

Effect of electrolytic lesion of the dorsal raphe nucleus on water intake and sodium appetite

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Abstract

The present study determined the effect of an electrolytic lesion of the dorsal raphe nucleus (DRN) on water intake and sodium appetite. Male Wistar rats weighing 290-320 g with a lesion of the DRN (L-DRN), performed two days before experiments and confirmed by histology at the end of the experiments, presented increased sensitivity to the dehydration induced by fluid deprivation. The cumulative water intake of L-DRN rats reached 23.3 ± 1.9 ml (a 79% increase, $N = 9$) while sham-lesioned rats (SL-DRN) did not exceed 13.0 ± 1.0 ml ($N = 11$, $P < 0.0001$) after 5 h. The L-DRN rats treated with isoproterenol ($300 \mu\text{g kg}^{-1} \text{ml}^{-1}$, *sc*) exhibited an increase in water intake that persisted throughout the experimental period (L-DRN, 15.7 ± 1.47 ml, $N = 9$ vs SL-DRN, 9.3 ± 1.8 ml, $N = 11$, $P < 0.05$). The L-DRN rats also showed an increased spontaneous sodium appetite during the entire period of assessment. The intake of 0.3 M NaCl after 12, 24, 36 and 72 h by the L-DRN rats was always higher than 20.2 ± 4.45 ml ($N = 10$), while the intake by SL-DRN was always lower than 2.45 ± 0.86 ml ($N = 10$, $P < 0.00001$). Sodium- and water-depleted L-DRN rats also exhibited an increased sodium appetite (13.9 ± 2.0 ml, $N = 11$) compared to SL-DRN (4.6 ± 0.64 ml, $N = 11$) after 120 min of observation ($P < 0.02$). The sodium preference of L-DRN rats in both conditions was always higher than that of SL-DRN rats. These results suggest that electrolytic lesion of the DRN overcomes a tonic inhibitory component of sodium appetite.

Key words

- Sodium appetite
- Water intake
- Dorsal raphe nucleus
- Serotonergic system
- Electrolytic lesion
- Rats

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Research supported by CAPES
and Departamento de Ciências
Fisiológicas, IB, UFRRJ.

Received February 19, 2003
Accepted August 7, 2003

Introduction

Serotonergic neurons of the mesencephalic dorsal raphe nucleus (DRN), located in its medial portion, project to prosencephalic regions involved in hydroelectrolytic and cardiocirculatory homeostasis (1-4). Recipro-

cal projections from structures of the terminal lamina constitute evidence that the monitoring of cardiocirculatory signals and changes in plasma tonicity and volume are sent to the DRN (4-7). Consistent with this, the angiotensinergic stimulation of the subfornical organ (SFO), which represents, in

the short and long term, the changes in volume, pressure and sodium concentration in the extracellular fluid, evokes electrophysiological changes in the DRN (7). In this context, it has been shown that the microinjection of serotonergic agonists of the 5HT_{2A} and 5HT_{2C} receptors into the SFO excites angiotensin II- (ANG II) sensitive neurons (8). In addition, structures of the lamina terminalis, including the SFO and those of the wall of the anteroventral region of the third ventricle (AV3V), the organum vasculosum laminae terminalis (OVLT), and the median preoptic nucleus (MnPO) constitute areas related to hydroelectrolytic and cardiovascular regulation (4,9-11). Relevant observations have shown that stimulation of the AV3V region by intracerebroventricular (*icv*) administration of serotonin, serotonin-releasing agents or 5HT_{2A/2C} agonists increases the urinary excretion of sodium (12,13). All of these stimulation conditions under fluid deprivation, cholinergic or angiotensinergic stimulation of the AV3V region and central or systemic β -adrenergic stimulation also caused a decrease in water intake (14-16). These observations were later confirmed in part using different experimental paradigms in which the 5HT_{2B/2C} agonist, 1-3-chlorophenylpiperazine (mCPP), was administered by the *icv* route (17). The authors reported an inhibition of water intake after dipsogenic challenges induced by fluid deprivation, hypertonic saline overload or hypovolemia. Furthermore, it has been shown that basal c-Fos expression in serotonergic neurons of the DRN was decreased after sodium depletion induced by peritoneal dialysis and was increased after spontaneous and induced sodium intake, suggesting that there is a tonic inhibition of sodium appetite by serotonergic cells of this nucleus (18).

