ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Research Paper Acta Neurobiol Exp 2013, 73: 326–337

Inhibition of natriuresis in median eminence polydipsia: Effects after intake of diets with different osmolalities and after hypertonic NaCl administration

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Lesions in the hypothalamic median eminence (ME) induce polydipsia and polyuria in male rats. A first experiment was designed to examine the effect of salt consumption (standard 0.25% Na^+ vs. low-salt 0.04% Na^+ diet) on the fluid-electrolytic balance (plasma sodium, urinary sodium excretion, urine osmolality) and water intake of ME polydipsic animals. In the first 6 h post-surgery, the natriuretic response was higher in ME-lesioned animals than in control groups. At 24 h post-surgery, however, less sodium was excreted by ME rats fed with a standard salt diet (ME/SS), despite showing no decrease in salt intake, and they evidenced an increase in plasma sodium concentration and water intake. Urine osmolality was significantly higher in control animals than in either ME-lesioned group. In experiment 2, hypertonic NaCl administration (2 ml/2M) increased the polydipsic behavior of ME-lesioned but not control rats (day 2). Animals deprived of food/salt showed a significant reduction (on day 2) in the initial (day 1) polydipsia, which increased on day 3 when the animals had access to a standard-salt diet. These results suggest that the reduced natriuretic response and the consequent sodium retention observed in ME animals may exacerbate the hydromineral imbalance of this polydipsic syndrome.

Key words: salt intake, natriuresis, polydipsia, plasma sodium, rat

INTRODUCTION

Polydipsic behaviors after damage to various brain regions, including the median eminence (ME) (Smith and Mc 1962, Rolls 1970, Grossman et al. 1977, Hennessy et al. 1977, Antunes-Rodrigues et al. 1990), were usually interpreted in terms of abnormalities in water retention mechanisms (Friedman et al. 1958, Smith and Mc 1962, Rolls 1970). However, it was recently demonstrated that other factors might also be involved in ME polydipsia (Mahía et al. 2008). Thus, the water intake of ME polydipsic animals was maintained and even increased (vs. controls) by the availability of standard food, whereas the polydipsia and polyuria observed in this disorder were reduced by deprivation of food or of dietary sodium (Mahía and Puerto 2006, Mahía et al. 2008).

The above findings are compatible with reports that ME lesions may interrupt some of the brain circuits (e.g., posterior hypothalamo-hypophyseal axis) involved in the neuroendocrine regulation of water and sodium metabolism (Friedman et al. 1958, McCann et al. 1989, McCann et al. 1997, Antunes-Rodrigues et al. 2004). In fact, polydipsia related to lesions of basal hypothalamic structures (medial ventral tuberomammillary nucleus, posterior hypothalamus) has been associated with a reduction in natriuretic activity (Morris et al. 1976, 1977, McCann et al. 1989, 1997, Antunes-Rodrigues et al. 1991, Mahía et al. 2009) as well as with an alteration in fluid retention mechanisms (Morris et al. 1977). This reduced capacity to excrete sodium chloride-concentrated urine tends to produce a rise in plasma sodium concentrations (Bealer 1983, Rose 1984).

With this background, the first experiment in this study was designed to examine whether ME lesions produce changes in water intake and fluid-electrolytic balance (plasma sodium, urinary sodium excretion, urine osmolality) and to determine how these physiological and behavioral processes are affected by the availability of diets with different salt concentrations

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Received 29 June 2012, accepted 1 March 2013

(standard 0.25% Na^+ vs. low-salt 0.04% Na^+ diet). In the case that ME-lesioned animals showed increased sodium retention and plasma sodium levels after the consumption of salt in their diet, with a resulting increase in water intake, a second experiment was designed to examine the effects of food-deprivation and the administration of hypertonic sodium chloride (2 ml/2 M), an osmoregulatory dipsogenic challenge, on the course of the polydipsia induced by the ME lesions.

METHODS

Experiment 1

Subjects

The study was performed in male Wistar rats (from the breeding colony of the University of Granada) weighing 250–340 g at the beginning of the experiment. Animals were individually housed in individual cages with *ad libitum* access to food (Mucedola, Debiomed S.L., Barcelona, Spain, 0.25% Na^+) and tap water. The room was maintained on a 12:00/12:00 h light/dark cycle (lights on at 08:00 AM) at approximately 22°C. All experimental protocols were performed according to the European Communities Council Directive of 24 November 1986 (86/609/EEC) on animal experimentation and were approved by the Ethical Committee for Animal Experimentation of the University of Granada (10-301). Every effort was made to minimize animal suffering and to reduce the number of animals used.

Surgical procedure

Rats (26 animals) were anesthetized with sodium thiopental (50 mg/kg i.p., Laboratory Abbot, Madrid, Spain) and held in a stereotaxic apparatus (Stoelting Co. 51.600, IL, USA). Bilateral lesions of the ME were made by passing an anodic electric current (1.5 mA) for 15 s through an 00 stainless steel electrode, insulated except at the tip, using a DCML-5 lesion-maker (Grass Instruments, Quincy, MA, USA). Anatomical coordinates were obtained from the Paxinos and Watson stereotaxic atlas (Paxinos and Watson 1986): 6.44 mm anterior to interaural line, 0.4 mm lateral to midline, and 0.2 mm dorsal to interaural line.

The non-lesioned control rats underwent the same surgical procedure except that the electrode was placed at 0.5 mm above the lesion coordinates and no current was applied.

At the end of the surgery, each rat received an i.m. injection of 0.1 mL penicillin (Penivel Retard, Laboratory Level, S.A., Barcelona, Spain) at 250 000 IU/mL.

Experimental procedure

All animals were given an adaptation period during the 10 days before surgery with *ad libitum* access to tap water and food (Mucedola, Debiomed S.L., Barcelona, Spain, 0.25% Na^+). Water and food intakes during the 4 days before surgery were recorded as baseline values.

