# Vasopressin: physiology, assessment and osmosensation

Lise Bankir, Daniel G Bichet, N. G. Morgenthaler

# Vasopressin: physiology, assessment, and osmosensation

Lise BANKIR [1, 2], Daniel G. BICHET [3, 4], Nils G. MORGENTHALER [5, 6]

1. INSERM Unit 1138, Centre de Recherche des Cordeliers, 75006 Paris, France;
2. Université Pierre et Marie Curie, 75006 Paris, France;
3. Université de Montréal, Montréal (Québec), Canada;
4. Départements de Pharmacologie, Physiologie et de Médecine, Hôpital du Sacré-Coeur de Montréal, Montréal (Québec), Canada;
5. Institut für Experimentelle Endokrinologie, Charité Universitätsmedizin Berlin, Berlin, Germany;

6 InVivo Biotech Services, Neuendorfstraße 24a, Hennigsdorf/Berlin, Germany.

Short title : **Vasopressin: physiology, assessment, osmosensation**

Corresponding author

Lise BANKIR, INSERM Unit 1138, Centre de Recherche des Cordeliers
15 rue de l'Ecole de Médecine, 75006 PARIS, France
Tel : 33 1 47 35 34 15 or 33 6 82 94 80 10
E-mail : lise.bankir@inserm.fr or lise.bankir@renalphy.com

Conflicts of interest

LB is an occasional consultant for Danone, France

DGB and NGM: none

## Abstract

Vasopressin (AVP) plays a major role in the regulation of water and sodium homeostasis by its antidiuretic action on the kidney, mediated by V2 receptors. AVP secretion is stimulated by a rise in plasma osmolality, a decline in blood volume, or stress. V1a receptors are expressed in vascular smooth muscle cells but the role of vasopressin in blood pressure regulation is still a matter of debate. AVP may also play a role in some metabolic pathways, including gluconeogenesis, through its action on V1a receptors expressed in the liver.

It is now understood that thirst and arginine-vasopressin (AVP) release are regulated not only by the classical homeostatic, intero-sensory plasma osmolality negative feedback, but also by novel, extero-sensory, *anticipatory* signals.

AVP measurement is time-consuming and AVP level in the blood in the physiological range are often below the detection limit of the assays. Recently, an immuno-assay has been developed for the measurement of copeptin, a fragment of the pre-pro-vasopressin molecule that is easier to measure. It has been shown to be a good surrogate marker of AVP.



## Keywords

Osmolality, Copeptin, Hypothalamus, Thirst, Kidney, Vasopressin receptors

# Introduction

The hormone vasopressin (AVP) or antidiuretic hormone (ADH) plays a major role in the control of body water homeostasis and associated disorders. His possible role in blood pressure regulation remains controversial. Recently its role in chronic renal, cardiovascular and metabolic diseases has been a matter of growing interest. This is mostly due to the availability of new tools. First, efficient orally active antagonists, the vaptans, offer the possibility of therapeutic interventions. Second, advances in the study of neurone biology has provided a new light in the mechanism of osmosensation and neuro-hormonal circuits leading to the release of the hormone. Third, a recently developed immuno-assay allows the measurement of copeptin, a surrogate of vasopressin that is more stable *in vitro* and much easier to measure than the hormone itself.

In this review, we will first provide a rapid survey of vasopressin physiology, receptors, target organs, and multiple biological functions (beyond its role in the control of body fluid homeostasis) [1, 2]. Then, we will present the new advancements in the understanding of osmosensation and the neuronal pathways that lead to the secretion of the hormone. Finally, we will describe the measurement of copeptin and the wide opening this has provided in epidemiologic studies and as a diagnostic tool.

# Overview of vasopressin physiology

## ***Vasopressin synthesis and secretion***

Vasopressin is a small peptidic hormone (MW = 1080) comprising 9 amino acids in a ring structure. It is synthetized in the hypothalamus and stored in the neurohypophysis. The pre-prohormone protein contains vasopressin, neurophysin, and copeptin that are cleaved in their course in the pituitary stalk, and released simultaneously in the blood in equimolar amounts.

The primary fonction of vasopressin is to maintain body fluid balance by keeping plasma osmolality within narrow limits and allowing the kidneys to adapt water excretion to the body's needs, in conjunction to thirst. No other hormone can replace vasopressin: the lack of vasopressin results in diabetes insipidus (10-15 L urine/d). Vasopressin has a very short biological half life (about 3 min) and it is cleared mostly by filtration in the kidneys. Accordingly, its effects are very prompt and promtly reversible.

The plasma level of vasopressin in the usual range is very low (0 to 3 pg/ml ≈ 3 x $10^{-12}$ M). Even the best assays are unable to quantify vasopressin in the low range of physiological values (the lowest threshold of most immuno assays is 0.5 pg/ml). Vasopressin concentration in the urine is several-fold higher than that in the blood, but its excretion rate does not truly reflect its concentration in the blood because it is largely influenced by the osmolar excretion.

The main stimulus for the release of vasopressin is dehydration resulting in an increase in plasma osmolality. But for the same change in osmolality, sodium has a larger influence on vasopressin secretion than does urea or glucose [3]. Other stimuli for vasopressin secretion are a reduction in circulating blood volume, and stress situations [4]. The threshold and slope for vasopressin secretion versus plasma osmolality is very reproducible in the same subjects, and shows strong heritability [5].

## ***Vasopressin receptors and target sites tissues***

Three different receptors for vasopressin have been characterized [6]. The effects following V2 receptor (V2R) activation are mediated by cyclic AMP while the effects mediated by V1a or V1b (V1aR, and V1bR also called V3) receptor activation are mediated by calcium signals. The commonly known target tissues for vasopressin are the kidney collecting duct (with V2R) and the vascular smooth muscle cells (with V1aR). But vasopressin receptors are expressed in a number of other organs and tissues (see **Figure 1**) [7]. Moreover, V1aR are also expressed in the kidney, and V2R are expressed in the endothelium (where they play a vasodilatory role by inducing the formation of NO).

The sensitivity to vasopressin most probably differs among target organs but this relative sensitivity is poorly documented. And this sensitivity may be modified by diverse physiologic and pathologic adaptations. As an example, the stimulation of V1aR in the kidney requires higher concentration of vasopressin than the V2 effects [8]. Actually, the kidney V2R is exquisitively sensitive. In healthy humans, the infusion of vasopressin at rates of 1, 5 or 25 pg/min per kg induced a significant, dose-dependent antidiuretic action, but only the highest rate induced a measurable rise in plasma vasopressin [9].

dDAVP (de-amino 8D-arginine vasopressin) is a peptidic V2R specific agonist, widely used in the diagnostic of disorders of water balance and in the treatment of central diabetes insipidus. Terlipressin (= glypressin), a (poorly) selective V1aR agonist is used in hepatorenal syndrome and oesophageal varices. Recently, potent, non-peptide, orally active, selective antagonists of V2R have been designed, such as tolvaptan [10].

## ***Vasopressin effects on the kidney***

V2 receptors are localized in the principal cells of the collecting duct (CD) and, to a lesser extent, in the thick ascending limb. Along the entire CD, vasopressin increases water permeability by promoting the insertion of aquaporin 2 (AQP2)-rich vesicles in the luminal membrane of the CD cells. This allows an increase in water reabsorption when a favorable osmotic driving force is present (generated by solute accumulation in the surrounding interstitium). In addition, vasopressin exerts two other effects on the CD through V2R (**Figure 2A**). 1. In the cortical and outer medullary CD, vasopressin stimulates sodium reabsorption by its action on the luminal sodium channel ENaC. This drives water iso-osmotically and thus helps concentrate all other solutes in the lumen. 2. In the terminal inner medullary CD, vasopressin increases the permeability to urea by activating the facilitated urea transporters UT-A1 and UT-A3. This allows concentrated urea to diffuse in the medullary interstitium and thus maintain in the interstitium a high urea concentration

that favors water reabsorption [11, 12]. In the thick ascending limb, vasopressin stimulates the Na-K-2Cl cotransporter NKCC2, and thus promotes sodium reabsorption. But this effect requires a higher concentration of the hormone than that on the CD [13]. Altogether these combined effects on several membrane transporters and channels contribute jointly to urine concentration.

These V2R-mediated effects are partially counteracted by V1aR effects in two ways. Luminal V1aR, exposed to urinary vasopressin, induce the production of prostaglandins by CD cells that, indirectly, attenuate the adenylate cyclase response to V2R stimulation. Second, V1aR are also abundantly expressed in interstitial cells of the medulla where they stimulate the production of prostaglandins that vasodilate the medullary vasculature. This opposes the possible vasoconstrictive effect of V1aR in the descending vasa recta and induces an increase in medullary blood flow that compromizes the osmotic gradient of the medulla. Thus, there is a subtle balance between V2R and V1aR effects in the kidney, as demonstrated in rats [8, 11].

Vasopressin action on the CD and thick ascending limb improve urine concentration, but they lead to some sodium and urea retention. The fractional excretion of sodium and urea (and most probably that of other solutes) is reduced when urine is more concentrated ((**Figure 2B**)). The vasopressin-dependent sodium retention may contribute to salt-sensitive hypertension and is compensated by the pressure-natriuresis mechanism [11]. The urea retention may lead to an increase in plasma urea concentration and, by an indirect mechanism, to a rise in glomerular filtration rate (GFR). It has been shown in both rats and humans that GFR increases with increasing urine osmolality (**Figure 3**) [14-16]. This "hyperfiltration" is propably an indirect consequence of the tubular action of vasopressin [17, 18]. In the long term, it may have adverse effects, as described elsewhere [17, 19]. An adverse effect of vasopressin is also observed in autosomal polycystic kidney disease, but through a different and more direct mechanism because AVP-dependent cAMP stimulates cyst enlargement (see [17] and other review by Olivier Devuyst et al in this journal).

There are two different options for reducing to vasopressin actions. Either a voluntary increase in fluid intake [20], or the use of selective vasopressin antagonists [10]. However, they differ in some aspects. An increase in water intake will lower

plasma osmolality and vasopressin secretion. In contrast, V2R antagonists, that induce a water loss, will increase plasma osmolality and thus stimulate vasopressin secretion. The effects of vasopressin on V1a and V1b receptors may thus be potentiated. However, no rise in blood pressure has been observed in healthy subjects or patients treated with vaptans.

## ***Effects on the liver and pancreatic islets***

The expression of vasopressin V1aR in the liver and of V1bR in pancreatic islets was known for a long time, based mostly on *in vitro* studies in isolated perfused liver or pancreas or in isolated hepatocytes. But very little attention was given to the possible *in vivo* consequences of vasopressin action on these organs. It is now recognized that vasopressin stimulates the secretion of either insulin or glucagon by beta and alpha cells of the pancreas, respectively (depending on the level of glycemia). In the liver, vasopressin stimulates several metabolic pathways including glycogenolysis, gluconeogenesis and ureagenesis, glutamine and proline metabolism. These effects may differ according to fed or fasted condition and to glycemia. They are very similar to those induced by glucagon. But they occur through the activiation of different second messengers ($Ca^{++}$ for AVP and cAMP for glucagon), suggesting that these effects might be additive.

