Brazilian Journal of Medical and Biological Research (1999) 32: 309-317
ISSN 0100-879X

The influence of septal lesions on sodium and water retention induced by Walker 256 tumor

F. Guimarães[1],
O. Rettori[2],
A.N. Vieira-Matos[2]
and G.A. Fernandes[3]

[1]Departamento de Fisiologia Animal e Biofísica, Instituto de Biologia,
[2]Laboratório de Pesquisas Bioquímicas,
Centro de Atenção Integral à Saúde da Mulher, and
[3]Núcleo de Medicina e Cirurgia Experimental,
Faculdade de Ciências Médicas, Universidade Estadual de Campinas,
Campinas, SP, Brasil

Abstract

In the course of studies on the effects of septal area lesions on neuroimmunomodulation and Walker 256 tumor development, it was observed that tumor-induced sodium and water retention was less marked in lesioned than in non-lesioned rats. In the present study possible mechanisms involved in this phenomenon were investigated. The experiments were performed in septal-lesioned (LW; N = 15) and sham-operated (SW; N = 7) 8-week-old male Wistar rats, which received multifocal simultaneous subcutaneous (*sc*) inoculations of Walker 256 tumor cells about 30 days after the stereotaxic surgery. Control groups (no tumor, sham-operated food-restricted (SFR), N = 7) and lesioned food-restricted (LFR, N = 10) were subjected to a feeding pattern similar to that observed in tumor-bearing animals. Multifocal inoculation of Walker 256 tumor rapidly induces anorexia, which is paradoxically accompanied by an increase in body weight, as a result of renal Na^+ and fluid retention. These effects of the tumor were also seen in LW rats, although the rise in fractional sodium balance during the early clinical period was significantly smaller than in SW rats (day 4: SW = 47.6 ± 6.4% and LW = 13.8 ± 5.2%; day 5: SW = 57.5 ± 3.5% and LW = 25.7 ± 4.8%; day 6: SW = 54.4 ± 3.8% and LW = 32.1 ± 4.4%; $P<0.05$), suggesting a temporary reduction in tumor-induced sodium retention. In contrast, urine output was significantly reduced in SW rats and increased in LW rats (LW up to -0.85 and SW up to 4.5 ml/100 g body weight), with no change in osmolar excretion. These temporary changes in the tumor's effects on LW rats may reflect a "reversal" of the secondary central antidiuretic response induced by the tumor (from antidiuretic to diuretic).

Correspondence
F. Guimarães
Laboratório de Pesquisas Bioquímicas
CAISM, UNICAMP
Caixa Postal 6151
13081-970 Campinas, SP
Brasil
Fax: +55-19-231-5273

Publication supported by FAPESP.

Received October 28, 1997
Accepted November 17, 1998

Key words
- Na^+ retention
- Secondary diuretic response
- Tumor systemic effects
- Septal area lesion
- Multifocal tumor inoculation
- Walker 256 tumor

Introduction

Human and experimental cancers usually grow for variable periods of time without disturbing the host's physiology. However, once the first signs of homeostatic disturbances become evident, they show a rapid, sometimes fulminant evolution (1-4). The homeostatic disturbances associated with cancer are usually manifested as alterations in food and water intake, as well as in water-electrolyte composition, temperature, neurovegetative functions, immunofunction, and so on (5-9).

Since the limbic system plays a key role in regulating the major homeostatic functions, and since the various systemic effects induced by tumors are initiated suddenly and simultaneously and are rapidly followed by irreversible general homeostatic alterations culminating in the host's death, it seems reasonable to suppose that the tumor's systemic effects could be a consequence of alteration of limbic system physiology.

Impaired salt-water excretion is a well-known systemic effect of cancer, and has been studied in several animal models including Walker 256 tumor (5,6,10,11). Recent evidence from our laboratory indicates that the sodium retention induced by Walker 256 tumor is a primary renal tubular effect (11) rather than being secondary to adrenal hyperfunction or salt sequestration by the growing tumor (12-15).

In the course of studies on the effects of septal area lesions on neuroimmunomodulation and Walker 256 tumor development, we observed that tumor-induced sodium and water retention was less marked in lesioned than in non-lesioned rats. This finding suggested a role for the septal area in tumor-induced sodium retention since the septal nucleus belongs to a supra-hypothalamic region involved in the behavioral control of water and sodium balance (16-18). In the present study, we have investigated the mechanisms involved in this phenomenon. The experiments were performed in septal-lesioned and control (sham-operated) rats receiving multifocal simultaneous subcutaneous (*sc*) inoculations of Walker 256 tumor cells. Use of the multifocal tumor model was critical in this work because it markedly anticipated and synchronized the onset of the tumor's systemic effects.

