Investigations on the Physiological Controls of Water and Saline Intake in C57BL/6 Mice

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Running Head: Water and Saline Intake in C57BL/6 Mice

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ABSTRACT

To examine the behavioral and neural control of body fluid homeostasis, water and saline intake of C57BL/6 mice was monitored ad libitum, after dipsogenic treatments or treatments that induce salt intake, and after ablation of the periventricular tissue of the anteroventral third ventricle (AV3V). Mice have nocturnal drinking that is most prevalent after the offset and before the onset of lights. When given ad libitum choice, C57BL/6 mice show no preference for saline over water at concentrations up to 0.9% NaCl and a progressive aversion to saline above that concentration. Systemic hypertonic saline, isoproterenol and polyethylene glycol treatments are dipsogenic, however, systemic angiotensin II (ANG II) is not. Both intracerebroventricular injections of hypertonic saline and of ANG II are dipsogenic, and diuretic treatment followed by a short period of sodium deprivation induces salt intake. After ablation of the AV3V, mice can be nursed to recovery from initial adipsia and like rats, show chronic deficits to dipsogenic treatments. Taken together, the data indicate that mechanisms controlling thirst in response to cellular dehydration in C57BL/6 mice are similar to rats, but that there are differences in the efficacy of extracellular dehydration-related mechanisms, especially for systemic ANG II, controlling thirst and salt appetite.

Keywords: AV3V ablation, drinking, homeostasis, rhythms, sodium appetite, thirst
INTRODUCTION

A major interest in the study of homeostasis has focused on the physiological and behavioral regulation of body fluid balance (28). A substantial body of research has characterized the mechanisms of fluid regulation in the rat, dog, and sheep. Current findings in these species support the double depletion hypothesis of thirst (18,21) as proposed by Epstein (18) and Fitzsimons (21) indicating that deficits of either the extracellular- or intracellular-fluid compartments will induce drinking. However, thirst is influenced by other factors. The amount of drinking induced experimentally by extracellular or intracellular deficits as well as drinking induced by food intake is modulated by the circadian system of the rat (29,30). Ad libitum water intake in rats is highly rhythmic, nocturnal, and associated with food intake (23,32,55).

Specific brain areas have been implicated in the control of drinking responses induced by humoral stimuli associated with cellular and extracellular dehydration. The periventricular tissue of the anteroventral third ventricle (AV3V) region, which includes the organum vasculosum of the lamina terminalis (OVLT), the ventral portion of the median preoptic nucleus (vMnPO), and the preoptic periventricular nucleus of the third ventricle (8), is necessary for thirst induced by angiotensin II (ANG II) and hypertonic saline treatments in several species.

An important component of body fluid homeostasis is electrolyte balance, and this has been extensively studied in the rat in relation to behavioral influences on sodium regulation (22). Sodium appetite is a behavioral means to defend extracellular fluid and may be produced by deficits in extracellular fluid volume. The regulation of sodium intake also has central components in that lesions of the AV3V, subfornical organ (SFO), and central amygdala all produce reductions in experimentally induced salt appetite (12,25,49,50,53,54), whereas lesions in the area postrema increase salt appetite (10,17,27).
Recent developments in the genetic manipulation of the regulation of body fluids and blood pressure of mice (6,9) have made it important to gain insight into the characteristics and control of water and salt intake in normal mice. Several studies have demonstrated that mice conform to the double depletion model of thirst in some respects, but differ from rats in others. For example, as found in rats, 24-h water deprivation or intracellular dehydration by subcutaneous injections of hypertonic saline results in robust water drinking in mice (43). However, manipulations of the peripheral renin-angiotensin system (RAS), a key humoral stimulus of thirst during extracellular dehydration, produce only weak or insignificant water intake in mice (15,31,43).

The regulation of salt intake in the mouse appears to be different than that of the rat. Unlike rats (24), mice do not prefer saline (at concentrations near physiological) compared to water (44), adrenalectomized mice do not increase their salt intake (43), and systemic treatment with deoxycorticosterone (DOCA) is reported to have no effect on salt intake (39,43,51; but see 2,5,42). Manipulations of the RAS are also equivocal in terms of their effects on salt intake in mice. Neither acute systemic ANG II treatments (14,31) nor chronic subcutaneous infusion s of ANG II (15) increase salt appetite. Captopril, an angiotensin converting enzyme (ACE) inhibitor, in small doses, which might be expected to increase central ANG II, when mixed with the food or water of mice (43,52) does not induce a salt appetite.