In recent years, several studies have revealed significant associations between high vasopressin levels (or its diverse surrogates, low fluid intake, low urine flow rate, high osmolality, or plasma copeptin concentration) and the prevalence or incidence of metabolic syndrome or diabetes (see other reviews in this journal). Interventional studies in humans and experimental studies in animal models are needed to further evaluate the contribution of vasopressin to renal and metabolic disorders. **Figure 4** summarises the multiple effects of vasopressin on the kidney and liver and their possible adverse consequences.

# Recent advances in thirst perception and coordination of eating, drinking and vasopressin release

Mammals are "osmoregulators". They have evolved mechanisms that maintain extracellular fluid osmolality near a stable value [21], yet values fluctuate around a set point : in humans, for example, 40 min of strenuous exercise [22, 23] or 24 h of water deprivation [24] increase plasma osmolality by more than 10 mosmol/kg $H_2O$. In a dehydrated individual, drinking the equivalent of two large glasses of water (~850 ml) lowers plasma osmolality by approximately 6 mosmol/kg $H_2O$ within 30 minutes [25] . Similarly, ingestion of 13 g of salt increases plasma osmolality by approximately 5 mosmol/kg $H_2O$ within 30 minutes [26].

It is now understood that thirst and arginine-vasopressin (AVP) release are regulated not only by the classical homeostatic, intero-sensory plasma osmolality negative feedback, but also by novel, extero-sensory, *anticipatory* signals.

## ***Intero-sensory and extero-sensory regulation of thirst and vasopressin release***

1. Deviations in intero-sensory stimuli

Differences between the extracellular fluid (ECF) osmolality and the desired set-point induce proportional homeostatic responses according to the principle of negative feedback [21, 27] (**Figure 5A**). ECF hyperosmolality stimulates the sensation of thirst [28] to promote water intake and the release of vasopressin [29] that will enhance water reabsorption in the kidney. By contrast, ECF hypo-osmolality suppresses basal vasopressin secretion in rats and humans [30]. Thirst and vasopressin release appear thus far as a purely homeostatic response to deviations in **intero-sensory** stimuli : blood osmolality, pressure or volume. The techniques used in the 1960's and 1970's to describe these intero-sensory stimuli lacked the

ability to track thirst neurons of the lamina terminalis and vasopressin neurons projecting to the posterior pituitary in real time in behaving animals, and so could not assess **extero-sensory** information regulating these processes [31].

## 2. Extero-sensory stimulation : anticipation for thirst stimulation and vasopressin release

Recent experiments using optogenetic tools in awake animals demonstrate that a substantial fraction of normal drinking behavior and vasopressin release is not regulated directly by changes in the blood, and instead, appears to anticipate homeostatic changes before they occur [32]. Anticipatory signals for thirst and vasopressin release converge on the same homeostatic neurons, subfornical organ neurons specifically, that monitor the tonicity of blood [28, 29]. Subfornical organ excitatory neurons ($SFO^{Nos1}$) (**Figure 6**) [33-36], activated by water restriction, had their activity rapidly returning to baseline after water access well before any measurable change in plasma osmolality occurs [28]. This rapid anticipatory response to drinking has been suggested by blood-oxygen-level-dependent (BOLD signal) measurements during thirst stimulation in humans. The BOLD signal from the anterior cingulate cortex area, known to be responsible for the conscious perception of thirst, decreased rapidly after water consumption, well before any systemic absorption of water [37]. There is a delay of around ten minutes [21] between the ingestion of water and its full absorption into the bloodstream. These new data explain how drinking can quench thirst within seconds, long before the ingested water has had time to alter the blood volume or osmolality.

The rapid anticipatory response to drinking has at least two components: an immediate signal that tracks fluid ingestion, and a delayed signal that reports on fluid tonicity, possibly generated by an oesophageal or gastric osmosensor. The alleviation of dry mouth information will flow up the fifth cranial trigeminal nerve, the taste of water will be relayed by the chorda tympani included into the seventh nerve, pharyngo-esophageal impulses metering volume swallowed, by the ninth nerve and lower esophageal and gastric sensation —including distension— by the 10th cranial nerve; but the exact sensing receptors of these afferents are unknown. On a Darwinian point of view, the rapid, volumetrically exact intake of water consequent

upon thirst or of a salt solution in case of sodium-depletion, carries high survival advantage: it permits animals to go to a water or salt source, to rapidly correct the deficit, and leave the place, reducing their exposure to predators that have learned to wait there [38].

## 3. Coordination of eating, drinking and vasopressin release

Eating increases the need for water for two reasons: 1) there is a need to replace the fluid utilized for swallowing (saliva) and digestion (water diverted from the circulation into the gastrointestinal tract); 2) to counteract the increase in blood osmolality caused by the absorption of salts and other osmoles from food. As described recently in a review on thirst [32], anticipatory signals about ongoing food ingestion are communicated to the lamina terminalis by multiple mecanisms. 1) Somatosensory signals from the oral cavity that report on food swallowing or its effects on the saliva. 2) Several hormones associated with eating and satiety have been proposed to modulate thirst neurons and vasopressin release, including amylin, cholecystokinin, ghrelin, histamines, insulin, and leptin. Some of these hormones might be increased in patients with diabetes mellitus and may explain their high vasopressin plasma concentration [39].

The responses to drinking and feeding are bidirectional, yet asymmetric : using electrophysiological recordings in genetically identified supraoptic nuclei pituitary-projecting vasopressin ($VP_{pp}$) neurons in water-restricted mice, Yael Mandelblat-Cerf et al. [29] observed rapid *decreases* in spiking within seconds of presentation of cues signaling water availability, beginning prior to water ingestion. In contrast, ingestion of dry food —a hyperosmotic challenge— elicited rapid *increases* in $VP_{pp}$ neuron activity, prior to any increase in plasma osmolality.

If prandial thirst is not quenched by drinking, then further food consumption is reduced, a phenomenon known as dehydration-induced anorexia that could be observed in young patients with congenital nephrogenic diabetes insipidus [40].

Altogether, these new data explain the speed of thirst satiation, the fact that oral cooling is thirst-quenching and the widespread coordination of eating, drinking and

vasopressin release.

### ***Osmosensitive cells shrinking during dehydration is mechanically coupled to the activation of delta-N TRPV1 channels***

All cells respond to dehydration or to hyperhydration by changing volume, but cells of the subfornical organ (SFO), organum vasculosum of the lamina terminalis (OVLT), and median preoptic nucleus (MnPO) of the hypothalamus are “perfect” osmoreceptors, that is, their changes in volume are maintained as long as the osmotic stimulus persists [21, 27]. Cell shrinking during dehydration is mechanically coupled to the activation of delta-N TRPV1 channels [36] (a molecular co-detector of body temperature and osmotic stress) through a densely interweaved microtubule network present only in osmosensitive cells (**Figure 5B**) [27], including excitatory thirst neurons from the SFO bearing angiotensin-I receptors.. This coupling allows dehydration and decreased systemic volume stimuli to be integrated because SFO neurons are outside of the blood-brain barrier [33]. Systemic hypotonicity might be perceived by TRPV4 channels [41].

## Copeptin measurement as a surrogate marker for AVP release

With the development of immunoassay for several other pituitary hormones in the early 1970s, measurement of AVP became an obvious goal. The first AVP assay was developed by Robertson’s group [42]. Like most other AVP assays [43], it was a competitive radioimmunoassay (RIA). However, until today, AVP measurement remains cumbersome and complex. AVP can be measured only in a few specialized

laboratories, and the data generated using existing assays have not convinced the clinical community to use AVP in their diagnostic workup studies.

## ***Measurement of Copeptin as alternative to AVP***

The discovery of the first prohormone by Donald Steiner in 1967 (see review in [44]) and the successful use of the C-peptide of the insulin precursor as a surrogate marker for insulin release paved the way for a suitable alternative. The solution was to replace the problematic measurement of a bioactive, rapidly cleared peptide hormone like AVP by the measurement of another larger peptide derived from its precursor and showing a better stability *in vitro*. Due to their stoichiometric generation, the amounts of pro-hormone fragments released reflect those of the respective mature hormones, offering an alternative way to assess the release of the hormone. Applying this approach to the AVP precursor, the C-terminal glycopeptide, termed “copeptin” by Roger Acher [45], is the ideal “shadow” fragment reflecting AVP release (**Figure 7A**).

Several copeptin assays are now available. The only assays with sufficient technical description and clinical data justifying their routine clinical use are 1. the original sandwich immunoluminometric assay (LIA) as described previously [46] except that the capture antibody was replaced by a murine monoclonal antibody directed to amino acids 137–144 of pro-AVP, and 2. its automated immunofluorescent successor (on the KRYPTOR platform).

Both assays are CE certified and therefore approved for clinical use in the EU and some other countries accepting the CE mark. Other “*for research use only*” assays are available in the USA and China, and individual “home brew” assays are being developed by research groups. The disadvantage of these assays, besides not being approved or certified for patient care, lies in their lack of technical and clinical validation. As there is still no official reference calibration, it would seem reasonable to use only clinically approved and certified assays for patient care.

These are the advantages of copeptin measurement compared to AVP.

**- Sample volume:** the copeptin assay requires only 50 µL serum or plasma, whilst AVP assays need one or more ml of plasma.

**- Extraction:** no extraction step or other pre-analytical procedures such as the addition of protease inhibitors is needed.

**- Time to results:** results are available in approximately 0.5 to 2.5 h, whereas many of the competitive AVP immunoassays require more than 48 h due to extensive incubation steps and the need for antibody equilibrium.

**- Sensitivity:** As a sandwich immunoassay, it is more sensitive than competitive AVP immunoassays, as demonstrated by the analytical detection limit of < 1 pmol/L. The assay can detect copeptin in plasma or serum even when plasma osmolality is low, whereas AVP is often not detectable in plasma samples within the low physiological range of osmolality.

**- Stability:** Copeptin, unlike AVP, is very stable *ex vivo*. Recovery was greater than 80% in serum and plasma samples for at least 7 days at room temperature, and 14 days at 4°C (**Figure 7B**).

## ***Normal range of copeptin***

The normal range for copeptin is now well defined. Data from the first study with healthy volunteers, which was carried out without prior fluid control or fasting, indicated a median copeptin plasma concentration of 4.2 pmol/L (range, 1–13.8 pmol/L) [46]. Other studies confirmed this initial report: range of copeptin values between 1 and 13 pmol/L (upper 97.5 percentile) with median values < 5 pmol/L [47, 48]. Men consistently show higher values than women, but the difference in median values is only about 1 pmol/L. Higher concentrations of AVP in males than females had also been reported in several studies, although in smaller number of subjects (see revue in [49]).

Copeptin and AVP levels have been compared in 500 subjects (equal numbers of each sex) of a French population [50]. A highly significant correlation was found between copeptin and AVP in the 319 people in whom both compounds were above the detection limit. ($r = 0.686$, $p < 0.001$). The copeptin values were systematically

higher than those for vasopressin, and even more so in the low range of vasopressin [50].

