Adenosine Triphosphate and Adenosine Triphosphatase in Hormone-Containing Granules of Posterior Pituitary Gland

Abstract. *Neurosecretory granules prepared from bovine posterior pituitary glands by cell fractionation methods contain adenosine triphosphate and adenosine triphosphatase activity. Addition of adenosine triphosphate to suspensions of granules stimulates release of vasopressin. It is suggested that adenosine triphosphate and adenosine triphosphatase participate in the storage and release of vasopressin.*

Both adenosine triphosphate (ATP) and adenosine triphosphatase are present in the hormone-containing granules of the chromaffin cells of the adrenal medulla, and there has been speculation that these substances are involved in storage (*1*), uptake (*2*), or release (*3*, *4*) of the medullary hormones. We find that ATP and adenosine triphosphatase are also present in the neurosecretory granules of the posterior pituitary gland which contain the hormones vasopressin and oxytocin.

Neurosecretory granules were prepared from posterior lobes of bovine pituitary glands by centrifugation in a density gradient (*5*), yielding a highly purified granule fraction. The concentration of vasopressin in the isolated neurosecretory granules (Table 1) was close to 10 units per milligram of protein, a value in agreement with those obtained previously (*5*, *6*). Dean and Hope (*5*) and La Bella *et al.* (*6*) obtained values of 10.73 ± 1.74 and 9.95 units per milligram of protein, respectively.

Adenosine triphosphate was present, as revealed by the firefly method (*7*), in each of seven different preparations of neurosecretory granules. The ratio of vasopressin to ATP was about 10 unit/nmole (Table 1). In the same granule fractions, the ratio of ATP to protein was close to 1 nmole/mg (Table 1), a value more than twice that (0.49 ± 0.07) found in the mitochondrial layer of the density gradient in these same experiments. Thus, the ATP in the neurosecretory granule fraction cannot be accounted for by mitochondrial contamination. The fraction containing neurosecretory granules also possessed adenosine triphosphatase activity (Table 1), and this was of the same order as that reported for chromaffin granules (*8*). The active material was insoluble in water and was supported by Mg^{2+} or Ca^{2+} and in these respects also resembled the adenosine triphosphatase activity of chromaffin granules (see *8* and *9*).

In neurosecretory granules, ATP may participate in hormone storage in the manner proposed for ATP in medullary chromaffin granules (*1*). However, the evidence for such a function in the neurosecretory granules is less persuasive. In the chromaffin granules the molar ratio of hormones (catecholamines) to ATP is close to 4:1, a ratio in which the negative charges on the ATP equal the positive charges on the catecholamines. But such stoichiometry is not present for the neurosecretory granules. Each vasopressin molecule carries two positive charges at physiological *p*H, one of which—that on the amino-terminal group—is probably not available for binding to ATP because of association with the carrier protein, neurophysin (*10*). It follows that a molar ratio of vasopressin to ATP of 4 : 1 would be expected if ATP were to neutralize all the available charges on the vasopressin. But we calculate the molar ratio of vasopressin to ATP in the neurosecretory granules to be about 20 : 1. Thus, the ATP present could neutralize only about one-fifth of the available charges on the vasopressin molecule. While this departure from stoichiometry does not rule out ATP as a constituent of a hormone storage complex, it raises the possibility that ATP has some other function. Adenosine triphosphate has been found not only in chromaffin granules but in noradrenaline-containing granules of adrenergic nerves (*11*) and in 5-hydroxytryptamine-containing granules of platelets (*12*). It is thus conceivable that it is a constituent common to secretory granules of different sorts.

The adenosine triphosphatase present in the neurosecretory granules may participate in hormone release. This is one of the functions that have been proposed for the adenosine triphosphatase of chromaffin granules, because ATP releases catecholamines and other constituents from granule suspensions in vitro (*3*, *4*), and because this effect is potentiated by ATP-regenerating systems (*13*) and blocked by inhibitors of adenosine triphosphatase (*4*, *14*). We have found that ATP added to suspensions of neurosecretory granules stimulates release of vasopressin. The granules were suspended in a medium containing (in millimoles per liter) KCl, 160; NaCl, 5; $MgCl_2$, 0.5; TES buffer [*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid] (*p*H 7.0), 10, maintained at 30°C; and ATP (0.5 m*M*) was added alone or with an ATP-regenerating system, phosphoenolpyruvate and pyruvate kinase (*13*). In three experiments where ATP was added alone, the rates of vasopressin release—measured over periods of 20 minutes—were, respectively, 1.8, 2.8, and 3.6 times higher in the presence of ATP than in its absence (*15*). In six other experiments, where the regenerating system was added with ATP, the corresponding values, measured over periods of 10 to 30 minutes, ranged from 2.2 to 12.4. This stimulant effect of ATP on release of vasopressin from isolated neurosecretory granules in vitro may offer a clue to the mechanism of secretion in the intact neurosecretory system. There is evidence that the normal

Table 1. Vasopressin, ATP, and adenosine triphosphatase in neurosecretory granules from bovine posterior pituitary glands. Vasopressin was bioassayed by its pressor effect on the blood pressure of the rat (*16*). Protein was measured by the method of Lowry *et al.* (*19*). Vasopressin was assumed to possess 500 pressor units per milligram (*20*). Hydrolysis of ATP was measured by appearance of inorganic phosphate [estimated by the method of Martin and Doty (*21*)] during a 30-minute incubation at 37°C in a medium containing (in millimoles per liter): KCl, 20; $MgCl_2$ 2.0; ATP (disodium salt), 2.0; TES buffer (*p*H 7.0), 30 (*22*).