The observation that the recruitment of ascending serotonergic pathways by flow of information originated from prosencephalic

structures implied hydroelectrolytic and cardiocirculatory regulation (4,5,7). Taken together with the evidence that electrolytic lesions of the DRN reduce the basal or stimulated plasma levels of atrial natriuretic peptide (ANP), these data suggest that ascending pathways of the raphe integrate signals concerned with the volume of body fluid homeostasis through the control of renal water and electrolyte excretion, as well as of water intake (3).

No systematic studies involving the direct manipulation of the DRN and its implication in the expression of sodium appetite and water intake have been reported. There has been only a single report which demonstrated that electrolytic lesion of the DRN caused a chronic increase in water intake when only this fluid was offered (3).

The present study was designed to assess the effect of electrolytic lesion of the DRN on water intake in the experimental paradigms that involve an increase of extracellular fluid tonicity by fluid deprivation (osmotic thirst) and brain signaling induced by the systemic production of ANG II in dl-isoproterenol-treated rats (4,19-21). In addition, spontaneous sodium appetite was investigated in DRN-lesioned rats following a simultaneous offer of water and hypertonic saline for three consecutive days.

Material and Methods

Animals

Male Wistar rats weighing 290-320 g were maintained in a room with lights on from 7:00 to 19:00 h, at a controlled temperature of 25°C and with free access to Purina chow and water. Before brain stereotaxic surgery the rats were housed in metabolic cages for at least 5 days. Electrolytic lesions of the DRN were produced in rats anesthetized *ip* with 2.5% tribromoethanol (Aldrich Chemical Company Inc., Milwaukee, WI, USA) and fixed in a stereotaxic frame.

Electrolytic lesions

In order to perform the electrolytic lesions, stereotaxic coordinates were obtained according to the parameters defined in the atlas of Paxinos and Watson (22), using the anteroposterior (AP) coordinates = 7.6-7.8 mm, posterior to bregma, lateral = 0 mm and vertical (V) = 6.4-6.6 mm, below the top of the skull.

The lesions were produced by passing an anodal current of 1 mA for 10 s. Sham-operated rats were used as controls. At the end of the experiment the rats were sacrificed under deep anesthesia and transcardiac perfusion was performed with 10% formaldehyde. The location of the lesions was confirmed by histological examination of serial coronal sections (10 μ m) through the brainstem stained by the Nissl method. A prophylactic dose of 30,000 IU penicillin (Fort Dodge Saúde Animal Ltda., Campinas, SP, Brazil) was administered *im* to operated rats. Data for rats with lesions outside the dorso-ventromedial region of the DRN were excluded from statistical analysis. All the experimental protocols and animal procedures were carried out in accordance with current Brazilian legislation.

Statistical analysis

Data were analyzed statistically by two-way analysis of variance with repeated measures, and the significance of differences between means was determined by the Newman-Keuls test. The difference between DRN-lesioned rats (L-DRN) and sham-lesioned rats (SL-DRN) was calculated in all experiments. The level of significance was set at 5%. Data are reported as mean \pm SEM.

Experimental procedures

Water intake. Water intake was determined in rats submitted to 16 h of fluid

deprivation and in rats treated with dl-isoproterenol (300 μ g kg⁻¹ ml⁻¹, *sc*; Aldrich) for 5 h at hourly intervals. Under these conditions, thirst and water intake are related to an increased tonicity of the extracellular fluid and the systemic production of ANG II, respectively (4). In this set of experiments rats were used with an electrolytic lesion of the DRN (L-DRN, N = 9) or sham lesion (SL-DRN, N = 11) produced 2 days before treatment with isoproterenol or isotonic saline.

Spontaneous (basal) sodium appetite. In order to assess the sodium appetite, 0.3 M NaCl was offered simultaneously with distilled water to the rats and the intake of fluids was determined at 3, 6, 9, 12 and 24 h (on the first day) and on a daily basis thereafter, up to 3 days post-lesion (N = 10 for SL-DRN and L-DRN rats).

