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BRIEF COMMUNICATION

Drinking and Subsequent Suppression of Vasopressin is Unaltered by Naloxone in Dogs[1]

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WADE, C E AND M M HUNT *Drinking and subsequent suppression of vasopressin is unaltered by naloxone in dogs* PHARMACOL BIOCHEM BEHAV 24(4) 1129–1132, 1986 —The effect of the opioid antagonist naloxone on drinking and the subsequent suppression of plasma vasopressin were evaluated in seven dogs following 24 hr of water deprivation Each animal underwent an intravenous injection of vehicle as a control and a low (0 05 mg/kg) and high (1 mg/kg) dose of naloxone Plasma vasopressin was significantly ($p<0$ 05) increased from a control value of 4 6±1 9 μU/ml to 9 9±3 1 μU/ml after the high dose of naloxone Fluid intake was not altered by naloxone, 42±6 ml/kg for the control, 45±8 ml/kg at the low dose, and 49±7 ml/kg for the high dose Six minutes after the onset of drinking vasopressin was reduced by 48% for the control, 41% for the low dose and 45% for the high dose, with no significant difference among treatments Thus, in dehydrated dogs naloxone presumably blocks endogenous opioids, elevates vasopressin following dehydration, but does not affect drinking behavior or the subsequent suppression of vasopressin after drinking

Opioids Dehydration Plasma osmolality Water deprivation

OPIOID antagonist administered to animals attenuates drinking induced by dehydration or hypertonic saline infusion [16]. Endogenous opioids may mediate thirst [16]. Endogenous opioids may also modulate the release of vasopressin Although controversial, recent work [9,14] suggests that the opioid agonists suppress vasopressin release, while the antagonist (e g , naloxone) produces an increase Thus, opioids appear to play a role in both thirst and the release of vasopressin. Endogenous opioids could therefore be an important component of fluid balance by modulating fluid intake by thirst and fluid loss by vasopressin's actions on the kidneys

In the last few years, several investigators [1, 8, 17] have shown that drinking suppresses vasopressin release in a variety of species including man. Thrasher *et al* [17] postulated that this response in dogs is mediated by an oropharyngeal reflex. Just as sham drinking is reduced in rats by naloxone, Rockwood *et al.* [13] suggest the suppression of drinking by naloxone may be mediated by a similar oropharyngeal reflex Thus, the modulation of drinking by naloxone administration and suppression of vasopressin with drinking may involve a common pathway. In the present study, using the opioid antagonist naloxone, we evaluated the possible role of endogenous opioids on drinking and their suppression of vasopressin following drinking in dogs deprived of water for 24 hr.

METHOD

Preparation

In this study we studied seven mongrel dogs, previously prepared with bilateral carotid loops. The dogs weighed 16 to 23 kg and were provided food and water ad lib. The animals were caged (1×2 m) individually All were housed in a room

[1]The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense In conducting the research described in this report the investigation adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animals Resources, National Research Council

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with constant temperature (21°C) and humidity (50%) and a light cycle of 14 hr/10 hr light/dark. All experiments were performed in the morning, two hr after lights were turned on Each dog was trained by repeated exposure and positive conditioning to stand quietly in a modified Pavlov sling (Alice King Chatham Medical Arts, Los Angeles, CA) Each animal underwent all experimental manipulations in a randomized manner, and a minimum of 10 days separated experiments.

Dehydration

In the morning, a dog in normal fluid balance was weighed, brought to the laboratory, and placed in the sling A saphenous vein was cannulated (Bard I-Cath, Murray Hill, NJ) Twenty min later a 20-ml blood sample was obtained, the volume replaced with normal saline, and the animal placed in a holding cage similar to their home cage for the next 24 hr and deprived of water The normal food ration was provided. The next morning, the animal was weighed again, brought to the laboratory, and placed in the sling Food was withheld until completion of the experiments as food consumption modifies fluid intake and may effect the oropharyngeal reflex modulating vasopressin release and thirst A saphenous vein was cannulated again to obtain a blood sample and a carotid artery was catheterized (Angiocath 22G Needle, Deseret Co , Sandy, UT) in order to monitor blood pressure. Blood pressure and heart rate were monitored continually throughout the experiment with mean values determined over a 2 min period Blood pressure and heart rate were measured before the injection, 30 min after treatment, and over the first 2 min of water presentation

An intravenous injection of saline (5 ml) as a vehicle control or naloxone in saline (0 05 or 1 0 mg/kg) (Endo Laboratories Inc., Garden City, NY) was administered The low dose of naloxone (0.05 mg/kg) was selected because it is a clinically effective dose for reversing exogenous opioid overdose [11] The high dose (1 0 mg/kg) was selected because it effectively attenuated induced drinking in other species [7, 11 14] Thirty minutes after the injection a blood sample (40 ml) was obtained from the saphenous vein Water was then presented to the animal for 6 min Blood samples (40 ml) were again obtained 6 min after the presentation of water Blood sample volumes were replaced with normal saline Upon completion of the experiment the animals were returned to their home cage and allowed free access to food and water.

