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# The effect of hypertonic saline administration or stalk transection on histamine and histamine N-methyltranferase in the rat posterior pituitary

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*Abstract*

**There is evidence to suggest that histamine is a neurotransmitter in the CNS and functions in the regulation of arg-vasopressin (AVP) secretion. The posterior pituitary contains high levels of histamine and histamine N-methyltransferase (HNMT). Therefore, posterior pituitary histamine could also modulate the release of AVP. Paralleling the effect on AVP levels, the concentration of histamine in the rat posterior pituitary decreased from 18.8 ± 2.7 ng/mg protein (x ± SEM) to 12.9 ± 1.9 ng/mg protein following 2 days of 2% (w/v) hypertonic saline administration and to 11.5 ± 0.9 ng/mg protein with 7 days of treatment. Conversely, posterior pituitary HNMT activity was significantly elevated after hypertonic saline administration. Pituitary stalk transection did not reduce the concentration of histamine in the rat posterior pituitary although HNMT activity was reduced from 18.8 ± 0.82 munits/gland to 9.22 ± 1.56 munits/gland (x ± SEM). These results indicate that histamine released from posterior pituitary mast cells could facilitate AVP release as part of the overall mechanism for osmotic stimulation of AVP secretion and support the concept that most posterior pituitary histamine is not neuronally derived from the brain. HNMT, on the other hand, may be contained in neurons disrupted by stalk section.**

## *Introduction*

High concentrations of histamine are present in the rat posterior pituitary when compared to the levels of norepinephrine, dopamine, and serotonin [1]. Morphologic and pharmacologic studies have shown that mast cells are present in the posterior pituitary [2–4]. Histamine is a putative neurotransmitter in the CNS and is stored in both neurons and mast cells in brain [5]. Therefore, the presence of mast cells in the posterior pituitary does not exclude a partial neuronal localization of histamine in this tissue. However, the occurrence of descending histaminergic neurons from the brain to the posterior pituitary has not been previously considered. Histamine N-methyltransferase (HNMT) is also present in the posterior pituitary and represents the primary route of histamine catabolism in brain [6]. In the monkey, posterior pituitary HNMT activity exceeds the level of HNMT in the hypothalamus and HNMT activity in the rat posterior pituitary is similar to whole brain levels [7, 8]. Therefore, a mechanism exists for the rapid inactivation of histamine in the posterior pituitary.

Recent evidence suggests histamine may modulate arg-vasopressin (AVP) secretion. Histamine was shown to excite antidromically identified supraoptic neurons [9] and intraventricular histamine administration or injection of histamine into the supraoptic nucleus stimulates AVP secretion [10, 11]. Posterior pituitary histamine may also stimulate AVP secretion. The concentration of histamine in the posterior pituitary of the AVP-deficient Brattleboro rat is lower than heterozygous controls [12]. Furthermore, exogenous histamine releases AVP from the median eminence and the posterior pituitary *in vitro* [13]. The objectives of the present study were two-fold. First, to determine if posterior pituitary histamine plays a role in regulating the release of AVP induced by osmotic stimulation, the levels of AVP, histamine, and HNMT in the posterior pituitary were evaluated following the chronic administration 2% (w/v) hypertonic saline to rats. Second, to define the cellular localization of histamine in the posterior pituitary, AVP, histamine, and HNMT levels were evaluated following pituitary stalk transection.

## *Methods*

### Oral hypertonic saline administration

Male Wistar rats (200–300 g) were randomly allocated

to three groups. The control group received normal drinking water, while the experimental groups received hypertonic 2% (w/v) sodium chloride solutions for either 2 or 7 days. All groups were given food *ad libitum*. The three groups were sacrificed by decapitation on the same day.

**Pituitary stalk transection**

Stalk-sectioned and sham-operated Sprague Dawley male rats (250 g) were purchased from Zivic Miller (Allison Park, PA). The animals were allowed a one-week recovery period. The animals were sacrificed by decapitation on the morning of the 8th day.

**Tissue preparation for histamine, HNMT and AVP assays**

The pituitary was removed and the posterior lobe was separated from the intermediate and anterior pituitary using a dissecting microscope. The two portions of the pituitary were sonicated in 12 mm × 75 mm polypropylene tubes that contained 250 μl of cold 0.25 M sucrose. For histamine determinations, 100 μl of the sucrose homogenate was deproteinated with 25 μl of 0.4 M perchloric acid and centrifuged at 40,000 × g for 10 min at 10°C. The resultant supernatant was diluted 4-fold with $H_2O$ and stored at −20° C. A 25 μl aliquot of diluted sample was assayed for histamine by a previously reported radioenzymatic assay [8, 14, 15]. The sensitivity of this assay, defined as the amount of histamine generating twice blank cpm, is 2.0 pg. The coefficient of variation for the assay is less than 5%. A 25 μl aliquot of the sucrose homogenate was diluted 25-fold with 0.1% bovine serum albumin (w/v) and assayed for HNMT activity by a previously described method [8]. HNMT activity was quantified by measuring the conversion of histamine to tritiated N-τ-methyl-histamine/hr. This assay is linear for 1 hr with 0.035 Units of HNMT activity. One Unit of HNMT activity catalyzes the formation of 1 nmole of N-τ-methyl-histamine/hr. The sensitivity of this assay, defined as the amount of posterior pituitary tissue necessary to generate twice blank cpm during a 30 min incubation, is 3 μg. For AVP determinations, 25 μl of the posterior pituitary sucrose homogenate was diluted 2-fold with 0.1 M HCl and stored at −20° C. Immediately prior to assay, samples were diluted 1:5000 with assay buffer and AVP was assayed by a previously reported radioimmunoassay [16]. There is less than 1% cross reactivity with oxytocin in this assay and levels over a range of 0.2 to 40 pg of standard vasopressin/assay tube can be detected. The intra-assay coefficient of variation is 3.9% at 1 pg/tube and 6.2% at 10 pg/tube. Total protein was determined from another 25 μl aliquot of the sucrose homogenate by the method of Bradford [17].

