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Cellular Dehydration and Hypovolemia: Effect of Acetylsalicylic Acid on Drinking[1]

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KENNEY, N. J. AND K. E. MOE. *Cellular dehydration and hypovolemia: Effect of acetylsalicylic acid on drinking.* PHARMAC. BIOCHEM. BEHAV. 17(1) 73–76, 1982.—Chronic oral administration of acetylsalicylic acid (ASA), an inhibitor of prostaglandin synthesis, reduces the latency with which rats begin drinking in response to hypovolemia but has no effect on the total amount of water consumed to this stimulus. When drinking is due to cellular dehydration, latency to drink is unaffected while total water intake is markedly augmented by ASA-pretreatment. Chronic, low-dose exposure to ASA or indomethacin has no effect on plasma levels of the dipsogen, angiotensin II. These data, taken in conjunction with previous work demonstrating a suppression of drinking following administration of exogenous prostaglandin E, support the contention that the E prostaglandins are involved in the physiological control of water intake, but suggest that the precise role of the prostaglandin in controlling consumption is dependent upon the stimulus eliciting the behavior.

Water intake Hypovolemia Cellular dehydration Prostaglandin E Acetylsalicylic acid Indomethacin Angiotensin II

THE E prostaglandins (PGE), naturally-occurring fatty acids which function as local hormones or paracrines [2], act both centrally and peripherally to reduce water ingestion to specific dipsogenic stimuli. The precise effect of these prostaglandins (PGs) on water intake is dependent upon both the site of application of the PGE and the specific conditions stimulating the drinking bout.

Either central [3, 6, 8, 9, 14, 15] or peripheral [11] administration of PGE effectively reduces water intake of rats elicited by angiotensin II (A II) treatment. Application of PGE directly to the brain also suppresses drinking resulting from polyethylene-glycol-induced hypovolemia [9]. Water intake due to such hypovolemia is resistant to intraperitoneal treatment with PGE, however, requiring a high, generally antidipsogenic, dose of the PG to elicit transient reductions of intake [11]. In contrast, drinking induced by cellular dehydration is markedly suppressed by peripheral administration of PGE [7,11], but is unaffected by even high-dose application of the PG to the brain [8,9].

Studies involving the suppression of endogenous PG synthesis, taken in conjunction with those involving administration of exogenous PGE, suggest that PGE may be involved in the physiological control of water ingestion. Intraperitoneal administration of indomethacin, a potent inhibitor of PG synthesis, elicits drinking by water-replete Sabra rats [4]. Chronic oral administration of this synthesis inhibitor markedly augments fluid intake in response to intravenous infusion of A II and reduces the latency to the onset of this drinking [10]. Some investigators [15,16] report that drinking induced by centrally administered A II is also augmented when PG synthesis is suppressed. Others, however, have failed to see any increase of water intake to intracranial A II following suppression of PG synthesis [6,10].

The effects of PG synthesis on drinking to polyethylene-glycol-induced hypovolemia and cellular dehydration are reported here. Prior to dipsogen testing, animals were pretreated with acetylsalicylic acid (ASA), an effective inhibitor of PG biosynthesis which has been reported to be more selective in inhibiting peripheral rather than central PGE levels than other PG synthesis inhibitors [1]. In addition, since many non-steroidal, anti-inflammatory agents which inhibit PG synthesis, including ASA, have been found to suppress renal renin release [18], the effect of chronic oral intake of low doses of ASA or indomethacin on plasma A II levels is reported.

METHOD

Subjects

Adult, male Long-Evans rats were housed individually in standard hanging cages. All animals had continuous access to food (Wayne pelleted lab chow) and water except as noted below. Testing was performed in the light portion of the 12:12 light:dark sequence.

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PG Synthesis Inhibition

Prostaglandin synthesis was inhibited by oral administration of ASA according to the method of Kenney and Moe [10]. The ASA solution (2.25 mg/ml de-ionized water) was offered to the animals in lieu of water for 4 or 5 days prior to dipsogen testing. This method of ASA administration suppresses plasma levels of the major circulating metabolite of PGE_2, 15-keto-13,14 dihydro PGE_2, by approximately 72% [10]. Levels of 15-keto-13,14 dihydro PGE_2 are reported to correlate directly with those of PGE_2 [13].

Hypovolemia

Eighteen rats were randomly assigned to one of two treatment groups. Rats in Group 1 (n=9, average body weight=376.7±18.4 g) received the ASA solution as their only fluid source for four days prior to testing. De-ionized water was provided as the drinking fluid for the animals in Group 2 (n=9, average body weight=393.6±21.6 g). Body weight and 24-hr fluid intake were recorded for each rat throughout the experiment.

