Osmotic and nonosmotic control of vasopressin release

SCHRIER, ROBERT W., TOMAS BERL, AND ROBERT J. ANDERSON. Osmotic and nonosmotic control of vasopressin release. Am. J. Physiol. 236(4): F321–F332, 1979.—While the existence of an osmotic control for vasopressin (AVP) release has been long recognized, development of a sensitive immunoassay has allowed for better understanding of factors affecting the threshold and sensitivity of AVP release. Individual variation, genetic, environmental, and species differences, and the nature of the solute providing the osmotic stimuli can significantly affect the release of the hormone by altering the threshold and/or the sensitivity of the osmoreceptor. In addition to the hypothalamic osmoreceptor, AVP secretion is also controlled by an anatomically separate pathway which is responsive to nonosmotic stimuli. It appears that both low-pressure (left atrial) and high-pressure (carotid and aortic) receptors via the parasympathetic pathways provide the major nonosmotic pathway for vasopressin release. Such pathways are activated in response to acute systemic hemodynamic changes, stress, and hypoxia. The precise interaction between osmotic and nonosmotic AVP release remains to be clarified. A model of osmotic and nonosmotic interactions, based on available electrophysiologic studies, is presented and its clinical implications are discussed.

hypothalamic osmoreceptor; parasympathetic pathways; immunoassay; threshold and sensitivity of AVP release; nonosmotic AVP release

ON JUNE 12, 1947, Professor E. B. Verney delivered his classic Croonian Lecture entitled “The antidiuretic hormone and the factors which determine its release” to the Royal College of Physicians in London (82). Some 30 years later, this treatise still provides much of the basis of our understanding of the regulation of antidiuretic hormone (ADH).

Osmotic Control of ADH Release

Professor Verney performed experiments in conscious, trained dogs undergoing a water diuresis. He demonstrated that the rapid injection of hypertonic saline over a 10-s period into a carotid artery was associated with a rapid fall in urine flow. Since this anti-diuresis could be produced by similar intracarotid injections of non-sodium-containing solutions, including hypertonic glucose, hypertonic sucrose, and hypertonic sodium sulfate, he proposed the existence of an “osmoreceptor.” Moreover, since the anti-diuresis produced by these intracarotid injections could be mimicked by the injection of posterior pituitary extract, he suggested that the osmoreceptor influenced the release of an ADH from the posterior pituitary gland. The general location of the osmoreceptor was delineated by experiments performed after ligation of the right internal carotid in “Pat,” one of Verney’s trained dogs. Subsequent to ligation of this blood vessel in Pat, injections of hypertonic solutions into the right carotid artery failed to produce an anti-diuresis, thereby suggesting that the osmoreceptor is located somewhere along the circulation of the internal carotid artery. Later studies localized this effect to the anterior hypothalamus (39).

For the next two decades after these classic experiments, considerable information was accrued about the anatomy and chemistry of the neurohypophysis, but the concepts of osmoregulation of ADH were not advanced to any great extent beyond those originally proposed by Professor Verney. More recently, however, the use of a sensitive radioimmunoassay for arginine vasopressin, the major mammalian ADH, has provided additional information which further extends and refines the original concepts of Verney (82).

Using this radioimmunoassay for measuring ADH, Robertson and associates (61) demonstrated a close correlation between plasma ADH levels and plasma osmolality in patients in various states of hydration (Fig. 1A). Using linear regression analysis, these authors defined the osmotic threshold for ADH release as the point of intercept on the horizontal axis (280 mosmol/kg H₂O) and the sensitivity of the osmoreceptor as the slope of the linear regression line. Because patients with the syndrome of primary polydipsia or compulsive water drinking (presumably with chronic ADH suppression) and patients with nephrogenic diabetes insipidus (presumably with chronic ADH stimulation) did not deviate
detectably from the linear regression line of the normal subjects, it was suggested that neither chronic suppression nor stimulation of the osmoreceptor grossly alters its sensitivity.

It is relevant to note that Weitzman and Fisher (84) have recently challenged the use of linear regression analysis to evaluate the functional properties of the osmoreceptor. These investigators suggest from their results derived in conscious sheep that the relationship between ADH and plasma osmolality is exponential rather than linear. If this is indeed the situation, then theoretical considerations dealing with an exact osmotic threshold for ADH release may be problematical. On reanalysis of the results of Weitzman and Fisher (84), Rodbard and Munson (63), however, have concluded that their results are compatible with either an exponential or threshold model. In practical terms, there may be very little functional difference between these two mathematical descriptions of the osmoreceptor as defined by the relationship between ADH and plasma osmolality. In the threshold model, there is an abrupt increase in ADH at a specific plasma osmolality, whereas ADH increases gradually (i.e., rheostat-like) in the exponential or geometric model. Thereafter, within the physiological range of plasma osmolality there is a relatively linear relationship between ADH and plasma osmolality with both models. At much higher levels of plasma osmolality, which were not examined by Robertson et al. (61), there appears to be a geometric relationship between plasma osmolality and ADH. The levels of plasma ADH in this range of plasma osmolality are, however, well above those necessary to achieve a maximal antidiuresis.