Material and Methods

Animals and brain surgery

Eight-week-old male Wistar rats were obtained from the UNICAMP Central Animal House. The rats were anesthetized with ether and fixed in a stereotaxic instrument for small animals (Kriegh-Johnson type). Brain lesions were produced by bilateral aspiration of the septal area with a cannula (1 mm in diameter) connected to a vacuum pump. The cannula was inserted into the brain 1 mm anterior to the bregma, 1 mm lateral to the midline, at a 5° angle to the sagittal plane and 6 mm under the dural surface, based on the stereotaxic coordinates of an atlas of the rat brain (19). When the cannula was in position, negative pressure (10 mmHg) was applied for 10 s. After surgery, the rats were handled as during the experimental period until their recovery (about 30 days), in order to familiarize them with the subsequent procedures that could otherwise be stressful to septal-lesioned animals (20).

At the end of the experiments, the brains were removed and frozen and coronal sections were obtained (20-30 μm). The extent of the lesions was examined in toluidine blue-stained sections (Figure 1). Control sham-operated rats were submitted to the same procedure, except for the vacuum pump aspiration.

Experimental conditions and parameters measured

The rats were housed in individual metabolic cages in a temperature-controlled room

(22 ± 1°C) on a 12-h light-dark cycle and were provided with a standard rat chow (Labina, Purina) and tap water *ad libitum*, except for the food-restricted groups described below. Body weight, food consumption and urine volume, osmolality and Na^+ excretion were determined daily. Urinary sodium levels were measured by flame photometry and osmolality by cryoscopic point changes. After natural death, autopsies were performed and the presence of possible metastases or other macroscopic abnormalities capable of interfering with the results was recorded.

Tumor

The Walker-256 line A (originally obtained from the Christ Hospital Line, National Cancer Institute Bank, Cambridge, MA, USA) was donated by Dr. M.C. Cintra-Gomes (Department of Physiology, IB, UNICAMP) and was stored in liquid nitrogen or grown through consecutive subcutaneous (*sc*) or intraperitoneal (*ip*) passages. After at least three consecutive *ip* passages, 4×10^6 viable ascitic cells, suspended in 0.25 ml of Ringer-lactate, were inoculated *sc* at each of eight sites in the dorsolumbar region.

The multifocal tumor model

In the usual experimental studies (one single *sc* tumor inoculation/rat), the systemic effects of tumors initiate unpredictably in individuals of the same group, between 6 and 40 or more days after inoculation. At this point, many rats bear large tumor masses and metastases, the mechanical effects of which may mask the sudden and rapid development of the pathognomonic systemic effects. Simultaneous multifocal *sc* inoculations were performed in the present study in an attempt to anticipate and synchronize the initiation of systemic effects among the animals of a given group. With this procedure, the pathognomonic systemic effects initiate in 100% of the animals within 3-5 days after tumor cell inoculation, at which point the animals bear tiny tumors, but no significant metastases. This approach does not alter the characteristics and duration of the subsequent final stages of the illness (10).

Data synchronization

Despite the marked increase in the synchronization of effects provided by the multifocal tumor model, a significant degree of non-synchronization still remains since the systemic effects are not strictly initiated on the same day, develop very rapidly and present two phases (moderate and severe) with different individual duration. Thus, a simple averaging of individual daily data would mask the real information at this critical stage of the illness. To circumvent this problem, we employed a "synchronized average", similar to that used in physiology to average parameters such as individual electrocardiograms or nerve action potentials. Details of this procedure have been described elsewhere (10).

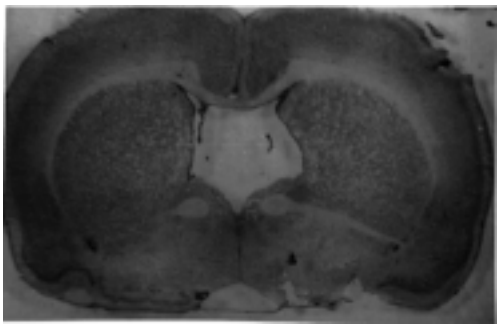
Data analysis

The results are reported as means ± SEM. Fractional sodium balances were calculated daily using the following formula: fractional Na^+ balance = (input-output) x 100/input, where input is the food intake (g/day) x diet ion content (mEq/g), and output is the urine volume (ml/day) x urine concentration (mEq/ml).

Post-hoc comparisons of means were performed using the least significant difference test (planned comparison) among multiple groups or the Student *t*-test for two groups. The level of significance was set at $P<0.05$.

