Proliferation of glial cells induced by lithium in the neural lobe of the rat pituitary is enhanced by dehydration

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Abstract. Rats dehydrated by 6 days of water deprivation had a low level of mitotic activity in the astrocytes ('pituicytes') of the neural lobe of the pituitary. Mitotic activity in the pituicytes was greatly increased when isotonic lithium was administered in the last 3 days of water deprivation. Rehydration on the last day of the experiment produced a further increase in mitoses. Isotonic solutions of sodium, potassium or rubidium chloride did not increase mitoses. This model of cell proliferation is of interest because the mitotic activity is related to a physiological attempt to maintain homeostasis rather than a response to injury or the development of neoplasia.

INTRODUCTION

Dehydration or administration of hypertonic solutions stimulate secretion of vasopressin by neurones of the supraoptic and paraventricular nuclei in the hypothalamus. This neuroendocrine secretion is transported to the neural lobe of the pituitary where it is discharged into the blood stream. The resident, specialized astrocytes of the neural lobe ('pituicytes') participate in this part of the neuroendocrine secretion process. The pituicytes are themselves stimulated by the neuroendocrine secretory process, as indicated by the occurrence of mitoses in the pituicytes of rats that have been deprived of water or given hypertonic NaCl.

Recently we reported that administration of lithium also induced mitoses in the pituicytes of the neural lobe even though the lithium chloride solution was isotonic (Levine & Saltzman 2000). That procedure for producing pituicyte mitoses involved bilateral nephrectomy which prevented excretion of the administered lithium and resulted in relatively constant, high levels of this cation. Persistent high levels of lithium would favour its penetration into cells. The presence of lithium within the hypothalamic neurones might be misinterpreted as a systemic osmotic signal, leading to vasopressin secretion and pituicyte mitoses. Reports of increased neurosecretion in hypothalamic nuclei (Ficek 1982) and increased serum vasopressin levels (Anai *et al.* 1997) in lithium-treated rats are compatible with this suggestion. If this hypothesis is correct, then lithium and dehydration produce pituicyte mitoses through the same path and might have additive effects. The present work concerns the interaction of lithium with systemic dehydration (water deprivation) for production of pituicyte mitoses.

MATERIALS AND METHODS

Lewis rats of either sex were kept in hanging shoebox type plastic cages with hardwood bedding. Laboratory Rodent Diet 5001 (PMI Feeds, St. Louis, MO) and tap water were freely available except as described below. The rats were used when 7–10 weeks old (180–260 g in weight).

Rats were deprived of water for 3 days before intraperitoneal (IP) injection of isotonic lithium chloride (LiCl) or other electrolytes (0.15 M in water, 2 mL/100 g body weight). The injections were repeated on the next 2 days. Water continued to be withheld except for the water contained in the various injections. In some experiments, water was restored for the last day, as suggested by Kawamoto & Kawashima (1984). Food was not withheld but consumption was low. Unlike our previous report, no surgical procedure was performed.

The rats were killed at intervals by exsanguination while under CO₂ anaesthesia. The brain was removed quickly and the base of the skull immersed in Bouin's fluid to fix the pituitary body *in situ*. The next day the pituitary was removed, embedded in paraffin in entirety, sectioned at 5 µm at three levels and stained with haematoxylin and eosin. The three levels of pituitary were separated from each other by 300 µm. The level of pituitary which presented the largest area of neural lobe was selected for study. Coded slides were studied at 400× magnification and mitoses in the entire area of neural lobe were counted. Unlike selection problems encountered in stereological cell counts in the brain, selection bias could not occur because the entire neural lobe area was counted. As stated by Chambers (1945): 'A mitotic figure was not considered such unless the nuclear membrane was absent and multiple chromosomes present.' Duplicate counts agreed within 1, 2 or 3 mitoses and are reported as the average, rounded to the nearest whole number. The area of the neural lobe was determined with the aid of an ocular reticule, calibrated by a stage micrometer. The total number of mitoses divided by the area in mm² constituted an indication of mitotic activity. Statistical analysis was done by Kruskal–Wallis one-way analysis of variance.

Reasons for preferring mitotic counts over other indices of cell proliferation have been discussed by Williams (1962) and Cavanagh (1970). One such index, increased DNA synthesis, is not always accompanied by detectable mitoses (Lawson et al. 1993), so its ultimate significance is uncertain. Also, note that mitotic pituicytes are larger and more readily identifiable than mitoses in most other cell types.