Substance	Units	Observations (No.)	Mean ± S.E.
Vasopressin	unit/mg protein	8	9.94 ± 0.57
ATP	nmole/mg protein	7	1.05 ± 0.12
Ratio of vasopressin to ATP	unit/nmole	7	10.31 ± 1.18
Ratio of vasopressin to ATP	mole/mole	7	20.6 ± 2.3
Adenosine triphosphatase	μmole of P/30 min per mg protein	7	1.91 ± 0.30

stimulus for release of posterior pituitary hormones is invasion of the neurosecretory terminals by impulses discharged down the hypothalamo-hypophysial tract (*16*); and, according to some authors (*17*), propagated impulses in excitable tissues cause the release of ATP from the plasmalemma. On this view, impulses in the neurosecretory terminals would make endogenous ATP available to interact with the adenosine triphosphatase of the secretory granules. Similar events could be involved in linking depolarization to release of transmitter in conventional neurons for there are reports that granules containing synaptic transmitter substances also show adenosine triphosphatase activity (*18*).

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Autoimmune Glomerulonephritis Induced in Sheep by Injections of Human Lung and Freund's Adjuvant

Abstract. *Sheep immunized with human lung and Freund's adjuvant develop progressive glomerulonephritis with deposition of autoantibodies and complement in the glomerular basement membrane. This nephritis appears to be the first autoimmune disease induced by antigens that are not organ-specific. By clinical and immunopathologic criteria, this nephritis appears identical to the nephritis induced in sheep by human glomerular basement membrane and greatly resembles certain nephritides of man, in particular, the nephritis in Goodpasture's disease.*

Sheep injected with heterologous glomerular basement membrane (GBM) and Freund's adjuvant (FA) invariably develop progressive, usually fulminating glomerulonephritis and form antibodies against the injected antigenic determinants (*1*). Though kidney shares antigens with other organs (*2*, *3*), our attempts to induce progressive glomerulonephritis in sheep by injections of various nonrenal tissues, such as human placenta (*1*), heart, synovia, and tonsil together with FA, have been unsuccessful.

By contrast, injections of human-lung basement membranes and FA have induced progressive glomerulonephritis in sheep. This finding is contrary to what would be expected as a result of previous efforts to induce experimental "autoimmune" disease (*4*) and may provide insight into the pathogenesis of Goodpasture's nephritis and other human diseases.

Shavings (60 μm) of human lung were cut on a freezing microtome (*2*), refrozen, and cut twice more. The shavings were washed with normal saline and centrifuged at 1800*g* for 10 minutes. This process was repeated twice. The sediment, suspended in saline, was disrupted in a sonic oscillator for 20 minutes and then washed and centrifuged (as above) three times. Microscopically, the sediment consisted predominantly of refractile plates of basement membranes and fibrillar material. The sediment was suspended in saline containing merthiolate (1:10,000) and homogenized by sonication; suspensions [50 mg of sediment (wet weight) per milliliter of saline] were emulsified with equal volumes of complete FA. Equal volumes of saline (without lung) and complete FA were emulsified for injection into control sheep. A similar emulsion containing 2 mg of heat-killed, lyophilized group A hemolytic streptococci per milliliter was injected into two sheep (*5*).

Every 2 weeks each sheep was injected with 5 ml of emulsion: 3 ml was given intradermally in the neck, axillary, and inguinal regions; 1 ml was given intramuscularly; and 1 ml was given subcutaneously. Periodic urinalyses and blood urea nitrogen determinations were made on all animals. Repeated urinalyses in untreated sheep rarely showed more than an occasional trace or 1-plus proteinuria. Hematuria was seen only in sheep with autoimmune nephritis.

Renal tissue was obtained by open-wedge biopsy or when the animals were killed and prepared by conventional methods for light and fluorescent microscopy. Fluorescent-conjugated rabbit antiserum to sheep immunoglobulin G (IgG) (*6*) and antiserum to sheep complement (β_{1C}-globulin) were prepared (*7*).

All five sheep injected with human lung and FA developed glomerulonephritis. One sheep that had received 15 ml of emulsion in 4 weeks developed proteinuria with azotemia on day 38 and died in uremia 8 days later. At autopsy, the kidneys were swollen and covered with many petechiae. The glomeruli had exudative, hemorrhagic, necrotizing, intra-, and extracapillary proliferative changes morphologically identical with those previously described (*1*). There was interstitial edema with mononuclear and polymorphonuclear cell infiltration but no fibrosis. Nearly all glomeruli were involved. A second sheep, receiving 25 ml of emulsion in 8 weeks, developed proteinuria on day 42 and azotemia on day 47 and died in uremia on day 91. Renal biopsy showed a progression from acute to subacute to chronic changes. At autopsy, only a few petechiae were seen on the kidneys. Interstitial fibrosis was diffuse, and most glomeruli were hyalinized. A third sheep received 30 ml of emulsion in 75 days, developed proteinuria on day 59 and azotemia on day 66, and died in uremia on day 126. Morphologic findings were similar to those of the second sheep. The remaining two sheep each received 60 ml of emulsion within 22 weeks and had hematuria, proteinuria, petechiae, and acute glomerular lesions on day 89. Both sheep were alive on day 160. Six controls (each receiving 50 ml of