Induced sodium appetite. Additionally, SL-DRN and L-DRN rats (N = 11 for both groups) were treated with a combination of furosemide (10 mg kg⁻¹ ml⁻¹, *sc*; Aventis Pharma, Suzano, SP, Brazil) + captopril (5 mg kg⁻¹ ml⁻¹, *sc*; Aldrich). After 60 min without fluids or food, water and 0.3 M NaCl were made available in metabolic cages. Fluid

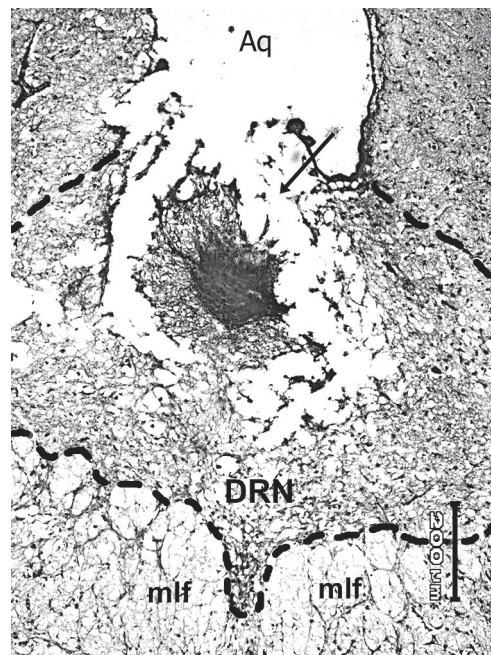


Figure 1. Photomicrograph of a histological section of the encephalon, at the level of the mesencephalon. The electrolytic lesion of the dorsal raphe nucleus (DRN, delimited by the traced line) is indicated by the arrow. Aq = aqueduct, mlf = medial longitudinal fasciculus. Nissl, 5X.

intake was determined over 2 h at 30-min intervals. In this situation, the effect of the ANG-converting enzyme inhibitor captopril increases plasma levels of ANG I available to the circumventricular organs of the lamina terminalis, probably the SFO, in which the local conversion to ANG II occurs, leading to the stimulation of water intake and of sodium appetite (4,23-25). Sodium preference was calculated according to the following formula: volume of 0.3 M NaCl intake/volume of 0.3 M NaCl intake + volume of water intake.

Results

The electrolytic lesions of the DRN suitable for the investigation of ingestive responses extended from the dorsomedial to the ventromedial portion of the nucleus between the coordinates AP = 7.6-8.4 mm and V = 6.2-6.6 mm (Figure 1).

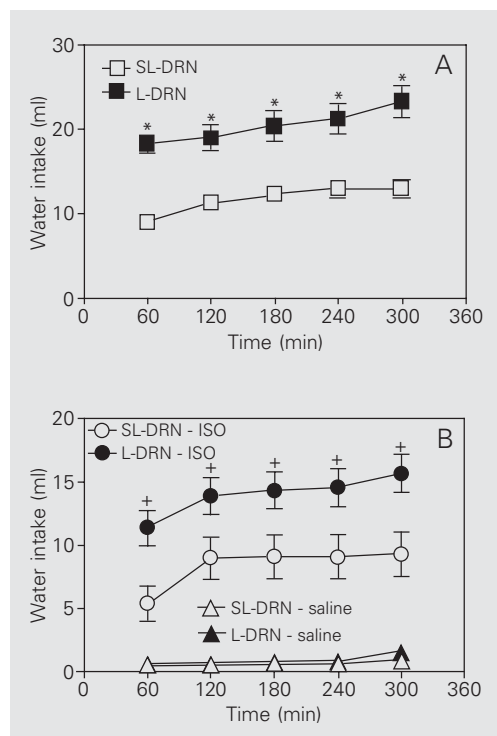
There was an increased sensitivity of L-DRN rats to the dehydration induced by fluid deprivation with increased water in-

take throughout the period of observation (Figure 2A). After 5 h the cumulative water intake by L-DRN rats reached 23.3 ± 1.9 ml (a 79% increase) while that of SL-DRN rats did not exceed 13.0 ± 1.0 ml ($P < 0.0001$). Similar results were observed in L-DRN rats treated with isoproterenol, in which the increase in water intake persisted throughout the experimental period (Figure 2B). After 5 h, L-DRN rats had ingested 15.7 ± 1.47 ml (a 69% increase, $P < 0.05$) and SL-DRN rats, 9.3 ± 1.8 ml.

