Chronic Hyponatremia Reduces Survival of Magnocellular Vasopressin and Oxytocin Neurons after Axonal Injury

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Axonal injury to hypothalamic magnocellular vasopressin (AVP) and oxytocin (OT) neurons causes degeneration of a substantial subpopulation of these neurons. In this study, we investigated the influence of osmolality on this injury-induced cell death. Normonatremic, chronically hypernatremic, and chronically hyponatremic rats received pituitary stalk compression (SC), which causes degeneration of AVP and OT terminals in the neurohypophysis. Twenty-one days after SC, rats were perfused and hypothalami were serially sectioned and alternately stained for AVP-neurophysin and OT-neurophysin immunoreactivities. Normonatremic and hypernatremic rats exhibited a triphasic pattern of water intake after SC, with peak intakes 3 times higher than those exhibited by sham-operated normonatremic rats. In contrast, hyponatremic SC rats exhibited peak water intakes of 600 ml/24 hr, ~9–10 times the water intakes of sham-operated normonatremic

rats. In normonatremic rats, SC caused degeneration of 65% of the AVP neuron population in the SON and 73% in the PVN, but only 31% of the OT neuron population in the SON and 35% in the PVN. Similar results were found in hypernatremic rats after SC. However, in hyponatremic rats SC caused degeneration of 97% of the AVP neuron population in the SON and 93% in the PVN, and 90% of the OT neuron population in the SON and 84% in the PVN. Our results, therefore, demonstrate that injury-induced degeneration of magnocellular AVP and OT neurons is markedly exacerbated by chronic hypo-osmolar conditions, but neuronal survival is not enhanced by chronic hyperosmolar conditions.

Key words: axonal injury; vasopressin neurons; oxytocin neurons; osmolality; neuronal death; hyponatremia; diabetes insipidus

Injury to magnocellular vasopressin (AVP) and oxytocin (OT) neurons induces marked changes in the morphology and function of the neurohypophysis. Neurolobectomy and transection of the pituitary stalk result in degeneration of the neural lobe of the pituitary gland and the development of diabetes insipidus (O'Connor, 1952; Moll and De Wied, 1962). In addition, disruption of the axons of the hypothalamo-neurohypophyseal tract also leads to retrograde degeneration of substantial numbers of magnocellular neurons in the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus (Hare, 1937; Raisman, 1973).

Degeneration of the perikaryon after axonal damage is not unique to magnocellular neurons, because this phenomenon has been observed throughout the CNS (Fry and Cowan, 1972; Brand and Mugnaini, 1976; Kawaguchi et al., 1981; Sofroniew and Isacson, 1988). However, only a few studies to date have ascertained possible factors influencing the survival of axonally damaged CNS neurons. Changes in hormonal milieu (Yu, 1989) and the volume of the axoplasm separated from the perikaryon (Fry and Cowan, 1972; Sofroniew and Isacson, 1988) have been suggested as factors influencing survival after axonal injury. Neurotrophic factors such as fibroblast growth factor (Grothe et al., 1989) and nerve growth factor (Hefti, 1986; Tuszynski et al., 1990), as well as fetal brain grafts (Sievers et al., 1989), have been reported to rescue CNS neurons from axotomy-induced degeneration. Fetal hypothalamic grafts have also been reported to rescue hypothalamic magnocel-

lular neurons after neurohypophysectomy (Marciano et al., 1989). In contrast, Herman et al. (1986) reported that AVP administration to neurolobectomized rats was associated with an increased rate of degeneration of magnocellular AVP neurons and prevented functional recovery of the vasopressinergic system (Herman et al., 1987). Interestingly, however, this treatment appeared to have little effect on the survival of magnocellular oxytocinergic neurons, which were not affected after neurolobectomy, as assessed by visual analysis.