## *Influence of plasma or serum osmolality*

In healthy subjects, copeptin (like mature AVP) is regulated within the normal range but may fluctuate according to physiological conditions. Copeptin increases towards higher values in the normal range during fasting, and declines rapidly *in vivo* towards low normal values after intake of water [46].

In a study of healthy volunteers, copeptin showed identical changes during disordered water states or osmolality as previously shown for AVP: water deprivation increased serum copeptin from 4.6±1.7 to 9.2±5.2 pmol/L ($p<0.0001$). Copeptin increased from 4.9±3.0 to 19.9±4.8 pmol/L ($p<0.0001$) with additional infusion of hypertonic saline. Conversely, copeptin decreased from 6.2±2.4 to 2.4±2.1 pmol/L ($p<0.01$) during hypotonic saline infusion [51] (**Figure 8A**).

A direct comparison between copeptin and AVP serum concentrations in relationship to serum osmolality in healthy subjects was performed using the AVP assay established by Gary Robertson [52]. This comparison showed a stronger correlation between copeptin and serum osmolality ($r = 0.77$) than between AVP and serum osmolality ($r = 0.49$).

## *Influence of blood pressure*

The effect of experimental hemorrhagic shock on copeptin was studied in a small number of baboons [53]. After induction of hemorrhagic shock, median copeptin increased sharply from 7.5 to 269 pmol/L. Copeptin dropped after one hour of reperfusion and continued to decline until it reached a plateau of 24 pmol/L at the end of the experiment. The mean arterial blood pressure followed inverse kinetics in all animals, decreasing during bleeding, and increasing slowly after reperfusion (**Figure 8B**) [53]. This demonstrates that the response of copeptin to critical conditions like shock is much more pronounced than to changes in osmolality.

### ***Influence of exercise***

In healthy individuals, serum copeptin concentration in the blood increased during exercise, but did not exceed the 99th percentile of the normal range [46]. The situation is different in patients with cardiovascular disease. In patients with a history of angina pectoris undergoing a diagnostic treadmill exercise test, serum copeptin can increase to levels far beyond the normal range [54].

### ***Conclusion: copeptin for the clinician***

The evidence to date shows that copeptin is a good surrogate marker for AVP release, and measuring copeptin is both practical and easy. Copeptin is now used in different clinical situations in which its measurement has helped in the diagnosis of disease or in management of the patients. This is reflected in the increasing number of copeptin publications since the development of the copeptin assay, and some of these studies will be presented in this special issue of JIM.

## ACKNOWLEDGEMENTS

None from any of the three authors

# Figure Legends

**Figure 1. The different target sites for vasopressin via its V2, V1a and V1b receptors. A.** In addition to its classical target organs, the kidney (with V2 receptors) and vascular smooth muscle cells (with V1a receptors), many other organs express receptors for vasopressin. In some organs, both V2 and V1a receptors are expressed, however in different cell types. **B. Localisation of vasopressin binding sites in the rat kidney and liver with selective V2 or V1a ligands.** *Top right* : binding of the $^{125}$I-labelled V2 receptor selective agonist dDAVP. Autoradiogram reproduced from a cover picture of Kidney Int (2009, 76(2) with permission of the authors, Robert C. Speth (College of Pharmacy, Nova Southeastern University, Fort Lauderdale, FL, USA) and Jia L. Zhuo (University of Mississipi Medical Center, Jackson, MS, USA). *Top left and bottom* : binding of the $^{3}$H-labelled V1a receptor selective antagonist SR49059. Adapted from [7].

**Figure 2. Vasopressin actions along the nephron and their consequences on water and solute handling. A**. Representative nephron showing the different transporters/channels influenced by vasopressin action through V2 receptors. Modified after [11]. **B**. With increasing urine osmolality, the fractional excretion of sodium and urea decline in parallel with urine flow rate. Results observed in normal rats, based on 24 h urine collections and measurement of glomerular filtration rate by inulin clearance. Data of individual rats from the study reported in [14].

**Figure 3. Hyperfiltration as a consequence of vasopressin V2 receptor-mediated action on the kidney. A and B**. Glomerular filtration rate (GFR) was measured by inulin clearance in healthy subjects on two occasions at a two week interval in random order: once with a high hydration (HH) and once with a low (normal) hydration (LH). In every subject, GFR was higher on the LH than on the HH condition (**A**). A significant correlation was observed between GFR and urine osmolality in the HH but not in the LH condition (**B**). Adapted from [15]. **C**. In 74 normal rats with different levels of diuresis (during usual short term clearance experiments under anesthesia), GFR is positively and linearly

correlated with urine osmolality. Reproduced from [16]. **D**. In normal rats in which urine concentration was either decreased or increased for one week (by increasing water intake or infusing dDAVP, respectively), GFR is positively and linearly correlated with urine osmolality. Adapted from [14].

**Figure 4. The multiple effects of vasopressin on the kidney and liver and their possible adverse consequences.**

**Figure 5. Cell autonomous osmoreception in vasopressin neurons**.

**A. Changes in osmolality cause inversely proportional changes in soma volume.** Shrinkage activates delta-N transient receptor vanilloid-type (TRPV1) channels. The ensuing depolarization increases the firing rate of action potential and vasopressin (VP) release from axon terminals in the neurohypophysis. Increased VP levels in blood enhance water reabsorption by the kidney (antidiuresis) to restore extracellular fluid osmolality toward the set point. Hypotonic stimuli inhibit TRPV1. The resulting hyperpolarization and inhibition of firing reduces VP release and promotes diuresis. Modified from [27]. **B. Shrinking of hypothalamic osmoreceptor neurons during dehydration is mechanically coupled to the activation of delta-N TRPV1 channels**. As a result of cell shrinking, the plasma membrane shifts inward (right), increasing the proportion of microtubules that push onto (and activate) delta-N Trpv1 channels [36]. Reproduced with permission from [36].

**Figure 6. Anticipatory thirst and central control of volemia.** Neurons in the subfornical organ (SFO) mediate anticipatory thirst (pathway indicated with blue arrows). SFO neurons are activated when mice are dehydrated. This activity is almost immediately inhibited by drinking, owing to unknown signals that stem from the oral cavity, and which might act through the trigeminal ganglion. Other extero-sensory stimulation, implicating oesophageal or gastric osmosensor, are not represented. SFO neurons project to other median-pre-optic (MnPO) and organum vasculosum of the lamina terminalis (OVLT) circumventricular nuclei and to vasopressin producing neurons in the SFO and para-ventricular nuclei (PVN) [33]. Dendritic release of vasopressin in the PVN is perceived by vasopressin V1a receptors on pre-autonomic neurons with consequent

stimulation of renal afferents, a central control of volemia [34]: the lamina terminalis and autonomic nervous system are separated by just two synapses: excitatory neurons in the lamina terminalis project to neurons in the PVN which, in turn, send descending projections to autonomic regions of the hindbrain and spinal cord. PP = posterior pituitary; ANS = autonomic nervous system. Modified from [35].

**Figure 7. Principle of prohormone processing and the copeptin assay and stability of copeptin measurement. A.** The sandwich immunoassay uses two antibodies to the amino acid sequence 132–164 of preprovasopressin in the C terminal region of the precursor. This assay offers considerable advantages over measuring AVP. **B.** Good *ex vivo* stability of copeptin in serum and plasma at room temperature [46].

**Figure 8. Changes in copeptin concentration as influenced by water intake, hypo- or hypertonic saline infusion, or bleeding. A.** In healthy volunteers, plasma copeptin increased or decreased after infusion of hypotonic or hypertonic saline infusion, respectively. Here, copeptin followed the well-established pattern of AVP. Modified after [51]. **B. Plasma copeptin concentration and mean arterial pressure (MAP) in four baboons before, during, and after hemorrhagic shock.** The baboons were anesthetized and placed on a ventilator. They were then bled down to a MAP of 40 mmHg in two stages. During the first hour of resuscitation, the MAP was brought to 100 mmHg by the infusion of 25% of the shed blood plus Ringer solution, and during the third hour of resuscitation, an additional 25% of the shed blood plus Ringer solution was administered to raise the MAP to baseline levels. The total duration in hours and the time points of blood draw during bleeding and reperfusion are indicated. R0 is the time point after 3 h of bleeding and immediately before reperfusion was started. Reproduced from [53].

# References


1 Robertson GL. Antidiuretic hormone. Normal and disordered function. *Endocrinology and metabolism clinics of North America* 2001; **30:** 671-94, vii.

2 Bockenhauer D, Bichet DG. Urinary concentration: different ways to open and close the tap. *Pediatric nephrology (Berlin, Germany)* 2014; **29:** 1297-303.

3 Robertson GL. The regulation of vasopressin function in health and disease. *Recent progress in hormone research* 1976; **33:** 333-85.

4 Antoni FA. Vasopressin as a Stress Hormone. In: Fink G, ed, *Stress: Neuroendocrinology and Neurobiology*: Academic Press,. 2015; 97-108.

5 Zerbe RL, Miller JZ, Robertson GL. The reproducibility and heritability of individual differences in osmoregulatory function in normal human subjects. *The Journal of laboratory and clinical medicine* 1991; **117:** 51-9.

6 Thibonnier M, Coles P, Thibonnier A, Shoham M. Molecular pharmacology and modeling of vasopressin receptors. *Prog Brain Res* 2002; **139:** 179-96.

7 Serradeil-Le Gal C, Raufaste D, Marty E, Garcia C, Maffrand JP, Le Fur G. Autoradiographic localization of vasopressin V1a receptors in the rat kidney using [3H]-SR 49059. *Kidney international* 1996; **50:** 499-505.

8 Perucca J, Bichet DG, Bardoux P, Bouby N, Bankir L. Sodium excretion in response to vasopressin and selective vasopressin receptor antagonists. *Journal of the American Society of Nephrology : JASN* 2008; **19:** 1721-31.

9 Andersen LJ, Andersen JL, Schutten HJ, Warberg J, Bie P. Antidiuretic effect of subnormal levels of arginine vasopressin in normal humans. *Am J Physiol* 1990; **259:** R53-60.

10 Verbalis JG. AVP receptor antagonists as aquaretics: review and assessment of clinical data. *Cleve Clin J Med* 2006; **73 Suppl 3:** S24-33.

11 Bankir L, Bichet DG, Bouby N. Vasopressin V2 receptors, ENaC, and sodium reabsorption: a risk factor for hypertension? *American journal of physiology Renal physiology* 2010; **299:** F917-28.

12 Fenton RA. Essential role of vasopressin-regulated urea transport processes in the mammalian kidney. *Pflugers Arch* 2009; **458:** 169-77.

13 Bankir L. Antidiuretic action of vasopressin: quantitative aspects and interaction between V1a and V2 receptor-mediated effects. *Cardiovasc Res* 2001; **51:** 372-90.

14 Bouby N, Ahloulay M, Nsegbe E, Déchaux M, Schmitt F, Bankir L. Vasopressin increases GFR in conscious rats through its antidiuretic action. *J Am Soc Nephrol* 1996; **7:** 842-51.