L-DRN rats also exhibited an increased spontaneous sodium appetite throughout the period of assessment, while their water intake was higher than that by SL-DRN rats only during the first 12 h (Figure 3, panels A and B). The intake of 0.3 M NaCl after 12, 24, 36 and 72 h was 20.2 ± 4.45 , 32.6 ± 8.6 , 27.7 ± 5.8 and 26.9 ± 4.7 ml in L-DRN rats, and 0.97 ± 0.31 , 3.3 ± 1.1 , 4.2 ± 1.3 and 2.45 ± 0.86 ml in SL-DRN rats, respectively ($P < 0.00001$ or less, at all time points). For comparative purposes, water and 0.3 M NaCl intake was measured cumulatively during the first 24 h. Spontaneous sodium preference was significantly higher in L-DRN rats than in SL-DRN rats, ranging from 0.39 ± 0.08 3 h post-lesion to 0.51 ± 0.12 24 h post-lesion, maintaining a level 0.45 up to the 72 h of assessment ($P < 0.02$ or less, at all time points) (Figure 4).

The L-DRN rats submitted to sodium and water depletion, through the combined administration of furosemide + captopril, also exhibited an increased sodium appetite throughout the experiment compared to SL-DRN ($P < 0.02$ or less, at all time points) (Figure 5A). After 120 min, the intake of 0.3 M NaCl reached 13.9 ± 2.0 ml, while among SL-DRN rats the intake was 4.6 ± 0.64 ml. The differences in mean water intake between SL-DRN and L-DRN rats were not significant (Figure 5B). Sodium preference ranged from 0.51 ± 0.045 at 30 min to 0.52 ± 0.047 at 120 min among depleted L-DRN rats, while it never exceeded 0.35 ± 0.044 (at

Figure 2. A, Water intake by dorsal raphe nucleus-lesioned (L-DRN, filled squares) and sham-lesioned (SL-DRN, open squares) rats deprived of water for 16 h. B, Water intake by isoproterenol (ISO)- (L-DRN, filled circles; SL-DRN, open circles) or isotonic saline- (L-DRN, filled triangles; SL-DRN, open triangles) treated rats. Data are reported as means \pm SEM. * $P < 0.0001$ compared to the SL-DRN group (panel A). + $P < 0.05$ compared to the SL-DRN group (panel B) (two-way ANOVA followed by the Newman-Keuls post-test).



120 min, $P < 0.002$, or less) among SL-DRN rats (Figure 6).

Discussion

The results of the present study reveal that electrolytic lesions of the DRN produced an increase in sensitivity to osmotic stimulation by dehydration induced by fluid deprivation and β -adrenergic stimulation with isoproterenol. This observation is consistent with the hypothesis that neurons of the DRN, which are probably serotonergic, exert a modulatory influence on the thirst related to an increase in the tonicity of extracellular fluid or on the thirst signaled by increased plasma levels of ANG II (4,19-21).

Neurons of the lamina terminalis (e.g., SFO, OVLT and MnPO) constitute the neuroanatomical substrate for the primary integration of thirst induced by osmotic stimulation and by β -adrenergic stimulation (4,26, 27). Serotonergic innervation originating in the mesencephalic raphe and receptors for serotonin have been identified throughout the lamina terminalis (1,2,4-6,8).

The effects induced by DRN lesion suggest that ascending serotonergic pathways are involved in the modulation of the thirst induced by dehydration and brain angiotensinergic stimulation. The present observations support previous reports, which demonstrated that *icv* administration of the 5HT_{2C} agonist, MK212, modulates water intake induced by fluid deprivation obtained by central microinjection of ANG II or carbachol as well as by central or systemic administration of the β -adrenergic agonist isoproterenol (14-16). More recently, these observations were extended through the use of other dipsogenic challenges in which the *icv* administration of a 5HT_{2B/2C} agonist, mCPP, inhibited water intake induced by fluid deprivation, acute overload with hypertonic saline and hypovolemia (17).

Similarly, homeostatic integration of sodium appetite depends on structures in the

lamina terminalis (4). The SFO and OVLT constitute convergent sites for signals related to volume depletion (4,18,28,29). As mentioned previously, these structures are innervated by serotonergic neurons, are rich in serotonin receptors and reciprocally transmit signals to the raphe (1,2,4-6,8). It is quite likely that these signals reflect adjustments concerning variations in volume, electrolytic composition of the extracellular fluid and cardiovascular parameters (4,5,7,30).

