Kidney International, Vol. 31 (1987), pp. 611–620

Physiological control of the urinary concentrating mechanism by peptide hormones

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Since Wirz's study [1] the mechanisms by which the kidney produces concentrated or dilute urine have been the object of much theoretical and experimental work. In the sixties, proof of the presence of a counter current concentrating process was progressively established. They led to a coherent idea of the functioning of the system under steady state conditions: the loops of Henle, especially the thick ascending limbs, carry out the necessary osmotic work of maintaining the longitudinal osmotic pressure gradient and, in the presence of ADH (antidiuretic hormone), the final concentration process is ensured along the collecting ducts by osmotic equilibration with the surrounding medium. Several aspects of the urinary concentrating mechanism, however, are not well understood. For instance, the exact way in which the longitudinal osmotic pressure gradient, which adjusts the urine osmolality, is established or suppressed, is unknown. Knowledge of the functional properties of the various nephron segments, although considerably advanced by in vitro physiological and biochemical studies of isolated tubules, which throw light on the potentialities of each segment, is still inadequate to explain the integrated functioning of all the mechanisms concerned. However, research in this field has recently derived a renewed stimulus from in vivo studies of the endocrine factors affecting tubular transport.

These in vivo studies were based on the discovery by Morel et al [2] that parathyroid hormone (PTH), calcitonin (CT), and glucagon stimulate the same cyclase pool in the cortical portion of the thick ascending limb (TALH), and that ADH, CT, and glucagon act similarly on the medullary portion of TALH. In other words, in both portions (which are homogeneous at the cell level), these hormones should all induce adenylate–cyclase activation in common target cells. Since the cell type determines the nature of the response, these authors predicted that these hormones should induce in a given portion the same physiological response.

From this conclusion, the need to develop a new approach to in vivo investigations of the renal effect of peptide hormones became evident [3]. To explore the effect of one of these hormones, it is not sufficient to abolish its presence in the circulating blood and then to administer it under appropriate conditions. It is also essential to eliminate the other hormones from the blood that could maintain the cyclase activity of the cells involved. We therefore created a model, the so-called "hormone–deprived" model, to satisfy these new requirements [3, 4]. Rats with hereditary diabetes insipidus which lack completely vasopressin production (Brattleboro strain) were used. They were acutely thyroparathyroidectomized to abolish PTH and CT and were infused with glucose or somatostatin to inhibit glucagon secretion.

In this review, we shall describe first the exact nature of the effects elicited in the superficial nephrons by each of the four hormones acting on the cyclase of the TALH. Subsequently, the effects of each of these hormones on the function of the long–looped nephrons will be examined. Then the physiological significance of the multiple hormonal control of the urinary concentrating mechanism will be discussed. It is important to note that in all experiments, the effects of plasma concentrations of peptide hormones similar to those obtained during stimulation of endogenous secretion of the respective hormone (so called "physiological doses") were studied.

Proximal tubule and loop of Henle of superficial nephrons

Early distal and late accessible proximal convolutions belonging to the same nephron were punctured in hormone–deprived (HD) rats and in HD rats receiving one of the four hormones activating the cyclase of the TALH [3, 5, 6]. The single nephron glomerular filtration rate (SNGFR) and the filtered load of electrolytes were almost unchanged by dDAVP (a synthetic analog of ADH devoid of pressure effects) and by PTH but, as expected, were significantly increased by human calcitonin (HCT) and glucagon, both of which also increase the glomerular filtration rate of the whole kidney. Neither dDAVP nor glucagon modified water and electrolyte reabsorption by the proximal tubule, which is in agreement with the lack of vasopressin or glucagon–sensitive adenylate–cyclase in that segment [7, 8]. PTH, in the dose (5 mU/min 100 g body wt) chosen to avoid pharmacological effects, did not greatly influence electrolyte transport rates along the proximal convoluted tubule [6]. With such an infusion rate of PTH, the most marked effect on phosphate transport was found in the loop, which confirms the demonstration that the pars recta is the major site of action of PTH on phosphate transport [9]. On the other hand, HCT slightly decreased fractional reabsorption of water and NaCl along the proximal tubule, as observed by Poujeol et al with salmon calcitonin [10]. It is possible that this effect of calcitonin does not involve the adenylate–cyclase system in the proximal tubule, since the proximal cyclase is stimulated in the mouse