There were several aims of the following studies examining the control of water and saline intake in normal mice (strain C57BL/6). First, the ad libitum intake of water and saline (2%) of mice was monitored to examine the 24-h rhythmic patterns, the relationship of those patterns to the light-dark (LD) cycles and the effects of phase shifts of the LD cycle. The ad libitum preference of mice for saline vs. water was examined using a two tube choice of water
with varying concentrations of saline. Second, the effects of experimental treatments to induce thirst and/or salt appetite were determined. The induction of water intake was attempted using peripheral and central injections of hypertonic saline to produce intracellular dehydration. Extracellular dehydration was produced by subcutaneous injection of polyethylene glycol (PEG), and was mimicked by peripheral injection of isoproterenol and ANG II and by central injection of ANG II. The RAS was also manipulated by the addition of captopril to the drinking water at varying concentrations. Induction of a salt appetite was attempted using diuretic treatment followed by sodium deprivation and also by diuretic treatment combined with captopril. Finally, the acute and chronic effects of AV3V lesions were studied in C57BL/6 mice. Mice received lesions designed to destroy the areas of the forebrain defined as the AV3V in studies of rats. Subsequently, the acute effects of the lesions were examined in relation to the recovery of ad libitum body fluid regulation, and the chronic effects of the lesions were studied in terms of the ability of mice to respond to body fluid challenges.

METHODS

Animals and Housing

Adult male C57BL/6J mice (60 days old) were purchased from Jackson Laboratories (Bar Harbor, ME). Mice were housed individually either in stainless steel hanging cages or in plastic basket cages in a temperature (22°C) and light (LD 12:12) controlled room. The light onset for the majority of the studies was at 0600 h. However, for one subset of mice the light onset was set at 0330 h to test the effect of an LD cycle phase shift on the ad libitum intake water pattern. The mice were not isolated from regular animal care activity nor the activity of the other scientific staff, although human activity in the colony was restricted to the light period as was
any experimental manipulation. Except where noted, the mice had water and food (7013 NIH-31 Modified diet; Harlan/Teklad; 0.33 % sodium) ad libitum. All mice were adapted to the room for at least 2 wks before experimentation. For any surgical procedure, mice were anesthetized with sodium pentobarbital (50 mg/kg bw, ip), and surgery was conducted under aseptic conditions. All experiments were conducted in accordance with the National Institutes of Health “Guide for the Care and Use of Laboratory Animals” and were approved by the University of Iowa Animal Care and Use Committee.

**Water Intake Measures**

In order to monitor the daily pattern of water and saline (2%) intake, lickometers were employed. The hanging cages were equipped with glass burettes (0.1 ml gradation) that were modified by producing a 1 mm hole in the side near the bottom end and running a thin wire down the inside of the tube. The bottom of the cage was connected to a wire such that when the mouse licked the tube opening, an electrical “switch-closure” occurred that could be detected and recorded by computer. Data were collected in 10-min bins. It should be noted that the lickometer data do not give an accurate indication of intake volume since the pattern and efficiency of licking of individual mice and the openings of different tubes are idiosyncratic. However, it does give a reliable measure of the pattern of intake. Individual tubes were maintained with each mouse throughout all measures.

For studies employing the acute homeostatic challenges, accurate quantitative measures of intake were read directly from a glass graduated burette (0.1 ml resolution). In some cases a second burette was also attached to the cage so that saline intake could be measured. When mice were housed in plastic basket cages, graduated burettes with attached stainless steel spouts (0.1
ml resolution) containing water and/or saline were inserted through the bars at the top of the cage with the spouts protruding about 5 cm into the cage.

**Saline Preference**

Over a series of consecutive weeks, a group of eight mice was studied for their preference or aversion to saline relative to water. Two tubes, one containing water and one containing saline of varying concentrations, were placed on the cage and the intakes were monitored for at least 48 consecutive hours. The position of the tubes was alternated every 24 h to control for placement preference. Percent saline preference was calculated as volume of saline intake divided by total fluid intake. The concentrations of saline studied were first presented in ascending series from 0.1% to 3.1%, then several concentrations were selected and sequentially introduced in a descending series, and finally 3.1% saline was presented at the conclusion of the tests.