15 Anastasio P, Cirillo M, Spitali L, Frangiosa A, Pollastro RM, De Santo NG. Level of hydration and renal function in healthy humans. *Kidney international* 2001; **60:** 748-56.

16 Bankir L, Ahloulay M, Bouby N, Trinh-Trang-Tan MM, Machet F, Lacour B, Jungers P. Is the process of urinary urea concentration responsible for a high glomerular filtration rate? *Journal of the American Society of Nephrology : JASN* 1993; **4:** 1091-103.

17 Bankir L, Bouby N, Ritz E. Vasopressin: a novel target for the prevention and retardation of kidney disease? *Nature reviews Nephrology* 2013; **9:** 223-39.

18 Bankir L, Roussel R, Bouby N. Protein- and diabetes-induced glomerular hyperfiltration: role of glucagon, vasopressin, and urea. *American journal of physiology Renal physiology* 2015; **309:** F2-23.

19 Clark WF, Sontrop JM, Huang SH, Moist L, Bouby N, Bankir L. Hydration and Chronic Kidney Disease Progression: A Critical Review of the Evidence. *American journal of nephrology* 2016; **43:** 281-92.

20 Wang CJ, Grantham JJ, Wetmore JB. The medicinal use of water in renal disease. *Kidney international* 2013; **84:** 45-53.

21 Bourque CW. Central mechanisms of osmosensation and systemic osmoregulation. *Nature reviews Neuroscience* 2008; **9:** 519-31.

22 Edwards AM, Mann ME, Marfell-Jones MJ, Rankin DM, Noakes TD, Shillington DP. Influence of moderate dehydration on soccer performance: physiological responses to 45 min of outdoor match-play and the immediate subsequent performance of sport-specific and mental concentration tests. *British journal of sports medicine* 2007; **41:** 385-91.

23 Saat M, Sirisinghe RG, Singh R, Tochihara Y. Effects of short-term exercise in the heat on thermoregulation, blood parameters, sweat secretion and sweat composition of tropic-dwelling subjects. *Journal of physiological anthropology and applied human science* 2005; **24:** 541-9.

24 Shirreffs SM, Merson SJ, Fraser SM, Archer DT. The effects of fluid restriction on hydration status and subjective feelings in man. *The British journal of nutrition* 2004; **91:** 951-8.

25 Geelen G, Greenleaf JE, Keil LC. Drinking-induced plasma vasopressin and norepinephrine changes in dehydrated humans. *The Journal of clinical endocrinology and metabolism* 1996; **81:** 2131-5.

26 Andersen LJ, Jensen TU, Bestle MH, Bie P. Gastrointestinal osmoreceptors and renal sodium excretion in humans. *American journal of physiology Regulatory, integrative and comparative physiology* 2000; **278:** R287-94.

27 Prager-Khoutorsky M, Bourque CW. Mechanical basis of osmosensory transduction in magnocellular neurosecretory neurones of the rat supraoptic nucleus. *Journal of neuroendocrinology* 2015; **27:** 507-15.

28 Zimmerman CA, Lin YC, Leib DE*, et al.* Thirst neurons anticipate the homeostatic consequences of eating and drinking. *Nature* 2016; **537:** 680-4.

29 Mandelblat-Cerf Y, Kim A, Burgess CR, Subramanian S, Tannous BA, Lowell BB, Andermann ML. Bidirectional Anticipation of Future Osmotic Challenges by Vasopressin Neurons. *Neuron* 2017; **93:** 57-65.

30 Claybaugh JR, Sato AK, Crosswhite LK, Hassell LH. Effects of time of day, gender, and menstrual cycle phase on the human response to a water load. *American journal of physiology Regulatory, integrative and comparative physiology* 2000; **279:** R966-73.

31 Watts AG. Great Expectations: Anticipatory Control of Magnocellular Vasopressin Neurons. *Neuron* 2017; **93:** 1-2.

32 Leib DE, Zimmerman CA, Knight ZA. Thirst. *Current biology : CB* 2016; **26:** R1260-r5.

33 Oka Y, Ye M, Zuker CS. Thirst driving and suppressing signals encoded by distinct neural populations in the brain. *Nature* 2015; **520:** 349-52.

34 Son SJ, Filosa JA, Potapenko ES*, et al.* Dendritic peptide release mediates interpopulation crosstalk between neurosecretory and preautonomic networks. *Neuron* 2013; **78:** 1036-49.

35 Bichet DG. Vasopressin at Central Levels and Consequences of Dehydration. *Annals of nutrition & metabolism* 2016; **68 Suppl 2:** 19-23.

36 Zaelzer C, Hua P, Prager-Khoutorsky M, Ciura S, Voisin DL, Liedtke W, Bourque CW. DeltaN-TRPV1: A Molecular Co-detector of Body Temperature and Osmotic Stress. *Cell reports* 2015; **13:** 23-30.

37 Egan G, Silk T, Zamarripa F*, et al.* Neural correlates of the emergence of consciousness of thirst. *Proceedings of the National Academy of Sciences of the United States of America* 2003; **100:** 15241-6.

38 Saker P, Farrell MJ, Adib FR, Egan GF, McKinley MJ, Denton DA. Regional brain responses associated with drinking water during thirst and after its satiation. *Proceedings of the National Academy of Sciences of the United States of America* 2014; **111:** 5379-84.

39 Kageyama K, Yamagata S, Akimoto K, Sugiyama A, Murasawa S, Suda T. Action of glucagon-like peptide 1 and glucose levels on corticotropin-releasing factor and vasopressin gene expression in rat hypothalamic 4B cells. *Molecular and cellular endocrinology* 2012; **362:** 221-6.

40 Bockenhauer D, Bichet DG. Nephrogenic diabetes insipidus. *Current opinion in pediatrics* 2017; **29:** 199-205.

41 Lechner SG, Markworth S, Poole K*, et al.* The molecular and cellular identity of peripheral osmoreceptors. *Neuron* 2011; **69:** 332-44.

42 Robertson GL, Mahr EA, Athar S, Sinha T. Development and clinical application of a new method for the radioimmunoassay of arginine vasopressin in human plasma. *The Journal of clinical investigation* 1973; **52:** 2340-52.

43 Kluge M, Riedl S, Erhart-Hofmann B, Hartmann J, Waldhauser F. Improved extraction procedure and RIA for determination of arginine8-vasopressin in plasma: role of premeasurement sample treatment and reference values in children. *Clinical chemistry* 1999; **45:** 98-103.

44 Steiner DF. On the discovery of precursor processing. *Methods in molecular biology (Clifton, NJ)* 2011; **768:** 3-11.

45 Levy B, Chauvet MT, Chauvet J, Acher R. Ontogeny of bovine neurohypophysial hormone precursors. II. Foetal copeptin, the third domain of the vasopressin precursor. *International journal of peptide and protein research* 1986; **27:** 320-4.

46 Morgenthaler NG, Struck J, Alonso C, Bergmann A. Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin. *Clinical chemistry* 2006; **52:** 112-9.

47 Bhandari SS, Loke I, Davies JE, Squire IB, Struck J, Ng LL. Gender and renal function influence plasma levels of copeptin in healthy individuals. *Clinical science (London, England : 1979)* 2009; **116:** 257-63.

48 Keller T, Tzikas S, Zeller T*, et al.* Copeptin improves early diagnosis of acute myocardial infarction. *Journal of the American College of Cardiology* 2010; **55:** 2096-106.

49 Perucca J, Bouby N, Valeix P, Bankir L. Sex difference in urine concentration across differing ages, sodium intake, and level of kidney disease. *American journal of physiology Regulatory, integrative and comparative physiology* 2007; **292:** R700-5.

50 Roussel R, Fezeu L, Marre M*, et al.* Comparison between copeptin and vasopressin in a population from the community and in people with chronic kidney disease. *The Journal of clinical endocrinology and metabolism* 2014; **99:** 4656-63.

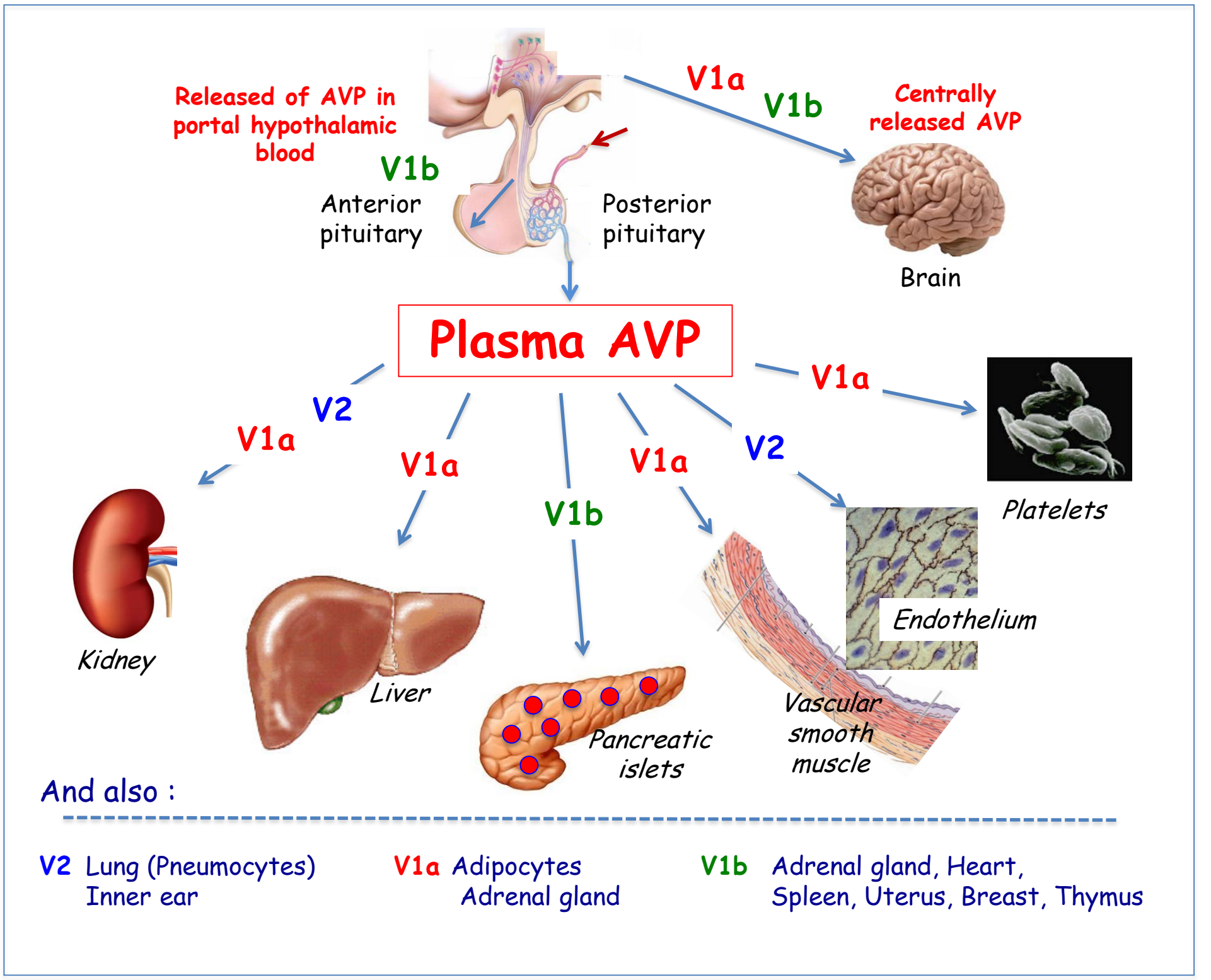
51 Szinnai G, Morgenthaler NG, Berneis K, Struck J, Muller B, Keller U, Christ-Crain M. Changes in plasma copeptin, the c-terminal portion of arginine vasopressin during water deprivation and excess in healthy subjects. *The Journal of clinical endocrinology and metabolism* 2007; **92:** 3973-8.