Received for publication July 9, 1986

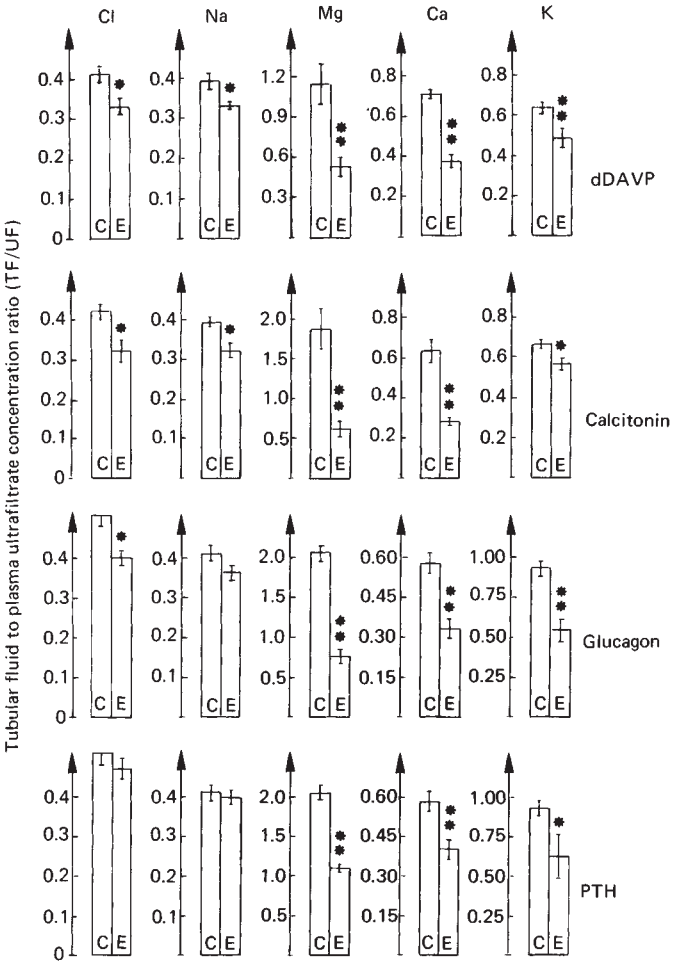

Fig. 1. *Effects of 1-desamino-8-D arginine vasopressin (dDAVP), calcitonin, glucagon and PTH on the Cl, Na, Mg, Ca and K tubular–fluid–to–plasma ultrafiltrate concentration ratios (TF/UF) in the early distal tubules.* Experiments were performed in hormone–deprived rats (Brattleboro D.I. rats which were acutely thyroparathyroidectomized and infused with somatostatin to inhibit glucagon secretion) and in such rats receiving one of the lacking peptides. Administration rates of the peptides were (per 100 g body wt): dDAVP, 20 pg/min; human calcitonin, 1 mU/min; glucagon, 5 ng/min; PTH, 5 mU/min. Abbreviations and symbols are: (C) control; (E) experimental; (*) $P < 0.05$; (**) $P < 0.01$. Each column represents the mean value obtained from 5 to 6 different animals. Data from [5, 6, 12].

[11] but not in the rat [7, 8]. To summarize the glomerular and proximal tubular effects of the peptide hormones, the most important facts to remember are that the absolute amount of electrolytes, especially NaCl, delivered to the loop of Henle was essentially unaltered by dDAVP and PTH, and increased proportionately to the increase in SNGFR with glucagon and slightly more than proportionately with HCT.

At the early distal site, all four hormones reduced the concentration of K, Mg and Ca in tubular fluid, as a consequence of enhanced reabsorption in the loop of Henle. In addition, dDAVP and HCT also reduced the tubular fluid NaCl concentration at this site, whereas PTH was without effect (Fig. 1). That glucagon stimulates NaCl transport in the loop first escaped attention because both the fractional reabsorption of Na and Cl by the loop and the Na luminal concentration at the early distal site were not significantly altered by the hormone in hormone–deprived rats [6]. It was later recognized, however, that the glucagon–mediated increase of glomerular filtration rate and consequent absolute deliveries of water, Na, and Cl to the loop masked this effect [13]. For a given delivery of water, the Na concentration at the beginning of the distal tubule was significantly lower in the presence than in the absence of glucagon, indicating that the hormone elicited the same effects as dDAVP and HCT in the loop of Henle. Thus the administration of any of the missing hormones to these hormone–deprived rats enhanced the reabsorptive capacity of the loop to K, Ca and Mg. In addition dDAVP, HCT and glucagon, but not PTH, enhanced NaCl reabsorption. The localization to TALH in the loop of the hormone–mediated effects on electrolyte transport is facilitated by the fact that the TALH is the main segment involved in magnesium reabsorption [14], and is the sole segment with a cyclase system sensitive to all four hormones. Therefore, the tubular localization of these physiological processes is unequivocal, the TALH being the only possible candidate.