Recently, we have characterized the effects of a brief (30 sec) compression of the pituitary stalk in rats, which include degeneration of the posterior pituitary and development of diabetes insipidus, but not damage to the anterior pituitary (Dohanics et al., 1992). Similar to neurolobectomy and transection of the pituitary stalk, stalk compression (SC) also caused degeneration of magnocellular neurons, which was apparent by a visible decrease in the number of AVP, but not OT, neurons by immunocytochemical staining of hypothalamic sections. In this study, hypothalamic AVP and OT neurons were counted after SC to provide a more quantitative assessment of the effects of axonal injury on survival of magnocellular neurons. In addition, we performed a similar analysis on rats that were made chronically hypo- or hypernatremic before SC, treatments known to down-regulate and upregulate neurohypophyseal secretion (Claybaugh, 1976; Verbalis et al., 1986; Verbalis and Dohanics, 1991) and synthesis (Sherman et al., 1986; Robinson et al., 1990), respectively, to evaluate whether the functional activity of magnocellular neurons before SC affected their subsequent survival.

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MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats weighing 250–275 gm (Zivic-Miller, Allison Park, PA) were housed individually in wire-mesh cages in a temperature-controlled room (21–23°C) with lights on from 7:00 A.M. to 7:00 P.M. Animals were fed solid food or liquid diet as described below. Daily fluid consumption was recorded throughout all studies.

Pituitary stalk compression

Stalk compression was performed as described previously (Dohanics et al., 1992). Briefly, rats were anesthetized with methoxyflurane (Metofane, Pitman-Moore, Washington Crossing, NJ) and mounted on a stereotaxic frame, then the skull was opened through an ~3 mm by 2 mm window. A triangle-shaped wire (~1.5 mm wide at the base) was lowered in the coronal plane 4.0 mm caudal to bregma in the midline until it touched the floor of the skull. The wire was held against the skull for 30 sec and then removed. For sham surgeries, the wire was lowered 9 mm beneath the surface of the brain. The wound was closed, and rats received 60,000 U of penicillin G (i.m.).

Induction of hypernatremia and hyponatremia

To induce hypernatremia, rats were given 2% NaCl solution *ad libitum* as their only drinking fluid for 7 d. Daily saline intake was monitored. Hyponatremia was induced as described previously (Verbalis and Drutarosky, 1988). Briefly, rats were fed with 40 ml/d (70 kcal/d) of a nutritionally balanced liquid diet (AIN-76, Bioserv, Frenchtown, NJ), which was presented daily each morning. After 2 d on liquid diet, osmotic minipumps (Alzet model 2002, Alza, Palo Alto, CA) containing 1-desamino-[8-D-arginine]-vasopressin (DDAVP; Rorer Pharmaceuticals, Fort Washington, PA) were implanted subcutaneously using methoxyflurane anesthesia to deliver DDAVP at a rate of 5 ng/hr. On the day of osmotic minipump implantation, the rats were given a more diluted preparation of the liquid diet (70 kcal in 60 ml), but thereafter resumed the more concentrated formula (70 kcal in 40 ml).

Treatment groups

The following four groups of rats were studied.

Sham-operated normonatremic rats. These rats received sham stalk compression and had access to either solid chow *ad libitum* or 40 ml of liquid diet daily. They also had unrestricted access to tap water, the consumption of which was monitored daily.

Stalk-compressed normonatremic rats. These rats received SC and had access to either solid chow *ad libitum* or 40 ml of liquid diet daily. They also had unrestricted access to tap water, the consumption of which was monitored daily.

Stalk-compressed hypernatremic rats. These rats were given 2% NaCl solution *ad libitum* as their only drinking fluid for 7 d. On the seventh day, these rats received SC. After the surgery, they were given tap water instead of 2% NaCl. Water and 2% NaCl intakes were monitored daily. These rats had unrestricted access to solid food.

Stalk-compressed hyponatremic rats. Seven days after the induction of hyponatremia, these rats received SC. Hyponatremia was then maintained for the next 7 d, after which the rats were allowed to return to normonatremia. Because rapid correction of chronic severe hyponatremia is known to cause severe neurological effects (Verbalis et al., 1991a), rats were slowly made normonatremic over several days to avoid such side effects. Seven days after surgery (14 d after the induction of hyponatremia), the DDAVP infusion was terminated by removing the osmotic minipumps, but the rats remained on the liquid diet for an additional 2 d before they were given solid food *ad libitum*.