After surgery, rats were placed in metabolic cages (3701M0-000; Tecniplast) for 24 h and were then randomly assigned to one of four experimental groups: a ME-lesioned or control (sham) ME group with *ad libitum* access to a standard-salt (SS) diet (Mucedola, Debiomed S.L., Barcelona, Spain, 0.25% Na^+) and tap water [ME/SS (n=7) or MEc/SS (n=6)] or a ME-lesioned or control (sham) ME group with ad lib access to a low salt (LS) diet (Mucedola, Debiomed S. L., Barcelona, Spain, 0.04% Na^+) and tap water [ME/LS (n=70) or MEc/LS (n=6)].

Data were collected on food, water, and sodium intake, urine volume, urine osmolality, urinary sodium excretion, and sodium balance (total salt intake minus urinary sodium excretion) of all animals at 6 h (0–6 h period) and 24 h post-surgery (data at 24 h was for the 18-h period between 6 and 24 h post-surgery), and plasma sodium levels were recorded at 24 h post-surgery. Selection of these time points was based on the time-course of behavioral effects observed in previous studies (Mahia and Puerto 2006, Mahia et al. 2007, 2008).

Salt intake was calculated by multiplying the amount of salt present in the chow (standard-salt diet, 0.05 mEq/g; low-salt diet, 0.007 mEq/g) by the amount of chow consumed.

Urine osmolality (mOsm/kg H_2O) was measured by means of an osmometer (OM-6020; Osmostat, Kyoto, Japan) based on the freezing-point method. Urinary sodium excretion (mEq) was determined by using an automatic analyzer (Synchron CX3 Delta; Bekman Instruments, California, USA). The urinary sodium excretion was calculated by multiplying the urine sodium by the urine volume. In order to study the plasma sodium

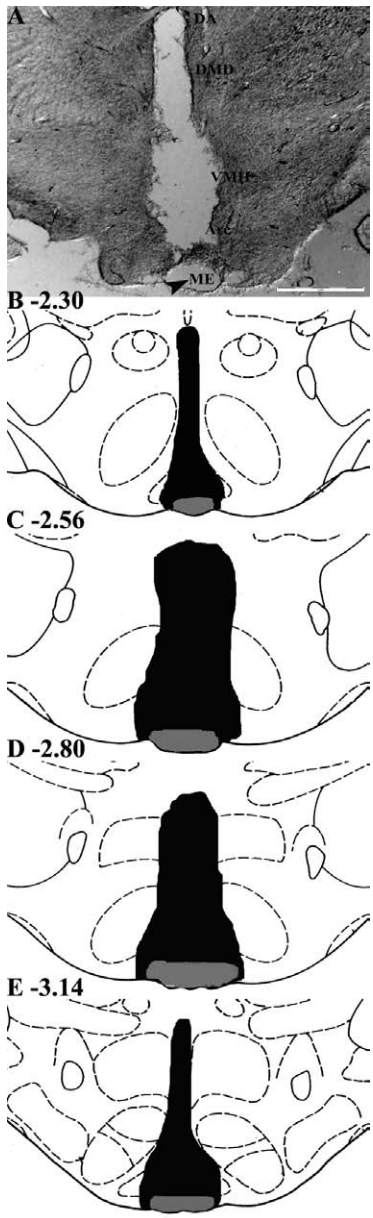

Fig. 1. The photomicrographs depict the damaged brain region (A) in a sequential series of schematic drawings of the smallest (grey areas) and largest (black areas) ME lesions from a representative ME-lesioned rat (B–E). Plates correspond to coronal sections approximately −2.30 (B), −2.56 (C), −2.80 (D) and −3.14 (E) caudal to bregma according to the atlas of Paxinos and Watson (1986). (Arc) arcuate hypothalamic nucleus; (DA) dorsal hypothalamic area; (DMD) dorsomedial hypothalamic nucleus, dorsal part; (ME) median eminence; (VMH) ventromedial hypothalamic nucleus. The arrow head indicates the lesion. Scale bar is 2 mm. Modified from Paxinos G, Watson C, The Rat Brain in Stereotaxic Coordinates (2nd Edition). Figures 27, 28, 29 and 30, Copyright (c) 1986, with permission from Elsevier.

levels (mEq/l), the rats were sacrificed by total exsanguination at 24 h post-surgery, extracting the blood by abdominal aorta puncture under the same anesthetic conditions as for the surgery. Blood samples were centrifuged at 3 000 rpm for 10 min, and the plasma was kept frozen until analysis.

Histology

On completion of the experimental protocol, the brains were removed, soaked in 4% formaldehyde for at least 24 h, frozen, and rostrocaudally sliced into 40-µm sections (Leitz 1320, Wetlar, Germany). Slides were mounted and stained with cresyl violet, examined under light microscope (stereoscopic microscope UMZ-4F, Olympus, Tokyo, Japan), and microphotographed (Olympus Optical, mod PM-G, Tokyo, Japan) to determine the localization of lesions.

DATA ANALYSIS

The data were expressed as means ± SEM and analyzed by using Statistica Software for Windows (6.0; StatSoft, Inc., OK, USA). Non-parametric tests were applied because the data were not normally distributed and showed large differences in variances between groups (Krauth 1988). The Kruskal-Wallis one-way ANOVA test was used to compare data on water, food, and sodium intake and on body weight, urine volume, water "balance" (water intake minus urine volume), urine osmolality, urinary sodium excretion, sodium balance (at 6 and 24 h post-surgery), and plasma sodium level (at 24 h post-surgery) among groups. The Mann-Whitney *U*-test was used for pairwise comparisons between groups. In all cases, the level of significance was set at $P<0.05$.

Experiment 2

Subjects and surgical procedure

Male Wistar rats (26 animals) from the breeding colony at the University of Granada (270–350 g) were housed for 10 days under the same conditions as in Experiment 1, with free access to tap water and food (Mucedola, Debiomed S.L., Barcelona, Spain, 0.25% Na^+). Food and water intake during the 4 days before surgery were recorded as baseline values. The surgical procedure described in experiment 1 was followed.

Experimental procedure

After surgery, the rats were maintained in metabolic cages (3701M0-000; Tecniplast) for 48 h and were randomly distributed among four groups: two food-deprivation (FD) groups (lesioned and non-lesioned) that received hypertonic NaCl and two FD groups (lesioned and non-lesioned) that did not.