The cells of the medullary and cortical portions of the TALH, although possessing the same main morphological organization, differ somewhat in that they contain cyclases of distinct responsiveness to hormones and are very different in size—the cells of the cortical portion being narrower with a simpler ultrastructural organization than those of the medullary portion [15]. Since the specificity of the biological response to intracellular cyclic AMP generation depends on the characteristics of the cell itself, it is possible that the responses elicited by the hormones in the cortical and medullary TALH are different. If we assume, on the basis of physiological data, that the hormones induce the same physiological effects on their respective target sites, it seems probable that, in the rat, ADH, calcitonin and glucagon stimulate NaCl transport in the medullary TALH, and these three hormones and PTH stimulate Mg, Ca and K transport in the cortical TALH. The lack of effect of PTH on NaCl transport would suggest, but does not prove, that none of the hormones stimulate NaCl transport in the cortical TALH, since, as already mentioned, PTH acts only in the cortical TALH. Whether ADH, calcitonin and glucagon stimulate Mg, Ca, and K transport in both the cortical and medullary TALH would depend on the transport mechanisms of these three ions, a problem still unsolved. If the reabsorption of these ions is linked to the NaCl transport in the medullary TALH, the hormones might increase Mg, Ca, and K reabsorption.

In vitro microperfusion studies should help to clarify the respective functions of these two nephron segments. Few in vitro studies have been performed in the rat, however. In this species, it is known that ADH increases the positive transepithelial potential difference (PD) [16, 17] and the net chloride reabsorption flux in the medullary TALH [17]. The mouse and the rabbit are the two species most extensively studied by in vitro microperfusion techniques. Unfortunately, the cyclase responsiveness of the mouse and rabbit TALH differs in some respect from that of the rat [7, 8]. Such in vitro studies showed that in the mouse, ADH [16, 18] and glucagon [19] increase the transepithelial PD in the medullary TALH and ADH increases chloride reabsorption [18, 20]. In the mouse cortical TALH, however, ADH does not change the transepithelial PD [20]. In the rabbit, ADH [16] and calcitonin [21] exert no measurable

effect on the TALH PD. Calcitonin stimulates calcium transport in the medullary TALH, but is inactive in the cortical portion [21]. Parathyroid hormone stimulates calcium [21–24] and magnesium [24] transport in the cortical TALH, but not in the medullary TALH [23]. Although these results using the microperfusion technique are fragmentary, all of the hormonal effects on electrolyte transport thus far demonstrated agree with the general conclusions drawn from studies on hormone–deprived animals.

It is clear that the hormone–deprived model has greatly facilitated the discovery of the potential effects of each hormone. For example, in the presence of the other hormones that act on the same cells, the effect of vasopressin on the loop is too weak to be easily detectable by free–flow micropuncture techniques. Some years ago, Schnermann et al examined the possible effects of ADH on the Na and K transport by the loop of Henle in Brattleboro rats [25]. They failed to detect any effect. Neither could Field et al [26], more recently, detect any modification of the Na and K luminal concentration in the early distal tubule following ADH administration in Brattleboro rats. Reexamination of this question by in situ microperfusion of the loop of Henle indicated that administering ADH to intact Brattleboro rats decreased the early distal chloride concentration by only 4.6 ± 1.5 mEq/liter [17]. By contrast, in two studies carried out in hormone–deprived rats, ADH elicited a 20% fall in the chloride concentration at the early distal site, which is equivalent to a 10 mEq/liter decrease [3, 12]. That ADH may exert some effect on NaCl transport by the loop in intact DI Brattleboro rats suggests that the hormone can control the rate of NaCl transport in the TALH, even in the presence of the other hormones that act on the TALH cyclase.

Our studies suggest that two hormones besides ADH, namely calcitonin and glucagon, by virtue of the similarity of their action on NaCl transport in the loop of Henle, may participate in the control of the urinary concentrating mechanism.