Experimental design

The study was performed on 39 animals



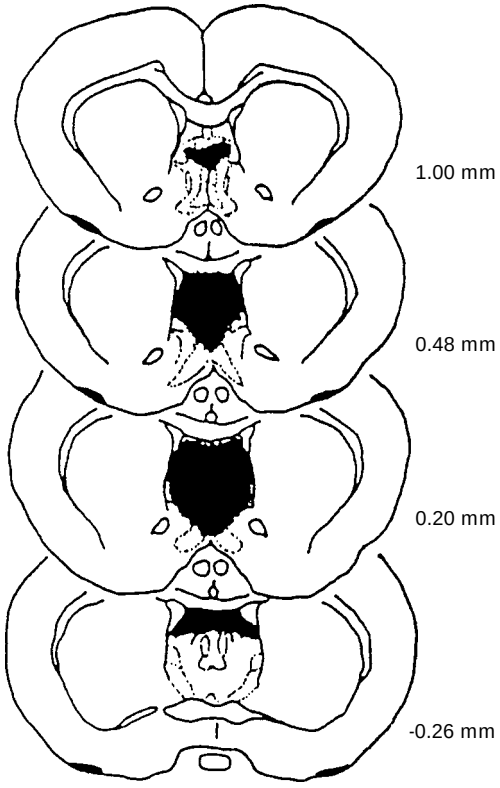


Figure 1 - Septal-lesioned rats. *Top*, Toluidine blue-stained coronal section (20-30 µm) and *bottom*, schematic representation of brain planes showing the extent of the lesion, which involved the medial and lateral (dorsal, intermediate and ventral) septal nucleus, besides part of the septofimbrial nucleus. In some rats, the lesion may have included the septohypothalamic nucleus, triangular septal nucleus, septal projection of the fornix and a small anterior portion of the corpus callosum.

Table 1 - Average duration (days) of the subclinical (sCP) and clinical (CP) periods and survival (SV) of rats multifocally inoculated with Walker 256 tumor, sham-operated (SW; N = 7) and septal-lesioned (LW; N = 15).

The values are reported as the means ± SEM for the number of rats indicated by N.

	SW (N = 7)	LW (N = 15)
sCP	3.0 ± 0.2	2.6 ± 0.1
CP	7.7 ± 0.6	7.0 ± 0.3
SV	10.7 ± 0.6	9.6 ± 2.5

divided into four groups, as follows: SW (sham-operated Walker), 7 animals; LW (septal- lesioned Walker), 15 animals; SFR (sham-operated food-restricted), 7 animals; LFR (lesioned food-restricted), 10 animals. Food-restricted animals (no tumor, SFR and LFR groups) were subjected to a feeding pattern similar to the average food ingestion observed in tumor-bearing animals during the hypophagic stage of the disease.

Results

Brain lesion

As expected, aspiration of the septal area produced a large lesion involving the medial and lateral (dorsal, intermediate and ventral) septal nucleus, in addition to part of the septofimbrial nucleus. In some animals, the lesion may have included the septohypothalamic nucleus, the triangular septal nucleus, the septal projection of the fornix and a small anterior portion of the corpus callosum (Figure 1).

Tumor and disease evolution

All tumor cell inoculations gave rise to continuously growing tumors which were palpable within 2-3 days. Independently of the group, in the tumor-bearing rats the disease followed its discontinuous pattern of evolution (10) with an initial period free of detectable systemic effects (subclinical period; sCP), that was suddenly interrupted by the symptomatic period (clinical period; CP). Table 1 shows the average duration of these periods. The onset of the CP occurred 2-3 days after tumor cell inoculation, independently of the lesion, and was characterized by pathognomonic and sudden development of alterations in fundamental homeostatic mechanisms, including appetite inhibition that was paradoxically accompanied by a gain in body weight due to sodium and fluid retention. The sudden manifestation and rapid

progression of these homeostatic alterations are illustrated in Figure 2. Body weight in the LW group did not rise as much as in the SW group, nor did the sodium excretion decrease as much in the LW group as in the SW group during the early days of CP. Thus, SW rats became significantly different from their non-tumor-bearing food-restricted controls (SFR) after the fifth day for body weight (Table 2) and after the fourth day for sodium excretion (Table 3), while in the LW group this only occurred (LW in relation to LFR) after the sixth day for body weight (Table 2) and after seventh day for sodium excretion (Table 3). Significant differences were also observed between the LW and SW groups at 6-7 days for body weight and at 4-6 days for sodium excretion (Figure 2).