**Ablation of the AV3V**

To produce lesions in the AV3V area, the scalp was shaved, disinfected with Betadine® and a midline incision made. A hole was drilled in the skull, the midsagittal sinus was retracted, and then a 22-ga nichrome wire electrode was stereotaxically positioned such that the tip was located 0.4 mm rostral to bregma, in the midsagittal plane and 4.8 mm below the dura (skull leveled between bregma and lambda). The coordinates were established for the mouse using a stereotaxic atlas (47) and empirically adjusted using several mice for pilot placements before the main experiments. The electrode was insulated except for the extreme tip. Once the tip of the electrode had been positioned, a ground electrode was attached at the scalp incision, and 1 mAmp of current was passed across the tip for 5 seconds. Mice receiving sham lesions had the same treatment except for the passage of current, and the electrode was lowered only 4.0 mm
below dura to avoid mechanical damage to the AV3V. Prior to surgery, all mice were accustomed to the taste of 10% sucrose to avoid neophobic reactions to the taste of sucrose since sucrose was often needed to facilitate hydration and therefore postsurgical recovery. All surgeries were completed in the morning or early afternoon so that the animals recovered from anesthesia well before the onset of the dark period of the LD cycle. It is important to note that recovery from anesthesia before light offset greatly facilitated post-lesion recovery. Water intake during the subsequent dark period was measured, and any mouse consuming less than about one-third of normal intake (i.e., less than 1 ml) was placed on 10% sucrose to facilitate orally maintained hydration and gradually, as required by the individual mouse, over a period of days (generally less than 3 wks) weaned to water by lowering the sucrose concentration. In extreme cases of adipsia, recovery was aided by subcutaneous injection of physiological saline. No experiments were conducted until mice had recovered for at least two wks.

When all the behavioral tests were completed, the mice were perfused transcardially with 10% formalin. The brains were removed, cryoprotected in 30% sucrose (dissolved in 10% formalin) for at least 24 h, and then sectioned (40 µm) on a freezing microtome and stained with cresyl violet for histological analysis.

**Lateral Ventricle Cannulas**

For injections into the lateral ventricle, under aseptic conditions a guide cannula was stereotaxically placed such that the tip was 0.3 mm caudal to bregma, 1.0 mm lateral to the midline and 3.0 mm below dura (skull level between bregma and lambda). The coordinates were estimated from an atlas (47) and the placement of the cannula in the lateral ventricle was confirmed by the injection of India ink (2 µl) in several test mice. The cannula (10 mm long 23-ga stainless steel tubing) was cemented in place using dental acrylic and a stainless steel screw in
the skull. At least 4 days were allowed for recovery before any injections were made, and all lateral ventricular injections, performed as aseptically as possible, were given as a 2 µl bolus. The injector was 11 mm long (so that it extended 1 mm beyond the guide cannula) 30-ga stainless steel tubing.

Behavioral Tests

Mice received systemic homeostatic challenges designed to produce intracellular dehydration or to mimic hypovolemia. To induce cellular dehydration, the mice were injected subcutaneously with hypertonic (0.5 ml of 3 or 6% w/v) or physiological (0.9%) saline, and their water intake was monitored for 2 h using inverted graduated (0.1 ml resolution) cylinders fitted with sipper tubes.

To mimic components of hypovolemia (i.e., hypotension and elevated renin-angiotensin levels) the same procedure was used except the mice received either isoproterenol (15 or 30 µg/kg) or vehicle (0.9% saline) and intake was monitored for 90 min. To test the effect of systemically administered ANG II in an initial study, one group of mice was injected (100 µg ANG II or vehicle in 0.5 ml, sc) and water intake was monitored for 1 h. Because of concerns that the initial dose of ANG II may have produced inhibition of drinking due to the pressor actions of the peptide, a second study was conducted using 4 independent groups injected with three doses of ANG II (10, 33, 100 µg or vehicle in 0.1 ml, sc). To induce hypovolemia, PEG (25% w/v, 1 ml/mouse) was injected subcutaneously and water and 2% saline intake were monitored for 2-6 h.

In addition to the systemic challenges, bolus (1-3 sec duration) central injections into the lateral ventricle were made using Intramedic PE 10 tubing attached to an injector and a 10 µl Hamilton syringe. One group received ANG II (20 ng/2 µl), or hypertonic saline (1 µl of 600
mM) or the combined challenges in tests conducted on separate days, and intakes were recorded for 30 min from a glass burette attached to the cage. Another group received repeated injections of ANG II counterbalanced at different dosages to obtain a dose-response curve. The doses were given on separate days and counterbalanced, and intakes were recorded for 20 min.