Robertson and colleagues (62) also have shown a very close relationship between urinary osmolality and radioimmunoassayable titers of plasma ADH in individuals with various states of hydration (Fig. 1B). The exception to this relationship is the patients with nephrogenic diabetes insipidus. Using these radioimmunoassay results, the exquisite sensitivity and gain of the osmoreceptor-ADH-renal reflex can be well demonstrated. For example, a normally hydrated man may have a plasma osmolality of 287 mosmol/kg H2O, a plasma ADH level of 2 pg/ml, and a urinary osmolality of 500 mosmol/kg H2O. With an increase of 1% in total body water, plasma osmolality will fall by 1% (2.8 mosmol/kg H2O), plasma ADH decreases to 1 pg/ml, and urinary osmolality will diminish to 250 mosmol/kg H2O. Similarly, it is only necessary to increase total body water by 2% to suppress plasma ADH maximally (<0.25 pg/ml) and maximally dilute urine (osmolality <100 mosmol/kg H2O). In the opposite direction, a 2% decrease in total body water will increase plasma osmolality by 2% (5.6 mosmol/kg), plasma ADH will rise from 2 to 4 pg/ml, and urine will be maximally concentrated (>1,000 mosmol/kg). Thus, in the context of these sensitivity changes, a 1-mosmol rise in plasma osmolality would be expected to increase plasma ADH by 0.38 pg/ml and urinary osmolality by 100 mosmol/kg H2O. Such small changes in plasma osmolality (measured by freezing-point depression) or plasma ADH (measured by radioimmunoassay) may be undetectable, yet be of extreme physiological importance. For example, a patient with a 24-h urinary solute load of 600 mosmol must excrete 6 liters of urine with an osmolality of 100 mosmol/kg to eliminate this solute; however,
if urine osmolality increases from 100 to 200 mosmol/kg (due to an undetectable rise of 1 mosmol in plasma osmolality and 0.38 pg/ml in plasma ADH), the obligatory 24-h urine volume to excrete the 600 mosmol decreases substantially from 6 to 3 liters. These examples, therefore, are presented both to emphasize the extreme sensitivity and gain of the osmoreceptor-ADH-renal mechanism and to demonstrate the limits of quantitating the system even with present day radioimmunoassay techniques.

Potential Factors Affecting the Initial Release of ADH During Rise in Plasma Osmolality (Osmotic Threshold)

Genetic or environmental factors may be important in the osmotic threshold for vasopressin release. Evidence in support of this possibility derives from the study of a large number of subjects from Japan, suggesting an osmotic threshold of approximately 265 mosmol/kg H₂O (76), a value considerably below the osmotic threshold of 280 mosmol/kg H₂O estimated from studies in the United States (62), France (29), and England (8). This difference, of course, could be methodological rather than environmental or genetic in nature, and, therefore, is in need of further study.

There does appear to be a species difference in osmotic threshold for ADH release. A number of studies in rat (26), dog (59), and monkey (35) indicate an osmotic threshold ranging from 285 to 292 mosmol/kg H₂O, values which are uniformly higher than the mean value of 280 mosmol/kg H₂O found in Occidental man (8, 29, 61). Even in Occidental man, however, there is significant individual variation in osmotic threshold (see below).

The intracellular solute concentration has been suggested, but not proven, to influence the osmotic threshold for ADH release (27, 62). For example, during hypokalemia, the main intracellular cation is decreased, an event which might decrease the volume of osmoreceptor cells and thereby sensitize them to changes in extracellular fluid (ECF) osmolality. Similarly, during starvation intracellular solutes, such as amino acids, might be diminished and thereby alter the cell volume and sensitivity of osmoreceptors.

The osmoreceptor pathways for ADH also seem to be influenced by nonosmotic stimuli (59) (Fig. 2). Hypovolemia of 15% was produced in rats by the intraperitoneal injection of polyethylene glycol, and this maneuver was found to shift the osmotic threshold to the left without altering the sensitivity (slope) of the relationship (26). Similarly, a 15% reduction in blood pressure produced by the subcutaneous injection of isoproterenol also shifted the osmotic threshold to the left without altering the slope or sensitivity of the relationship (60). The dotted horizontal line in Fig. 2 represents the theoretical situation if the nonosmotic stimulus would have been only additive to the osmoreceptor system without shifting the osmotic threshold. In this setting, the base-line ADH would be elevated but the additional osmotic rise in ADH would occur at the normal osmotic threshold, in the range of approximately 290 mosmol/kg H₂O.

Potential Factors Affecting the Sensitivity (Slope) of the Osmotic Release of ADH

Among the several factors that may affect the sensitivity of the osmoreceptor, Helderman et al. (36) have recently examined the potential influence of age. Two
groups of subjects (younger group, mean age 35 yr, vs. older group, mean age 50 yr) were used to examine the response of ADH and negative free water clearance to a hypertonic saline infusion. The older group of subjects were found to have a greater rise in ADH for the same degree of osmotic stimulus; however, the osmotic threshold for ADH release was no different in the two groups of subjects. It is also interesting to note that the end-organ response to ADH as assessed by negative free water clearance was no different in the two groups. It also should be emphasized that this finding by Helder- man et al. (36) does not provide an explanation for the clinical impression of increased hyposmolar complications in the elderly. Such a defect would necessitate either a defect in osmotic suppression of ADH or hypersensitivity to nonosmotic stimuli for ADH release, or a combination thereof, in the elderly population. The effect of age on these responses remains to be examined.