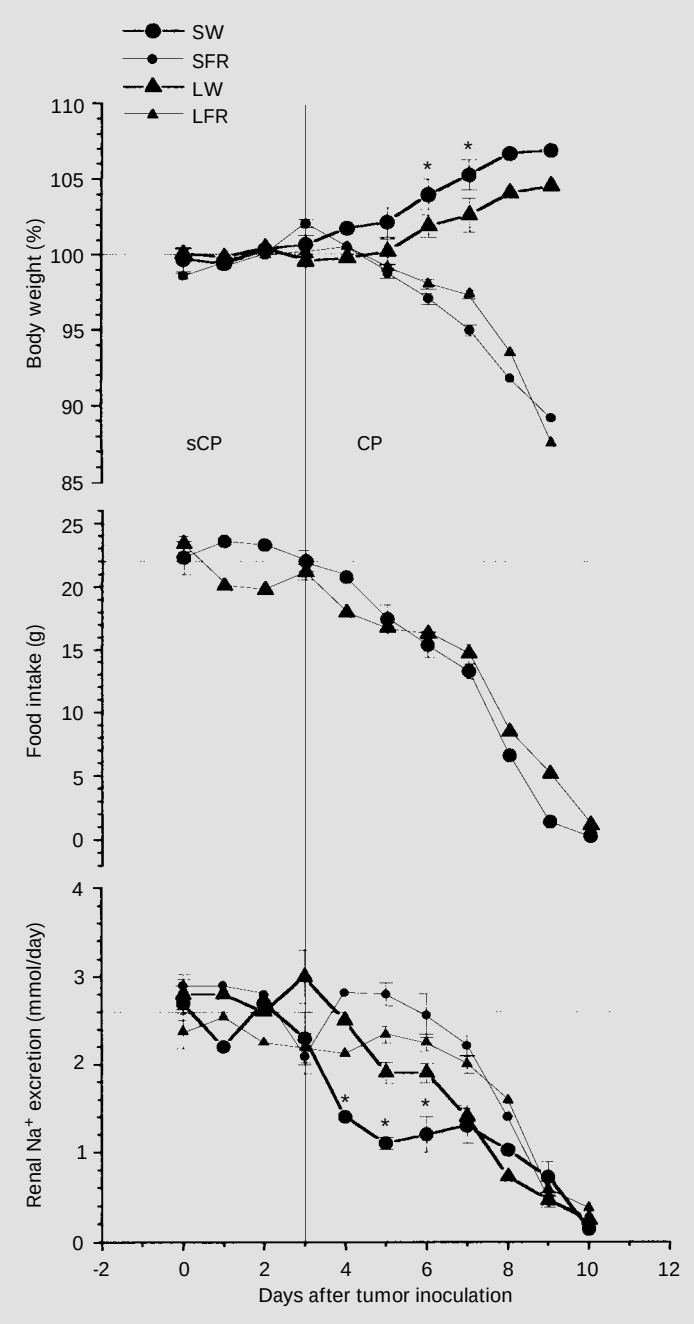


Figure 2 - Time course of the changes in body weight and renal Na^+ excretion in septal-lesioned tumor-bearing rats (LW; N = 15) compared with sham-operated tumor-bearing rats (SW; N = 7) and their respective non-tumor-bearing controls, sham-operated food-restricted (SFR; N = 7) and lesioned food-restricted (LFR; N = 10) rats. Food intake is given for the SFR and LFR groups. During the clinical period (CP), body weight gain and renal Na^+ excretion in LW rats were temporarily altered in relation to the SW rats (LSD test, *P<0.05 for SW x LW). sCP, Subclinical period.

Fractional sodium balance

Before the onset of systemic effects (up to the third day), sodium handling in tumor bearers was not different from that of food-restricted rats (SFR and LFR groups) (Figure 3). During this period (sCP), the fractional sodium balance was approximately 25%, indicating that about 75% of the ingested sodium was excreted through the kidneys. After the third day, when the systemic effects became apparent in tumor bearers and food intake decreased in all groups, the differences in sodium handling became very marked. In SFR and LFR rats, the fractional sodium balance decreased, indicating an increase in the fraction of ingested sodium excreted through the kidneys, which was well above 100% after day 7 (Figure 3). This progressive increase in the relative renal sodium excretion which was observed in both the SFR and LFR groups represented a physiological response to the body weight loss seen in these animals.