In the hypertonic NaCl groups, the ME-lesioned (ME/NaCl+FD, n=7) and ME control (MEc/NaCl+FD, n=6) groups were administered i.p. with 2 ml 2M NaCl (Acros Organics, NJ, USA) immediately after the surgery. On day 1 post-surgery, the animals were food deprived with *ad libitum* access to tap water. On day 2, the animals remained under the same conditions as on day 1 but without receiving hypertonic NaCl. From day 3 post-surgery, the animals had *ad libitum* access to food and tap water in their individual cages.

In the ME-lesioned (ME/FD, n=7) and ME control (MEc/FD, n=6) FD groups that received no NaCl injection, the needle was inserted into the peritoneal cavity; these animals had free access to tap water but received no food during the first two postoperative days (0–48 h). From day 3 post-surgery, the animals had *ad libitum* access to food and tap water in their individual cages.

The water and food intakes of each animal were recorded every 24 h during the 7-day experiment. Values were obtained for different study periods (day/s 1, 2, 3 and 4/7) for analyses of quantitative (fluid intake volume) and qualitative (polydipsia progression) differences among the study groups (Mahía and Puerto 2006, Mahía et al. 2007).

The urine volume, urine osmolality, and urinary sodium excretion of animals were measured at 6 h (for 0–6 h period), 24 h (between 6–24 h), and 48 h (for 24–48 h period) post-surgery. Urine osmolality (mOsm/kg H_2O) and urinary sodium excretion (mEq) were determined as in Experiment 1. Urinary sodium excretion was calculated by multiplying the urine sodium by the urine volume.

Histology

The histological procedure described in Experiment 1 was followed.

Data analysis

Because the data were not normally distributed, non-parametric methods were applied in the statistical analyses (Krauth 1988) using Statistica 6.0 software (StatSoft, Inc., OK, USA). Results are expressed as means ± SEM. The Kruskal-Wallis one-way ANOVA was used to compare data on body weight, water and food intake, urine volume, urine osmolality, and urinary sodium excretion among all groups (in all time periods). When a significant H value was obtained, the Mann-Whitney U-test was used for *post-hoc* comparisons between groups. In all cases, the level of significance was set at $P<0.05$.

RESULTS

Experiment 1

Histological analysis

Figure 1 displays a series of coronal planes adapted from Paxinos and Watson (1986), showing the largest and smallest lesions in the two ME-lesioned groups. The lesions in these groups were characterized by their caudal extension throughout the base of the brain, with damage in some cases to midline and basal hypothalamic structures (arcuate nucleus, dorsomedial hypothalamus and ventromedial hypothalamus). No damage to supraoptic or paraventricular nuclei was observed. The ME was completely lesioned from its midposition to its most posterior region, caudal to the site of stalk separation. The behavior of these animals did not differ according to the extent of ME damage; therefore, no animal was excluded from the study.

Baseline ingestive behavior

At baseline (pre-surgery, day-1), there was no overall group effect on body weight or on water or food or salt intake (Table I).

Ingestive behavior between 0 and 6 h post-surgery

Kruskal-Wallis ANOVA results showed a significance difference among the groups in water intake ($H_{3,26}$=19.20, $P<0.001$) and sodium intake ($H_{3,26}$=14.85, $P<0.002$) during the 6 h post-surgery. No differences in food intake or water balance were observed during this period. Pairwise comparisons (Mann-Whitney U-test) revealed a higher water intake by ME-lesioned animals *versus* their respective controls between 0 and 6 h (ME/SS: 8.14 ± 1.01 vs. MEc/SS: 1.66 ± 0.3 ml,

Table I

Water, food, and salt intakes and body weight at baseline, and water balance food and salt intake at 6 h (for 0–6 h period) and 24 h (for 6–24 h period) post-surgery (Experiment 1)

		ME/SS	MEc/SS	ME/LS	MEc/LS
		n=7	*n*=6	*n*=7	*n*=6
	Body Wt (g) Baseline	314.00 ± 5.77	317.00 ± 10.26	314.90 ± 4.80	316.50 ± 4.10
	Water intake (ml) Baseline	30.86 ± 1.06	29.83 ± 0.55	30.40 ± 0.40	27.83 ± 0.89
Water "balance" (ml)	Post-surgery 6 h	−5.00 ± 1.69	−5.00 ± 1.00	−3.60 ± 1.40	−3.20 ± 0.90
	Post-surgery 24 h	42.14 ± 9.39[a]	4.83 ± 3.52	21.60 ± 8.30[a]	3.80 ± 2.40
	Baseline	24.50 ± 0.70	26.00 ± 0.69	23.40 ± 0.50	24.20 ± 0.60
Food intake (g)	Post-surgery 6 h	2.00 ± 0.40	0.50 ± 0.20	1.30 ± 0.30	0.50 ± 0.20
	Post-surgery 24 h	28.29 ± 1.71	22.67 ± 1.19	26.40 ± 2.20	21.30 ± 0.80
	Baseline	1.22 ± 0.03	1.30 ± 0.10	1.17 ± 0.02	1.21 ± 0.03
Salt intake (mEq)	Post-surgery 6 h	0.10 ± 0.02[a]	0.03 ± 0.01	0.01 ± 0.00	0.01 ± 0.00
	Post-surgery 24 h	1.41 ± 0.08[a]	1.13 ± 0.06[b]	0.18 ± 0.02	0.14 ± 0.01

Values are means ± SEM for ME-lesioned and control rats on a standard-salt (ME/SS, *n*=7; MEc/SS, *n*=6) or low-salt (ME/LS, *n*=7; MEc/LS, *n*=6) diet. (*n*) number of animals. [a]Significantly different from control group, $P<0.05$ (Mann-Whitney *U* test). [b]Significantly different from MEc/LS group, $P<0.05$ (Mann-Whitney *U* test).

$P<0.002$; ME/LS: 8 ± 0.8 vs. MEc/LS: 1.66 ± 0.19 ml, $P<0.002$) (Fig. 2A). As expected, sodium consumption was higher in the ME/SS lesioned group than in the ME/LS lesioned group (0.1 ± 0.02 vs. 0.009 ± 0.001 mEq, respectively; $P<0.002$; Mann-Whitney test) (Table I).