The rate of change of the osmotic stimulus also may affect the sensitivity of ADH release (59, 62). Evidence for such an effect was obtained in studies in human subjects infused with either 3 or 5% hypertonic saline. At comparable infusion rates, a plasma osmolality of 315 mosmol/kg was reached more rapidly during infusion of the 5 than the 3% solution, the plasma ADH levels for this comparable level of osmotic stimulus were significantly greater with the 5 versus the 3% infusion rates. Since this effect of rate of change of osmotic stimulus is only demonstrable with changes greater than 2%/h, it is probably not of considerable physiological importance. However, these studies do emphasize that the rate of change of osmotic stimulus must be considered in experimental studies examining ADH release.

The individual variations in osmotic threshold for ADH release, which have been found to range from 276 to 291 mosmol/kg H$_2$O, also are associated with differences in sensitivity (slopes) of the osmoreceptor mechanism. The slopes of the regression lines in 16 patients studied during an infusion of hypertonic saline ranged from 0.15 to 0.98 (mean value, 0.38), and there was no consistent difference found between males and females (62). Although there is a wide range of individual variations both in osmotic threshold and sensitivity of the osmoreceptor mechanism, it has been claimed that any specific individual demonstrates similar values when re-studied over a period of time (62). Results to support this contention, however, have not been published but are important both to demonstrate the accuracy and reproducibility of the radioimmunoassay results and to allow the use of subjects as their own control over a period of time.

As emphasized in Verney’s studies (82), the nature of the solute providing the osmotic stimulus must be considered. Athar and Robertson (5) and Robertson et al. (59) have recently confirmed Verney’s findings that nonsodium solutes may provide potent osmotic stimuli for ADH release (Fig. 3). In their study, comparable increases in plasma osmolality during hypertonic saline and hypertonic mannitol infusions provided the same degree of rise in plasma ADH. Since ECF sodium concentration increases with hypertonic saline and decreases with hypertonic mannitol, these studies provide indirect evidence against the “sodium receptor” hypothesis (3, 4) and support Verney’s original proposal of an osmoreceptor controlling ADH release (82). It still could be argued, however, that because mannitol crosses the blood-brain barrier quite slowly the resultant osmotic shift of water out of the cerebrospinal fluid (CSF) may actually cause a rise in CSF sodium concentration surrounding the osmoreceptors. This would be compatible with a CSF sodium receptor for ADH release. The major support for the CSF sodium receptor hypothesis derives from studies in goats in which the intraventricular injection of hypertonic sodium but not hypertonic sucrose stimulated ADH release (4, 55). Intraventricular studies, however, are difficult to interpret, as excessive changes in electrolyte composition may activate periventricular neurons in an unphysiological manner. Moreover, it is important to note that other workers have found that intraventricular injections of sucrose do stimulate ADH release when the sucrose is contained in an artificial cerebrospinal fluid solution (51).

As with Verney’s studies (82), Athar and Robertson (5) and Robertson et al. (59) also found that hypertonic urea provides a poor osmotic stimulus for ADH release. This finding is apparently due to the fact that urea readily penetrates cell membranes and thus does not provide an “effective” osmotic stimulus. Another important aspect of the hypertonic urea studies deals with the proposal that the blood-brain barrier constitutes the semipermeable membrane for osmoregulation (3, 4, 55). If such were the case, then hypertonic urea might be expected to provide a potent osmotic stimulus for ADH release, since urea does not readily cross the blood-brain barrier. As already stated, this is not the case. One might, therefore, deduce from these results that the osmoreceptors for ADH release reside in those portions of the anterior hypothalamus that are outside of the blood-brain barrier, such as the subfornical organ and the organum vasculosum (83). It also has been shown recently in dogs that...
cerebrospinal fluid sodium concentration increases substantially during intracarotid infusion of hypertonic saline, hypertonic sucrose, or hypertonic urea, and yet only hypertonic saline and sucrose caused a potent antidiuresis (52). This is a critically important result that provides a near-fatal blow to the CSF's sodium receptor hypothesis for ADH release. Rather, as proposed by Verney (82), cells which respond to "effective" extracellular osmolality rather than changes in CSF sodium concentration seem most likely to mediate the osmotic release of ADH.