In many experiments, blood was taken from the inferior vena cava, and serum prepared for assay of lithium, electrolytes, total protein and osmolality. Lithium was assayed by atomic absorption spectrophotometry. Other serum assays utilized a Roche Hitachi model 740–100 analyser (Indianapolis, IN, USA). Forebrains (hereinafter designated 'brains') were assayed as described previously (Levine *et al.* 1993). The brain was used as a surrogate for the neural lobe because of the small size of the neural lobe.

RESULTS

Pituicytes from neural lobes from rats that had been deprived of water for 6 days and received only sham injections had only a few mitoses (Table 1). A few mitoses were found in similarly thirsted rats that had received IP injections of H₂O or isotonic solutions of NaCl, KCl, RbCl, or a mixture of equal parts of NaCl and KCl, on each of the last 3 days of dehydration. Far more mitoses were detected in the rats given isotonic LiCl on those last 3 days of dehydration (first

Table 1. Mitoses in neural lobe after 6 (or 5) days of dehydration and 3 days of electrolyte treatment

Treatments ^a		Mitotic cells/mm ² ± SD without rehydration ^b	Mitotic cells/mm ² ± SD after rehydration ^c
Dehydration	LiCl	14.7 ± 4.1 (23)	20.7 ± 11.8 (27)
Dehydration	NaCl	5.6 ± 3.7 (14)	5.5 ± 4.0 (6)
Dehydration	KCl	6.3 ± 2.0 (4)	4.7 ± 0.3 (4)
Dehydration	RbCl	5.8 ± 0.5 (4)	3.0 ± 1.3 (4)
Dehydration	KCl/NaCl	2.0 ± 0.5 (4)	6.0 ± 0.7 (4)
Dehydration	H ₂ O	2.5 ± 1.3 (4)	4.3 ± 2.3 (4)
Dehydration	Sham	2.5 ± 1.7 (4)	5.4 ± 4.0 (8)
—	LiCl	0.9 ± 0.3 (16)	NA

^aAll injections were isotonic (except H₂O) 2 mL/100 g body weight, administered IP on each of the last 3 days of dehydration. Deprivation of water started 3 days before injections.

^bAverage number of mitoses per mm² of neural lobe in paraffin sections of pituitary ± standard deviation. Number of rats in parentheses. Mitoses after dehydration plus LiCl were significantly higher than combined control groups ($P < 0.001$).

^cAccess to drinking water allowed only for last day of experiment (when last dose of electrolyte was injected). Therefore, these rats were dehydrated only for 5 days.

NA, not applicable.

line of Table 1); this difference was statistically significant ($P < 0.001$). In contrast, 3 days of LiCl treatment of rats with free access to water yielded very few mitoses (last line of Table 1). Therefore, both lithium and dehydration were necessary to achieve high mitotic rates.

The rats that had been dehydrated for only 5 days with rehydration on the 6th day had a few mitoses as had dehydrated/rehydrated rats also given three daily IP doses of H₂O or isotonic NaCl, KCl, RbCl, or the NaCl/KCl mixture. Again, LiCl on those last 3 days was much more effective in eliciting mitoses; this difference was statistically significant ($P < 0.01$) (Table 1). The mitotic counts in some of the lithium-treated rehydrated rats were very high; the highest count was 55. In these rats, almost every high power field had several mitoses (Fig. 1). However, such extremely high counts were not found in every experiment.

Mitoses occurred in all parts of the neural lobe and they were not related to blood vessels. The mitotic cells were large, with abundant, well demarcated cytoplasm, sometimes striated (Fig. 1). The chromosomes were widely dispersed. These features are typical of astrocyte mitoses (Cavanagh 1970; Kawamoto & Kawashima 1984) and they indicated that the mitoses were in pituicytes.

Despite the mitotic activity, there was no evidence of increased cell density. Total non-endothelial cell nuclei were counted in four uniformly defined portions of a high power (400× magnification) field from each of eight control rats and eight lithium-treated and dehydrated rats. The total counts averaged 101 (range 80–128) for the controls and 97 (range 64–120) for the lithium-treated rats, an insignificant difference.

Dehydration and LiCl injections caused slight increases in total protein and osmolality of the serum (5.9–6.3 g/dL and 315–323 mosm/L) compared to rats injected with lithium but not deprived of water (5.7–5.8 g/dL and 307–308 mosm/L). Serum urea did not show any change. Serum lithium levels in thirsted rats were higher than lithium levels in rats allowed to drink and these levels remained fairly constant during the 3 days of lithium treatment (Table 2). An extra pair of rats had access to drinking water for rehydration during the last day of the experiment. As expected, the lithium levels in serum decreased in these rats (Table 2).