Distal tubule of superficial nephrons and collecting ducts

The adenylate–cyclase system of the rat distal tubule is also sensitive to many hormones or agonists, including ADH, PTH, CT, glucagon, and isoproterenol [7, 8]. However, no additive experiments on the cyclase responsiveness have been made on this segment.

To determine the hormonal effects on the distal tubule, early and late accessible distal convolutions of the same superficial nephrons were punctured either in hormone–deprived rats or in such rats receiving one of the missing hormones [12, 27, 28]. These studies revealed that ADH elicited the expected increase in water permeability, whereas HCT, PTH and glucagon had no detectable effects.

Regarding electrolytes, all four hormones stimulated Ca transport and, except for dDAVP, Mg transport. HCT, PTH and dDAVP enhanced NaCl reabsorption, whereas glucagon had no effect. In the hormone–deprived rat, K secretion (net addition) along the distal tubule still persists. Human calcitonin inhibited K secretion, PTH and glucagon did not modify the amount of K delivered to the collecting duct, and dDAVP stimulated the net addition of K between early and late accessible distal convolutions.

Here again, the use of the hormone–deprived model made it possible to discover new physiological effects of peptide hormones on the portion of the distal tubule accessible to micropuncture. It is relevant to recall that in the intact Brattleboro rat, Field et al [26] failed to disclose any effect of ADH on K secretion in free–flow micropuncture studies. This could have been because the presence of the other hormones, especially calcitonin, partly masked the effect of ADH, and because AVP reduced the amount of water delivered to the distal tubule. When the authors microperfused an isotonic fluid, to maintain the same tubular fluid flow rate in early distal tubules in the presence and absence of ADH (which the free–flow micropuncture technique does not) a vasopressin mediated increase of K secretion was observed. In addition, in the TPTX Wistar rat, ADH enhanced Na but not Ca reabsorption [29]; it is possible that the Ca response was obscured by the presence of glucagon, which also stimulates Ca transport along the distal tubule.

Regarding the physiological significance and/or consequence of the hormonal control of distal tubule function, the following suggestions can be considered: 1) it is possible that two hormones other than ADH, namely PTH and calcitonin, may affect the urinary concentrating ability owing to their effect on NaCl reabsorption in the distal tubule. Indeed, when the permeability to water of the collecting ducts is increased by ADH, the effect of increased NaCl transport would enhance the amount of water to be reabsorbed in the cortex before the tubular fluid reached iso-osmolality at the cortico–medullary junction. Such a process would reduce the amount of water delivered to the medullary collecting duct, a reduction known to be a key factor in enhancing the urinary concentrating ability. 2) According to Jamison et al [30], K undergoes medullary recycling from the collecting ducts to the pars recta or descending thin limbs of the juxtamedullary (JM) nephrons. This hypothesis stipulates that the fraction of K remaining at the end descending limb will be dependent on the net potassium secretion rate in the nephron terminal segments. We may therefore anticipate that in hormone–deprived rats, the fraction of K remaining at the hairpin turn of the JM nephrons should be unchanged by either PTH or glucagon, increased by dDAVP, and decreased by human calcitonin.

Turning to the cortical collecting ducts, it was demonstrated that salmon CT and vasopressin produced non-additive effects on the cyclase activity [2]. The administration of salmon CT to hormone–deprived rats [4] reduced urinary flow and increased urinary osmolality (as also observed in Wistar rats [31]) as would be expected if CT was able to elicit the same effect on the cortical collecting duct as vasopressin, that is, an increase in the permeability to water. The physiological significance of this effect of calcitonin is unknown.

Loop of Henle of juxtamedullary nephrons

Relatively young (4 to 5 weeks old, weight range 60 to 90 g) hormone–deprived rats were used to perform micropuncture in Henle's loop of the juxtamedullary nephrons. The SNGFR of these nephrons did not vary significantly under the influence of any of the four hormones, administered at the same rate (per 100 g body wt) as in the preceding studies. Since the plasma electrolyte concentrations were virtually unaltered, the effects observed at the tip of the loops were necessarily of tubular origin. The phosphate delivery rate at the hairpin turn was increased by PTH, as expected from its effects on the pars