Blood Sample Analysis

Blood samples were placed into chilled centrifuge tubes containing heparin and placed in ice Samples were centrifuged at 5°C, plasma samples were removed and aliquots of plasma for hormone determination were immediately frozen Samples of plasma were then tested for osmolality by freezing point depression (Advanced Instruments, Model 3DII, Needham MA) For determination of hormone concentrations, all samples for one dog were analyzed within the same assay Plasma arginine vasopressin levels were measured by radioimmunoassay following a bentonite extraction Mean recovery following extraction was 60% The standard curve was generated by using standard arginine vasopressin (97 3 U/ml, Sigma Corp , St Louis, MO), and iodinated arginine vasopressin (New England Nuclear, Boston, MA) Oxytocin was 106 times less effective in dissociating the iodinated arginine vasopressin from the antibody than vasopressin Interassay coefficient of variability was 13% (n=10)

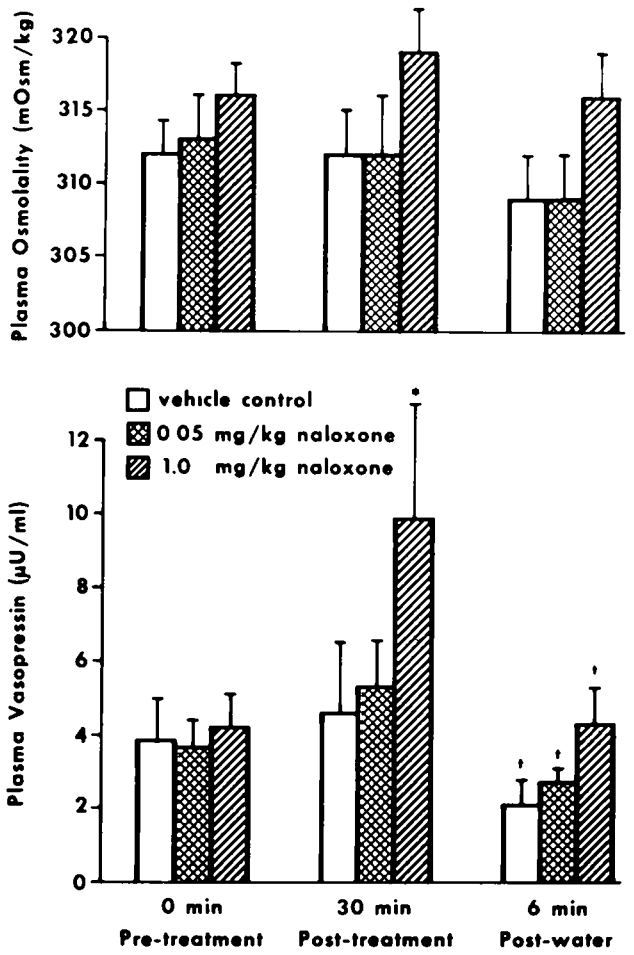

FIG 1 Plasma osmolality and vasopressin levels of seven dogs fluid deprived for 24 hr at rest following naloxone administration and drinking *Significantly different from pretreatment control (0 min) $p<0$ 05, †Significantly different from 30 min after treatment, $p<0$ 05

Statistical Analyses

A two-way analysis of variance for repeated measures was used to determine significant differences Differences between means were determined by using a Newman-Keul's test A probability at the level of less than 0 05 was considered significant Values in the text are mean plus or minus one standard error of the mean

RESULTS

Twenty-four hours of water deprivation significantly increased mean plasma osmolality by 14 mOsm/kg and vasopressin by 2 7 μU/ml from euhydrated values of 298±1 mOsm/kg and 1 2±0.2 μU/ml, respectively

Following dehydration there was no difference in plasma osmolality, prior to treatment, after treatment, or following drinking (Fig. 1) However, plasma vasopressin levels were significantly, $p<0$ 05, $F(2,12)=6$ 66, elevated with the high