An opposite, non-physiological response was observed in SW and LW rats when the fractional sodium balance suddenly started to increase, indicating that the fraction of

sodium excreted through the kidneys decreased to values close to 40%. Nevertheless, the rise in fractional sodium balance during the early days of CP was smaller in LW than in SW rats, since SW rats became significantly different from their non-tumor food-restricted controls (SFR) by the fourth day after tumor cell inoculation (SW = 47.6 ± 6.4% and SFR = 4.5 ± 8.3%; $P<0.05$), while in the LW group (LW in relation to LFR) this only occurred after day 5 (LW = 25.7 ± 4.8% and LFR = 10.5 ± 3.9%; $P<0.05$). Besides, the fractional sodium balance of the LW group was significantly lower than in the SW group from day 4 to 6 (day 4: SW = 47.6 ± 6.4% and LW = 13.8 ± 5.2%; day 5: SW = 57.5 ± 3.5% and LW = 25.7 ± 4.8%; day 6: SW = 54.4 ± 3.8% and LW = 32.1 ± 4.4%; $P<0.05$) (Figure 3). All of these results suggest that during this period a reduction in tumor-induced sodium retention had taken place, although later on the full tumor effect also developed in the septal-lesioned rats.

Table 2 - Body weight (%) from the fourth to the seventh day after tumor cell inoculation in sham-operated tumor-bearing (SW; N = 7) and septal-lesioned tumor-bearing (LW; N = 15) rats.

Data are compared to their respective non-tumor-bearing controls, i.e., sham-operated food-restricted (SFR; N = 7) and septal-lesioned food-restricted (LFR; N = 10) rats. ^{abc} $P<0.05$ (least significant difference test, SW x SFR and LW x LFR).

Day	Body weight (%)			
	SW (N = 7)	SFR (N = 7)	LW (N = 15)	LFR (N = 10)
4	101.8 ± 0.6	100.5 ± 0.4	99.8 ± 0.5	100.5 ± 0.4
5	102.2 ± 1.0^{a}	98.7 ± 0.3^{a}	100.2 ± 0.9	99.2 ± 0.2
6	104.0 ± 1.0^{ac}	97.1 ± 0.3^{a}	101.9 ± 0.7^{bc}	98.1 ± 0.3^{b}
7	105.3 ± 1.1^{ac}	95.3 ± 0.3^{a}	102.7 ± 1.1^{bc}	97.4 ± 0.3^{b}

Table 3 - Renal sodium excretion (mmol/day) from the fourth to the seventh day after tumor cell inoculation in sham-operated tumor-bearing (SW; N = 7) and septal-lesioned tumor-bearing (LW; N = 15) rats.

Data are compared to their respective non-tumor-bearing controls, i.e., sham-operated food-restricted (SFR; N = 7) and septal-lesioned food-restricted (LFR; N = 10) rats. ^{abc} $P<0.05$ (least significant difference test, SW x SFR and LW x LFR).

Day	Renal Na^+ excretion (mmol/day)			
	SW (N = 7)	SFR (N = 7)	LW (N = 15)	LFR (N = 10)
4	1.4 ± 0.2^{ab}	2.8 ± 0.2^{a}	2.6 ± 0.2^{b}	2.1 ± 0.1
5	1.1 ± 0.1^{ab}	2.8 ± 0.2^{ac}	1.9 ± 0.1^{b}	2.3 ± 0.1^{c}
6	1.3 ± 0.2^{ab}	2.5 ± 0.2^{a}	1.8 ± 0.1^{b}	2.2 ± 0.1
7	1.3 ± 0.2^{a}	2.2 ± 0.1^{a}	1.4 ± 0.1^{b}	2.1 ± 0.1^{b}

Changes in body fluids

Indirect evidence of a change in water balance accompanying sodium retention was obtained by observing body weight and urine volume changes. As shown in Figure 4, food-restricted rats (SFR and LFR groups) presented a rise in urine output during CP as a consequence of the body weight loss seen in these animals. However, in tumor-bearing rats, although urine output had been significantly reduced in SW rats (up to -0.85 ml/100 g body weight) and increased in LW rats (up to 4.5 ml/100 g body weight), both groups presented a body weight gain which indicated water retention. Figure 5 shows the fluid retention of the SW and LW groups by plotting the differences between body weight of tumor-bearing groups and their respective controls (SW minus SFR and LW minus LFR).

Osmolar excretion

The fractional sodium balances and indirect water balances just presented show that during the initial days of the systemic effects LW rats retained less sodium and excreted more urine than tumor-bearers that were not lesioned (SW). To determine whether an alteration in the glomerulo-tubular balance could account for the reduction in tubular sodium reabsorption in these animals, osmolar excretion was assessed (Table 4). There was no increase in osmolar excretion during the CP of the disease in any group, indicating that only water diuresis, and not osmotic diuresis, had occurred in rats of the LW group.

Autopsy findings

Autopsies revealed only incipient lymph node metastases that could not have interfered with the present results.

