

Brief Reports

Effects of Endogenous Antidiuretic Hormone (ADH) on Macrophage Phagocytosis

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Although several studies have indicated that antidiuretic hormone (ADH) enhances the phagocytic function of the reticuloendothelial system (RES) in shock syndromes, it remains unknown what influence ADH exerts upon the individual phagocytic components of this system. The present investigation was designed to evaluate the effects of endogenous ADH on the phagocytic activity of peritoneal macrophage cells. As a phagocytic stimuli, fluorescent methacrylate microbeads were injected intraperitoneally into Brattleboro (ADH deficient) and normal Long Evans rats in the presence and absence of exogenous ADH. Peritoneal cells were harvested 19–22 hr after the administration of the microbeads and the percent phagocytosis was determined in macrophage cells using a fluorescence-activated cell sorter (FACS II). Our results indicate that

the percentage of peritoneal macrophages ingesting the fluorescent methacrylate microbeads was significantly reduced in the absence of ADH (Brattleboro rats: $5.4 \pm 0.6\%$ versus Long Evans rats: $16.8 \pm 2.3\%$; $p < 0.001$). In addition, our data demonstrate that exogenous administration of ADH significantly enhanced macrophage phagocytosis in Brattleboro ($14.7 \pm 2.2\%$) and normal Long Evans ($49.6 \pm 4.5\%$) rats. These data suggest, for the first time, that endogenous ADH might play a modulatory role in the phagocytic activity of a specific component of the RES, namely, the macrophage cell.

KEY WORDS: Antidiuretic hormone; Macrophage; Phagocytosis; Reticuloendothelial system; Brattleboro rats; Long Evans rats; FACS II.

Introduction

Previous investigations have shown that in shock syndromes the phagocytic activity of the reticuloendothelial system (RES) can be influenced by antidiuretic hormone (ADH). Studies by Altura demonstrate that pretreatment of rats with synthetic analogues of ADH results in stimulation of the RES phagocytic function (1) and an increased survival to circulatory shock (4). In addition, rats that survived severe hemorrhage or lethal intestinal ischemic shock exhibited hyperfunctional RE systems (2). Based on these data a close correlation between RES phagocytic capacity and survival or tolerance to shock has been postulated (1,2,4). Interestingly, several types of circulatory shock, such as hemorrhage and trauma, are characterized by an enhanced release of ADH from the posterior pituitary gland in cats (5), dogs (11), and rats (7). Recent findings demonstrate that the mortality of Brattleboro rats with hereditary hypothalamic diabetes insipidus (i.e., lacking ADH) is significantly higher than that of normal Long Evans rats after hemorrhage or bowel ischemic shock (3). Moreover, a complete blockade of RES phagocytic function was observed in Brattleboro, but not in Long Evans, rats after circulatory shock (3).

Although the findings described above strongly suggest that endogenous ADH regulates the RES phagocytic function in shock, experimental evidence is still lacking regarding the effects of ADH on the specific phagocytic components of the RES (i.e., macrophage,

polymorphonuclear leukocytes). Thus, the present investigation was undertaken to evaluate the effects of endogenous and exogenous ADH on peritoneal macrophage phagocytosis. For this purpose, fluorescent methacrylate microbeads were injected intraperitoneally into untreated and ADH-treated Brattleboro and Long Evans rats. The Brattleboro rats are characterized by an absence of ADH (12) and therefore offer a unique opportunity to study the effects of the lack of this hormone on macrophage phagocytosis without introducing other physiological disturbances. The percentage of macrophage cells ingesting the microbeads in untreated and ADH-treated Brattleboro and Long Evans rats was determined using a fluorescence-activated cell sorter (FACS II).

Materials and Methods

Experiments were performed in 25 Long Evans hooded rats of the Brattleboro strain homozygous for the hypothalamic diabetes insipidus trait (Brattleboro rats) and in 18 normal Long Evans hooded rats (Long Evans rats). Body weight of the Brattleboro rats (279 ± 15 g) was not significantly different from that of the Long Evans rats (289 ± 25 g). The rats were placed in individual metabolism cages for an equilibration period of 6 days and were allowed free access to food and water.