Figure 1. Cluster of mitotic pituicytes in neural lobe from a dehydrated rat treated with lithium. Three mitotic cells are located near or below the arrow; there are three more mitoses on the other side of the photomicrograph. The mitotic cells are large, with distinct cell borders and abundant cytoplasm. The chromosomes are large and widely dispersed. Most of the other cells are pituicytes with oval or round nuclei, and capillary endothelial cells with smaller, darkly stained, elongated nuclei. Haematoxylin and eosin (magnification $\times 400$).

Table 2. Dehydration increases lithium levels in serum of rats given daily lithium injections

Exp.	Pretreatment	Lithium levels (mEq/L) in serum after 1, 2 or 3 daily lithium injections ^a			
		1	2	3	3 ^b
1	Dehydration (duration)	0.38 (4 days)	0.45 (5 days)	0.32 (6 days)	0.21 (5d + drink 1 d)
	No dehydration	0.13	0.15	0.12	
2	Dehydration (duration)	0.53 (6 days)	0.62 (6 days)	0.67 (6 days)	
	No dehydration	0.25	0.16	0.25	

^aSerum obtained 24 h after the last of one, two or three daily lithium injections. Each value is the average of a pair of rat serums. The duration of dehydration is indicated in parentheses.

^bThis pair of rats had access to drinking water for the last day of the experiment.

Lithium levels were measured in brain as well as serum. Brain levels were lower than serum after the first dose because lithium enters the brain slowly, but the levels were similar to serum after the second and third doses (Table 3). Interestingly, the extra, rehydrated rats maintained high brain levels even though their serum levels had declined considerably, because lithium

Table 3. Dehydration increases lithium levels in brain of rats given daily lithium injections

Pretreatment	Lithium levels (mEq/kg) in brain after 1, 2 or 3 daily lithium injections ^a			
	1	2	3	3 ^b
Dehydration (duration)	0.27 (4 days)	0.45 (5 days)	0.38 (6 days)	0.33 (5d + drink 1 day)
No dehydration	0.13	0.24	0.23	

^aBrains obtained 24 h after the last of one, two or three daily lithium injections (same rats as Experiment 1 in Table 2). Each value is the average of a pair of rat brains. The duration of dehydration is indicated in parentheses.

^bThis pair of rats had access to drinking water for the last day of the experiment.

leaves the brain slowly. This finding provides a partial explanation for the high mitotic rates in rehydrated rats, provided that the brain is a suitable surrogate for the neural lobe.

DISCUSSION

Dehydration is known to stimulate pituicyte mitoses (Leveque & Small 1959; Kawamoto & Kawashima 1984) but only to a small degree in our experiments. Lithium alone, in normal rats that were capable of renal excretion, had little or no mitogenic effect. However, the combination of lithium and dehydration increased lithium levels and was a strong mitogenic stimulant (Tables 1 and 2). This finding may be caused by the summation of the two independent stimuli, each acting on the hypothalamic neurones. Alternatively, the dehydration may have reduced renal function, causing extrarenal azotaemia and preventing excretion of lithium. In other words, dehydration might have done in these experiments what bilateral nephrectomy did in our previous work. However, measurement of serum urea did not show azotaemia even though serum osmolality and proteins were slightly elevated.

Our mitotic counts were made on stained paraffin sections which are well known to undergo some shrinkage during preparation. Shrinkage might be increased by the deprivation of water for 5 or 6 days. Shrinkage could cause an artifactual increase in cell density, including an increase in mitotic cells. Although this might mean that our counts were too high, it would not change our interpretation of the results provided that the shrinkage affected lithium-treated and control rats in identical fashion. The possibility of selective shrinkage of lithium-treated tissues was excluded by our counts of total nuclei, which showed no difference between experimental and control groups. Therefore, our interpretations are valid even if all the counts are slightly high because of shrinkage.

Theoretically, increased visible mitoses could be caused by increased *duration* of mitosis. However, the very low or absent mitotic activity in unstimulated neural lobes make it unlikely that prolonged duration of mitoses could cause a detectable increase of mitotic figures, and certainly could not approach the very high mitotic counts in some of our lithium-dehydrated–rehydrated rats.

Pituicytes are astrocytes. Astrocytes in the brain undergo mitoses in the vicinity of diverse injuries (Cavanagh 1970). This leads to gliosis (astrocytosis) at sites of brain injury, a situation analogous to scar formation in non-neural tissues. Brain astrocytes are also responsible for the most common primary brain tumour, the astrocytoma. In the neural lobe the situation is different. The mitogenic stimulus is physiological, not pathological. Perhaps a comparison of pituicytes

and brain astrocytes could lead to better understanding of the differing responses of these two related cell types to diverse stimuli. For any further study of pituicyte mitosis, the method described here has an advantage over our earlier study (Levine & Saltzman 2000) in that no surgery is required.

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