Lesions of the DRN induced an increase in sodium appetite both under basal conditions and in the paradigm of sodium and

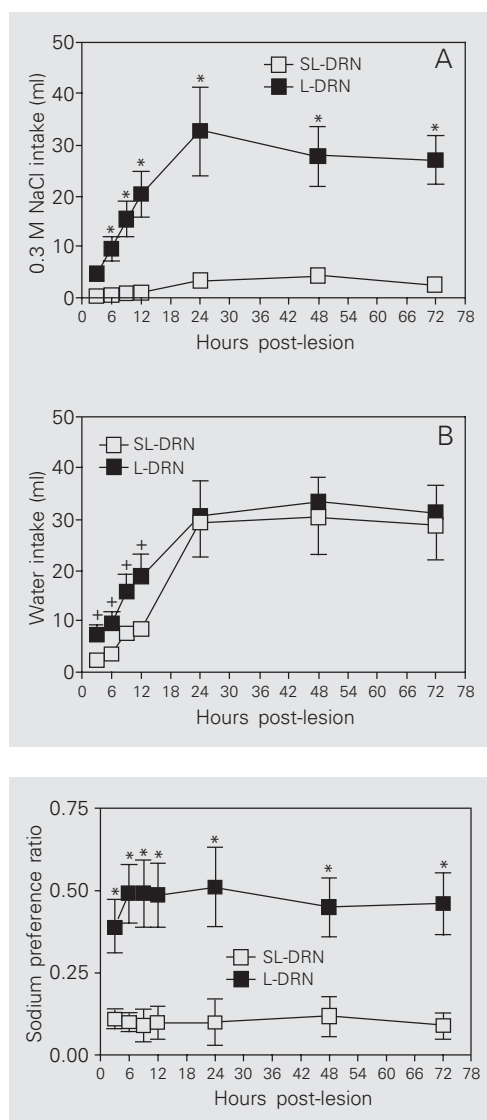


Figure 3. A, Intake of 0.3 M NaCl by dorsal raphe nucleus-lesioned (L-DRN, filled squares) and sham-lesioned rats (SL-DRN, open squares) under basal conditions. B, Water intake of L-DRN (filled squares) and SL-DRN (open squares) rats under basal conditions. Data are reported as means \pm SEM. * $P < 0.00001$ for 0.3 M NaCl intake and * $P < 0.05$ for water intake, compared to the SL-DRN group (two-way ANOVA followed by the Newman-Keuls post-test).

Figure 4. Sodium preference of dorsal raphe nucleus-lesioned (L-DRN, filled squares) and sham-lesioned rats (SL-DRN, open squares) under basal conditions. Data are reported as means \pm SEM. * $P < 0.02$ compared to the SL-DRN group (two-way ANOVA followed by the Newman-Keuls post-test).

water depletion provoked by combined administration of furosemide and a low dose of captopril. These data show that under basal conditions without a natriorexigenic challenge, electrolytic lesion of the DRN promotes the suppression of a tonic modulatory pathway of sodium appetite. Recent evidence has shown that sodium depletion induced by the combination of furosemide with a low dose of captopril causes an increase in c-Fos expression in the nucleus tractus solitarii (NTS), which is suppressed by renal deafferentation (31). Following the same line of reasoning, evoked potentials were recorded in serotonergic neurons of the DRN after the

isoosmotic loss of volume by hemorrhage and, more specifically, induction of c-Fos expression was determined in serotonergic neurons of the DRN and in structures of the lamina terminalis after sodium depletion by peritoneal dialysis (7,18).

These observations permit us to propose that moment-to-moment adjustments of renal sodium load and extracellular fluid volume generate signals that are transmitted to the NTS and, from there, to the DRN. Alternatively, other pertinent observations support the hypothesis that volume depletion (and possibly the serial changes in arterial pressure) activates neurons of the SFO sensitive to ANG II, which project to form synapses with serotonergic neurons of the DRN (7,30). As a result of this monitoring of the circulating levels of ANG II, signals would be generated and transmitted by ascending serotonergic pathways of the DRN with the objective of modulating sodium appetite, with subsequent regulation of extracellular fluid volume. According to this hypothesis, the removal of a tonic modulatory pathway would imply a greater sensitivity/activity of angiotensinergic mechanisms in the lamina terminalis implicated in the central control of dipsogenesis and of sodium appetite.