Discussion

Tumor and disease evolution

In the present study we confirmed that, although the time course of Walker 256 tumor followed its usual pattern of evolution (10) with an sCP and CP in both SW and LW rats, in the latter animals tumor-induced sodium and water retention was temporarily reduced. The multifocal tumor inoculation model was fundamental to this study since it dramatically hastened the onset of tumor effects and abrogated individual differences in the time course of the disease. In contrast to single site tumor inoculation (10), the tumor started very early in all animals, independent of the presence of septal lesions. At this stage the mechanical effects of tumor masses or metatases were insignificant, allowing the effects observed to be interpreted as systemic or remote effects of the tumor.

Effect of septal lesions on tumor-induced alterations in water and electrolyte balances

The simple analysis of the time course changes in body weight and sodium excretion in intact, sham-operated and septal-lesioned rats confirmed that the tumor had a reduced effect on salt-water balance in the last group (Figures 2 and 5). In SW rats, tumor-induced anorexia was paradoxically accompanied by a body weight gain because of sodium and water retention (10,11), while in LW animals the initial effect on sodium and water retention was temporarily reduced. As expected, SFR and LFR animals showed a reduction in body weight.

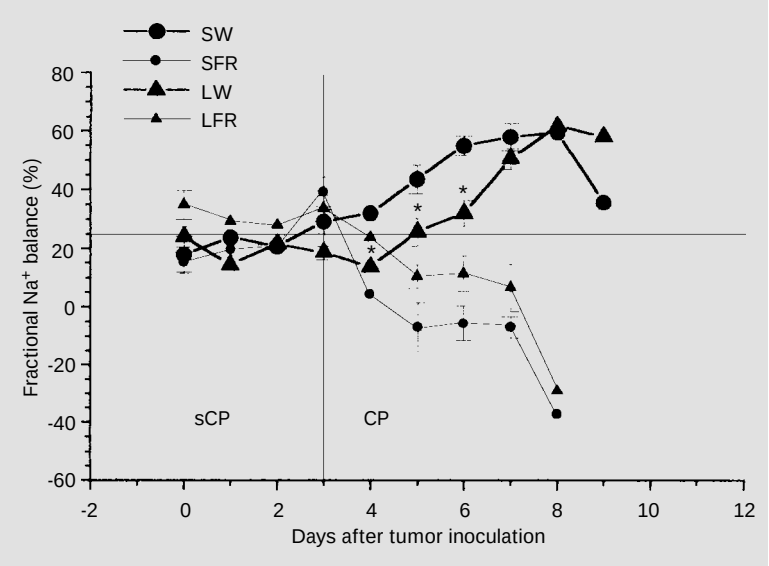


Figure 3 - Time course of sodium retention induced by the tumor in septal-lesioned tumor-bearing rats (LW; N = 15) compared with sham-operated tumor-bearing rats (SW; N = 7) and their respective non-tumor-bearing controls, sham-operated food-restricted (SFR; N = 7) and lesioned food-restricted (LFR; N = 10) rats. The fractional sodium balance was calculated as (input-output) x 100/input, where input is the food intake (g/day) x diet sodium content (mEq/g), and output is the urine volume (ml/day) x urine sodium concentration (mEq/ml). During the clinical period (CP), the tumor-induced Na^+ retention was temporarily reduced in LW rats compared with SW rats (least significant difference test, *P<0.05 for SW x LW). sCP, Subclinical period.

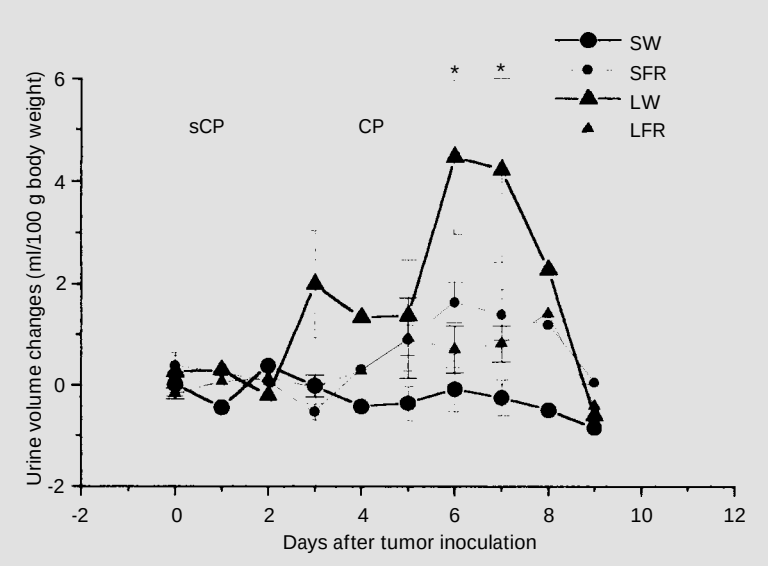


Figure 4 - Time course of urine output observed in septal-lesioned tumor-bearing rats (LW; N = 15), compared with sham-operated tumor-bearing (SW; N = 7) and their respective non-tumor-bearing controls, sham-operated food-restricted (SFR; N = 7) and lesioned food-restricted (LFR; N = 10) rats. During the clinical period (CP), the urine production was significantly increased in LW (least significant difference test, *P<0.05 for SW x LW). sCP, Subclinical period.