52 Balanescu S, Kopp P, Gaskill MB, Morgenthaler NG, Schindler C, Rutishauser J. Correlation of plasma copeptin and vasopressin concentrations in hypo-, iso-, and hyperosmolar States. *The Journal of clinical endocrinology and metabolism* 2011; **96:** 1046-52.

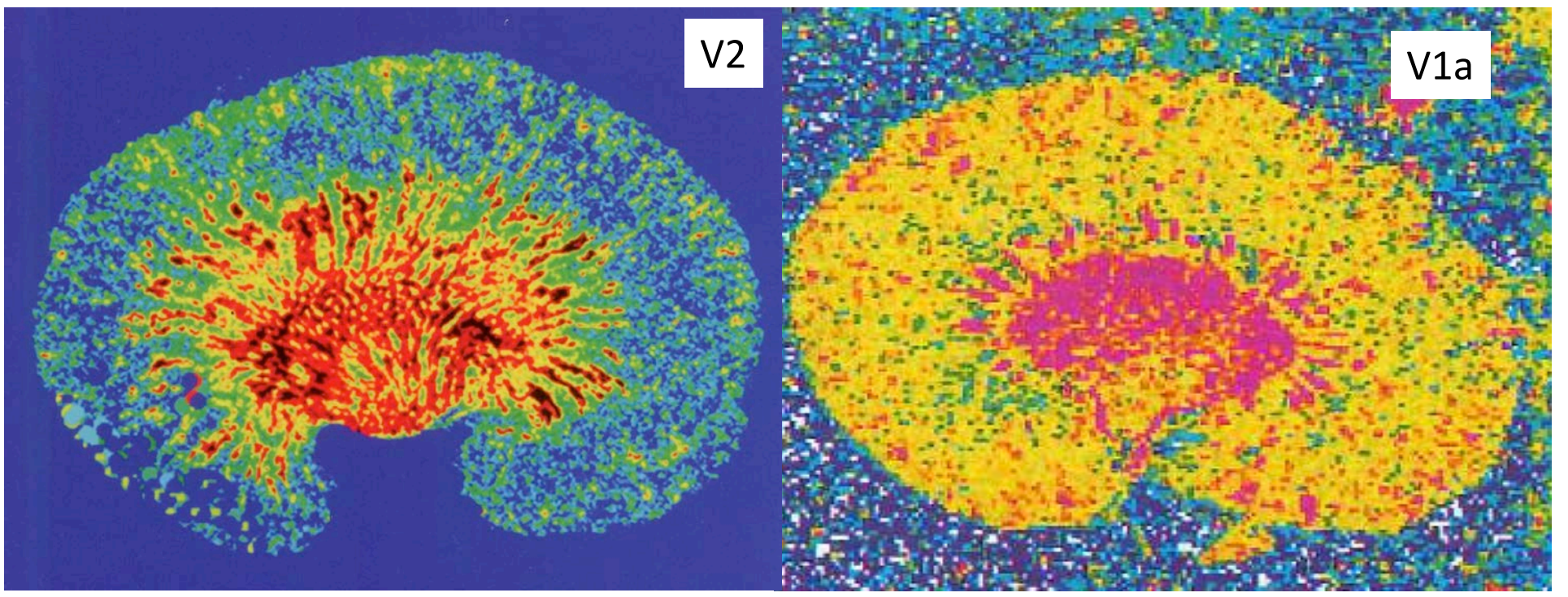
53 Morgenthaler NG, Muller B, Struck J, Bergmann A, Redl H, Christ-Crain M. Copeptin, a stable peptide of the arginine vasopressin precursor, is elevated in hemorrhagic and septic shock. *Shock (Augusta, Ga)* 2007; **28:** 219-26.

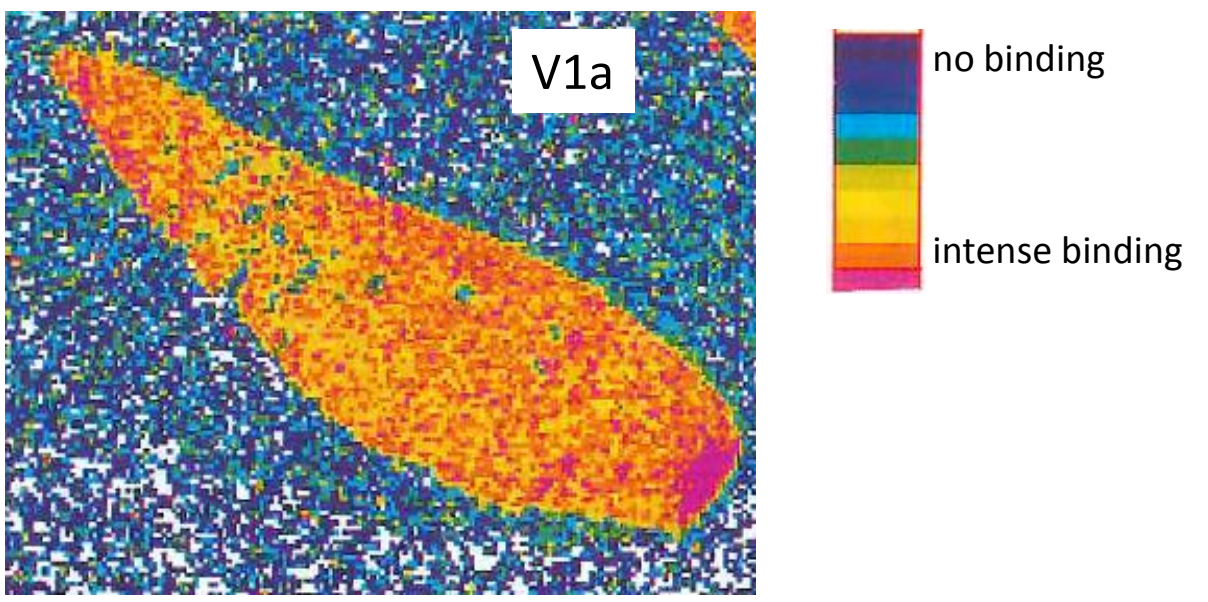
54 Staub D, Morgenthaler NG, Buser C*, et al.* Use of copeptin in the detection of myocardial ischemia. *Clinica chimica acta; international journal of clinical chemistry* 2009; **399:** 69-73.

A


Released of AVP in portal hypothalamic blood
V1b
Anterior pituitary
Posterior pituitary
V1a
V1b
Centrally released AVP
Brain
Plasma AVP
V1a
V2
V1a
V1a
V1b
V1a
V2
Platelets
Endothelium
Kidney
Liver
Pancreatic islets
Vascular smooth muscle
And also :
V2 Lung (Pneumocytes)
Inner ear
V1a Adipocytes
Adrenal gland
V1b Adrenal gland, Heart,
Spleen, Uterus, Breast, Thymus