On the day of the SC surgery, a blood sample was drawn from all rats via jugular puncture for measurement of plasma sodium concentrations ($[Na^+]$) using an ion-specific electrode (Beckman Electrolyte 2 Analyzer, Brea, CA).

Histology

Twenty-one days after SC surgery, rats received a lethal dose of pentobarbital (100 mg/kg). After an intracardiac injection of heparin (300 U), rats were perfused through the ascending aorta with 0.9% NaCl containing 2% sodium nitrite (total volume, 200 ml). The perfusion solution was then switched to a fixative consisting of 4% paraformaldehyde and 1.4% picric acid in 0.1 M phosphate buffer (total volume, 100 ml). Perfused rats were decapitated, and the brains and pituitaries were stored in 25% sucrose at 4°C until sectioning. Hypothalami were cut with a freezing

microtome into 25 μ m sections in the coronal plane. Alternate sections were processed for AVP-neurophysin (AVP-NP) and OT-neurophysin (OT-NP) immunohistochemical stainings using antisera produced by immunizations with N-terminal fragments of these peptides (Verbalis et al., 1991b). Stained sections were mounted on glass slides and coverslipped. Pituitaries were stored in 25% sucrose for 24 hr at 4°C and then immersed in 15% gelatin (J. T. Baker Chemical Company, Phillipsburg, NJ) for 15 min at 37°C. Gelatin-embedded pituitaries were fixed in the perfusion solution for 24 hr at 4°C and then stored in 25% sucrose for 24 hr at 4°C. Pituitaries were cut with a freezing microtome into 40 μ m sections. Sections were stained for AVP-NP and OT-NP immunoreactivities. Some hypothalamus and pituitary sections were stained with cresyl violet. Rats with incomplete SC (as determined by pituitary histology) were excluded from further evaluation.

Cell counting

AVP-NP- and OT-NP-immunoreactive cells in the PVN and SON were counted on all sections. Neurons were counted at 40 $\times$ magnification by identifying them visually or by visual identification combined with mapping of the position of identified neurons. The position of individual neurons was recorded using a microscope stage connected to a Macintosh computer that recorded the x - y coordinates of the stage position. In each group, neurons in sections with cresyl violet staining only were also counted over an 80 $\times$ 80 μ m² area of the SON ($n = 4$). Raw counts were corrected for double-counting errors using Abercrombie's method (Abercrombie, 1946).

Data analysis

All results are expressed as mean $\pm$ SE. Statistical analysis was performed on logarithmic transforms of the data using ANOVA followed by Tukey's protected t tests for multiple comparisons where appropriate.

RESULTS

Plasma $[Na^+]$ of the hypernatremic and hyponatremic rats at the time of SC was significantly higher and significantly lower, respectively, than in normonatremic rats (Table 1).

Water intake of the sham-operated normonatremic rats remained relatively constant over the entire period of observation. As reported previously, stalk-compressed normonatremic rats exhibited a triphasic pattern of water intake: a sharply increased intake during the first 24 hr after surgery (phase 1), followed by 2–3 d of water intake levels comparable with those observed in sham-operated rats (phase 2), after which water intake increased again and remained elevated throughout the remainder of the observation period (phase 3) (Fig. 1).

Stalk-compressed hypernatremic rats that drank 2% NaCl only during the 7 d immediately preceding SC consumed increasingly larger amounts of the saline solution during that period. The water intake of the hypernatremic rats during the first 24 hr after SC was significantly higher ($p < 0.01$) than that of sham-operated rats during the same period and, thereafter, the stalk-compressed hypernatremic rats exhibited a pattern of water intake similar to that of SC normonatremic rats (Fig. 2).

As expected, the stalk-compressed hyponatremic rats consumed very small amounts of water as long as hyponatremia was maintained, and stalk compression did not have any immediate effect on the water intake of these rats during this period. However, water intake began to increase from day 8 after SC, the first day

Table 1. Plasma $[Na^+]$ (mmol/l; mean $\pm$ SE) at the time of surgery

Sham-operated	SC/normonatremic	SC/hypernatremic	SC/hyponatremic
144.1 $\pm$ 0.8	142.4 $\pm$ 1.0	179.7 $\pm$ 4.5	103.9 $\pm$ 0.6
(6)	(7)	(7)	(6)
	NS	†††	†††

NS, $p > 0.05$ compared with sham-operated; †††, $p < 0.001$ compared with SC/normonatremic by one-way ANOVA.