Another hypothesis is based on the widely accepted concept that ANP is involved in the modulation of thirst induced by dehydration or by central angiotensinergic stimulation, and of sodium appetite provoked by sodium depletion (32,33). This same group showed that lesions of the wall of the AV3V region drastically reduce the plasma levels of ANP, mediating the expansion of blood volume (34). Taken together, these observations lead to the conclusion that structures in the lamina terminalis constitute the substrate which integrates signals designed to regulate the release of ANP. In view of the observations cited above, regarding the neural interactions between structures of the lamina terminalis and mesencephalic raphe, we suggest

Figure 5. Intake of 0.3 M NaCl and water (panels A and B, respectively) by dorsal raphe nucleus-lesioned (L-DRN, filled squares) and sham-lesioned rats (SL-DRN, open squares) submitted to sodium and water depletion induced by treatment with furosemide + captopril. Data are reported as means \pm SEM. * $P < 0.02$ for 0.3 M NaCl intake and * $P < 0.05$ for water intake compared to the SL-DRN group (two-way ANOVA followed by the Newman-Keuls post-test).

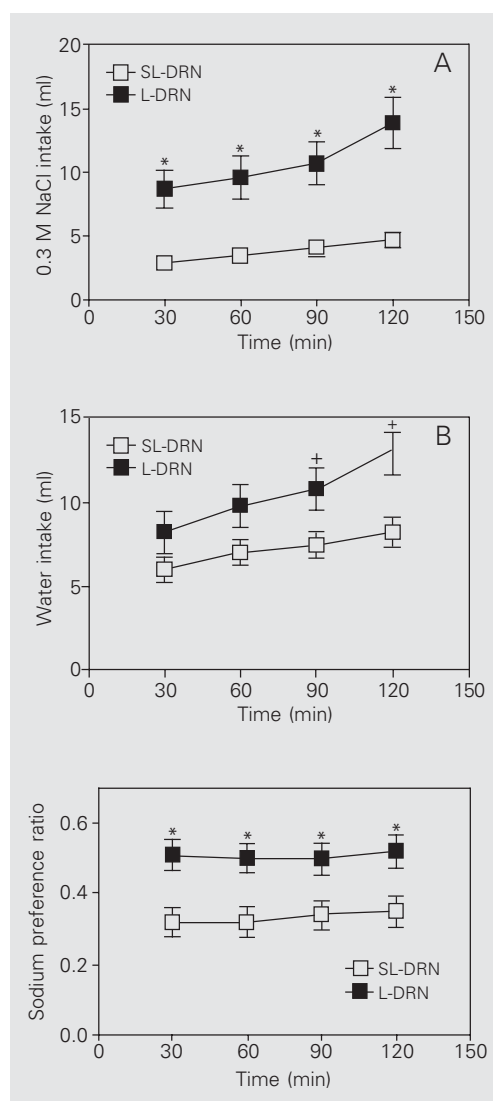


Figure 6. Sodium preference of dorsal raphe nucleus-lesioned (L-DRN, filled squares) and sham-lesioned rats (SL-DRN, open squares) submitted to sodium and water depletion induced by treatment with furosemide + captopril. Data are reported as means \pm SEM. * $P < 0.002$ compared to the SL-DRN group (two-way ANOVA followed by the Newman-Keuls post-test).

that electrolytic lesions of the DRN remove an excitatory serotonergic component involved in the release of ANP and, thus causing dysfunction of the system responsible for adjustments in volume and the maintenance of homeostasis, with a subsequent increase in sodium preference (3,35).

The oxytocin produced by the paraventricular nucleus is a candidate for a central mediator of satiety for sodium (4,36-38). Alternatively, this may constitute another modulatory pathway for sodium appetite dependent on serotonergic ascending activation, since serotonergic innervation of the paraventricular nucleus has been well established (1,2,8,39). In addition, an increase in Fos immunoreactivity was shown in oxytocinergic neurons during the process of sodium satiety in rats previously depleted of sodium by peritoneal dialysis (37).

These conclusions are consistent with the current literature. The existence of re-

dundancy of multiple effector reactions concerned with the process of sodium satiety might be explained by the insertion of serotonergic circuits in a polymodal system of recruitment/homeostatic activation at different levels of physiological disturbances of tonicity and extracellular fluid volume. In view of the hypotheses presented, the results obtained are compatible with the assumption that electrolytic lesion of the DRN suppresses a tonic inhibitory component of sodium appetite.

Acknowledgments

The authors are grateful to Dr. Karla Consort Ribeiro, Laboratório de Cardiologia Celular e Molecular, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, for preparing the photomicrographs, and to Dr. Wellington da Silva Côrtes for help with the English text.

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