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Effects of Norepinephrine Applied to the Lateral Hypothalamus on Schedule Induced Polydipsia[1]

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SINGER, G., S. ARMSTRONG AND M. J. WAYNER. *Effects of norepinephrine applied to the lateral hypothalamus on schedule induced polydipsia.* PHARMAC. BIOCHEM. BEHAV. 3(5) 869–872, 1975. – Intrahypothalamic injections of 3 doses of norepinephrine were administered to rats under conditions of 80 percent body weight reduction (prepolydipsia), 80 percent body weight reduction (schedule induced polydipsia), and normal body weight (postpolydipsia). The only significant reduction in water intake occurred with the highest dose of norepinephrine, under the prepolydipsic condition. The fact that norepinephrine failed to block schedule induced polydipsia indicates that this behavior is regulated by a different biochemical system than that of deprivation induced drinking.

Schedule induced polydipsia Norepinephrine Lateral hypothalamus Adjunctive behavior Drinking

CENTRALLY applied cholinergic and adrenergic agonists and antagonists affect eating and drinking [1,8]. Intrahypothalamic norepinephrine elicits eating in food satiated rats and intrahypothalamic carbachol elicits drinking in water satiated rats [7] and similarly applied phentolamine blocks norepinephrine induced eating [10]. Atropine as well as norepinephrine reduces carbachol produced, deprivation induced, salt arousal of drinking, and phentolamine as well as carbachol reduces norepinephrine and deprivation induced eating [14]. Several intermittent schedules of food delivery to partially food deprived animals have been shown to induce excessive drinking. This phenomenon which was originally described as psychogenic polydipsia [6] is more commonly referred to as schedule induced polydipsia. Schedule induced polydipsia is only one of a general class of behaviors which appear under a variety of intermittent reinforcement schedule conditions [6, 15, 16] and, since these behaviors are not necessary to obtain the reinforcement, have therefore been called adjunctive [6]. The experimental conditions under which schedule induced polydipsia is observed most readily involve schedule controlled bar pressing followed by eating and adjunctive drinking. Intraperitoneal injections of the cholinergic blocking agents atropine sulphate and atropine methyl nitrate reduced schedule induced polydipsia [2]. However, since atropine as the methyl nitrate salt dose not cross the blood brain barrier it was not clear whether a peripheral or central site of drug action is involved. When both salts of atropine are injected into the lateral hypothalamic or lateral preoptic area schedule induced polydipsia is reduced [3]. Since both lick rate and bar pressing were affected, it is possible that the high doses of atropine which were employed had a general sedating effect either directly in the brain or through diffusion into the peripheral nervous system.

The results of preliminary studies in our laboratory [17] indicated that intrahypothalamic injections of atropine and norepinephrine had no effect on bar pressing or schedule induced polydipsia. Only the two lower doses of atropine of a previous study [3] were used and the dose of norepinephrine was one which blocked deprivation induced and salt arousal of drinking [14]. Deprivation induced drinking in these same animals was blocked by higher doses of atropine and norepinephrine. Phentolamine, 650×10^{-4} M, also failed to affect schedule induced polydipsia. Carbachol increased lick rate but had no effect on water intake. These data do not seem to agree with the atropine–carbachol relation found in the other studies. However, lick patterns which seem indistinguishable from normal polydipsic licking as well as the failure to increase water intake suggest that carbachol might have a general activating effect on adjunctive licking. The fact that atropine and norepinephrine fail to block schedule induced

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polydipsia indicates that this behaviour has a different biochemical basis than normal drinking. The increase in activation following carbachol is in agreement with other data which show that carbachol increases drinking as well as mouse killing and suggests a nonspecific increase in motor excitability where the specific behaviour elicited depends upon the prepotency of environmental stimulation [16]. In the present experiment the effects of three dose levels of norepinephrine on deprivation, schedule induced and prandial drinking were compared.

METHOD

Animals

Eleven naive male Wistar derived rats weighing approximately 300 g and 90–120 days old were prepared with bilaterally implanted cannulae in the lateral hypothalamus (LH) according to previously described procedures [13]. Histological post-mortem examination revealed that the chemicals were injected into the anterior LH in at least one side at the anterior–posterior plane corresponding to Plates 39b to 32b of the König and Klippel atlas [9]. Rats lived in individual cages in a continuously illuminated room at 22°C. Testing was carried out in an adjacent room under similar conditions. A standard test chamber, 35 x 34 x 23 cm, with lever and a 45 mg pellet dispenser and drinking spout 6 cm away in the adjacent wall was employed. The test chamber was situated within a larger ventilated sound attenuating and illuminated enclosure.