Figure 1. Water intake of sham-operated (closed circles; $n = 6$) and SC rats (open circles; $n = 7$). Mean $\pm$ SE are shown.

after the termination of DDAVP infusion. During the subsequent days, water intake then increased further to extremely high levels, with peak intakes often exceeding 600 ml/24 hr. The rate of increase in water intake varied considerably in individual rats, peaking as early as 12 d after SC (5 d after the cessation of DDAVP infusion) or as late as 15 d after SC (8 d after the cessation of DDAVP infusion). Because the water intake curves of these rats were shifted by several days, individual examples are shown in Figure 3 instead of the group means. However, the mean peak water intake of the stalk-compressed hyponatremic rats was markedly higher than that of the stalk-compressed normonatremic ($p < 0.001$) and hypernatremic rats (Fig. 4).

In normonatremic rats, SC caused degeneration of a significant portion of the AVP neuron population in both the SON and the PVN, resulting in the survival of only ~ 35 and $\sim 27\%$ of AVP neurons in these nuclei, respectively (Figs. 5, 6; Table 2). In contrast, a much greater portion of the OT neuron population in the magnocellular nuclei survived SC, $\sim 69\%$ in the SON and $\sim 65\%$ in the PVN (Figs. 7, 8; Table 2).

Figure 2. Fluid intake of sham-operated/hypernatremic (closed circles; $n = 6$) and SC/hypernatremic rats (open circles; $n = 7$). Before surgery on day 0, all rats had access to 2% NaCl only. After surgery, all rats had unrestricted access to tap water. Mean $\pm$ SE are shown.

Figure 3. Water intake of SC/normonatremic rats (open circles; $n = 6$; mean $\pm$ SE) and individual SC/hyponatremic rats (#31: open triangles; #38: open squares). The SC/normonatremic rats were fed the same liquid diet as the SC/hyponatremic rats but did not receive DDAVP infusions (see Materials and Methods).

In hypernatremic rats, the extent of magnocellular neuronal degeneration after SC was similar to that observed in normonatremic rats. The portion of the surviving AVP neuron population was ~ 35 and $\sim 23\%$ in the SON and PVN, respectively (Figs. 5, 6; Table 2), and the portion of the surviving OT neuron population was ~ 61 and $\sim 60\%$, respectively (Figs. 7, 8; Table 2).

Hyponatremia greatly exacerbated the extent of magnocellular neuronal degeneration caused by SC. In the hyponatremic rats, only ~ 3 and $\sim 7\%$ of the AVP neurons in the SON and in the PVN survived, respectively (Figs. 5, 6; Table 2). The survival rates of OT neurons in the SON and PVN also decreased significantly to ~ 10 and $\sim 16\%$, respectively (Figs. 7, 8; Table 2).

Counting of magnocellular neurons in sections stained with

Figure 4. Maximum 24 hr water intake in sham-operated (Sham), stalk-compressed normonatremic (SC/normo), stalk-compressed hypernatremic (SC/hyper), and stalk-compressed hyponatremic rats (SC/hypo). Mean $\pm$ SE are shown. ***, $p < 0.001$ compared with sham; NS, $p > 0.05$; +++, $p < 0.001$; +, $p < 0.05$ for the paired comparisons indicated.

Figure 5. Topographically correct representation of AVP-NP neurons at three different rostrocaudal levels in the SON from a normonatremic sham-operated rat (A), a normonatremic SC rat (B), a hypernatremic SC rat (C), and a hyponatremic SC rat (D). oc, Optic chiasm.

Figure 6. Topographically correct representation of AVP-NP neurons at three different rostrocaudal levels in the PVN from a normonatremic sham-operated rat (A), a normonatremic SC rat (B), a hypernatremic SC rat (C), and a hyponatremic SC rat (D). 3v, Third ventricle.