Ingestive behavior between 6 h and 24 h post-surgery

The groups significantly differed in water intake ($H_{3,26}=21.41$, $P<0.001$), water balance ($H_{3,26}=9.82$, $P<0.02$), and salt intake ($H_{3,26}=20.87$, $P<0.001$) but not in food intake during this period (6–24 h post-surgery). Pairwise comparisons showed that ME-lesioned rats had a higher water intake (ME/SS: 110 ± 15.55 vs. MEc/SS: 22.17 ± 2.52 ml, $P<0.003$; ME/LS: 82.3 ± 9.98 vs. MEc/LS: 22.7 ± 3.79 ml, $P<0.003$) (Fig. 2A) and a more positive water balance (ME/SS: 42.14 ± 9.39 vs. MEc/SS: 4.83 ± 3.52 ml, $P<0.004$; ME/LS: 21.6 ± 8.29 vs. MEc/LS: 3.8 ± 2.36 ml, $P<0.02$) during this period in comparison to controls (Table I). Water intake was higher in ME/SS than in ME/LS rats (110 ± 15.55 vs. 82.3 ± 9.98 ml, respectively; $P<0.003$) but did not differ between the control groups (MEc/SS: 22.17 ± 2.52 vs. MEc/LS: 22.7 ± 3.79 ml, n.s; Mann-Whitney test) (Fig. 2A).

As expected, the sodium intake of the ME/SS lesioned group was higher than that of the ME/LS lesioned group during this period (6–24 h) (1.41 ± 0.08 vs. 0.18 ± 0.02 mEq, respectively; $P<0.002$) but did not differ with that of the non-lesioned control group (ME/SS: 1.41 ± 0.08 vs. MEc/SS: 1.13 ± 0.06 mEq, respectively; n.s.; Mann-Whitney test) (Table I). Sodium intake was also higher in MEc/SS sham-lesioned *versus* MEc/LS sham-lesioned animals (1.13 ± 0.06 vs. 0.14 ± 0.01 mEq, respectively; $P<0.003$) (Table I).

Urine measurements between 0 and 6 h post-surgery

Kruskal Wallis test results showed an overall group effect for urine volume ($H_{3,26}=14.37$, $P<0.002$), osmolality ($H_{3,26}=11.23$, $P<0.010$), sodium excretion ($H_{3,26}=21.94$, $P<0.001$), and sodium balance ($H_{3,26}=15.92$, $P<0.002$).

The urine volume during this period (0–6 h post-surgery) was higher in ME-lesioned groups than in their respective controls (ME/SS: 13.14 ± 1.88 vs. MEc/SS: 6.67 ± 1.22 ml, P<0.015; ME/LS: 11.6 ± 1.85 vs. MEc/LS: 4.8 ± 0.98 ml, P<0.008; Mann-Whitney test) (Fig. 2B). No difference was observed between ME-lesioned groups.

Urine osmolality was lower in ME/SS and ME/LS lesioned groups than in their controls (ME/SS: 697 ± 86.15 vs. MEc/SS: 1136 ± 53.88 mOsm/kg H_2O, P<0.015; ME/LS: 788.4 ± 101.72 vs. MEc/LS: 1219.5 ± 65.87 mOsm/kg H_2O, P<0.03; Mann-Whitney test) (Fig. 2C). No difference was observed between ME-lesioned groups during this period.

Urinary sodium excretion was higher in ME-lesioned rats than in their controls between 0 and 6 h post-surgery (ME/SS: 0.35 ± 0.05 vs. MEc/SS: 0.11 ± 0.01 mEq, P<0.003; ME/LS: 0.3 ± 0.04 vs. MEc/LS: 0.1 ± 0.01 mEq, P<0.002) (Fig. 2D). ME/SS and ME/LS lesioned animals developed a more negative sodium balance in comparison to their controls (ME/SS: −0.25 ± 0.05 vs. MEc/SS: −0.09 ± 0.01 mEq, P<0.04; ME/LS: −0.3 ± 0.04 vs. MEc/LS: −0.01± 0.00 mEq, P<0.002; Mann-Whitney test) (Fig. 3A).

Urine measurements between 6 h and 24 h post-surgery

Kruskal Wallis test results showed overall differences among groups in urine volume ($H_{3,26}$=19.17, P<0.001), osmolality ($H_{3,26}$=18.12, P<0.001), sodium excretion ($H_{3,26}$=21.81, P<0.001), and sodium balance ($H_{3,26}$=19.15, P<0.001).

Between 6 h and 24 h post-surgery, the diuretic response was higher in ME-lesioned rats than in their controls (ME/SS: 67.86 ± 12.21 vs. MEc/SS: 17 ± 3.78 ml, P<0.002; ME/LS: 60.7 ± 10.27 vs. MEc/LS: 18.8 ± 2.9 ml, P<0.004; Mann-Whitney test) (Fig. 2B). No significant difference was observed between the ME-lesioned groups.

Urine osmolality was lower in ME/SS and ME/LS lesioned groups than in their controls at 24 h (6–24 h period) (ME/SS: 803.5 ± 93.8 vs. MEc/SS: 1873 ± 168.9 mOsm/kg H_2O, P<0.002; ME/LS: 757.7 ± 108.29 vs. MEc/LS: 1603.9 ± 149.19 mOsm/kg H_2O, P<0.006; Mann-Whitney *post-hoc* test) (Fig. 2C). No significant difference was observed between the ME-lesioned groups.