To examine the effect of peripheral inhibition of ACE, captopril was chronically added to the drinking water at varying concentrations (0.1, 0.5 and 1.0 mg/ml drinking water) and water and saline intakes were monitored over 5 days, and the intakes of the last 2 days were averaged and compared to those of concurrently run controls. Baseline intakes were established for 4-6 days between tests. In an attempt to induce a salt appetite, water and salt sated mice were injected with the diuretic, furosemide (10 mg/kg sc), followed by the ACE inhibitor, captopril (5 mg/kg sc). Mice were deprived of water and saline for 1 h, and then water and saline (2%) were returned and intakes were measured for 2 h. In a further attempt to induce salt appetite, mice received two injections of furosemide (1 mg/kg) 2 h apart and received only sodium deficient diet (sodium deficient diet, rat modified; ICN Biomedicals, Inc., Aurora, OH) and distilled water for the next 21 h. Then, the intakes of distilled water and 1.2% saline were measured for 2 h.

**Statistical Analysis and Animal Grouping**

Data for the assessment of daily patterns of intake were gathered from two groups of mice (4 mice for 7 days and 8 mice for 5 days) that were eventually used in the following studies, but before any manipulations commenced. A separate group of mice (n=8) was tested repeatedly for preference of differing concentrations of saline compared to water.

For homeostatic challenges where repeated measures were made from individual mice, at least two days were allowed between measures for systemic manipulations and at least one day for central manipulations. For the challenges, an initial group of mice (n=8; 7 viable) was used
to test the effects of multiple concentrations of systemic hypertonic saline, ANG II (1st test), and PEG. A new group of mice (n=8) was used to test the effect of multiple concentrations of systemic isoproterenol. The attempt to induce salt appetite using combined treatment with furosemide and captopril was carried out in mice of both of the above groups (total n=16). Another four independent groups of mice (n=6 each) were used to test for effects of differing doses of systemic ANG II (2nd test) over a more complete range. Another group of mice (n=9; 7 viable) received cannulas for repeated testing of central NaCl and ANG II, and yet another group (n=10; 9 viable) received cannulas for repeated testing to establish a dose-response relationship to central ANG II. The attempt to induce salt appetite by treatment with furosemide followed by overnight salt deprivation was carried out in one group of mice (n=8) and later replicated on a second group (n=8) with an independent control group (n=8). Two independent groups of mice (n=6 each) received either repeated testing of response to captopril in their drinking water or concurrent control measurements.

For statistical analysis, in the case of multiple comparisons, data were first submitted to a repeated measures ANOVA (or completely randomized ANOVA if independent groups were compared) and, if significance was found, followed by the appropriate t-tests using the Bonferroni correction for multiple comparisons. In cases of only one treatment level, a simple t-test was used (independent means, repeated measures or comparison to predicted population means as applicable).

A separate group of mice (n=15, 11 viable) was used to assess the effects of an AV3V lesion. Behavioral data for these groups were analyzed with two separate two-way mixed design ANOVA’s (repeated measures on saline or isoproterenol) and mean differences isolated with a Tukey test to control α inflation.
Significance for all statistical tests was set at $P < 0.05$ (one-tailed in the cases of directional hypotheses).

RESULTS

The daily water intake pattern across the 24-h of an LD cycle is shown in Fig. 1 for two groups of mice on different phases of an LD 12:12 cycle. Since drinking is episodic in nature, which makes daily intake patterns difficult to discern, the data sets have been statistically smoothed as described in the figure caption. Both groups showed a major peak of water intake occurring shortly after light offset that falls to a minor trough in the mid-dark phase. Drinking then rises in anticipation of light onset to drop sharply after light onset to the major daily trough during the light phase. Although both groups show the common trimodal pattern on LD 12:12 cycles, the patterns differ somewhat in the height of the peaks associated with the LD transitions, perhaps because of the nearness of the LD transition to the regular daily caretaking in the case of light onset at 0600 h. In one group of eight mice, the average daily intake was $4.03 \pm 0.25$ ml of water and $0.11 \pm 0.03$ ml of 2% saline based on the grand mean of six days. The ad libitum intake of saline was so low that no meaningful assessment of the 24-h pattern could be made.

Saline Preference

There was little indication of either saline preference or aversion at concentrations of saline less than the physiological level (Fig. 2), but there was progressive aversion at concentrations greater than 0.9%. A post-hoc examination of the saline preference of the individual mice over the various concentrations indicated that the average preference pattern described most individual patterns. However it should be noted that there were idiosyncrasies in
that one mouse demonstrated an aversion to the saline immediately, and several mice showed a rather marked preference for saline near 0.9% (physiological).