After 4 days of exposure to ASA or water, all rats received subcutaneous injections of 5 ml of a 20% polyethylene-glycol (PEG) solution. Thus, rats treated with ASA received an average of 1.35±0.06 ml PEG/100 g body weight while the control rats received an average of 1.30±0.06 ml PEG/100 g body weight.

The PEG was dissolved in isotonic saline and injected between the scapulae while the animals were under light ether anesthesia. Rats were returned to their cages immediately after the PEG injection. All drinking fluids were withheld for the next 6 hours. After the 6-hr delay, all rats were given access to calibrated burets containing water. Water intake was measured every 10 minutes during the first hour of water access and at the end of the 120-min test session.

Cellular Dehydration

Nine rats (average body weight=307.3±9.8 g) were assigned to the PG-synthesis-inhibition group and were given the ASA solution as their only fluid source for 5 days prior to testing. Another nine rats (average body weight=310.1±9.5 g) were not treated with ASA and had ad lib access to de-ionized water as their drinking fluid.

On the test day (Day 6), each rat received a subcutaneous injection of 1 M NaCl (0.75 cc/100 g body weight) after which they were returned to their cages for 15 min. No water was available during this time. Following the delay, water was presented in calibrated burets. Latency to the onset of drinking was noted and water intake was measured every 5 minutes for the first 30 min of water access and at the end of the 1-hr water-presentation period.

Plasma A II Measurement

Twenty-eight adult, male Long-Evans rats (346–416 g) were assigned to one of four treatment groups matched for average body weight. Group 1 (n=8) received a 2.25 mg ASA/ml de-ionized water solution as their drinking fluid. Rats in Group 2 (n=8) received a solution of 0.01 mg/ml indomethacin in 1.2% ethanol as their drinking fluid, while rats in Groups 3 and 4 (n=6 for each group) received 1.2% ethanol in water or de-ionized water as their drinking fluids. The solutions and pelleted laboratory chow (Wayne) were provided ad lib for four days. Fluid intake and body weights were measured daily.

After the 4 days of exposure to the ASA, indomethacin or control solutions, all rats were sacrificed by decapitation and trunk blood was collected in heparinized tubes containing EDTA. Plasma A II was measured by radioimmunoassay according to the methods described in Reid *et al.* [17].

Data Analysis

Overall two-way (drug × time) repeated measures ANOVAs were conducted for all drinking tests. Planned orthogonal comparisons were performed in some cases to locate the source of treatment effect with regard to both time and drug. These comparisons were conducted on components of the overall treatment sum for each time interval. The F value was computed using a conservative error term specific to each comparison rather than a composite error term derived from the overall ANOVA [12]. In cases where tests other than ANOVA were used, the specific test is noted in the text.

RESULTS

Hypovolemia

During the 4 days of ASA exposure prior to PEG testing, rats in the ASA group ingested through their drinking fluid an average of 20.4±1.6 mg ASA per 100 g body weight daily. Total daily fluid intakes of these animals averaged 34.6±4.1 ml and did not differ significantly from the 38.5±2.4 ml reported by Kenney and Moe [10] for their animals showing a 72% decrease in plasma 15-keto-13,14 dihydro PGE_2. Average 24-hr intakes of the ASA-treated rats did not differ from the 24-hr intakes of rats receiving water (average water intake over the 4 days prior to testing=38.2±2.6 ml, $t(16)=0.749$, $p>0.20$).

Rats treated with the PG synthesis inhibitor drank more water than did controls during the first 10 min of the 2-hr test session (Fig. 1). During this first test-session interval, water intake of the ASA-treated animals averaged 6.3±1.0 ml compared to 3.3±0.7 ml for the controls, $F(1,16)=5.918$, $p<0.05$. (Rats not treated with PEG, but merely deprived of water for 6-hr after light ether exposure drank an average of 1.5±0.6 ml in the same time period). The elevation of hypovolemia-induced water intake observed for the ASA-treated rats was transient. Control animals drank slightly, though not significantly, more than the ASA-treated rats throughout the remainder of the test session. After 40 minutes of water access the mean cumulative water intake of control rats equalled that of the ASA-treated animals. At the end of the 2-hr session, total intakes of the ASA rats averaged 8.6±1.0 ml while that of the control rats averaged 7.4±0.8 ml (Fig. 1). (Again, both groups of PEG-treated rats drank more water during the 2-hr session than did animals not exposed to PEG but deprived of water for 6-hr. Intakes of these animals averaged 3.2±0.9 ml during the same 2-hr period).