Peritoneal exudate cells were obtained from untreated and ADH-treated Brattleboro rats and Long Evans rats 19–22 hr after receiving an intraperitoneal injection of 3 ml of a suspension containing 1.0×10^6 fluorescent methacrylate microbeads/ml of phosphate buffered saline (PBS). Ten of the Brattleboro rats and six of the normal Long Evans rats received an intraperitoneal injection of 250 mU/100 g body wt of ADH (Pitressin; Parke-Davis, Morris Plains, NJ) 5 min prior to the injection of the microbeads. Peritoneal macrophage cells were harvested as previously described (6) by washing out the peritoneal cavity with 20 ml of PBS (pH 7.4). The harvested cells were centrifuged and resuspended in 4.0% paraformaldehyde (Fisher Scientific Co., NJ).

The percent of phagocytic macrophages was determined using the FACS II system (Becton Dickinson Co., Sunnyvale, CA). A total of 4×10^4 cells were analyzed from each rat at a count rate of 500–1000 cells/sec. The percentage of peritoneal macrophages capable of ingesting the microbeads was determined by the FACS II by counting the number of cells giving a fluorescence signal and dividing by the total number of cells examined. Macrophage cells were identified by light microscopy.

Statistical significance was evaluated using Student's *t*-test for unpaired observations. Differences with a probability of <0.05 were accepted as significant.

Results

Light Scatter Profile

The light scatter profile of peritoneal exudate cells harvested from Long Evans and Brattleboro rats is illustrated in Figure 1. Preparations of peritoneal exudate cells yielded scatter histograms that have one peak, indicating the presence of only one cell population. Light scatter analyses revealed that the macrophages constitute $98 \pm 3\%$ and $97 \pm 5\%$ of the total cell population in the preparations from Long Evans and Brattleboro rats, respectively. The purity of the macrophage cell suspension was also confirmed by light microscopy. Exogenous administration of ADH did not alter the percentage of macrophage cells in the preparation.

Percent Phagocytosis

Figure 2 depicts the effect of endogenous ADH on the percent phagocytosis of peritoneal macrophage cells. It is evident from the results that there is a significant reduction in the percent phagocytosis of peritoneal macrophages in the absence of endogenous ADH (Brattleboro rats: 5.4 ± 0.6) compared to values obtained in the presence of endogenous ADH (Long Evans rats: 16.8 ± 2.3 ; $p < 0.001$).

As illustrated in Figure 3, exogenous administration of ADH (250 mU/100 g body wt) significantly enhanced basal macrophage phagocytosis in both Brattleboro ($14.7 \pm 2.2\%$) and normal Long Evans ($49.6 \pm 4.5\%$) rats. It should also be noted that exogenous administration of ADH restored the percent phagocytosis in Brattleboro rats to normal values (normal Long Evans: 16.8 ± 2.3 ; Brattleboro + ADH: $14.7 \pm 2.2\%$; $p > 0.05$).