The studies of Athar and Robertson (5) and Robertson et al. (59) differ somewhat from those of Verney (82) with respect to the effects of hypertonic glucose on water excretion and ADH release. Verney demonstrated that an acute intracarotid injection of hypertonic glucose over 10 s, which raised plasma osmolality by approximately 100%, was associated with an antidiuresis of a magnitude comparable to hypertonic saline. However, during constant-infusion studies over 40 min which raised plasma osmolality by approximately 2%, hypertonic saline but not hypertonic glucose caused an antidiuresis. In contrast, Athar and Robertson (5) and Robertson et al. (59) suggest from their studies in man that the infusion of hypertonic glucose actually suppresses ADH release and thus, perhaps, may contribute, along with the osmotic diuresis, to the polyuria associated with the hyperglycemia of uncontrolled diabetes mellitus. If alterations in cell volume provide the signal for osmoreceptor cells regulating ADH release, then the following sequence of events might be proposed to explain a suppression of ADH release with hyperglycemia. With glucose not penetrating rapidly into extracellular cells, osmotic water movement would occur from these cells into the ECF compartment and, thereby, cause hyponatremia. Since brain cells are more readily permeable to glucose, the decrease in ECF plasma sodium concentration will constitute the main osmotic gradient for water movement into osmoreceptor cells in the hypothalamus. According to the osmoreceptor hypothesis, this cell swelling would suppress ADH release. Although attractive, this hypothesis remains to be tested. Moreover, it should also be remembered that it is much easier to demonstrate a stimulation than a suppression of ADH, since base-line levels of blood ADH are quite low.

The role of angiotensin in stimulating ADH release has been a matter of intense interest since this possibility was originally proposed by Bonjour and Malvin (13). Using a bioassay for ADH, these authors suggested that intravenous angiotensin II stimulated ADH release. Support for this concept, however, was not forthcoming, either from studies from our laboratory (18) or others (21). The recent study in conscious dogs by Ramsay et al. (56), however, strongly supports the original proposal by Bonjour and Malvin (13). Using a radioimmunoassay, these workers found a dose-response curve between plasma ADH and doses of intravenous angiotensin ranging from 5 to 20 ng·kg⁻¹·min⁻¹. These increases in plasma ADH occurred despite rises in blood pressure which, via the baroreceptor mechanism (see below), should have suppressed ADH release. This study, therefore, might seem to demonstrate conclusively a physiological relation-
maintain an open mind about an important physiological role of angiotensin-induced stimulation of ADH release.

Although the studies in the rat cited earlier (26, 60) did not demonstrate an effect of nonosmotic stimuli on the sensitivity of the osmoreceptor for ADH release, other studies in the same species from the same laboratory do suggest that hypovolemia may enhance the sensitivity of the osmoreceptor-induced release of ADH (27). Results examining whether hypovolemia may attenuate the osmotic release of ADH are not available. It is interesting to note, however, that the hypovolemia associated with primary hyperaldosteronism in man generally is associated with increased plasma sodium concentrations.

**Nonosmotic Control of ADH Release (66–69)**

Although the major focus of Verney's Croonian lecture (82) dealt with the osmotic control of ADH, studies also were presented which examined the effect of "emotional stress" on urine flow in water-diureting dogs. The mechanism of producing "emotional stress" was by electrically stimulating the flanks of the animals. This maneuver produced only a small and transient antidiuresis, but it was accompanied by a rather large and consistent increase in blood pressure. O'Connor and Verney (54), therefore, reasoned that perhaps sympathetic stimulation during this "emotional stress" might produce a conflicting stimulus and thereby obscure a more profound antidiuresis. Studies, accordingly, were done on an animal named "Nicki" before and after bilateral section of the splanchnic nerves as well as denervation of the kidneys and adrenal glands. After the denervation procedure, a much larger antidiuresis was observed with the emotional stress. On the basis of these results, Verney and associates (54, 82) reasoned that either an infusion of tyramine or norepinephrine might interfere with the antidiuresis produced during electrical stimulation in the denervated animal. This was indeed found to be the case. These workers then sought to examine whether such an effect of sympathetic stimulation on water excretion was due to suppression of ADH release or interference with ADH at the level of the kidney. Since the antidiuretic effect of injections of posterior pituitary extract were unaltered by prior injection of tyramine, they concluded that sympathetic stimulation alters renal water excretion by suppressing the release of ADH. They proposed that such an effect of sympathetic stimulation was not due to the accompanying pressor effect, since bilateral occlusion of the common carotid arteries elevated blood pressure to the same degree as did either intravenous norepinephrine or tyramine and yet did not interfere with the antidiuresis associated with electrical stimulation.

The possibility remained, however, that common carotid occlusion by itself might induce ADH release and cause an antidiuresis. Some 25 yr later, studies by Share and Levy (74) confirmed this possibility. Later studies in our laboratory which demonstrated that bilateral cervical vagotomy causes an ADH-mediated antidiuresis (64) further focused our interest on the role of the autonomic nervous system in renal water excretion. This effect of vagotomy was due to interruption of afferent neural pathways, since effenter blockade of parasympathetic pathways with atropine did not alter renal water excretion.