Possible mechanism of temporary reduction in sodium reabsorption in LW rats

Since the osmolar excretion did not increase in LW rats (Table 3), it seems unlikely that an alteration in the glomerulo-tubular balance could account for the reduction in sodium reabsorption in these rats. Thus, the elevated urine output in LW rats observed during CP reflected an increased water excretion (water diuresis). Furthermore, it seems reasonable to suppose that the decrease in sodium and fluid reabsorption in septal-lesioned rats could reflect the development of a secondary diuretic response which would be a "reversal" of the secondary antidiuretic response seen in SW rats (10,11). In this case antidiuretic hormone (ADH) may be involved. The main effect of ADH is water retention, but this hormone also induces some sodium retention (21). Thus, in intact rats, ADH may potentiate the tumor-induced sodium retention, while in lesioned rats, inhibition of ADH release due to the lesion would result in a large volume of diluted urine accompanied by a reduction in sodium reabsorption.

In our working hypothesis, one or more humoral factors of tumoral origin would induce renal Na^+ retention which would elevate the plasma Na^+ concentration followed by water retention (10,11). In fact, during CP we have found a significant rise in plasma Na^+ concentration in multifocally inoculated rats when compared with food-restricted controls (plasma [Na^+] in tumor-bearing rats = 156 ± 2.3 and in food-restricted controls = 142 ± 2.6, $P<0.01$; Rettori O, unpublished data). Thus, we suppose that this combination might lead to conflicting physiological signals for ADH release when a high extracellular Na^+ concentration (and osmolality) acting through hypothalamic receptors induces ADH release, while a high extracellular volume acting through the vascular volume receptor would lead to ADH inhibition. In SW rats, the net result would be a rise in ADH (low volume of concentrated urine accompanied by some sodium retention), while in LW rats the result would be a marked ADH inhibition (large volume of diluted urine with some decrease in Na^+ reabsorption), possibly as a result of lesions in the septal area pathways involved in ADH release (22). This proposition supports the hypothesis described previously that one septal role in water regulation is to stimulate supraoptic cells during dehydration which then release more ADH, causing the animal to conserve water (23). To confirm our proposition, future experiments with ADH concentration studies should be planned.

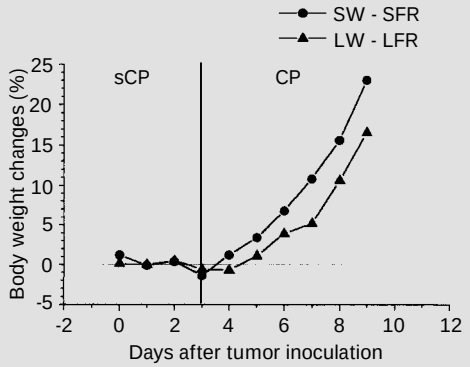


Figure 5 - Fluid retention changes of sham-operated (SW) and septal-lesioned (LW) tumor-bearing groups shown by plotting the differences between body weight of tumor-bearing groups and their respective controls (Figure 2; SW minus sham-operated food-restricted (SFR) and LW minus septal-lesioned food-restricted (LFR)). sCP, Subclinical period; CP, clinical period.

Table 4 - Comparison of osmolar excretion (mOsm/day) during the clinical period (CP; average of values obtained on days 5 to 7) and the subclinical period (sCP) in septal-lesioned tumor-bearing (LW; N = 15), sham-operated tumor-bearing (SW; N = 7), sham-operated food-restricted (SFR; N = 7) and septal-lesioned food-restricted (LFR; N = 10) groups.

	sCP (mOsm/day)	CP (mOsm/day)
SW (N = 7)	35.2 ± 2.1	33.3 ± 2.9
SFR (N = 7)	35.4 ± 2.8	36.3 ± 2.3
LW (N = 15)	45.3 ± 2.1	41.3 ± 1.6
LFR (N = 10)	32.6 ± 2.1	31.1 ± 1.7

Conclusion

Sodium and fluid retention induced by

the Walker 256 tumor was temporarily reduced in animals with septal area lesions. This effect was possibly a result of an inhibition of ADH release caused by the lesion rather than the elimination of a tumor-induced antinatriuretic effect.