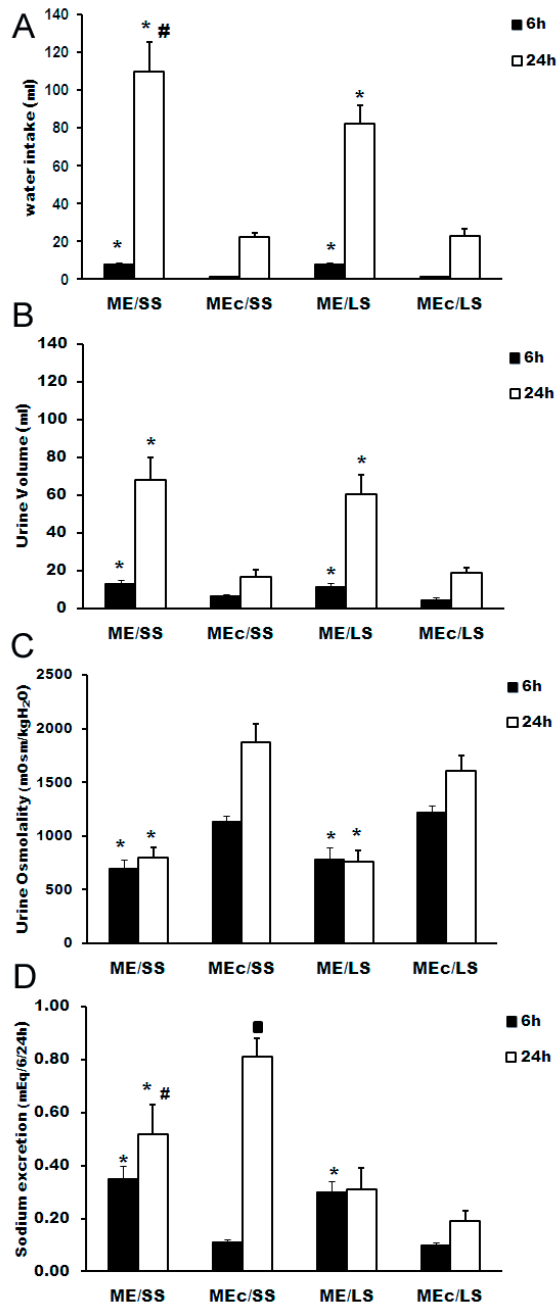

Fig. 2. Water intake (A), urine volume (B), urine osmolality (C), and sodium excretion (D) of ME-lesioned and control rats on a standard-salt (ME/SS, n=7; MEc/SS, n=6) or low-salt (ME/LS, n=7; MEc/LS, n=6) diet (Experiment 1). Measurements were taken at 6 h (for 0–6 h period) and 24 h (for 6–24 h period) post-surgery (see Methods text for more details). Values are means ± SEM. When the SEM is very small, it is not visible on the graph. *Significantly different from control group, P<0.05 (Mann-Whitney U test). #Significantly different from ME/LS group, P<0.05 (Mann-Whitney U test). ▪Significantly different from MEc/LS group, P<0.05 (Mann-Whitney U test).

A lower natriuretic response was observed in ME/SS lesioned *versus* control animals during this period (0.52 ± 0.11 vs. 0.81 ± 0.07 mEq, respectively; $P<0.006$) (Fig. 2D). Urinary sodium excretion was higher in ME/SS and MEc/SS groups than in ME/LS or MEc/LS groups (ME/SS: 0.52 ± 0.11 vs. ME/LS: 0.31 ± 0.08 mEq, $P<0.004$; MEc/SS: 0.81 ± 0.07 vs. MEc/LS: 0.19 ± 0.04 mEq, $P<0.003$; Mann-Whitney test) (Fig. 2D). The ME/SS lesioned group developed a more positive sodium balance in comparison to the ME/LS lesioned animals (0.99 ± 0.12 vs. −0.12 ± 0.06 mEq, $P<0.001$) and MEc/SS animals (0.99 ± 0.12 vs. 0.35 ± 0.13 mEq, $P<0.006$; Mann-Whitney test). The sodium balance was more negative in MEc/LS controls than in MEc/SS controls (−0.02 ± 0.01 vs. 0.35 ± 0.13 mEq, $P<0.03$) (Fig. 3A).

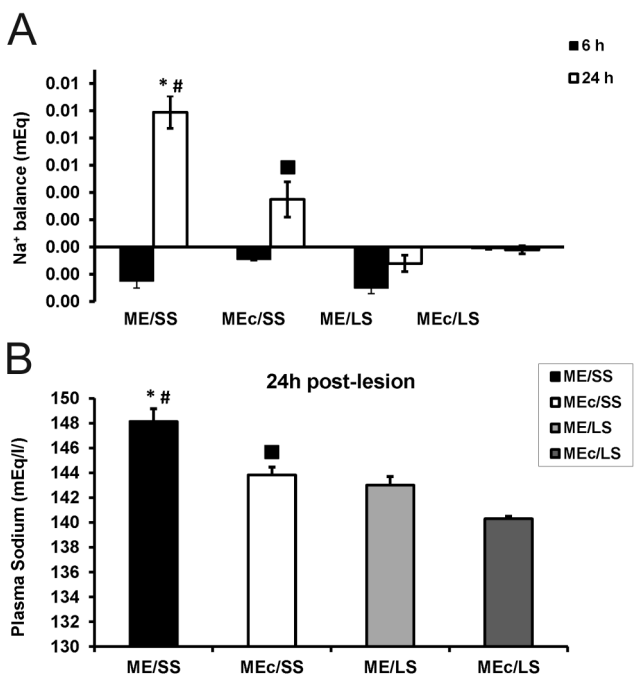

Fig. 3. Sodium Balance (A) and Plasma sodium levels (B) of ME-lesioned and control rats on a standard-salt (ME/SS, n=7; MEc/SS, n=6) or low-salt (ME/LS, n=7; MEc/LS, n=6) diet (Experiment 1). Measurements were taken at 6 h (for 0–6 h period) and 24 h (for 6–24 h period) post-surgery (sodium balance) and 24 h post-surgery (plasma sodium levels) (see Methods text for more details). Values are means ± SEM. When the SEM is very small it is not visible on the graph. *Significantly different from control group, $P<0.05$ (Mann-Whitney U test). #Significantly different from ME/LS group, $P<0.05$ (Mann-Whitney U test). ▪Significantly different from MEc/LS group, $P<0.05$ (Mann-Whitney U test).

Plasma sodium measurements at 24 h post-surgery

Plasma sodium concentrations significantly differed among groups at 24 h post-surgery ($H_{3,26}$=18.19, $P<0.001$).