Table 2. Cell counts (mean $\pm$ SE) of hypothalamic AVP and OT neurons

<i>AVP neurons in the SON</i>			
Sham-operated	2672 $\pm$ 268	(6)	
SC/normonatremic	936 $\pm$ 102	(7)	**
SC/hypernatremic	948 $\pm$ 279	(7)	***
SC/hyponatremic	97 $\pm$ 9	(6)	***†††
<i>AVP neurons in the PVN</i>			
Sham-operated	994 $\pm$ 141	(6)	
SC/normonatremic	270 $\pm$ 28	(7)	***
SC/hypernatremic	229 $\pm$ 41	(7)	***
SC/hyponatremic	69 $\pm$ 23	(6)	***†††
<i>OT neurons in the SON</i>			
Sham-operated	1958 $\pm$ 212	(6)	
SC/normonatremic	1361 $\pm$ 135	(7)	*
SC/hypernatremic	1198 $\pm$ 164	(7)	*
SC/hyponatremic	205 $\pm$ 9	(6)	***†††
<i>OT neurons in the PVN</i>			
Sham-operated	2417 $\pm$ 161	(6)	
SC/normonatremic	1585 $\pm$ 107	(7)	***
SC/hypernatremic	1459 $\pm$ 105	(7)	***
SC/hyponatremic	392 $\pm$ 36	(6)	***†††

*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ compared with sham-operated; †††, $p < 0.001$ compared with SC/normonatremic; the numbers in parentheses indicate the numbers of animals in each group.

cresyl violet only over limited areas of the SON revealed an $\sim 56\%$ survival rate in normonatremic SC rats (306 ± 23 vs 545 ± 22 in sham operated rats, $p < 0.01$). Corresponding magnocellular survival rates were $\sim 67\%$ in hypernatremic SC rats (366 ± 29 , NS compared with normonatremic SC) and $\sim 17\%$ in hyponatremic SC rats (98 ± 5 , $p < 0.001$, compared with normonatremic SC).

DISCUSSION

Retrograde degeneration of magnocellular neurons is well known to occur after hypophysectomy (Hare, 1937) or neurolobectomy (Bodian and Maren, 1951). Although magnocellular degeneration after axonal damage has also been demonstrated quantitatively (Bodian and Maren, 1951), these studies did not attempt to evaluate survival of AVP and OT neurons differentially. Only a recent report by Herman et al. (1987) using qualitative immunohistochemistry suggested that AVP rather than OT neurons are predominantly affected by axonal injury. Our quantitative results demonstrate that in normonatremic rats axonal injury affects the AVP neuron population more than twice as severely as it does the OT neuron population. Although this significant loss in the number of AVP neurons was also readily apparent by simple visual inspection, in our previous report in which magnocellular neurons were not counted (Dohanics et al., 1992) we were unable to detect any obvious effect of SC on the number of surviving OT neurons, similar to the results reported by Herman et al. (1987). The present study now shows that smaller but still significant degrees of degeneration also occur in hypothalamic OT neurons. It also shows that the apparent loss in the number of magnocellular neurons exhibiting AVP or OT staining reflects a decreased number of cells after surgery and likely is not the result of a temporary or permanent inability of magnocellular neurons to produce AVP- or OT-like immunoreactivity. This observation is in agreement with numerous earlier reports (O'Connor, 1947; Bodian and Maren, 1951; Raisman, 1973).

The factors that might account for the greater resilience of OT

neurons are unknown, but this observation is consistent with several other studies that strongly suggest that OT neurons also regenerate more readily than AVP neurons. Oxytocin but not AVP content of the stalk–median eminence is significantly increased after SC (Dohanics et al., 1992), and sprouting of OT but not AVP fibers into the external zone of the median eminence in PVN-lesioned rats has been observed (Antoni et al., 1988). One possible factor conferring a greater survival advantage to OT neurons could be a higher basal metabolic activity. In support of this, synthesis and secretion of OT appears to be less inhibited than AVP under conditions of chronic hyponatremia (Robinson et al., 1990; Verbalis and Dohanics, 1991). Another potential factor could be a more robust reparative response. In this regard, we have observed recently that after SC, intense expression of the immediate early gene product c-Jun occurred in magnocellular OT neurons, but staining intensity in the few c-Jun-expressing AVP neurons was much weaker (Dohanics et al., 1993b).