Several years after Verney's studies, several investigators demonstrated that intravenous norepinephrine causes a water diuresis in both man (6, 79) and animals (45, 48). In vitro experiments in anuran membranes that demonstrated an effect of norepinephrine to interfere with the hydroosmotic effect of vasopressin suggested a potential mechanism of action at the level of the renal tubule epithelium (7, 32, 80). Studies from our laboratory performed on dog (65), rat (50), and man (11), however, demonstrated that the effect of intravenous norepinephrine to cause a water diuresis was dependent on an intact source of endogenous vasopressin (AVP) (69). Specifically, acutely hypophysectomized dogs, Brattleboro rats suffering from diabetes insipidus, and patients with central diabetes insipidus, all receiving a constant infusion of vasopressin, failed to demonstrate a diuretic response to a dose of norepinephrine which increased water excretion in the intact species. These results, therefore, are compatible with Verney's interpretation that sympathetic stimulation may interfere with "the chain of chemical reactions initiated in the central nervous system by the electrical stimulus and ending in release of posterior pituitary antidiuretic substance" (82). In fact, Verney (82) suggested that the intracarotid injection of norepinephrine might be much more effective in causing a water diuresis than intravenous norepinephrine. Almost 30 yr later, Berl et al. (12) performed experiments in dogs using an intracarotid dose of norepinephrine which was estimated to equal the amount reaching the cerebral circulation during the administration of intravenous norepinephrine. The intravenous, but not the intracarotid, norepinephrine caused an increase in solute-free water excretion. This finding suggested that the effect of norepinephrine to suppress ADH release was not direct but rather involved an extracerebral reflex mechanism. Studies, therefore, were performed to examine whether intravenous norepinephrine caused a water diuresis in animals with baroreceptor denervation (bilateral cervical vagotomy and carotid sinus denervation) (12). In spite of an intact pituitary source for ADH release, intravenous norepinephrine failed to cause a water diuresis in these baroreceptor-denervated animals (12). Since other investigators had shown that the effect of intravenous norepinephrine to increase water excretion is abolished by alpha-adrenergic blockade (48), the results of the above experiments indicated that alpha-adrenergic stimulation primarily alters solute-free water excretion by suppressing ADH release via a baroreceptor mechanism. In view of the work of Turtle and Kipnis (81) that suggested opposing effects of alpha- and beta-adrenergic stimulation in various tissues, the effect of beta-adrenergic stimulation on water excretion was examined. In these studies, beta-adrenergic stimulation with isoproterenol caused an antidiuresis in intact rat (50), dog (70), and man (11), and this effect on water excretion was not demonstrable in states of ADH deficiency in any of these
species (11, 50, 70). These results and the failure of intracarotid isoproterenol to induce an antidiuresis (10) suggested a baroreceptor-mediated effect on ADH release similar to, but in the opposite direction of, alpha-adrenergic stimulation. Berl and colleagues (10), therefore, performed experiments in which the low- (atrial) and high- (carotid sinus) pressure baroreceptors were sequentially denervated. A primary importance of the high-pressure baroreceptors was implicated in this reflex, since bilateral carotid sinus denervation, but not bilateral cervical vagotomy, abolished the antidiuretic response to beta-adrenergic stimulation (10). Taken together, therefore, these series of experiments indicate that alpha- and beta-adrenergic stimulation alter renal water excretion by modulating ADH release via baroreceptor pathways (69).

The above studies cannot exclude totally a modest direct tubular effect of alpha- and beta-adrenergic stimulation to alter the response of the mammalian collecting duct to vasopressin. It is nevertheless clear that there is little need to invoke such a tubular mechanism in in vivo experiments. Studies by Beck et al. (9) in dog medullary tissue, however, demonstrated that norepinephrine interferes with, and isoproterenol enhances, the effect of vasopressin to increase cyclic 3',5'-adenosine monophosphate, the hormone's intracellular secondary messenger. On the other hand, determinations of adenylate cyclase concentration (20) and cyclic AMP (46) in individual rat medullary collecting ducts have failed to demonstrate any effect of isoproterenol. Moreover, McDonald et al. (50) have demonstrated stimulation of medullary cyclic AMP by isoproterenol only in the intact rat but not in Brattleboro rats suffering from diabetes insipidus. Recently, Rayson et al. (57) have perfused isolated papillary collecting ducts in vitro and have failed to find an effect of isoproterenol to alter the effect of vasopressin on water transport. Accordingly, there is little in vitro or in vivo evidence for a direct effect of beta-adrenergic stimulation on medullary collecting duct water transport.

The experimental evidence with alpha-adrenergic stimulation with norepinephrine on medullary collecting duct is less definitive. Kurokawa and Massry (46) did find that norepinephrine interferes with the cyclic AMP response to vasopressin during in vitro studies using rat medullary collecting ducts. McDonald et al. (50), however, could only demonstrate an in vivo effect of norepinephrine to decrease vasopressin-induced increases in cyclic AMP in intact rats but not in Brattleboro rats suffering from diabetes insipidus and receiving exogenous ADH. Similarly, only the intact rats demonstrated a norepinephrine-induced water diuresis. Last, during in vitro perfusion of isolated collecting ducts, Rayson et al. (57) demonstrated an effect of norepinephrine to inhibit vasopressin-induced cyclic AMP, but no effect of norepinephrine on water transport could be demonstrated.