Pairwise comparisons showed a higher plasma sodium concentration in the ME/SS group than in the MEc/SS control group (148.14 ± 1.02 vs. 143.83 ± 0.64 mEq/l, respectively; $P<0.015$) and ME/LS group (148.14 ± 1.02 vs. 143 ± 0.7 mEq/l, respectively; $P<0.006$; Mann-Whitney test) (Fig. 3B). No difference was found between the ME/LS lesioned group and its control group. Plasma sodium levels were higher in the MEc/SS control group than in the MEc/LS control group (143.83 ± 0.64 vs. 140.3 ± 0.19 mEq/l, respectively; $P<0.006$).

Experiment 2

Histological analysis

Histological examination of brains from the 14 lesioned rats showed similar extensive rostrocaudal ME lesions to those observed in Experiment 1 (Fig. 1).

Ingestive behavior

At baseline (pre-surgery, Day-1), there was no overall group effect (Kruskal-Wallis ANOVA) on body weight or on water or food intake (Table II).

Post-surgery, there was an overall group effect on water intake in all time periods studied (Day 1: $H_{3,26}$=19.55, $P<0.001$; day 2: $H_{3,26}$=20.58, $P<0.001$; day 3: $H_{3,26}$=18.95, $P<0.001$; days 4–7: $H_{3,26}$=19.22, $P<0.001$) and on food intake only on days 4–7 ($H_{3,26}$=19.59, $P<0.001$).

On day 1 post-surgery, water intake was higher in ME-lesioned groups than in their respective controls (ME/NaCl+FD: 114.29 ± 13.6 vs. MEc/NaCl+FD: 28.33 ± 3.19 ml; ME/FD: 93.57 ± 12.91 vs. MEc/FD: 20.33 ± 2.94 ml, all $P<0.01$ by Mann-Whitney test). No difference was observed between ME-lesioned groups at 24 h post-surgery (Fig. 4A).

On day 2 post-surgery, water intake remained higher in ME-lesioned *versus* control groups (ME/NaCl+FD: 134.14 ± 17.44 vs. MEc/NaCl+FD: 22.33 ± 2.84 ml; ME/FD: 75.29 ± 7.86 vs. MEc/FD: 26.83 ± 1.77 ml, all $P<0.01$ by Mann-Whitney test) (Fig. 4A).

Water intake was higher in ME/NaCl+FD lesioned rats than in ME/FD lesioned rats (P<0.02) but did not differ between their respective control groups.

On day 3 post-surgery, with *ad libitum* access to food, water intake was higher in the ME-lesioned groups than in their respective controls (ME/NaCl+FD: 106.71 ± 7.97 vs. MEc/NaCl+FD: 39.17 ± 2.15 ml; ME/FD: 96 ± 10.51 vs. MEc/FD: 39.17 ± 1.86 ml, all P<0.01 by Mann-Whitney test) (Fig. 4A).

On days 4–7, with *ad libitum* access to food, the water intake of ME-lesioned groups was higher than on previous days and was higher *versus* their respective controls (ME/NaCl+FD: 110.39 ± 4.96 vs. MEc/NaCl+FD: 31.67 ± 1.97 ml; ME/FD: 110.57 ± 5.27 vs. MEc/FD: 28.17 ± 0.72 ml, all P<0.01 by Mann Whitney test) (Fig. 4A). Food intake was higher in the ME-lesioned groups than in their respective controls (Days 4/7, all P<0.05 by Mann-Whitney test, Table II).

Urine measurements at 6 h (0–6 h), 24 h (6–24 h), and 48 h (24–48 h) post-surgery

There was a significant overall effect for urine volume among groups in all study periods (0–6 h: $H_{3,26}$=19.16, P<0.001; 6–24 h: $H_{3,26}$=18.82, P<0.001; 24–48 h: $H_{3,26}$=20.00, P<0.001; Kruskal-Wallis test).

A greater diuretic response was observed in the ME-lesioned groups than in their respective controls in all study periods (all P<0.01; Mann-Whitney test) (Fig. 4B). No difference in urine volume was observed between the lesioned groups.

Statistical analysis showed an overall group effect on urine osmolality at all measurement time points (0–6 h: $H_{3,26}$=22.06, P<0.001; 6–24 h: $H_{3,26}$=18.60, P<0.001; 24–48 h: $H_{3,26}$=21.10, P<0.001).

Urine osmolality was lower in ME-lesioned groups than in their respective control groups at all time points (all P<0.05). It was higher in ME/NaCl+FD *versus* ME/FD lesioned groups (ME/NaCl+FD vs. ME/FD: all, P<0.05; Mann-Whitney test) (Fig. 4C).

Sodium excretion differed among groups in all time periods (0–6 h: $H_{3,26}$=16.60, P<0.001; 6–24 h: $H_{3,26}$=16.92, P<0.001; 24–48 h: $H_{3,26}$=16.79, P<0.001).

Between 0 and 6 h post-surgery, urinary sodium excretion was higher in ME-lesioned *versus* control groups (ME/NaCl+FD: 0.46 ± 0.07 vs. MEc/NaCl+FD: 0.19 ± 0.03 mEq; ME/FD: 0.27 ± 0.05 vs. MEc/FD: 0.1 ± 0.02 mEq, all P<0.05 by Mann-Whitney test) (Fig. 4D). A greater natriuretic response was observed in the