Time between water presentation and the onset of drinking varied greatly for rats in both the ASA and water groups. Median latency to drink was significantly shorter for the ASA-treated animals, however (Med=2 sec for ASA-treated animals and Med=167 sec for controls, $\chi^2=3.776$, $p<0.05$).

Cellular dehydration

During the 5 days of ASA exposure prior to dipsogen

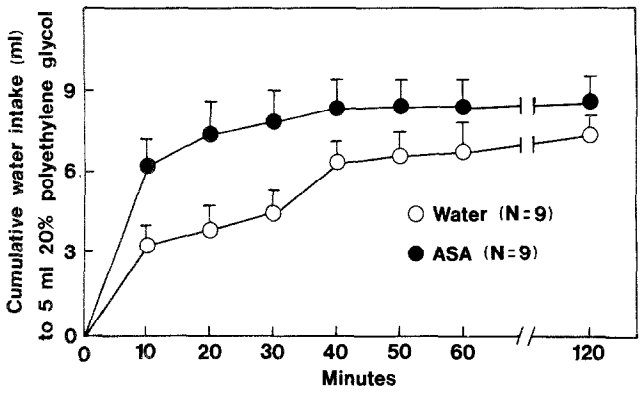

FIG. 1. Cumulative water intake (mean±standard error) following a subcutaneous injection of 5 ml 20% polyethylene glycol for rats pretreated with acetylsalicylic acid and for control animals not exposed to the prostaglandin synthesis inhibitor.

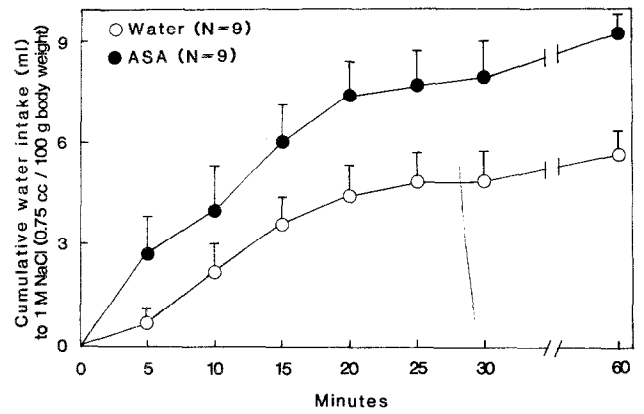

FIG. 2. Cumulative water intake (mean±standard error) of rats pretreated with acetylsalicylic acid and of control rats not exposed to the prostaglandin synthesis inhibitor in response to a subcutaneous injection of hypertonic saline.

testing, ASA-treated rats did not differ from control animals in total 24-hr fluid intake. ASA-treated rats consumed an average of 32.3±1.9 ml of the ASA solution daily while controls drank an average of 35.8±2.6 ml of water per day, $t(16)=1.063$, $p>0.20$. The average dose of ASA consumed daily by animals in the PG-synthesis-inhibitor group was 23.8±0.6 mg/100 g body weight. Again, this does not differ from the amount consumed by rats showing a marked reduction of plasma 15-keto-13,14 dihydro PGE_2 as reported by Kenney and Moe [10].

Rats pretreated with ASA drank significantly more water than controls following subcutaneous injection of hypertonic saline (Fig. 2). Overall comparison of water intakes of ASA-treated and control rats during the six 5-min intervals immediately following the saline injections indicated a significant augmentation of intake of ASA-treated rats, $F(1,16)=11.25$, $p<0.01$. During this first half of the test session, ASA-treated animals consumed an average of 7.9±1.1 ml while control rats drank only 4.8±0.9 ml. Animals exposed to the PG synthesis inhibitor continued to ingest slightly, though insignificantly, more water than control animals during the second half-hour of the test session (average intake=1.3±0.7 ml for the ASA rats and 0.8±0.4 ml for the controls). Thus, at the end of the test period, total intakes of the ASA rats were 64% greater than those of animals not exposed to the synthesis inhibitor. Average total intake across the entire test period was 9.2±0.6 ml for the ASA rats and 5.6±0.7 ml for the control animals, $F(1,16)=14.11$, $p<0.01$.

Latency to the onset of drinking varied greatly within both the ASA and control groups. Median latency to the onset of drinking in response to cell dehydration was not affected by ASA treatment (Med=530 sec for ASA-treated rats and Med=410 sec for controls, $\chi^2<1$).