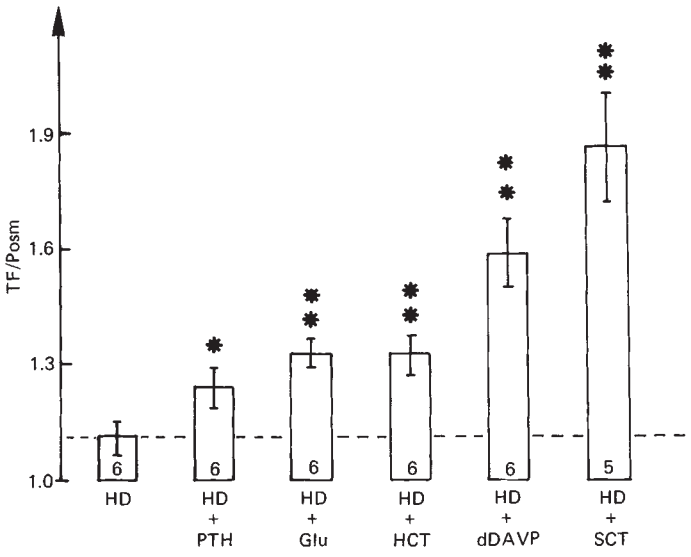

Fig. 2. *Tubular-fluid–to–plasma concentration ratios of total solutes (TF/P_{Osm}) at the tip of the loop of Henle of the juxtamedullary nephrons.* Experiments were performed in hormone-deprived (HD) rats or in HD rats receiving either PTH, glucagon, human calcitonin (HCT), dDAVP or salmon calcitonin (SCT). Administration rates of the peptides were as indicated in Fig. 1. SCT was administered at 2.5 mU/min. 100 g body wt. Numbers in the column indicate the number of rats studied. Significantly different: (*) $P < 0.05$; (**) $P < 0.01$ from HD (control) rats. Data from [4, 32–34].

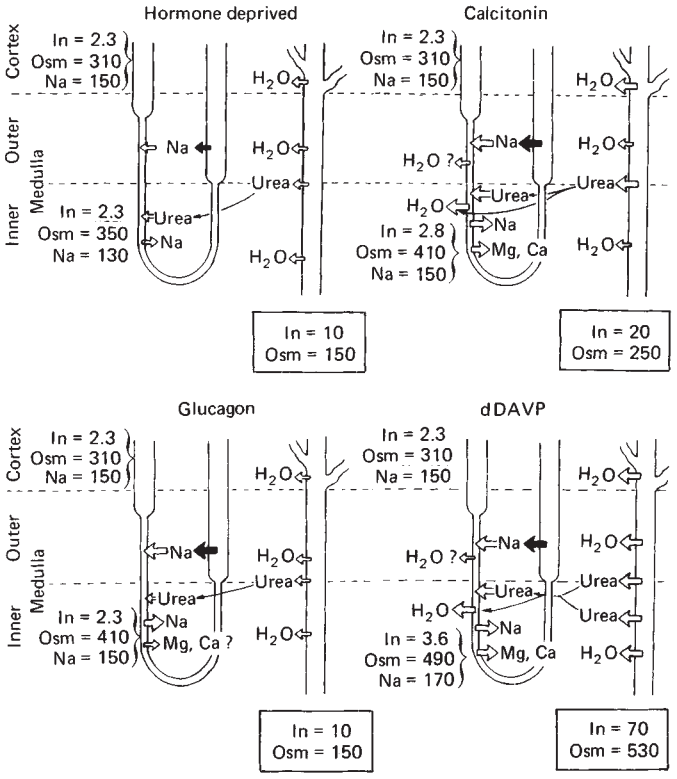

Fig. 3. *Effects of calcitonin, glucagon and dDAVP on the concentrating mechanism along the descending limb of the juxtamedullary nephrons.* This diagram is based on studies of the effects of the peptides in rats deprived of endogenous ADH, calcitonin, PTH and glucagon, four hormones which stimulate the adenylate-cyclase activity of common target cells in the distal nephron. The values indicated in the figure are those measured at the end of the descending limb or in ureteral urine under each experimental condition. The presumed values at the end of the proximal tubules of the nephrons are also indicated. Administration rates of the peptides were as indicated in Fig. 1. Abbreviations are: (In) tubular fluid or urine–to–plasma concentration ratio for inulin; (Osm) tubular fluid or urinary osmolality (mOsm/kg); (Na) tubular fluid Na concentration (mEq/liter). Black and white arrows refer to active and passive transports, respectively. The thin black urea arrow indicates diffusion into the interstitium. Small arrows refer to unstimulated transport. Large arrows mean that the solute and water movements were increased by the peptides, either directly or indirectly. Data from [32, 33, 34].

recta, whereas the other hormones were without significant effect on this variable [32–34].