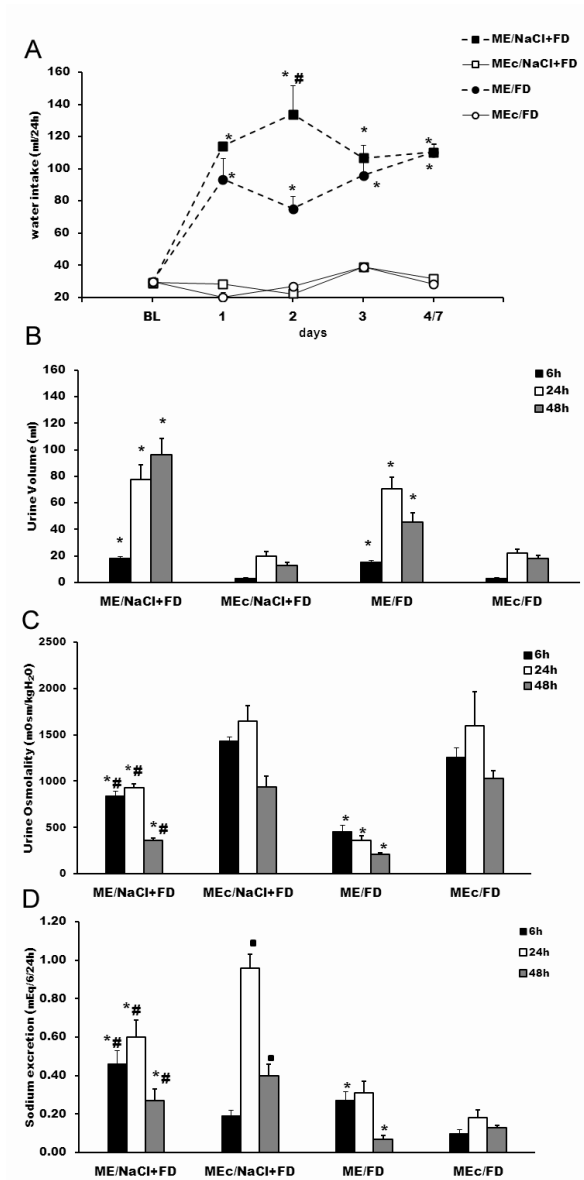

Fig. 4. Water ingested throughout different time periods (A), urine volume (B), urine osmolality (C) and sodium excretion (D) of ME-lesioned and control rats in response to i.p. hypertonic NaCl administration (ME/NaCl+FD, n=7; MEc/NaCl+FD, n=6), or food-deprivation (ME/FD, n=7; MEc/FD, n=6) (Experiment 2). (A) BL shows baseline (pre-surgical) data. Water intake (A) was measured every 24 h. Measurements (B, C and D) were taken at 6 h (for 0–6 h period), 24 h (for 6–24 h period) and 48 h (for 24–48 h period) post-surgery (see Methods text for more details). Values are means ± SEM. When the SEM is very small, it is not visible on the graph. *Significantly different from control group, P<0.05 (Mann-Whitney U test). #Significantly different from ME/LS group, P<0.05 (Mann-Whitney U test). ▪Significantly different from MEc/LS group, P<0.05 (Mann-Whitney U test).

Table II

Water and food intakes and body weight at baseline and food intake at different time periods post-surgery (day 3, day/s 4/7) (Experiment 2)

		ME/NaCl+FD	MEc/NaCl+FD	ME/FD	MEc/FD
		n=7	*n*=6	*n*=7	*n*=6
	Body Wt (g) Baseline	323.00 ± 6.80	326.00 ± 7.20	319.00 ± 8.10	324.00 ± 7.40
	Water intake (ml) Baseline	28.86 ± 0.98	29.50 ± 0.57	30.14 ± 0.50	29.83 ± 0.44
Food intake (g)	Baseline	26.14 ± 0.79	25.67 ± 0.45	27.29 ± 0.80	27.00 ± 1.03
	Day 3	33.57 ± 2.24	27.83 ± 1.71	40.00 ± 1.81	29.17 ± 1.38
	Days 4/7	35.71 ± 2.05[a]	25.33 ± 0.73	39.71 ± 1.40[a]	25.50 ± 0.66

Values are means ± SEM for ME-lesioned and control rats administered i.p. with hypertonic NaCl (ME/NaCl, *n*=7; MEc/NaCl, *n*=6), or food-deprived (ME/FD, *n*=7; MEc/FD, *n*=6) diet. (*n*) number of animals. [a]Significantly different from control group, $P<0.05$ (Mann-Whitney *U* test).

ME/NaCl+FD group than in the ME/FD group (0–6 h: 0.46 ± 0.07 vs. 0.27 ± 0.05 mEq, respectively, $P<0.002$). After the first 24 h post-surgery, the urinary sodium excretion was lower in ME/NaCl+FD animals than in their controls (ME/NaCl+FD vs. MEc/NaCl+FD, 6–24 h: 0.6 ± 0.09 vs. 0.96 ± 0.07 mEq, $P<0.05$; 24–48 h: 0.27 ± 0.06 vs. 0.4 ± 0.06 mEq, $P<0.05$). Between 24 h and 48 h post-surgery, the urinary sodium excretion was lower in ME/FD animals than in their controls MEc/FD, 24–48 h: 0.07 ± 0.02 vs. 0.13 ± 0.01 mEq, $P<0.05$; Mann-Whitney test) (Fig. 4D).

At 24 h (6–24 h) and 48 h (24–48 h) sodium excretion was higher in the ME/NaCl+FD group than in the ME/FD group (ME/NaCl+FD vs. ME/FD, 6–24 h: 0.6 ± 0.09 vs. 0.31 ± 0.06 mEq; 24–48 h: 0.27 ± 0.06 vs. 0.07 ± 0.02 mEq, both $P<0.01$). Sodium excretion was also higher in the MEc/NaCl+FD control group than in the MEc/FD control group (6–24 h: 0.96 ± 0.07 vs. 0.18 ± 0.04 mEq; 24–48 h: 0.4 ± 0.06 vs. 0.13 ± 0.01 mEq, both $P<0.01$; Mann-Whitney test) (Fig. 4D).