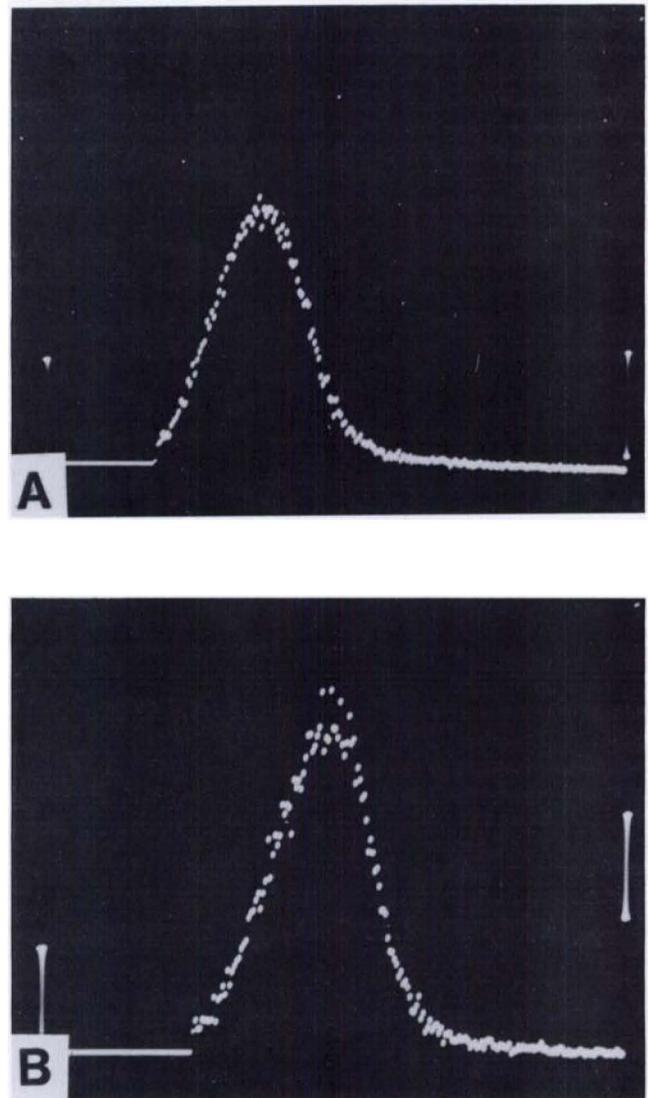


Figure 1. Light scatter profile of peritoneal exudate cells from Long Evans (A) and Brattleboro (B) rats. The abscissa represents the forward light scatter intensity, which is proportional to the cell size and shape, and the ordinate represents the relative cell number. Light scatter was collected at a forward angle of $1-15^\circ$ from the incident beam.

Discussion

The results of the present study show that the phagocytic activity of peritoneal macrophage cells harvested from ADH-deficient Brattleboro rats was significantly lower than that measured in normal Long Evans rats. The effect of ADH on macrophage phagocytosis was evident in the absence of other physiological disturbances such as shock or hemorrhage, which are known to stimulate ADH secretion and RES phagocytic function (1–4). Since ADH levels were not measured in our experiments, the possibility of enhanced ADH release in response to the intraperitoneal injection of the fluorescent

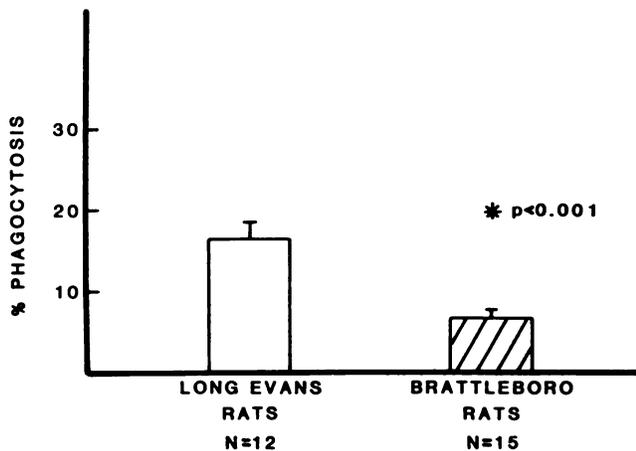
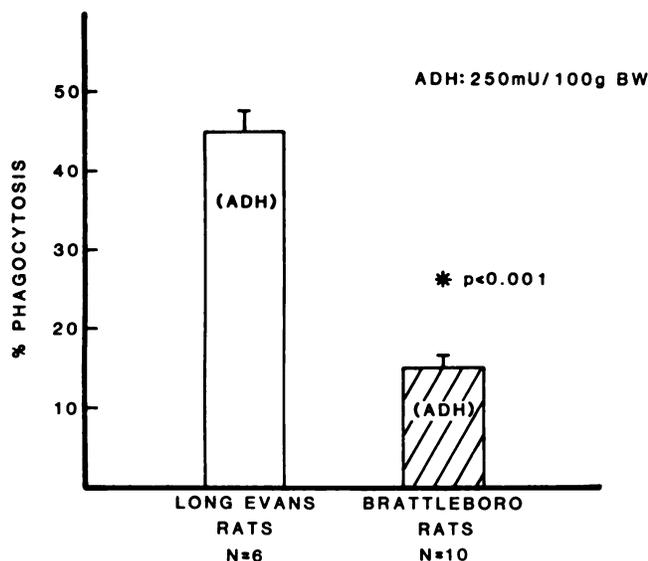


Figure 2. Percent macrophage phagocytosis in Long Evans and Brattleboro rats. Values are mean percentages of macrophage cells ingesting the fluorescent methacrylate microbeads \pm SEM.

methacrylate microbeads can not be ruled out in the control animals. An increase in plasma ADH concentration has been reported in certain pathological conditions involving inflammatory responses (8,9). This possibility, however, seems unlikely in view of the magnitude of the increase in macrophage phagocytosis observed in the normal Long Evans rats after exogenous ADH administration. On the other hand, the differences in percent phagocytosis detected between Brattleboro and normal Long Evans rats could be due to morphological differences in the macrophage cell populations.