**Dipsogenic Challenges in Normal Mice**

Subcutaneous injection of hypertonic saline produced a dose-dependent increase in water intake as did isoproterenol (Fig. 3). In an initial study, ANG II (0.1 mg/mouse, sc) did not produce significant water intake compared to vehicle injections (0.08 ± 0.05 ml for 0 mg vs. 0.05 ± 0.06 ml for 0.1 mg ANG II). A second study testing ANG II over a range of doses (Fig. 3) also produced no significant drinking to subcutaneous peptide. Subcutaneous injection of PEG produced a significant increase in water intake in a 2-h test (Fig. 3). However, PEG did not induce saline (2%) intake in any mouse during the 2-h period, and even at 6 h post-injection when total water intake had risen to 1.31 ± 0.03 ml, saline intake was still negligible (0.06 ± 0.03 ml).

When administered independently into the lateral ventricle, both ANG II and NaCl produced a significant short-latency water intake (Fig. 3). However, the treatment of NaCl combined with ANG II injection did not produce significantly more drinking than either treatment alone. A dose-response curve for centrally injected ANG II is shown in Fig. 3.

A robust salt intake was induced by the protocol of furosemide injections followed by 21 h of sodium deprivation (n=8). In the first study, the intake of saline (1.2%) during a 2 h test was significantly greater (0.73 ± 0.10 ml; P < 0.05) than that predicted (i.e., zero), but the water intake (0.15 ± 0.05 ml) was comparable to baseline intake. In a second test using the same procedure as above but including an explicit control group, the saline intake was also significantly greater in mice injected with furosemide (0.87 ± 0.05 ml, P < 0.05) than vehicle (0.9% saline; 0.05 ± 0.01 ml). The protocol that combined injections of furosemide with
captopril (n=16) failed to induce either water intake (0.12 ± 0.03 ml) or saline intake (intake nil) above that expected for baseline over a 2-h test.

For the study using captopril in the drinking water, a two-way mixed design ANOVA with repeated measures on captopril concentration indicated a significant interaction of group (captopril vs. control) by concentration (F(2,20) = 19.15, P < 0.01) in water intake. Captopril did not increase water intake significantly over the control measures at the lowest dose (0.1 mg/ml), but did significantly increase water intake progressively at 0.5 and 1.0 mg/ml (Table 1). There was no significant effect of captopril on saline intake.

**Acute Effects of AV3V Lesions**

Eleven of 15 mice that received the ablation recovered ad libitum water intake. Mice that died within 24-48 h had a water intake (ml) during the postsurgical dark period of 0.47 ± 0.44 compared to 0.17 ± 0.11 in mice subsequently identified as having complete AV3V lesions, 0.40 ± 0.18 in those identified as having partial AV3V lesions, and 2.88 ± 0.21 in those receiving sham ablations. The mice that recovered drinking to ad libitum amounts required gradual weaning from 10% sucrose to tap water over a period of 2-3 wks (sucrose concentration was progressively halved when 24-h intake was on the order of 1 to 2 ml of fluid intake).

**Histological Analysis of AV3V Lesions**

Coronal sections through the forebrain were assessed for cell loss or gliosis specifically focusing on the structures that produce behavioral deficits when ablated in the rat (8). Seven mice had lesions that included the OVLT, vMnPO, and preoptic periventricular nuclei that were judged to be complete lesions of the AV3V. In these cases the damage was essentially restricted to this area although some of these mice also had damage to the dorsal MnPO. Four mice had lesions that did not include all of the above structures or were unilateral, and these were
considered to be partial lesions. Two independent observers rating the lesions as complete or partial and blind to the fluid intake results showed 91% agreement (Fig. 4).

**Effect of AV3V Lesions on Dipsogenic Responses**

Mice that “recovered” from the acute effects of AV3V lesions were examined for responses to either subcutaneous hypertonic saline or isoproterenol injections. Drinking responses were grouped based on the histological analysis.

Using a two-way mixed design ANOVA with repeated measures on saline concentration, analysis of the intake after saline injection showed a significant interaction of group by saline concentration ($F_{(2,15)} = 26.85, P < 0.001$; Fig. 5). When the analysis was partitioned by concentration, a one-way ANOVA indicated that group differences occurred in intakes following 6% saline ($F_{(2,15)} = 20.73, P < 0.001$), and Tukey’s HSD ($P < 0.05$) indicated that the group with complete AV3V lesions drank significantly less than either the group with partial or sham lesions, but the intakes of the groups with the partial and sham lesions did not significantly differ from each other. No significant difference ($F_{(2,15)} = 1.64, P > 0.05$) was detected between the intakes for any of the groups following injection of isotonic saline (Fig. 5A). Furthermore, the intake of the AV3V groups was not significantly greater after the 6.0% compared to the 0.9% injection.