DISCUSSION

Lesions of the ME produced a consistent and early polyuric/polydipsic response in these rats on the first post-lesion day. Both ME-lesioned groups showed a marked diuretic and natriuretic response at 6 h post-surgery, confirming the presence of fluid retention abnormalities (Friedman et al. 1958, Smith and Mc 1962, Rolls 1970, McCann et al. 1989, Antunes-Rodrigues et al. 2004), which may explain the higher intake of hypertonic saline solutions by these animals during the first few hours post-surgery (Mahía et al. 2008). This higher urinary sodium excretion (natriuresis) has been observed in numerous animal species in states of dehydration (Bianca et al. 1965, Luke 1973, McKinley et al. 1982, McKinley et al. 1983, present study). However, although the fluid balance of our ME-lesioned animals was negative in the first few hours post-surgery, the same effect was also observed in the control animals (no significant between-group difference, Table I). This suggests that the greater natriuretic response of the lesioned groups could be attributable to other mechanisms besides those underlying dehydration. We propose that the electrolytic lesion may produce a transient activation of the affected neural tissue during the first few hours post-surgery (Schallert et al.1978, Blessing et al. 1982, Blessing and Willoughby 1985, Ramos et al. 1988), stimulating natriuretic mechanisms that could produce a higher urinary excretion of sodium in lesioned rats (Friedman et al. 1958, Conrad et al. 1993). The ingestion of higher salt amounts, without a proportional enhancement in water intake, may be beneficial to the animals because it would facilitate water reabsorption and thereby increase extracellular volume (Rose 1984, Geerling and Loewy 2008).

At 24 h (6–24 h) post-surgery, the water intake was significantly higher in lesioned animals fed with standard salt diet (ME/SS) than in those fed with a low-salt diet (ME/LS), whereas no difference was observed between their respective control groups. Plasma sodium concentrations were higher in the ME/SS group than in the non-lesioned control group, despite their similar food intake level, whereas they did not differ between ME/LS rats and their controls.

The low urine osmolality shown by ME-lesioned animals in comparison to the control groups may reflect an inability to excrete sodium-concentrated urine in an appropriate manner (Fried and Palevsky 1997, Verbalis 2002). In fact, ME/SS animals showed a lower sodium excretion versus their controls at 24 h (6-24 h) post-surgery, despite the former's higher urine excretion volume. This lesser natriuretic response in the lesioned ME/SS group produced a more positive sodium balance in comparison to the ME/LS lesioned group and MEc/SS control animals. This finding may explain the increased plasma sodium concentration in ME/SS rats at 24 h after surgery.

In this context, the median eminence is known to possess receptors for various peptides (e.g., ANP or OT) that are involved in the control of body salt and water homeostasis (Blackburn et al. 1995, De Luca et al. 2003), promoting a greater natriuretic response (Huang el al. 1995, Huang and Dellman 1996, Sjoquist et al. 2005, Bernal et al. 2007).

These findings are consistent with reports that hypothalamic lesions (e.g., posterior hypothalamus-hypophyseal axis) (Morris et al. 1977, Bealer 1983, McCann et al. 1989, Antunes-Rodrigues et al. 1991, McCann et al. 1997) not only disrupt the arginine vasopressin regulatory system (Mc and Brobeck 1954, Marubayashi et al. 1987, McKinley et al. 2001) but may also alter the natriuretic mechanisms involved in regulating and maintaining body salt and fluid homeostasis, thereby augmenting sodium excretion (Elias et al. 1997, McCann et al. 2003, Sjoquist et al. 2005, Bernal et al. 2007).

In fact, previous results obtained in our laboratory demonstrated that the i.p. administration of the natriuretic peptide oxytocin blocked the polydipsic and polyuric response in male rats with lesions in other hypothalamic structures (medial ventral tuberomammillary nucleus, posterior hypothalamus) (Mahía et al. 2009). Blockade of the polydipsia was accompanied by greater urine sodium excretion and increased urinary osmolality (Mahía et al. 2009), confirming the natriuretic capacity of this neurohormone (Verbalis et al. 1991, Huang et al. 1994, 1995, Bernal et al. 2007) and resulting in an estimated plasma sodium concentration that was lower in the OT-treated animals than in the controls (Bernal et al. 2010).

The water and mineral regulation abnormalities observed in the ME-lesioned animals may possibly explain the relationship between polyuria/polydipsia and the higher or lower concentration of plasma sodium levels. The consumption of salt through chow intake tends to increase plasma sodium concentrations, which would be higher in ME/SS animals because of their reduced natriuretic activity. In combination with the polyuria suffered by these animals, this increase would account for the higher water intake by the ME/SS group than by the ME/LS group or MEc/SS control group between 6 h and 24 h post-surgery.

In Experiment 2, the hypertonic NaCl administration exacerbated the hydromineral imbalances observed in the ME-lesioned animals in the first experiment, which manifested in a lower urinary sodium excretion between 6 h and 48 h post-surgery and an increase in polydipsic behavior. These results contrast with the data obtained in food-deprived animals that did not receive i.p. administration of hypertonic NaCl. Food deprivation (first 24 h post-surgery) reduced or at least did not increase plasma sodium concentrations in ME-lesioned animals (Experiment 1, plasma sodium at 24 h post-surgery) and reduced the polydipsic response in ME/FD animals (day 2) (see also Mahía et al. 2008).

These results are consistent with findings of a reduction of around 50% in the polydipsic and polyuric response of adrenalectomized animals (with reduced plasma sodium levels) with no effect on food intake (Friedman et al. 1962, Balment et al. 1976).

The extent of the lesions in the present animals may have involved midbasal-periventricular hypothalamic nuclei that critically participate in food intake (Hakansson et al. 1996, Flier and Maratos-Flier 1998), which could explain the elevated food consumption of the ME-lesioned animals on days 4/7, already observed in previous experiments (Mahía and Puerto 2006, Mahía et al. 2007, 2008). This behavior, combined with the reduced natriuretic response shown by the ME-lesioned animals, may have increased the plasma sodium concentration and produced a higher water intake to compensate for the osmotic challenge.

CONCLUSIONS

The present results suggest that ME polydipsia does not appear to depend exclusively on abnormalities in the retention of body fluids. Anomalies in body sodium regulation, especially in systems responsible for inducing an appropriate natriuretic response, may exacerbate the hydrational deterioration in ME polydipsia. A greater salt content in the diet can produce a higher plasma sodium concentration in the lesioned animals due to their lesser natriuretic response, which may further exacerbate the polydipsic/polyuric behavior of ME-lesioned animals.

ACKNOWLEDGMENTS

We are grateful to Richard Davies for his assistance with the English version of this paper. This research was supported in part by the University of Granada and the Spanish Ministry of Education and Culture (National R+D Plan: BSO2003-06627, SEJ2006-06710, PSI2010-17400 and FEDER/SEJ2007-61839).

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