For hypothalamic histamine and HNMT determinations, the hypothalamus was removed [18] and homogenized in 250 μl of cold 0.25 M sucrose. A 25 μl aliquot was diluted 50-fold with 0.1% bovine serum albumin and assayed for HNMT activity. For histamine determinations, 100 μl of the sucrose homogenate was diluted 2-fold with 0.2 M perchloric acid and centrifuged at 40,000 × g for 10 min at 10° C. The supernatant was diluted 10-fold with $H_2O$ and assayed for histamine.

**Statistical analysis**

The data were analyzed by means of a two-tailed Student's t-test or by analysis of variance followed by a two-tailed Student's t-test. The 0.05 level of probability was used as the minimum criterion of significance.

## *Results*

### Hypertonic saline administration

The administration of hypertonic saline had no effect on the total protein content of the posterior pituitary or the anterior pituitary (Fig. 1). Hypertonic saline treatment was a potent stimulus for AVP release as evidenced by the significant reduction ($p < 0.001$) of posterior pituitary AVP levels. Histamine concentrations

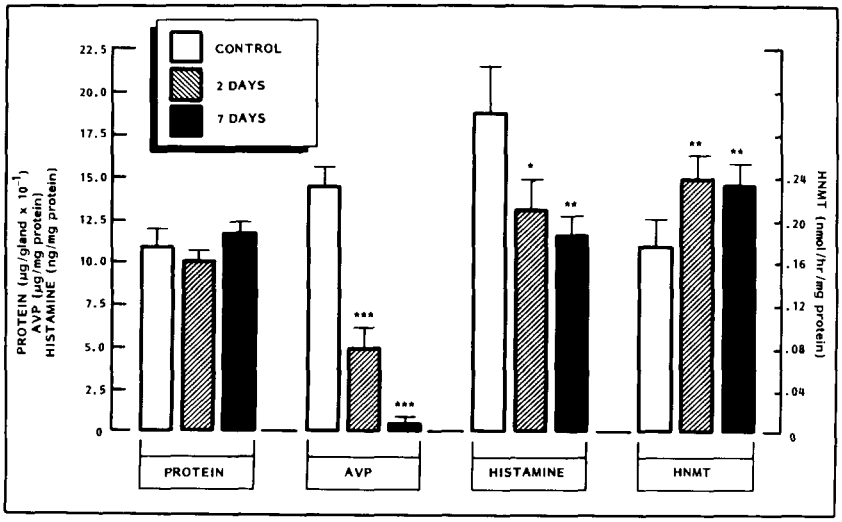


*Figure 1*
Effect of the administration of 2% (w/v) hypertonic saline for 2 and 7 days or on total protein, and the concentration of AVP, histamine, and HNMT in the posterior pituitary of the rat. Values are $\bar{x} \pm$ SEM, n = 6 for each group. Significantly different from control: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

*Table 1*
Effect of Stalk Transection on the Rat Posterior Pituitary

| Treatment Group | Protein (μg/gland) | AVP (ng/gland) | Histamine (ng/gland) | HNMT (munits/gland) |
|---|---|---|---|---|
| Sham-operated | 100.4 ± 5.7 | 820 ± 53.6 | 1.54 ± 0.21 | 18.8 ± 0.82 |
| Stalk-transected | 64.6 ± 12.5* | 21.4 ± 8.8** | 1.75 ± 0.26 | 9.22 ± 1.56** |

Stalk section significantly reduced size and protein content of the posterior pituitary. AVP, histamine, and HNMT are expressed on an organ basis. Values are $\bar{x} \pm$ SEM for 6 stalk sectioned animals and 10 sham-operated controls. Significantly different from control: *$p < 0.05$; **$p < 0.001$.

in the posterior pituitary were also significantly reduced after 2 days ($p < 0.05$) and 7 days ($p < 0.001$) of hypertonic saline inbibition. Conversely, HNMT activity was significantly elevated ($p < 0.01$) in the posterior pituitary with hypertonic saline treatment. When treated animals were compared to controls, the levels of histamine and HNMT in the posterior pituitary were inversely related. The concentration of histamine in the anterior pituitary was unaffectd by hypertonic saline as evidenced by control levels of 2.5 ± 0.2 ng/mg protein (x ± SEM) versus 2.3 ± 0.15 ng/mg protein (x ± SEM) following two days of treatment and 2.2 ± 0.1 ng/mg protein (x ± SEM) following seven days of treatment.