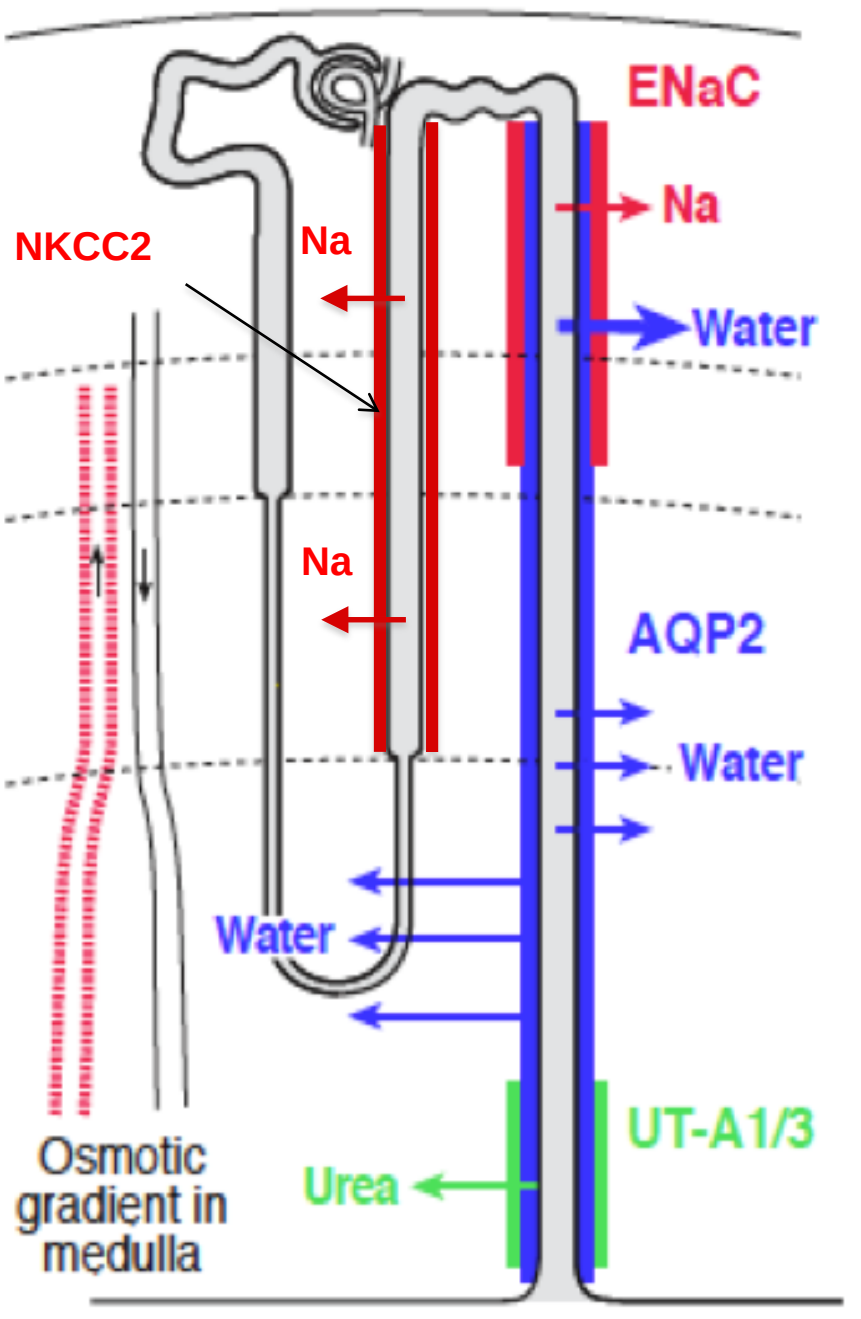
B


V2
V1a

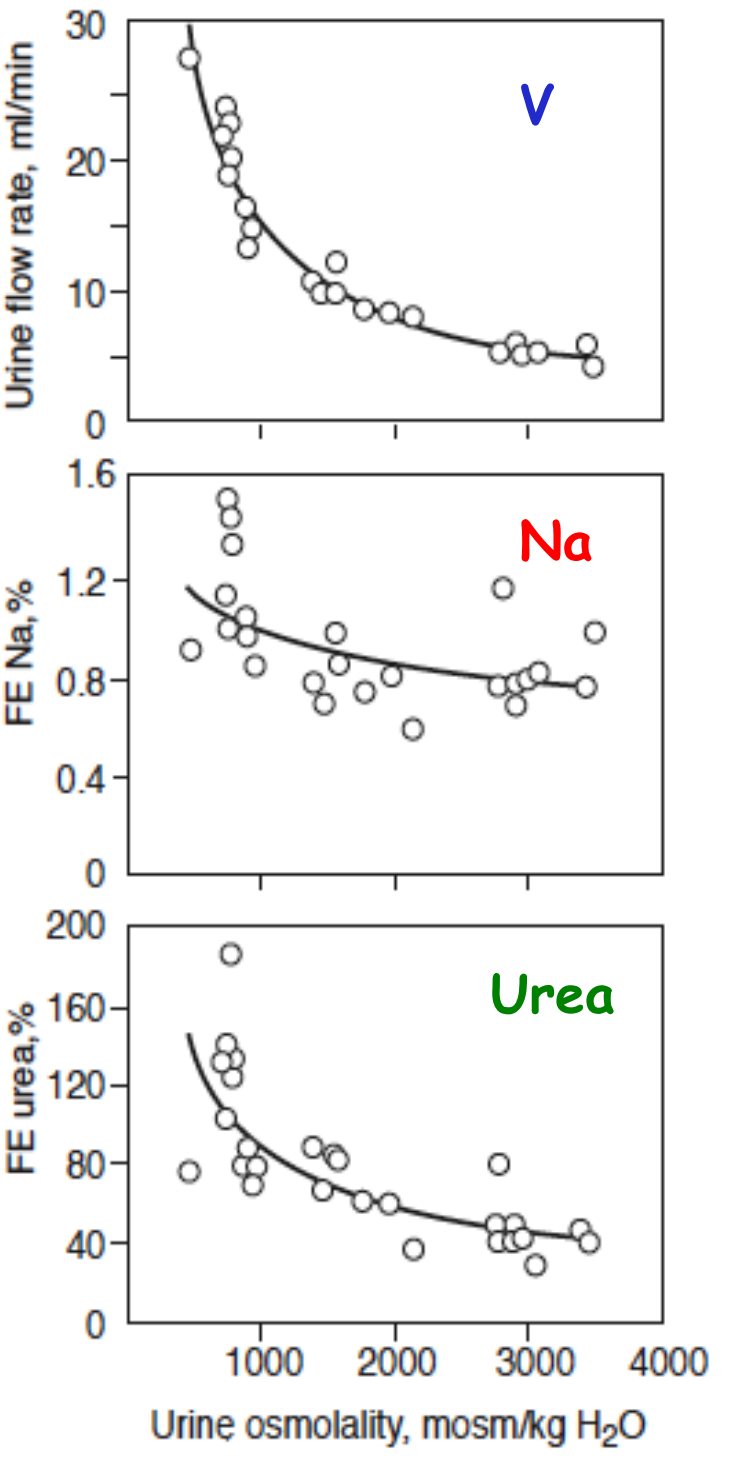

V1a
no binding
intense binding

Bankir et al, Figure 2

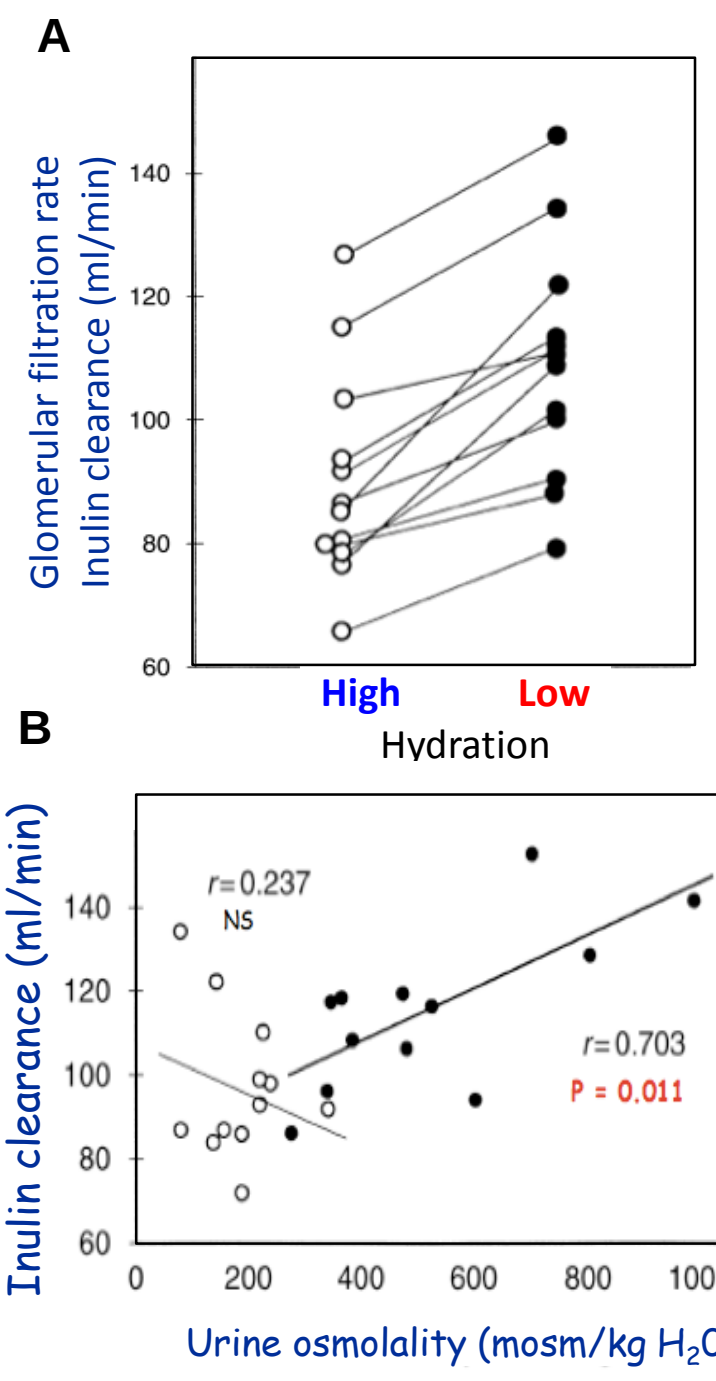
A



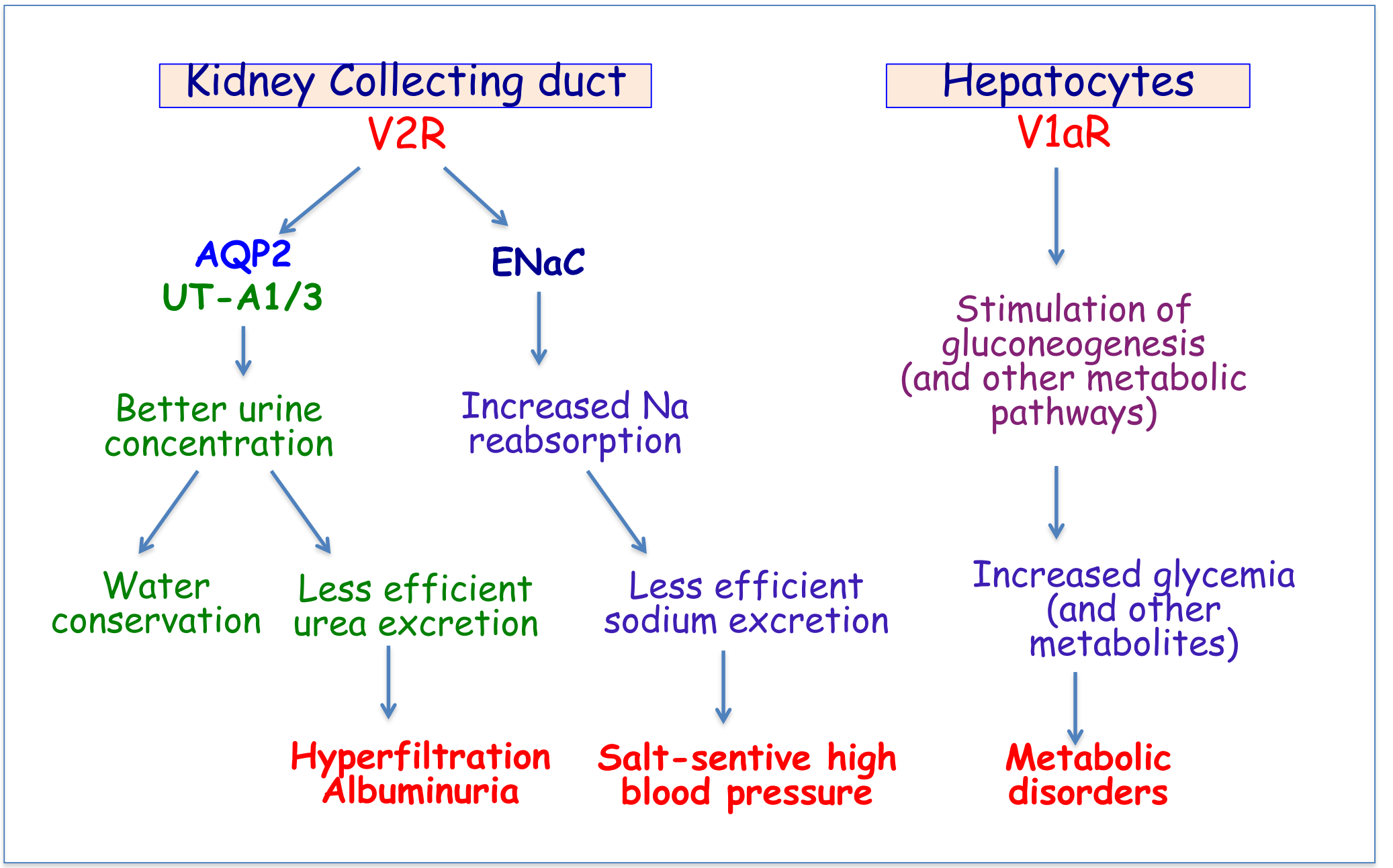