Although our experiments do not reveal the mechanism(s) responsible for the extent of neuronal degeneration caused by axonal injury, several previous studies have suggested various factors that influence the survival of injured neuronal populations. The first hypothesis put forward to explain retrograde degeneration after axonal injury of CNS neurons indicated the importance of surviving axon collaterals and the amount of axoplasm retained by the injured neuron. In an elegant experiment, Fry and Cowan (1972) demonstrated that the larger the axonal arborization separated from the perikaryon, the more likely that neurons of the lateral mamillary nucleus would die. This likely accounts for the long-standing observation that transection of the hypothalamo–hypophyseal stalk at more rostral levels causes more extensive neuronal degeneration in magnocellular nuclei and greater degrees of diabetes insipidus (O'Connor, 1947). Although there may have been a small degree of variability in the placement of SC in these studies, this factor could not have caused the wide differences in the survival rates between AVP and OT neurons in the same animals, nor is it likely to account for the large differences in survival rates of all magnocellular neurons between normonatremic and hyponatremic rats.

A previous study has also suggested that hormones such as anabolic steroids could also influence survival of neurons after injury (Yu, 1989), but because in our studies we used male rats exclusively, this factor also seems unlikely to account for the effects we observed. However, because the hyponatremic rats in our experiments were exposed to DDAVP, the possibility that this vasopressin analog itself influenced magnocellular neuronal survival cannot be excluded. Reports on the effects of AVP and DDAVP on magnocellular neuronal function have not been consistent. Although some investigators have reported decreased AVP secretory responses to physiological stimuli in rats treated with AVP or DDAVP (Cheng and North, 1989), others have found no such inhibitory effects (Lundin et al., 1985; Shimizu and Nakao, 1992). Moreover, although there is autoradiographic evidence for the existence of AVP-binding sites in the neural lobe (Bunn et al., 1986), AVP binding in the neural lobe appears to reflect the presence of neurophysins rather than AVP receptors (Freund-Mercier et al., 1991). Nonetheless, even if AVP receptors are located on AVP or OT terminals in the neural lobe, a direct action of DDAVP at this site would only be likely if those receptors were of the V_2 type, which has not been reported in neural tissue (Antoni, 1984). Although there is therefore little evidence supporting the likelihood of DDAVP acting directly on AVP and OT neurons at the level of the neural lobe, it is even less

Figure 7. Topographically correct representation of OT-NP neurons at three different rostrocaudal levels in the SON from a normonatremic sham-operated rat (A), normonatremic SC rat (B), hypernatremic SC rat (C), and hyponatremic SC rat (D). oc, Optic chiasm.

Figure 8. Topographically correct representation of OT-NP neurons at three different rostrocaudal levels in the SON from a normonatremic sham-operated rat (A), normonatremic SC rat (B), hypernatremic SC rat (C), and hyponatremic SC rat (D). 3v, Third ventricle.

likely that DDAVP could act directly at the level of the perikarya because DDAVP does not cross the blood–brain barrier in appreciable amounts (Stegner et al., 1983); but it is possible that permeability of the blood–brain barrier could have changed in our experiments. It is also theoretically possible that DDAVP could influence magnocellular neurons by acting at circumventricular organs, such as the subfornical organ or the organum vasculosum of the lamina terminalis.

A more likely cause of the excessive loss of magnocellular neurons in the SC hyponatremic rats is the greatly decreased functional activity of magnocellular neurons during induced hyponatremia. During chronic hyponatremia, stimulus-induced AVP and OT secretion is blocked or greatly suppressed (Verbalis et al., 1986; Verbalis and Dohanics, 1991). In addition, in chronically hyponatremic rats, synthesis rates of magnocellular hormones are decreased to 5–25% of the levels in normonatremic rats (Robinson et al., 1990). Consequently, it seems very likely that the metabolic rates in magnocellular neurons are also much lower during chronic hyponatremia. In support of this, we recently observed that morphological changes in oxytocin axonal structure that normally occur in rats after PVN lesions failed to occur in chronically hyponatremic rats (Dohanics et al., 1993a). A potentially equally important effect of chronic hyponatremia that may be relevant for magnocellular survival rates after SC is the lack of osmotic stimuli that are constantly present under normonatremic conditions. Hyponatremia, therefore, may directly impair the intracellular mechanisms necessary for structural and functional repairs after SC, or these intracellular reparative mechanisms may be compromised by the hyponatremia-induced inactivity of afferent neuronal inputs to magnocellular neurons that contribute to the baseline activity levels of AVP and OT neurons under normonatremic conditions.