Acknowledgments

The authors are indebted to Dr. T.C. Cavalcanti for assistance and helpful discussions of this paper and to Dr. S. Hyslop for valuable assistance in the preparation of the text.

References

1. Eastern Cooperative Oncology Group (1980). Prognostic effect of weight loss prior to chemotherapy in cancer patients. *American Journal of Medicine*, 69: 491-497.
2. Graf W, Glimelius B, Pahlman L & Bergström R (1991). Determinants of prognosis in advanced colorectal cancer. *European Journal of Cancer*, 77: 1119-1123.
3. Pasqualetti P, Casale R, Collacciani A & Colantonio D (1991). Prognostic factors in multiple myeloma: a new staging system based on clinical and morphological features. *European Journal of Cancer*, 27: 1123-1126.
4. Rustig VK (1987). Epidemiology of hepatocellular carcinoma. *Gastroenterology Clinics of North America*, 16: 545-551.
5. Tacaks FJ (1974). Fluid and electrolyte problems in patients with advanced carcinoma. *Medical Clinics of North America*, 59: 449-457.
6. Blackburn SL, Maini BS, Bistrian BR & McDermott WV (1977). The effect of cancer on nitrogen, electrolyte, and mineral metabolism. *Cancer Research*, 37: 2348-2353.
7. Garattini S, Bizzi A, Conelli MG, Guaitani A, Samanin R & Spreafico F (1980). Anorexia and cancer in animals and man. *Cancer Treatment Reviews*, 7: 115-140.
8. Baillie P, Millar FK & Pratt AW (1965). Food intake and Walker tumor growth in rats with hypothalamic lesions. *American Journal of Physiology*, 201: 293-300.
9. Scott OCA (1991). Tumor transplantation and tumor immunity: A personal view. *Cancer Research*, 51: 757-763.
10. Rettori O, Vieira-Matos AN & Tahin QS (1995). Variability and discontinuity of the pathognomic systemic effects caused by the Walker-256 tumor progression in rats. *Tumori*, 81: 370-377.
11. Rettori O, Vieira-Matos AN & Gontijo JAR (1996). Reduced renal sodium excretion in Walker-256 tumor-bearing rats. *Acta Physiologica Latino Americana*, 46: 111-118.
12. Morrison SD (1968). Effect of growth of a tumor on the regulation of water intake. *Journal of the National Cancer Institute*, 41: 1241-1248.
13. Morrison SD (1971). Water intake and exchange and hydration of rats during growth of Walker 256 carcinoma. *Journal of the National Cancer Institute*, 46: 825-830.
14. Morrison SD (1974). Sodium and the cachexia and hypophagia of tumor growth. *Journal of the National Cancer Institute*, 52: 869-874.
15. Toal JN, Millar FK, Brooks RH & White V (1960). Sodium retention by rats bearing the Walker carcinosarcoma 256. *American Journal of Physiology*, 200: 175-181.
16. Harvey JA & Hunt HF (1965). Effect of septal lesions on thirst in the rat as indicated by water consumption and operant responding for water reward. *Journal of Comparative and Physiological Psychology*, 59: 49-56.
17. Negro Vilar A, Gentil CG & Antunes-Rodrigues J (1965). Influência do sistema límbico na ingestão de cloreto de sódio e água no rato. *Ciência e Cultura*, 17: 253 (Abstract).
18. Covian MR (1966). Fisiologia del'área septal. *Acta Physiologica Latino Americana*, 16: 119-152.
19. Paxinos G & Watson C (1986). *The Rat Brain in Stereotaxic Coordinates*. 2nd edn. Academic Press, New York.
20. Paplowsky A & Isaacson RL (1990). Nimodipine accelarates recovery from the hyper-emotionality produced by septal lesions. *Behavioral and Neural Biology*, 53: 133-139.
21. Pitts RF (1968). Regulation of volume and osmolar concentration of extracellular fluid. In: Pitts RF (Editor), *Physiology of the Kidney and Body Fluids*. Year Book Medical Publishers Incorporated, Chicago.
22. Haibara AS, Saad WA, Camargo LAA, Menani JV, Renzi A, De Luca Jr LA & Antunes-Rodrigues J (1992). Opiate activation suppresses the drinking pressor and natriuretic responses induced by cholinergic stimulation of the medial septal area. *Brain Research Bulletin*, 28: 155-160.
23. Bridge JG (1976). Unit activity in the septal nuclei during water deprivation, drinking, and rehydration. In: De France JF (Editor), *Advances in Behavioral Biology. The Septal Nuclei.* Plenum Press, New York.