Based on the background of the studies with catecholamines (10, 12, 69) and other studies during hemorrhage (71), a tenable hypothesis was that the baroreceptors constitute the major nonosmotic pathway for regulation of ADH release (66-68). Additional studies, therefore, were undertaken to test this hypothesis. Anderson et al. (1) demonstrated that acute constriction of the thoracic vena cava was associated with an antidiuresis which also was primarily due to baroreceptor release of ADH. Because of earlier studies suggesting a direct central effect of nicotine to release ADH (17), further studies were performed with this agent (19). Unexpectedly, however, intravenous, but not intracarotid, nicotine caused an ADH-mediated antidiuresis in the anesthetized dog; moreover, intravenous nicotine did not decrease water excretion in baroreceptor-denervated animals (19). These studies demonstrate another stimulus for the nonosmotic release of ADH which was dependent on the integrity of the baroreceptors. The nicotine studies also indicated that a fall in arterial pressure is not necessary to activate this baroreceptor pathway for ADH release. In fact, the ADH release with nicotine administration was associated with a pressor response, most likely related to the agent's known ganglionic-stimulating properties. More recently, acute hypoxia also has been shown to stimulate baroreceptor-mediated release of ADH in the absence of a fall in arterial pressure (2). It is, therefore, possible that increased sympathetic stimulation even in the absence of a fall in arterial pressure will activate baroreceptor pathways for the nonosmotic release of ADH. Such an effect, therefore, might explain the ADH release which occurs without a fall in arterial pressure. As shown by O'Connor and Verney (54, 82), however, sympathetic stimulation which is sufficient to raise arterial pressure may provide conflicting stimuli for ADH release. In the absence of a pressor or depressor response, various alarm stresses such as fright, temperature changes, or physical pain may alter baroreceptor tone by causing sympathetic stimulation, which then causes a reciprocal diminution in parasympathetic afferent tone with resultant ADH release.

Nonosmotic release of ADH without a fall in arterial pressure also might occur by decreasing the activity of low-pressure (left atrial) baroreceptors. This pathway has been suggested to stimulate ADH release during nonhypotensive hemorrhage (72, 73). However, since denervation of the carotid (high-pressure) baroreceptors was necessary to abolish the nonosmotic release of ADH in response to catecholamines (10, 12), nicotine (19), and thoracic caval constriction (1), it seemed possible that left atrium (low-pressure) receptors are relatively unimportant in the nonosmotic regulation of ADH. In this regard, Kappagoda et al. (42, 43) have suggested that ADH does not mediate the diuresis associated with left atrial distension. This conclusion was based on studies during left atrial distension in which bioassayable titers of ADH were not found to fall (43) and cauterization of the pituitary failed to abolish the diuresis of atrial distension (42). This conclusion concurred with the earlier studies of Ledsome and Mason (47), but conflicted with the original proposal of Henry et al. (38) as well as other investigators who reported a
fall in bioassayable ADH during left atrial distension with inflation of a balloon (40, 78). Surgical removal of the pituitary with administration of large doses of exogenous ADH, however, has recently been found to abolish the diuresis associated with left atrial distension; also, a fall in radioimmunoassay titers of ADH was found to occur during left atrial distension (23). Since pacemaker-induced atrial tachycardia increased left atrial pressure, the mechanism of the associated water diuresis was examined in the dog (14). The atrial tachycardia-induced diuresis was abolished by either acute hypophysectomy, which removed the source of ADH release, or bilateral cervical vagotomy (14). More recently it also has been suggested that acute pulmonary hypertension might cause an antidiuresis in the conscious dog through activation of left atrial receptors and stimulation of ADH release (85). In these studies, radioimmunoassay titers of ADH rose and left atrial pressure fell; however, it was not determined whether interruption of cervical vagal pathways abolishes the antidiuresis.

Taken together, therefore, a series of experiments from our laboratory, which are shown schematically in Fig. 5, have demonstrated nonosmotic release of ADH via either high- or low-pressure baroreceptor pathways (66–69). It should not be concluded, however, that there are not other pathways whereby the nonosmotic release of ADH might occur. In this regard, other pathways for the nonosmotic release of ADH have been proposed, including chemoreceptors (75), a cerebral emetic center (58), and a cerebral pain center (34). The recent results of Anderson et al. (2), however, failed to demonstrate any involvement of the chemoreceptors in ADH release during acute hypoxia; baroreceptor, but not chemoreceptor, denervation abolished the effect of hypoxia to stimulate ADH release. There is no question that both nausea (58) and pain (34, 82) are associated with ADH release; these stimuli, however, could be mediated through baroreceptor pathways rather than it being necessary to incriminate other as yet undefined pathways for nonosmotic release of ADH. It also should be pointed out that recent stress studies in the rat, including ether anesthesia, immersion, and pain, failed to stimulate ADH (15). Whether conflicting stimuli, as shown by Verney (82), obscured the effect of stress to stimulate ADH release was not examined in these studies. Additional studies are necessary to determine whether nonbaroreceptor pathways also modulate nonosmotic ADH release.

**Relationship Between Osmotic and Nonosmotic Pathways for ADH Release**

The exact details of how the osmotic and nonosmotic pathways interact have not been clarified. However, there are some recent results in human subjects and experimental animals that provide some insight into this relationship. The recent study of subjects who had been classified as having "essential hypernatremia," has been quite revealing (31). These patients had been demonstrated to have near absence of osmoreceptor mediated ADH release (as tested during hypertonic saline infusion), while nonosmotic release of ADH (as tested during hypotension with trimethaphan) was normal. These patients with osmoreceptor ablation had adipsia and hypothalamic lesions. Thus, the thirst center and osmoreceptors appear to have anatomically adjacent locations in the hypothalamus. Also, the osmoreceptors must be anatomically separate from the baroreceptor input to the hypothalamus.