A parallel analysis of the data resulting from the isoproterenol injections also indicated a two-way interaction of group by isoproterenol or vehicle ($F_{(2,10)} = 14.16, P < 0.001$). Group differences were found after injection of isoproterenol ($F_{(2,10)} = 19.60, P < 0.001$), and Tukey’s HSD ($P < 0.05$) indicated that the group with complete AV3V lesions drank significantly less than either of the other groups, which did not significantly differ from one another. Again, there was no significant difference ($F_{(2,10)} = 0.58, P > 0.05$) in intake detected between any of the
groups following vehicle injections (Fig. 5B), and the intake of the group with AV3V lesions did not differ significantly between vehicle and isoproterenol injection.

**DISCUSSION**

Much of our understanding of the behaviors implicated in body fluid regulation has been based on studies of rats, dogs and sheep (13,22,36,40,48). More recently, interest has developed in the body fluid regulatory aspects of mice (14,15,43,52), and specifically an interest in the genetic manipulation of the RAS of mice (6,9). However, the analysis of much of the regulatory control in normal mice remains to be conducted. Our studies focused on normal C57BL/6J mice because of their widespread use in studies employing genetic manipulations of this strain (37,38).

The present report shows that mice demonstrate a close similarity to rats (29,30) in their daily pattern of water intake while on an LD cycle. Both species show an anticipatory rise in water intake before light offset that peaks 1-2 h after light offset. Both species show a mid-active period trough (i.e., in the dark period) in intake and a striking rise in intake beginning several hours before light onset. There is very little water intake during the light phase of the cycle. It should be noted that the pattern observed in the dark phase in the present study is free from disturbance, however during the light phase there was some level of disruption, especially early in the light phase when the daily caretaking in the colony occurred. The general trimodal pattern matches that seen in rats both in “smoothed” analog analyses and in “actogram” plots (29,30). Others have noted idiosyncratic bimodal or trimodal patterns of daily water intake in individual mice (37). However, given the episodic, stochastic nature of water intake in rodents, it can be argued that the smoothed bimodal pattern provides the best model of predicting when
water intake will occur over the course of a daily LD cycle. Interestingly, a bimodal pattern
similar to the above described pattern (i.e., peaks near the LD transitions and a major trough in
the mid-light phase and minor trough in the mid-dark phase) is also evident in “smoothed” data
of blood pressure and heart rate rhythms of mice on LD cycles (33). The clear shift of the
pattern of intake that follows the phase of the LD cycle in the present study suggests a circadian
rhythm that entrains to a light Zeitgeber. Other studies have clearly demonstrated the circadian
character of drinking by demonstrating free-running rhythms of mice in constant conditions
(16,34,37).

The saline-water preference study clearly indicates that on average mice of the C57BL/6J
strain have very little preference or aversion for saline concentrations up to physiological (0.9%)
saline and a marked aversion for concentrations much above physiological. At the
concentrations that were lower or at the physiological level where the preference hovers about
50%, it is difficult to state whether this is due to a true lack of preference or a lack of detection of
the difference. Hoshishima et al. (26) report that the taste threshold for C57 mice to be 2% NaCl
(and that it ranges from 1-3% in other strains). However, the marked aversion seen in
concentrations above 1% in the present data suggested that the detection threshold is at least as
low as physiological saline. Research reviewed by Rowland and Fregly (44) examined saline-
water preference in other strains (NA-II, white; SM, white; aa, white; C_{3}H, black; C_{57}, black; 0-
61, black; CD-1; wild type stock) of mice also reports little indication for a saline preference.
More recent studies of up to 28 strains of mice indicates that, although some strains show saline
preferences at low concentrations, there is little indication of a saline preference in C57BL
(1,3,4). Thus, mice are clearly different from rats, since rats have a preference for saline
solutions near physiological concentrations (reviewed by 44). However, the reduced preference
for saline observed in the descending series following the ascending series of concentrations in the present study has been noted in rats and described as a hysteresis (45) and in C57BL mice receiving separate ascending and descending series (3).

The water intake induced by systemic challenges of cellular dehydration via hypertonic saline injections or hypotension and increased RAS activity induced by isoproterenol injections indicates that C57BL/6 mice, like rats, respond to stimuli associated with either cellular or extracellular fluid dehydration (18,21). Previous research had established that systemic hypertonic saline induces a robust water intake in mice (43). However, the same report failed to detect an effect of isoproterenol except a weak induction of water intake at very high doses in CD1 mice (43). The present study found a clear dose-dependent effect of isoproterenol on water intake at relatively low doses (similar to those effective in rats). Although drinking in response to isoproterenol was modest compared to the water intake induced by hypertonic saline, responding to the $\beta$-adrenoceptor agonist was reliably detected in two separate groups of C57BL/6 mice. We can only speculate that the difference in findings between the present and previous report (41) may be a result of strain differences.