Figure 3. Percent macrophage phagocytosis in Long Evans and Brattleboro rats in the presence of exogenous ADH. Values are mean percentages of macrophage cells ingesting the fluorescent methacrylate microbeads \pm SEM.



Preliminary morphological data appear to indicate that this is not the case. For example, the size of the macrophage cells of the Brattleboro rats ($10.4 \pm 1.7 \mu$) was found to be not significantly different from that of the normal Long Evans rats ($9.4 \pm 1.3 \mu$). Additional morphological studies, however, are necessary in order to completely rule out this possibility. Our data also indicate that in the ADH-deficient Brattleboro rat exogenous administration of the hormone was able to restore macrophage phagocytosis to normal levels. Taken together, these findings suggest that ADH might play a role in the regulation of the phagocytic activity of the macrophage cells.

Previous investigations have demonstrated an increase in the phagocytic activity of the RES with exogenous administration of ADH (2) or stimulation of endogenous levels of ADH (3) in shock and trauma. However in these studies (2,3), the role of ADH on RES phagocytic function was assessed by the blood clearance of intravenously infused carbon particles. Although this technique offers an estimate of total RES phagocytic function, it does not provide information regarding the cellular elements of the RES involved in the phagocytic response. A further limitation of the technique is the inability to determine whether the alterations in the clearance of the carbon particles are a result of changes in RES phagocytic activity and/or in the blood flow to the organs responsible for the removal of these particles (i.e., liver, spleen). This observation requires special consideration due to the vasopressor effects of ADH (10).

Thus, the present experiments represent the first instance in which a stimulatory effect of ADH on a specific phagocytic component of the RES, namely, the macrophage cell, has been observed. This finding adds a new aspect to the role of ADH as a modulator of the activity of the RES. Functional and morphological studies are still required to elucidate the mechanism responsible for the effects of ADH on macrophage phagocytosis as well as the pathophysiological implications of this action.

Literature Cited

1. Altura BM: Use of reticuloendothelial phagocytic function as an index in shock therapy. *Bull NY Acad Med* 43:259, 1967
2. Altura BM: DPAVP: A vasopressin analog with selective microvascular and RES actions for the treatment of circulatory shock in rats. *Eur J Pharmacol* 37:155, 1976
3. Altura BM: Evidence that endogenous vasopressin plays a protective role in circulatory shock: role for the reticuloendothelial system using Brattleboro rats. *Experientia* 36:1080, 1980
4. Altura BM, Hershey SG: Reticuloendothelial function in experimental injury and tolerance to shock. *Adv Exp Med Biol* 33:545, 1973
5. Clark BJ, Rocha e Silva M: An afferent pathway for the selective release of vasopressin in response to carotid occlusion and hemorrhage in the cat. *J Physiol (Lond)* 191:529, 1967
6. Fernandez-Repoller E, Mittler RS, Tiffany S, Schwartz A: In vivo effects of prostaglandin E₂ and arachidonic acid on phagocytosis of fluorescent methacrylate microbeads by rat peritoneal macrophages. *J Histochem Cytochem* 30:466, 1982

7. Gingsburg M, Heller H: Antidiuretic activity in blood obtained from various parts of the cardiovascular system. *J Endocrinol* 9:274, 1953
8. Harrington JT, Cohen JJ: Clinical disorders of urine concentration and dilution. *Arch Intern Med* 131:810, 1973
9. Rosenow EC, Segar WE, Zehr JE: Inappropriate antidiuretic hormone secretion in pneumonia. *Mayo Clin Proc* 47:169, 1972
10. Saameli K: *Handbook of Experimental Pharmacology*. Springer-Verlag, Berlin, 1968, p 545-624
11. Shade RE, Share L: Vasopressin release during nonhypotensive hemorrhage and angiotensin II infusion. *Am J Physiol* 228:149, 1975
12. Valtin H, Schroeder HA: Familial hypothalamic diabetes insipidus in rats (Brattleboro strain). *Am J Physiol* 206:425, 1964