The findings in these patients, therefore, seem to exclude the possibility that the nonosmotic baroreceptor release of ADH always occurs indirectly via alteration of the osmotic release of ADH. Perhaps in the strictest sense such a situation would constitute true "resetting of the osmoreceptor." In addition, these results indicate that the osmoreceptors for ADH can be virtually totally ablated without altering the capacity of magnocellular neurosecretory cells to secrete large amounts of ADH in response to nonosmotic stimuli. Accordingly, two theories about the anatomical and functional relationship between osmotic and nonosmotic pathways for ADH release are consonant with these findings. The first is that the nonosmotic baroreceptors might alter the secretion of ADH in a totally separate population of hypothalamic neurosecretory cells from those related to the osmotic release of ADH. Alternatively, the osmoreceptor and baroreceptor pathways may have discrete and anatomically separate inputs into the same population of magnocellular neurosecretory cells. Recent electrophysiological studies in the rat provide some results in support of the latter possibility. Kannan and Yagi (41) have assessed electrical activity of single supraoptic neurons. In these studies a single cell was demonstrated to respond to both osmotic (intracarotid hypertonic saline) and nonosmotic stimuli (carotid occlusion) but not to intracarotid isotonic saline (Fig. 6). From these studies and the studies in patients with "essential hypernatremia" a potential model for the interaction between osmotic and nonosmotic pathways can be proposed. The schema for this model is shown in Fig. 7. In this model, separate osmotic and nonosmotic pathways enter the same magnocellular

![Parasympathetic Afferent Pathways](image-url)
neurosecretory cells. Further studies will, of course, be necessary to test the validity of this model. In addition, this arrangement does not exclude the possibility that baroreceptor and osmoreceptor pathways also may relate to totally separate populations of neurosecretory cells.

If osmoreceptor and baroreceptor pathways stimulate the same population of hypothalamic neurosecretory cells, as suggested by electrophysiological studies (33, 41), then perhaps different neurotransmitters mediate these separate pathways. Most studies into the nature of central neurotransmitters for ADH release have involved in vivo hypothalamic injections of large concentrations of potential neurotransmitters, or have used in vitro preparations that have interrupted the hypothalamo-neurohypophysial pathways. In these studies, an important role for catecholamines (16), cyclic nucleotides (49), potassium (25), calcium (24), and acetylcholine (30) all have been suggested. In a recent study in conscious rats, a central role of catecholamines as potential neurotransmitters in the osmotic and nonosmotic release of ADH has been suggested (53). In this study, intraventricular 6-hydroxydopamine was used in doses that depleted brain catecholamines by 67%, but heart catecholamines were not significantly altered. The control animals and the animals given 6-hydroxydopamine were studied during both osmotic (intravenous hypertonic saline) and nonosmotic (intraperitoneal dextran) stimuli. The plasma osmolality, volume status, and systemic and renal hemodynamics were comparable in the two groups of rats both before and during the osmotic and nonosmotic stimuli. All the animals were undergoing a water diuresis in the control period, and during both the osmotic and nonosmotic stimuli the rise in urinary osmolality was significantly less in the rats given 6-hydroxydopamine. These blunted responses in urinary concentration also were associated with smaller rises in radioimmunoassayable titers of ADH in the 6-hydroxydopamine-treated rats. Although these preliminary studies suggest a potential role for catecholamines in the central release of ADH for both the osmoreceptor and baroreceptor pathways, it must be remembered that 6-hydroxydopamine depletes both dopamine and norepinephrine. Accordingly, it is still theoretically possible that the osmoreceptor and baroreceptor pathways have different neurotransmitters. Moreover, it is possible that catecholamines constitute only one step in the ultimate release of ADH, and factors such as calcium influx into the neurosecretory cells with activation of intracellular cyclic AMP also may be important.

In summary, the development of a sensitive radioimmunoassay has permitted the determination of several potential factors which affect the “threshold” and sensitivity of the relationship between plasma osmolality and plasma arginine vasopressin. Individual variations, the rate of osmotic change, age, and species are examples of factors that may alter these relationships.

The reflection coefficient for solute penetration into cells is an important determinant of the osmotic release of vasopressin. Since urea penetrates into cells readily but crosses the blood-brain barrier slowly, the results using this solute are particularly important. Because hypertonic urea does not cross the blood-brain barrier readily, water moves out of the cerebrospinal fluid and the sodium concentration in the CSF rises. In this setting the failure of hypertonic urea to stimulate arginine vasopressin release provides strong evidence against the proposed “sodium-sensitive” CSF receptor for AVP release. Additionally, because hypertonic saline and hypertonic mannitol cause ECF sodium to change in the opposite direction and yet stimulate AVP to a comparable degree, an osmoreceptor and not a “sodium-sensitive” ECF receptor must be involved. The available results suggest that the osmoreceptor cells lie outside the blood.
brain barrier in the anterior hypothalamus.