B



Bankir et al, Figure 3

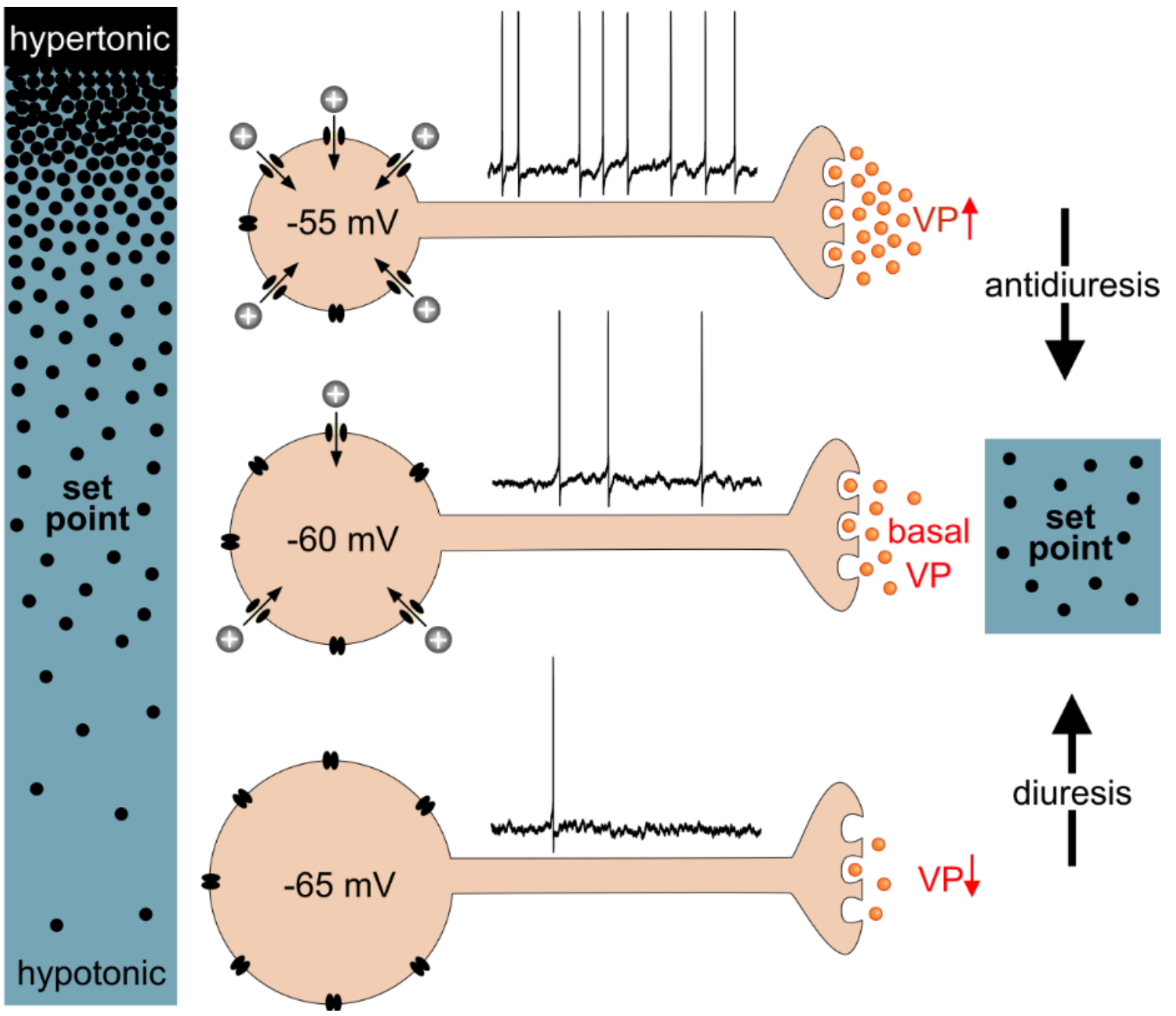


Bankir et al, Figure 4

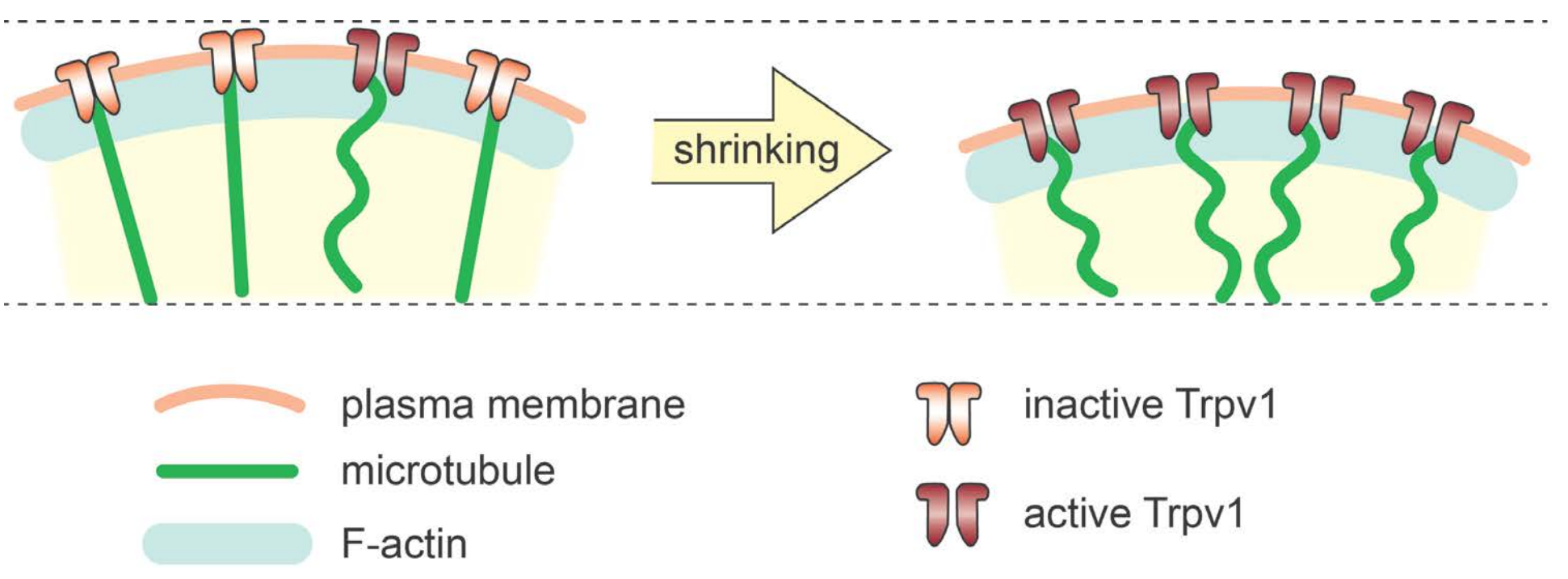


Bankir et al, Figure 5

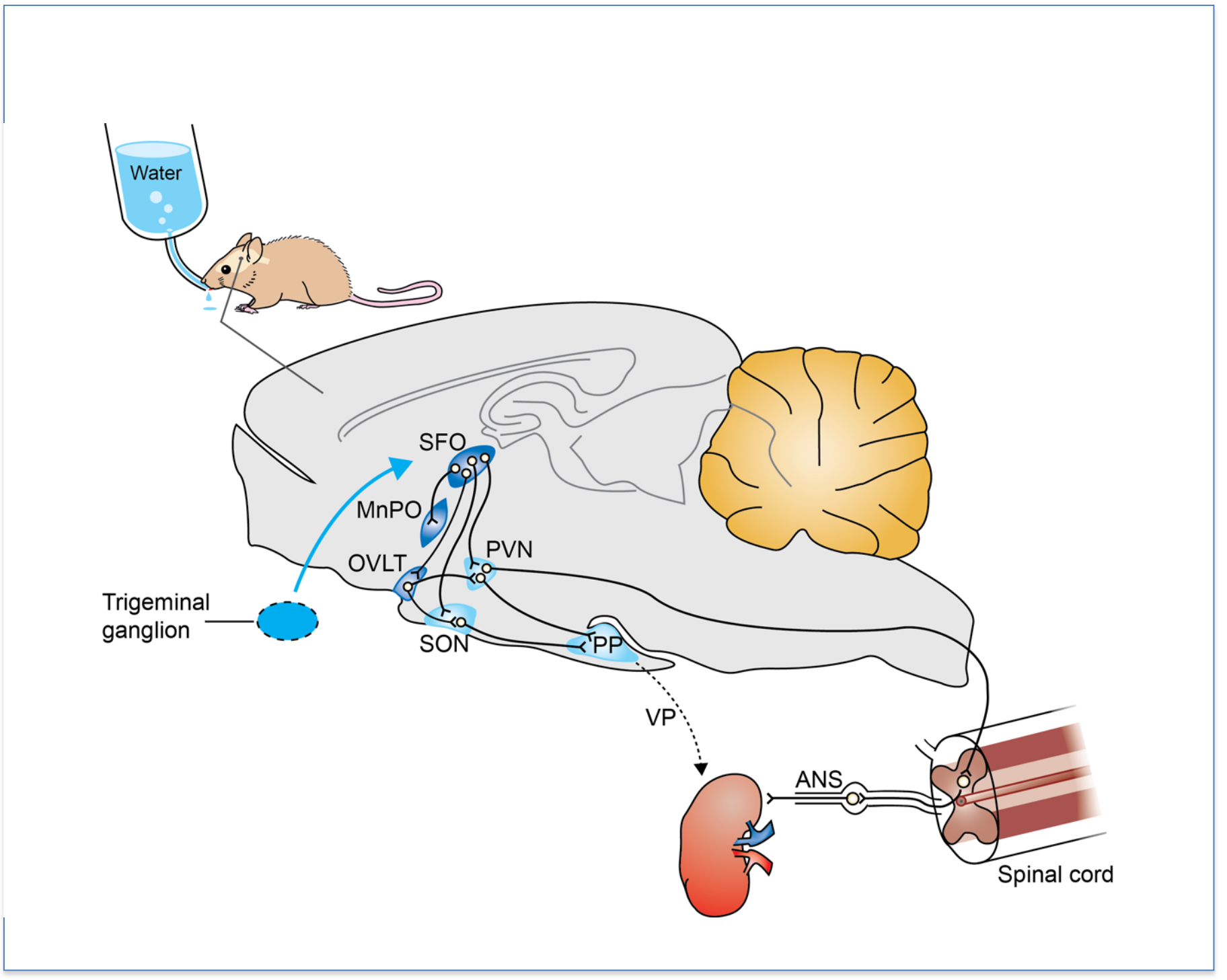