Procedure

The experimental design consisted of three phases; prepolydipsic testing, polydipsic testing, and postpolydipsic testing.

The purpose of the prepolydipsic testing was to determine the effects of LH injections of norepinephrine on food and water intakes of animals reduced to 80 percent of pretest body weight. Rats were reduced to 80 percent body weight over a 6 day period and then maintained at this level by restrictive food intake. Each day animals were weighed, placed in an injection apparatus, and then placed in the test chamber for 1 hr where they were fed 45 mg pellets from a bowl and water was available at the spout. Animals were returned to the living cages and were given supplementary food to maintain 80 percent body weight levels. Water was available ad lib. Food and water intakes were recorded. Animals were given 5 days to adapt to this regime and then the injection sequence was carried out over the next 2 weeks.

Polydipsic testing consisted of essentially the same procedures except that during the 1 hr test session the 45 mg food pellets could be obtained only by bar pressing on a fixed interval 60 sec (FI 60 sec) reinforcement schedule. Excessive drinking usually began in the second session. Number of bar presses and water consumed were recorded at 30 and 60 min. After 4 days, when bar pressing and water intakes appeared to stabilize, animals were subjected to the injection sequence for the next 2 weeks.

Postpolydipsic testing began after the animals were placed on ad lib food and water and body weights returned to pretesting levels. Animals were subjected again to a 2 week injection sequence with food and water always available, thus the effects of LH administration of norepinephrine on normal food and water intakes were ascertained.

All injections were given bilaterally in a volume of 1.0 μl. After removal of the injection needle, the inner plunger was reinserted and the rat was placed either in the test chamber during prepolydipsic or polydipsic testing or back in the home cage during postpolydipsic testing. In each of the 3 phases of the experiment rats received 4 LH injections consisting of 1 injection of isotonic saline and 3 of norepinephrine. Norepinephrine, (1-arterenol bitartrate monohydrate, Sigma) in 72, 216 and 648 x 10^{-4}M concentrations were employed. These doses were selected from a previous report [11]. Norepinephrine was dissolved in distilled water and the concentration was adjusted with NaCl to 0.154M. Consequently, 0.154 NaCl was used as a control. Injections into the LH were given every third day. On the 2 intervening days animals were subjected to the same procedure except that the injection needle was not inserted. Therefore, the data from noninjection days served as an additional control and permitted the effects of isotonic saline to be determined.

RESULTS

The data are summarized in Fig. 1. The mean 1 hr water intakes for the 11 animals are presented for each dose of norepinephrine and the saline injections during the 3 phases of the experiment in the upper part of the figure. There was a significant reduction of prandial water intake (for the dose of 648 x 10^{-4}M) when compared to saline during the prepolydipsic phase when the animals were reduced to 80 percent of bodyweight, binomial test, $p<0.003$ [12]. The other 2 doses produced no significant change when compared to the saline injection. Although schedule induced polydipsia occurred during the second phase of the experiment and water intakes were elevated by 100 percent, there were no differential effects produced by any doses of norepinephrine compared to the saline injection. When animals were returned to ad lib eating and drinking and body weights recovered, there were no differential effects of norepinephrine compared to saline injections on food and water intakes. There were no significant differential effects of norepinephrine on bar pressing during the polydipsic phase or body weight throughout the course of the experiment.

The differences between the test day mean 1 hr water intakes and those of the previous sham control test are presented in the lower part of Fig. 1. Positive values indicate increases in water intakes and negative values indicate decreases in intakes as compared to intakes in noninjection sham control tests. A comparison between sham injection and saline tests shows that saline had little measurable effect on drinking under all 3 conditions. The only significant difference occurred for the large 648 x 10^{-4}M dose of norepinephrine, Fisher Exact Probability Test, $p = 0.025$ [12] during the prepolydipsic phase of the experiment. The remainder of the data indicate clearly that the 3 doses of norepinephrine utilized in this experiment had no effect on schedule induced water intake. There were no significant effects on bar pressing during the polydipsic phase.