Consistent with the efficacy of isoproterenol as a dipsogen found in the present studies, volemic deficits created by PEG induced water intake as previously reported in another strain (43). The previous study also found that PEG produced a robust intake of saline (0.15 M), however the present study failed to find an increase in salt appetite as tested with a 0.34 M (2.0%) NaCl solution. Both the present work and the previous study (41) used similar concentrations of PEG. In the present study, significant water intake was induced within 2 h. However, PEG treatment proved lethal to the majority of mice in our study by 24-h. Perhaps the sodium appetite induced by PEG requires a longer latency to develop than thirst, and the PEG
was having detrimental effects by that time in our group of mice. It is not clear why the PEG injection produced mortality in our mice although renal pathology has been a concern in rats after PEG administration.

We failed to induce drinking using systemic injections of ANG II. The above finding is in agreement with other studies in mice in which either acute systemic injections or chronic systemic infusions failed to increase water intake (15,44,52). However, the contribution of systemic ANG II to drinking may be complicated by peripheral effects of ANG II on the vasculature (19,41).

Although it has been reported that centrally administered ANG II is an ineffective dipsogen in mice (44), we found that induction of water intake in mice with this octapeptide is remarkably consistent with a short latency (1-2 min), and a consistent volume of intake, followed by rapid cessation and with few behavioral side effects (mice were back in their typical mid-day resting posture clearly within 15 min after the injection). Consistent with our results, another report has demonstrated that central infusion of ANG II acts as an effective dipsogen (15). Central injection of ANG II also produces cardiovascular responses in mice (11). The dose response-curve for intraventricular ANG II corresponds roughly to that seen in rats (46). The present intraventricular injection data indicate that central injection of hypertonic saline is also an effective dipsogen. However, the combined injection of hypertonic saline and ANG II failed to produce an additive effect as reported in rats (7). One must consider the possibility that both ANG II and NaCl individually were producing near maximal drinking, especially considering that the dose response curve for icv ANG II reaches maximum at about 0.5-0.6 ml (a volume that might produce inhibitory stomach distention). However, mice are capable of drinking larger volumes in a short time. Post-hoc examination of the current data on drinking induced by 6%
hypertonic saline indicated that mice drank 0.72 (± 0.05) ml in 15 min and a cumulative 1.11 (± 0.05) ml in 30 min.

In summary the water regulatory aspects of mice appear to be very similar to those found in rats. In contrast, the control of salt intake appears to be somewhat different in rats and mice. First, as stated above, mice of the C57BL strain do not have an innate preference for the ad libitum intake of saline solutions near physiological concentrations. Captopril, an ACE inhibitor, when administered via the drinking water causes increased salt intake in rats (50). In the present study, captopril administered in the drinking water of mice at concentrations that are effective in rats (0.1 mg/ml) produced no increase in fluid intake, but reliably increased daily water intake but not 1.8% saline intake at higher concentrations (0.5, 1.0 mg/ml). Given the body weight differences of rats and mice even the lowest concentration of captopril used in the water would have produced a daily dose in mice (28 mg/kg/day) that is much higher than the estimated effective dose (11 mg/kg/day) of studies in rats (50). Other studies have also shown that salt appetite is not induced in mice by captopril administered via the food or water (43,52). A reliable salt appetite is induced in C57BL/6 mice by combined treatment of furosemide followed by sodium restriction as has been shown in rats and other strains of mice (14,28). However, the combined treatment with furosemide and captopril, which is another procedure that reliably induces a rapid salt appetite in rats (20,35), did not induce salt intake in mice in the present studies.