**Pituitary stalk transection**

The total protein content of the posterior pituitary was significantly reduced ($p < 0.05$) following stalk transection (Table 1). The content of histamine in the posterior pituitary was not affected by stalk transection. In contrast, the content of AVP and HNMT activity in the posterior pituitary were significantly lower ($p < 0.001$) in stalk-transected animals when compared to sham-operated controls. The concentration of histamine in the hypothalamus of stalk-transected animals was 4.89 ± 0.39 ng/mg protein (x ± SEM, n = 6) and did not represent a significant change from the hypothalamic histamine concentration of 5.76 ± 0.07 ng/mg protein (x ± SEM, n = 10) observed for sham-operated controls. There was also no significant change in hypothalamic HNMT which was 17.7 ± 3.3 units × $10^{-3}$/ml protein before stalk section and 18.3 ± 2.5 after section. Finally, stalk transection reduced the total protein content of the anterior pituitary from 1083 ± 45 μg/gland to 455 ± 72 μg/gland. The histamine content of 1.60 ± 0.47 ng/gland (x ± SEM, n = 6) in the anterior pituitary of stalk-transected animals was not significantly different from the anterior pituitary histamine content of 2.15 ± 0.19 ng/gland (x ± SEM, n = 10) observed for sham-operated controls.

*Discussion*

Histamine may be involved in the regulation of fluid balance since the peripheral or central administration of this compound increases water intake and decreases urine output [19, 20]. AVP appears to mediate the effect of centrally administered histamine on urine formation [5, 10, 19, 20]. The antidiuretic response to peripherally administered histamine is not affected by hypophysectomy, however, others have attributed this observation to the stimulation of AVP release from non-degenerated or regenerated magnocellular neurons projecting to the median eminence [19, 20]. Histamine stimulates the release of AVP from the posterior pituitary and median eminence *in vitro* [13].

Plasma hyperosmolarity is a potent stimulus for the secretion of AVP although the neuronal nature of the osmoreceptive process remains obscure. The chronic ingestion of 2% (w/v) hypertonic saline caused a substantial reduction in the AVP content of the posterior pituitary. This treatment also reduced posterior pituitary histamine levels. Therefore, during conditions designed to physiologically deplete posterior pituitary AVP levels, the concentration of histamine was also reduced. Similarly, histamine levels are decreased in the posterior pituitary of the AVP-deficient Brattleboro rat [12]. An elevation in posterior pituitary HNMT activity was also observed with hypertonic saline treatment. HNMT levels in the hypophysis may be dynamically regulated since this enzyme activity is reported to undergo a diurnal variation [21]. Furthermore, HNMT activity in the kidney can be induced by pharmacologic and pathologic

stimuli [22, 23]. Thus, the current study may be the first to demonstrate the induction of HNMT in the posterior pituitary by a physiologic stimulus. Reduced histamine concentrations and elevated HNMT activity following hypertonic saline administration may reflect increased histamine turnover and suggests posterior pituitary histamine could facilitate AVP release as part of the overall mechanism mediating osmotic stimulation of AVP secretion.

The cellular localization of histamine in the posterior pituitary is an important consideration for elucidating the mechanisms responsible for the reduction of histamine concentrations observed after hypertonic saline administration. Pituitary stalk transection had no effect on the content of histamine in the posterior pituitary. Additionally, high $K^+$ concentrations, known to release neuronal histamine from brain slices [24], failed to release histamine from the posterior pituitary *in vitro* (unpublished observation). These results, in conjunction with previous studies [2–4], indicate mast cells are the primary if not exclusive repository of histamine in the rat posterior pituitary. Therefore, stimulation of histamine secretion from mast cells would appear to be a possible means for regulation of AVP secretion by histamine at the level of the posterior pituitary.

Mast cells contain little or no HNMT activity [25] and unlike the effect observed for histamine, there was a significant reduction in the level of posterior pituitary HNMT following stalk transection. This observation implies that HNMT is located in descending neurons or in pituicytes that are not viable following stalk transection. There is evidence for glial and neuronal localization of HNMT in the CNS and the results of lesion studies indicate HNMT does not reside exclusively in histamine-containing neurons [26, 27]. Therefore, the absence of posterior pituitary histaminergic neurons does not preclude a neuronal localization of HNMT in the posterior pituitary.

In summary, the concentration of histamine is reduced and HNMT activity is elevated in the rat posterior pituitary with chronic 2% (w/v) hypertonic saline administration. Stalk transection had no effect on posterior pituitary histamine concentrations but substantially reduced HNMT levels. In conjunction, these observations suggest histamine is released from mast cells in the posterior pituitary following elevations in plasma osmolarity and suggest that intra-pituitary histaminergic systems may modulate AVP secretion.

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