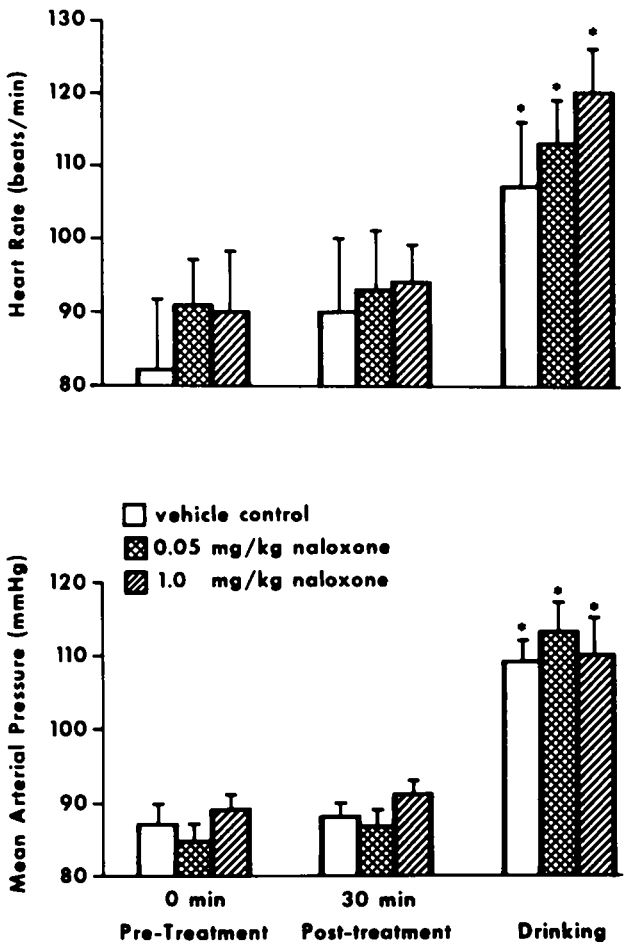

FIG 2 Heart rates and mean arterial pressures of seven dogs fluid deprived for 24 hr and the response to naloxone administration and drinking *Significantly different from pretreatment control, $p<0$ 05

dose of naloxone (1 mg/kg) compared to control values Plasma vasopressin levels were reduced by 48±7% for the control, 41±8% for the low dose, and 45±9% with the high dose of naloxone, 6 min after the presentation of water The animals began drinking immediately upon the presentation of water There was no significant difference in the percent reduction of plasma vasopressin 6 min following drinking among treatments

Fluid intake following water deprivation was not altered by the administration of naloxone. Fluid intake in response to vehicle injection was 42±6 ml/kg compared to 45±8 ml/kg for the low doses and 49±7 ml/kg for the high doses of naloxone The volume of water consumed over the six minutes of presentation was equivalent to the weight loss during the 24 hr of fluid deprivation. The volumes were. control 893±183 g vs 936±131 ml; low dose 1144±183 g vs. 984±136 ml, high dose 800±201 g vs. 1091±50 ml (change in body weight vs. water intake) The animals were able to rehydrate rapidly irrespective of naloxone administration

Fluid intake was related to the increase in plasma osmolality during dehydration for the individual animal ($r=0.66$, $n=21$, $p<0.001$).

Blood pressure and heart rate before drinking were not altered by naloxone (Fig. 2). During drinking, blood pressure, $F(2,12)=74.71$, and heart rate, $F(2,12)=61.67$, were increased with no apparent effect of naloxone

DISCUSSION

Fluid intake following increases in plasma osmolality in rats is reduced after treatment with opioid antagonists and potentiated by agonists [3, 5, 15, 16] These findings suggest endogenous opioid systems may be involved in drinking behavior. However, in mice the reduction in drinking following opiate antagonist administration was less pronounced than that observed in rats [2], while in pigeons a suppression of drinking was not apparent [6]. In hamsters the administration of agonist failed to increase water intake as noted in rats [10]. The dogs in the present study also showed no effect of naloxone on fluid intake following dehydration (at a dose effective in rats and cats [7,15]), which suggests that endogenous opiates do not alter dehydration-induced drinking behavior in the dogs

Ramsay *et al* [12] found dogs deprived of water for 24 hr to rehydrate by 89% within the first two minutes following the presentation of water Thrasher *et al* [17] noted similar results in dogs and found the animals satiated for 60 min after the initial intake of water Thus, the presentation of water for six minutes to the dogs in the present study, and the intake volume being equal to the body weight loss over the 24 hr of fluid deprivation suggest the effects of naloxone on drinking may be evaluated effectively in our preparation

The opiate antagonists elevate plasma vasopressin levels [9,14]. In the present study of dogs, plasma vasopressin was increased following the highest dose of naloxone, 1 mg/kg. A rise in vasopressin has been suggested to account for reduced drinking following naloxone treatment; however, it seems not to be necessary as naloxone suppresses fluid intake following dehydration in rats deficient in vasopressin due to genetic diabetes insipidus or hypophysectomy [3,4].

Recent work [1, 8, 17] has shown that the act of drinking following dehydration caused a rapid decline in plasma vasopressin levels in dogs, monkeys, and man. The reduction in plasma vasopressin concentration precedes absorptive changes in plasma osmolality or volume [17] These observations led Thrasher *et al* [17] to postulate that an oropharyngeal mechanism was operative. We postulated that the oropharyngeal response suppressing vasopressin during drinking could be mediated by endogenous opioids, and thus altered by naloxone However, no effect of opioid blockade with naloxone was noted in the suppression of vasopressin with drinking In fact, our findings support the observation by Thrasher *et al* [17] that the release of vasopressin must be rapidly and fully suppressed with the onset of drinking inasmuch as the time course of decline in plasma vasopressin corresponds to the half-life of the hormone in dogs (5 to 8 min). In our animals, irrespective of the initial vasopressin level, drinking reduced vasopressin values by more than 40% Thus, the act of drinking appears to suppress the release of vasopressin independent of regulation by endogenous opioids

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