Our results clearly showed that hyponatremia significantly decreases the likelihood of magnocellular neuronal survival after axonal injury, but hypernatremia, which is well known to upregulate magnocellular afferent stimulation, synthetic activity, and secretion, did not appear to confer any increased survival advantage. This indicates that the effects of neuronal activity on magnocellular survival are not symmetrical, and suggests that at baseline levels of plasma osmolality optimum conditions for magnocellular regeneration after axonal injury may already exist, in which case hypernatremia would not further enhance the likelihood of cell survival. Perhaps more interesting, however, is the finding that the markedly increased metabolic activity produced by the chronic hyperosmolality did not itself further compromise neuronal survival after SC. However, because hypernatremia lasted only a little over 24 hr after SC, direct comparisons between the effects of hypo- and hyperosmolality on magnocellular survival cannot be extended to the entire postoperative period.

It is also important to note that the results of the cell survival studies are consistent with the functional deficits in neurohypophyseal secretion produced by the SC as reflected by the drinking behavior of the rats. The postoperative triphasic pattern of water intake observed in SC normonatremic rats was essentially identical to that observed in SC hypernatremic rats. In contrast, stalk-compressed hyponatremic rats exhibited a very different pattern of water intake. After surgery, the continued DDAVP infusion prevented any increase in the water intake of these rats despite the loss of their ability to secrete AVP. However, after cessation of DDAVP infusion, the water intakes of these rats increased dramatically and reached levels much higher than those of either SC normonatremic or SC hypernatremic rats. Furthermore, the

peak water intakes observed in SC hyponatremic rats were more than twice as high as the water intakes of Brattleboro rats, which lack AVP immunoreactive neurons (Valtin et al., 1965), and also of neurolobectomized AVP-treated rats in the experiments of Herman et al. (1986, 1987). This observation suggests a complete lack of urine-concentrating capability in SC hyponatremic rats. In Brattleboro rats that develop diabetes insipidus early in postnatal life, increased OT secretory activity has been suggested to compensate partially for the deficiency of AVP secretion. A similar mechanism could account for the relatively milder polydipsia observed in neurolobectomized AVP-treated rats, which lose most of their AVP magnocellular neurons but appear to retain their OT neuron populations relatively intact (Herman et al., 1987). In contrast, after SC in hyponatremic rats both AVP and OT neuronal populations undergo substantial degeneration, thereby leaving no hormonal secretion capable of exerting anti-diuretic effects at the kidneys.

In summary, our results indicate that injury-induced degeneration of magnocellular neurons is amplified by chronic hyponatremia, either by removing osmotic stimuli that would normally impinge on magnocellular neurons or by downregulating the metabolic and synthetic activity of the neurons. These results are potentially relevant for considering treatment strategies for human patients with postoperative or post-traumatic injuries to the hypothalamo–neurohypophyseal system. Such patients are usually treated with DDAVP to provide symptomatic relief of their diabetes insipidus. However, this therapy may in fact be detrimental to long-term survival of the injured AVP and OT neurons by virtue of decreasing the basal activity of these neurons. In this case, prospects for functional recovery of neurohypophyseal secretion might be best enhanced by treatment of ongoing urinary water losses simply by free water replacement, thereby allowing a continual stimulation of the activity of the injured neurons. Further studies will be necessary to understand better the relationship between osmotic status and the regenerative ability of magnocellular neurons, and the cellular mechanisms that are responsible for these effects. Similarly, the degree to which these observations generalize to other neuronal systems is conjectural at this time, but it is interesting to note that a conceptually similar hypothesis regarding the importance of ongoing neuronal activity for neuronal survival has been advanced recently as a possible explanation for the neuronal loss in Alzheimer's disease (Sofroniew, 1991).

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