urine Na/K ratio
			1	2	3 hr	1	2	3 hr			
Estradiol benzoate											
Control	6	281 ± 5	8.8 ±1.4[a]	9.9 ±1.3	10.2 ±1.3	5.1 ±0.9	6.6 ±1.0	7.9 ±0.9	1.5 ±0.2	0.74 ±0.09	2.18 ±0.14
Estradiol benzoate (21 μg/kg/day)	6	272 ± 5	4.3 ±0.9*	6.3 ±1.4	6.5 ±1.4	5.7 ±0.9	8.1 ±1.3	10.5 ±1.5	1.5 ±0.2	0.91 ±0.12	1.64 ±0.14
Estradiol benzoate (42 μg/kg/day)	6	262 ± 5	3.7 ±0.8**	4.4 ±1.2**	5.2 ±1.2*	5.5 ±0.7	7.8 ±1.1	8.6 ±1.2	1.4 ±0.2	0.73 ±0.09	1.89 ±0.19
Estradiol											
Control	6	309 ± 6	8.2 ±2.2	8.7 ±2.2	9.0 ±2.2	7.7 ±1.3	9.8 ±1.3	10.6 ±1.3	2.0 ±0.2	1.06 ±0.06	1.91 ±0.11
Estradiol-17β (6.5 μg/kg/day)	6	326 ±11	0.9 ±0.3**	1.3 ±0.4**	2.0 ±0.6**	6.2 ±0.6	10.8 ±0.6	14.7 ±1.4	2.3 ±0.2	1.13 ±0.04	2.00 ±0.18
Estradiol-17β (16.5 μg/kg/day)	6	314 ± 8	1.4 ±0.8**	1.7 ±0.8**	2.2 ±0.7**	4.7 ±1.0	8.8 ±1.2	11.2 ±1.4	2.2 ±0.2	0.96 ±0.06	2.32 ±0.18

[a] 1 SEM.

* Significantly different from control ($P < 0.05$).

** Significantly different from control ($P < 0.01$).

when carbachol, another parasympathomimetic agent, was microinjected into the septal area of the brain of the rat.

Chronic treatment with estrogens has long been known to inhibit the daily water intakes of rats (13–16), rabbits (5, 17), and goats (18). In addition, onset of estrus is accompanied by a reduction in the daily water intakes by rats (19–21), ewes (22), and cattle (23). The effect of estrogens on water intake may be related, in part at least, to a reduction in food intake (13, 15).

The mechanism by which chronic administration of either estradiol benzoate or estradiol-17β attenuates water intake accompanying administration of pilocarpine is unknown. It is also unknown whether this mechanism is the same as that by which estrogens attenuate the drinking response of rats to isoproterenol, renin and angiotensins I and II (1–4). The contribution of these attenuated responses to the reduction in daily water intake by estrogen-treated rats is unknown. While it has been known for many years that application of parasympathomimetic agents to several areas of the brain of the rat can stimulate drinking (24–26), Fitzsimons and Setler (27) recently reported that carbachol-induced drinking was blocked by either intracranial or subcutaneous doses of atropine while angiotensin-induced thirst was unaffected. On the other hand, haloperidol and spiroperidol, both dopamine antagonists, blocked angiotensin-induced drinking but not carbachol-induced drinking in rats. Similar results were reported by Gardina and Fisher (28). However, Swanson *et al.* (29) were unable to show that microinjections of haloperidol into the medial septum and medial preoptic area had an inhibitory effect on angiotensin-induced drinking. Moreover, both Swanson and Sharpe (30) and Myers *et al.* (31) reported that the areas of the brain that most readily elicit thirst in response to application of angiotensin II and parasympathomimetic agents overlapped considerably. Some disagreement thus exists regarding the uniqueness of central transmitters necessary to induce these two thirsts, as well as the separateness of their anatomical location. It is therefore difficult to know whether chronic treatment with estrogenic agents may attentuate pilocarpine-induced thirst by a peripheral or a central effect. Recent evidence suggests that angiotensin II-induced thirst is attenuated by estrogens (4). Whether estrogens may inhibit passage of both pilocarpine and angiotensin II into brain or whether it affects the responsiveness of receptors for each in the central nervous system is also unknown. Alternatively, the possibility that estrogens may attenuate responsiveness at some central site common to both thirst pathways remains for further study.

Summary. Acute ip administration of graded doses of the parasympathomimetic agent, pilocarpine, to female rats increased their water intake, urine output and urinary sodium excretion rate in a graded fashion. Of the three responses, urine output and urinary sodium excretion rate increased on administration of a dose of pilocarpine that had minimal effect on water intake. Chronic treatment of female rats with either estradiol benzoate (21 and 42 μg/kg/day) or estradiol-17β (6.5 and 16.5 μg/kg/day) attenuated significantly the drinking response to pilocarpine (3 mg/kg, ip) but failed to affect the increase in urine output or urinary sodium excretion rate. The mechanism by which chronic treatment with estrogens attenuates pilocarpine-induced water intake is unknown and awaits additional study.

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