DISCUSSION

These results indicate that the 3 doses of norepinephrine injected into the anterior LH had no effect on schedule

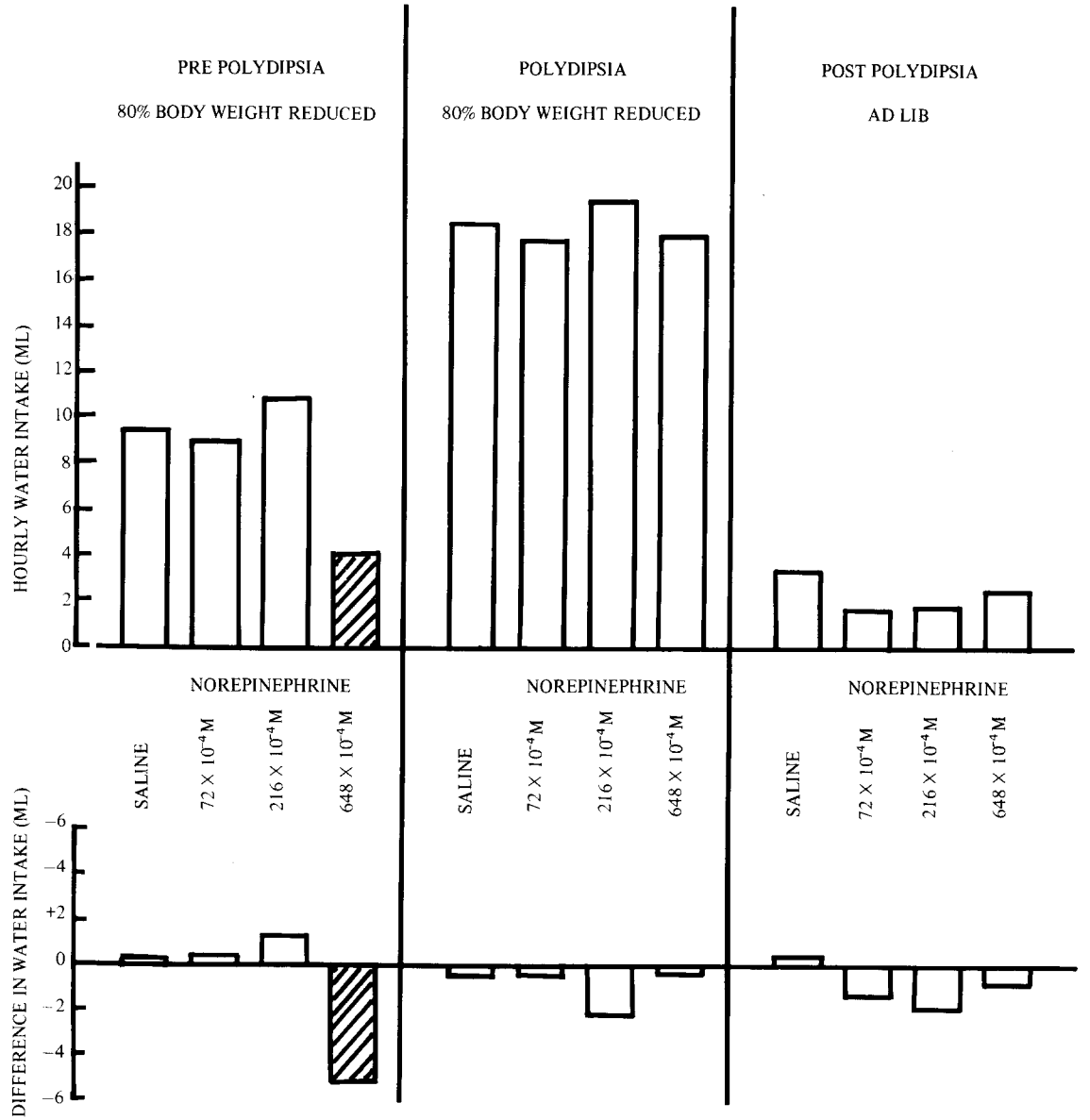

FIG. 1. Upper part: Mean 1 hr water intakes in ml following LH administration of saline, 72, 216 and 648 X 10^{-4}M of norepinephrine during prepolydipsic, polydipsic, and postpolydipsic conditions. (N = 11) Lower part: Mean differences between 1 hr water intakes following injections of saline and norepinephrine and intakes for previous day sham injection tests. Crosshatching indicates significantly different intakes. (N = 11).

induced polydipsia. The largest dose of norepinephrine decreased the prandial drinking which occurred during the prepolydipsia phase of the experiment when animals were maintained at 80 percent initial body weight by partial food deprivation. The postpolydipsic drinking which occurred when animals on ad lib eating and drinking and at normal body weight levels were placed in the test chamber for 1 hr after sham injections appeared to be depressed by the 3 doses of norepinephrine. These relatively tenuous data should be interpreted carefully because these same doses of norepinephrine administered to more posterior regions of the LH or to other parts of the brain might have different effects.

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