Finally, brain structures defined as the AV3V in rats, when destroyed in mice, produce acute and chronic syndromes similar to those seen in rats. Immediately after the ablation of the AV3V, mice showed adipsia characteristic of that in rats. Also similar to rats, mice showed the recovery pattern when nursed to hydrate by increasing the hedonic properties of ad libitum water. An obvious difference between rats and mice during the acute phase of the AV3V lesion is that mice, probably
because of their size compared to rats, seem more vulnerable to the debilitating effects of dehydration. Mice also show chronic deficits characteristic of rats with AV3V lesions in responding to both cellular and extracellular homeostatic challenges.
ACKNOWLEDGMENTS

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FIGURE LEGENDS

Figure 1. The daily pattern of water intake in two groups of mice on LD 12:12 cycles of differing phases is shown. Drinking was monitored by electronic lickometers. Each pattern shows the average of 30 min bins of data (± SEM) from mice on two different phases of the LD cycle. The rhythm in the top plot is the 7-day average of 4 mice, and that in the bottom plot is the 5-day average of 8 mice. The phase difference of the LD cycle for the two groups is 2.5 h, and time of the dark phase for each plot is shown by the black bar along the x-axis.

Figure 2. The preference-aversion function for varying saline concentrations is shown. First an ascending series of concentrations were presented (open circles) followed by a descending series (solid circles). Percent saline preference is defined as volume saline intake in 24 h divided by saline plus water intake. The points shown are means (± SEM) of 8 mice. The order of testing over the course of the study was first an ascending series (0.1, 0.3, 0.5, 0.7, 0.9, 1.1, 1.5, 1.9, 2.3, 2.7, 3.1% w/v NaCl); then a descending series (2.3, 1.5, 0.9, 0.3%); and finally 3.1%.

Figure 3. Mean water intake (± SEM) in response to systemic and central dipsogens. Length of the test periods is indicated in the y-axis label and dipsogen and mode of administration is indicated in the x-axis label. *P < 0.05 compared to control mean.

Figure 4. Coronal sections are shown at the level of the organum vasculosum of the lamina terminalis (OVLT, left column) and at the level of the ventral median preoptic nucleus (vMNPO, right column). The top pair of photomicrographs (A&B) is from a mouse that received a sham lesion. The OVLT and the vMNPO are intact. These structures are damaged in the bottom two
pairs of photomicrographs (C&D and E&F) from mice that were judged to have complete AV3V lesions. The scale bar in E is 1000 µm and applies to A-F.

Figure 5. When injected with 6% saline (A), both the mice identified as having only partial AV3V lesions and the mice that had received sham ablations drank significantly more water than when injected with 0.9% saline (*P < 0.05). However, those mice of the group histologically identified as having a complete AV3V lesion drank much less than either the group with sham or partial lesions. Following the isoproterenol injections (B) all groups showed the same significance pattern as above.
Table 1. Effect of Captopril in Drinking Water on Fluid Intakes (ml/24 h)

<table>
<thead>
<tr>
<th>Captopril Concentration</th>
<th>Water Intake</th>
<th>Saline (1.8%) Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 mg/ml</td>
<td>4.47 ± 0.28</td>
<td>0.52 ± 0.16</td>
</tr>
<tr>
<td>control</td>
<td>4.15 ± 0.09</td>
<td>0.55 ± 0.08</td>
</tr>
<tr>
<td>0.5 mg/ml</td>
<td>4.22 ± 0.27 *</td>
<td>0.57 ± 0.17</td>
</tr>
<tr>
<td>control</td>
<td>3.58 ± 0.05</td>
<td>0.48 ± 0.10</td>
</tr>
<tr>
<td>1.0 mg/ml</td>
<td>5.37 ± 0.17 *</td>
<td>0.50 ± 0.06</td>
</tr>
<tr>
<td>control</td>
<td>4.00 ± 0.09</td>
<td>0.45 ± 0.03</td>
</tr>
</tbody>
</table>

* P < 0.05 intake of experimental compared to corresponding control group
Figure 1
Saline Concentration (% w/v)  

0.3  0.7  1.1  1.5  1.9  2.3  2.7  3.1  

% Saline Preference  

0  10  20  30  40  50  60  70  80  90  100  

Saline Concentration (% w/v)  

Figure 2
Systemic Manipulations

NaCl (% w/v, 0.5 ml/mouse sc)

<table>
<thead>
<tr>
<th>Isoproterenol (µg/kg sc)</th>
</tr>
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<tbody>
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<td>0.0</td>
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<table>
<thead>
<tr>
<th>PEG (25% w/v/1 ml/mouse sc)</th>
</tr>
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<tbody>
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<td>0.0</td>
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</table>

Central Manipulations

ANG II (20 ng/2 µl icv); NaCl (600 mM/1 µl icv)

ANG II (ng/2 µl/mouse icv)

<table>
<thead>
<tr>
<th>Water Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>ml/0.5 h</td>
</tr>
</tbody>
</table>

| ml/1 h     |

| ml/2 h     |

Figure 3
Figure 4
**Figure 5**

A  
![Graph A](image)

B  
![Graph B](image)