It is also clear that the osmoreceptor cells in the hypothalamus in the supraoptic and paraventricular areas are separate from the neurosecretory cells associated with nonsomatic release of AVP. This conclusion is based on the finding in human subjects with hypothalamic lesions who are unresponsive to osmotic stimulation but demonstrate a normal release of AVP in response to a nonsomatic stimulus. These patients also frequently have hypodipsia, suggesting a close anatomical relationship between the thirst center and osmoreceptor cells.

A number of studies now provide conclusive evidence that the major nonsomatic pathway for vasopressin release involves the autonomic nervous system. Low-pressure left atrial receptors and high-pressure carotid receptors which communicate to the hypothalamus via parasympathetic pathways appear to be the primary receptors. Since changes in arterial pressure are not mandatory to activate the high-pressure receptors, changes in the balance between sympathetic and parasympathetic tone, as well as arterial pressure, appear adequate to activate these nonsomatic pathways for AVP release. Indeed, many circumstances in which AVP is released without increases in plasma osmolality are associated with increased sympathetic tone, and, therefore, probably a decrease in parasympathetic afferent tone. It is clear that the release of parasympathetic afferent tone to the hypothalamus is associated with AVP release. This nonsomatic pathway is probably involved in AVP release during various acute stress circumstances such as pain, psychiatric disturbances, rapid decreases in cardiac output, acute hypoxia, and acute adrenal insufficiency.

It is possible that these interactions between increased stress and vasopressin release may have developed phylogenetically as an integral part of the alarm reaction. There is now some support for this hypothesis. The recent availability of a specific competitive inhibitor of the pressor effect of AVP has demonstrated a role for AVP in maintenance of blood pressure in the fluid-deprived state (G. Aisenbrey, W. Handelman, M. Manning, and R. Schrier, unpublished observations). The development of a specific competitive antagonist of the antidiuretic effect of AVP is needed to examine a cause and effect relationship between plasma AVP levels and renal water excretion in man. Experiments done before and after acute ablation of the source of AVP have allowed the demonstration of such a cause and effect relationship in animals, but this approach is obviously not applicable to human studies.

The relationship between osmotic and nonsomatic pathways for AVP is now becoming better delineated. Electrophysiological studies suggest that a single magnocellular neurosecretory cell in the hypothalamus may respond both to osmotic and nonsomatic stimuli. This information, along with the demonstration of preservation of nonsomatic without osmotic release of AVP, suggests the model presented in Fig. 7. Although, given our present state of knowledge, this model is no doubt oversimplified, it does have several implications. The term "reset" osmostat has heretofore been suggested to describe some intrinsic change in the osmoreceptor cells which leads to these cells "sensing" a lower or higher (reset) level of plasma osmolality as normal. With this new model (Fig. 7) involving an intimate relationship between "baroreceptor" and "osmoreceptor" input into the same population of neurosecretory cells, a competitive input between these two pathways may explain many clinical findings without involving an intrinsic "resetting" of the osmoreceptor cells. For example, in the presence of increased baroreceptor input to stimulate AVP release, a greater decrement in plasma osmolality may be necessary to allow the dominance of hyposmolality in suppressing AVP release. This relationship may explain why some patients with hyposmolality associated with cardiac failure, cirrhosis, and the syndrome of inappropriate ADH secretion (SIADH) may dilute their urine in response to an acute water load. The osmoreceptor cells in these patients may not be "reset" by any intrinsic alteration in their osmoreceptor properties, but rather a larger, and perhaps more acute, decrement in plasma osmolality may be necessary to overcome a nonsomatic stimulus for ADH release. There are, of course, patients with these diseases in whom an acute water load causes no urinary dilution. These may be patients with the most potent nonsomatic stimuli for ADH release, so that no degree of hyposmolality will allow the osmoreceptor input into the neurosecretory cells to predominate. In this context, to describe the patient with lung disease who dilutes his urine during an acute water load as possessing a "reset osmostat" and the individual who does not demonstrate urinary dilution during a similar water load as a patient with SIADH may be an example of using different diagnostic terminology to describe the spectrum of the same disorder.

Last, recent evidence from our laboratory indicates that catecholamines are important in both the osmotic and nonsomatic release of ADH in vivo. It seems likely, however, that catecholamines are only one link in a chain of events which remains to be unraveled. The high concentration of cyclic AMP in supraoptic nuclei in the hypothalamus and the reported importance of calcium in ADH release have led us to formulate an hypothesis whereby catecholamines initiate calcium movement into hypothalamic neurosecretory cells, which then activate 3',5'-adenosine monophosphate (cyclic AMP) and a chain of events leading to AVP synthesis and release. Considerable work will, of course, be necessary to test this hypothesis.

Thus, in the past decade considerable new knowledge has been contributed to the original studies by Verney of factors that alter ADH release, but there still remain many questions yet to be answered. Nevertheless, given the background of this recent work, Verney's statement (82) about the mammalian water regulatory system seems even more true now: "One can scarcely conceive an arrangement more elegant in apparent design or indicative in purport in an animal order whose developmental succession from intimacy with the external environment has depended upon the concurrent acquisition of control over the internal environment."
REFERENCES


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