Plasma A II Measures

During the four days of access to ASA, rats in Group 1 ingested an average of 20.3±0.6 mg ASA/100 g body weight per day. Rats in Group 2 took an average of 0.10 mg indomethacin/100 g body weight daily.

Although both the ASA and the indomethacin treatments have been reported to significantly suppress plasma 15-keto-13,14 dihydro PGE_2 (and presumably PGE_2) levels [10], these treatments had no significant effect on plasma A II levels. After 4 days of ASA treatment, plasma A II levels averaged 93.5±41.7 pg/ml (MED=46). For rats exposed to indomethacin, plasma A II levels averaged 47.9±5.9 pg/ml (MED=45). Control plasma A II levels averaged 62.8±5.4 pg/ml (MED=63) for rats offered water as their drinking fluid and 70.2±15.0 pg/ml (MED=59) for animals given 1.2% ethanol in water.

DISCUSSION

Previous data have indicated a potential role for peripheral, but not central, PGE in controlling water ingestion in response to cellular dehydration. Peripheral administration of either PGE_1 [7] or PGE_2 [11] suppresses water consumption in response to a hypertonic saline load. Centrally administered PGE has no effect on drinking to this stimulus [8,9].

The present data add support to the hypothesis that peripheral PGE levels are involved in the control of total fluid consumption in response to cell dehydration. Suppression of endogenous PG levels through chronic oral administration of ASA results in a marked augmentation of total water intake but no change of latency to the onset of drinking following subcutaneous injection of 1 M NaCl. Thus, endogenous peripheral PGE may act as a satiety indicator which limits the size of a drinking bout initiated in response to reduced intracellular fluid volume.

The role of peripheral PGs in controlling intake in response to hypovolemia remains uncertain. Kenney *et al.* [11] reported that intake induced by polyethylene-glycol treatment was unaffected by peripheral PGE_2 administration unless high, generally antidipsogenic doses of the PG were employed. Similarly, we report here that suppression of endogenous PG levels by ASA has only transient effects on the amount of water consumed to hypovolemia. We did find, however, that the latency to the onset of drinking to this stimulus was markedly lower for rats exposed to ASA. If peripheral PGE levels do play a role in controlling fluid ingestion to hypovolemia, its role must be that of modifying

the rate of onset of drinking rather than limiting total ingestion.

ASA, in addition to blocking PG biosynthesis, reduces renin-A II secretion under a variety of conditions [18]. The failure of ASA-pretreated rats to increase water consumption in response to polyethylene-glycol treatment apparently is not due to suppression of plasma levels of the potent dipsogen, A II, however. Chronic oral administration of either ASA, as used in the dipsogen tests reported here, or indomethacin, another effective PG synthesis inhibitor, has no reliable effect on basal plasma A II levels. Although it is possible that such PG-synthesis-inhibitor treatment might reduce A II release induced by the polyethylene-glycol administration, the reduction of stimulated A II release very likely would not be sufficient to counteract any major influence of PG inhibition on drinking. Complete elimination of renal renin secretion through nephrectomy has negligible effects on water intake to polyethylene-glycol induced hypovolemia [19].

In concurrence with the report of Kenney and Moe [10], 24-hr fluid intakes of the animals studied here, independent of specific dipsogenic challenges, were not affected by exposure to the PG synthesis inhibitor. Prior to both the polyethylene-glycol and the hypertonic saline tests, 24-hr intakes of rats exposed to ASA did not differ from those of control animals. The failure of chronic PG synthesis inhibition to augment 24-hr fluid intake does not preclude a role for PGE in the control of spontaneous water intake. Long-term, ad lib water intake is under the control of a multitude of factors including many of a non-homeostatic nature [5,20]. Thus, it is likely that the effect of manipulation of any single controlling factor, such as plasma PGE levels, would result in compensatory actions by other fluid-intake control mechanisms. Any effect of single-factor manipulations would more likely be observable through a thorough analysis of the short-term units of fluid ingestion, bout size and bout frequency. Such an analysis has not been conducted for animals treated with inhibitors of PG synthesis.

While the question of the role of PGE in spontaneous water ingestion remains unanswered, the data reported here, taken in conjunction with previous studies demonstrating the antidipsogenic effects of exogenous PGE [7, 9, 11], provide a strong evidence for a role of endogenous peripheral PGE in determining total fluid consumption in response to cellular dehydration and suggest that the PG may also slow the onset of drinking in response to hypovolemia.

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