A




B

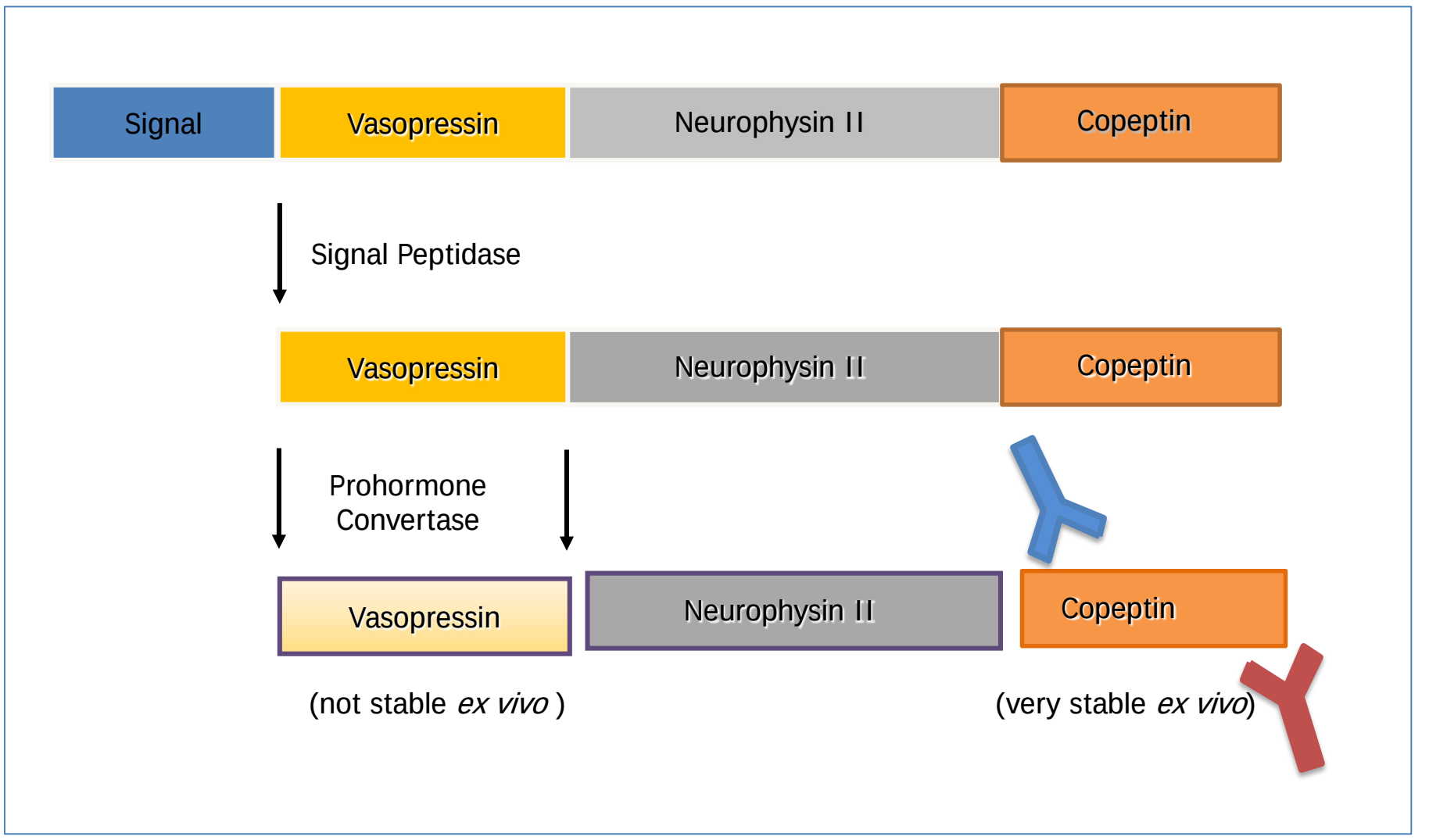


Bankir et al, Figure 6

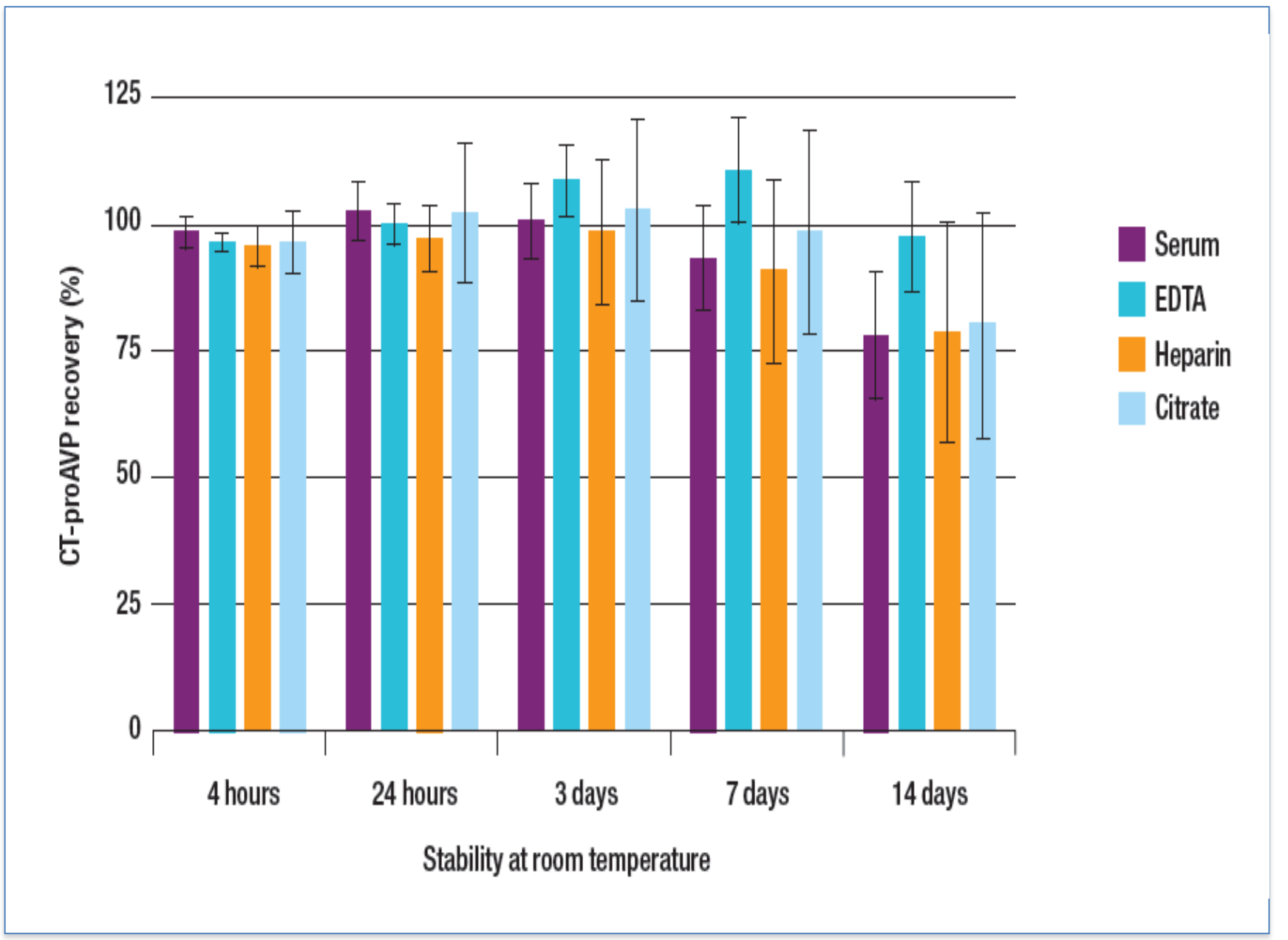


Bankir et al, Figure 7

A

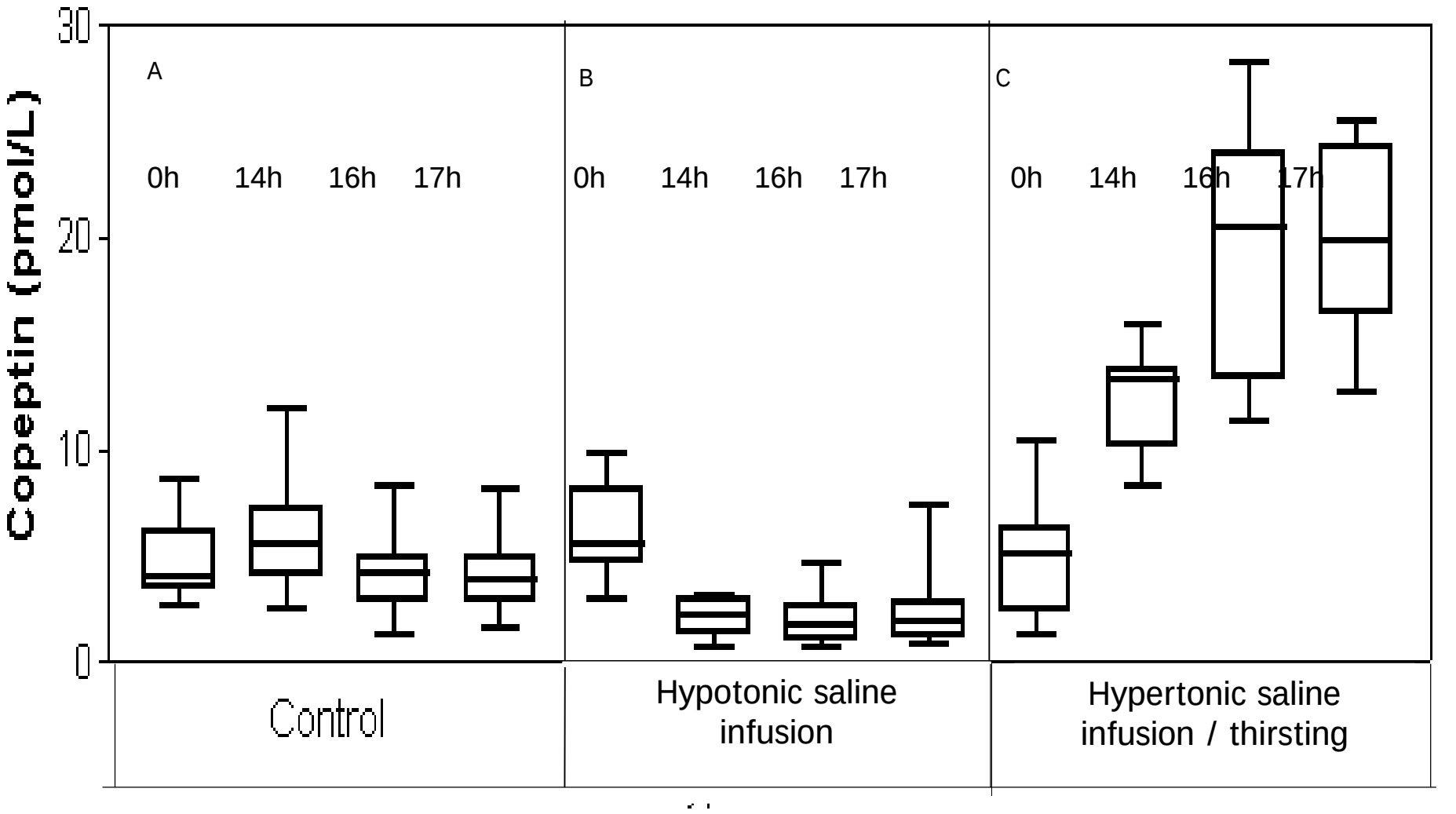



B



Bankir et al, Figure 8

A




B