Physiological consequence of suppression of the peptide hormones modulating the activity of the thick ascending limb and the water permeability of the collecting ducts

Figure 2 shows that, in hormone-deprived rats, the TF/Posm of the fluid at the tip of Henle's loops of the longest nephrons was no greater than 1.10 to 1.15. The corresponding osmotic pressure of fluid at this level was thus about 350 mOsm · kg^{-1}. This implies that the cortico-medullary concentration gradient is greatly reduced, though not abolished, in animals deprived of the four hormones modulating the activity of the thick ascending limb. This reduction was not only due to extreme water diuresis, since in intact Brattleboro rats, as diuretic as the present experimental animals, the tubular fluid at the tip of the papilla reached 500 to 600 mOsmol · kg^{-1} [35, 36]. Suppression of the four hormones thus leads to a major disturbance of the function of the renal medulla, hence the interest of studying each of the four hormones for their effects on the cortico-medullary concentration gradient. The fact that the tubular fluid at the tip of the loops of hormone-deprived animals was close to isotonicity indirectly gives the value for TF/Pin at the end of the proximal tubule of the JM nephrons. In the absence of a longitudinal concentration gradient, the fluid flowing down the descending limb of Henle's loop is subjected to limited (if any) osmotic water removal. In hormone-deprived rats, the TF/Pin at the tip of the loop was between 2.2 and 2.4 (Fig. 3), a value which should thus be found at the end of the proximal tubule of JM nephrons in this species.

Two additional points are of interest: 1) as shown in Figure 3, in hormone-deprived rats the $(TF/UF)_{Na}$ can be lower than one at the hairpin turn. In the less concentrated of the samples collected in thin descending limbs ($N = 10$) the $(TF/P)_{Osm}$ was strictly equal to unity (0.99 ± 0.02), but the TF/UF Na was only 0.84 ± 0.04 (significantly different from 1.0, $P < 0.001$). This indicates that some Na can be reabsorbed along the descending limb if, as is likely, the Na concentration at the end of the proximal tubule was equal to the plasma Na concentration. Such Na reabsorption was certainly caused by a favorable transepithelial concentration gradient between the lumen and the inner medullary interstitium. In fact, we found in hormone-deprived rats that the vasa recta–to–plasma concentration ratio for Na was only 0.62 ± 0.03 (11 samples in 3 rats). 2) The fluid at the tip of the loop was slightly but significantly hyperosmotic, and its osmolality was notably higher than that calculated from

the electrolyte concentrations alone. An osmotically active solute should be responsible for this difference. It is generally assumed that this solute is urea. Since almost no water reabsorption should occur along the descending limb in HD rats and since the $(TF/UF)_{Na}$ is lower than one, the tubular fluid hypertonicity should be mostly due to urea entry (Fig. 3).

Physiological effects of peptide hormones on the water, total solutes and NaCl deliveries at the hairpin turn

All four hormones increased the osmolality of the fluid reaching the hairpin turn (Figs. 2 and 3), thereby confirming that the washout of the cortico-medullary concentration gradient in hormone–deprived rats was due to the hormonal depletion. The effect of PTH was the smallest. It is clear that neither glucagon nor HCT alone were capable of restoring completely the cortico-medullary concentration gradient existing in intact DI Brattleboro rats ($1.73 < TF/P_{Osm} < 1.96$ [35, 36]). This was obtained only with SCT infused at 2.5 mU/min · 100 g body wt. Surprisingly, the effect of dDAVP, although higher than that of glucagon or HCT, was not so marked.

The mechanism responsible for the increase of the tubular fluid osmolality at the hairpin turn was not the same for all the hormones (Fig. 3). Glucagon enhanced both the osmolality and the NaCl concentration of the tubular fluid, but left unchanged the absolute and fractional water delivery. Its effects, therefore, were entirely attributable to net NaCl entry (secretion) in the tubular lumen. This was supported by a positive correlation between the Na (or Cl) fractional delivery and the tubular fluid osmolality, indicating that the more concentrated this fluid the greater the amounts of NaCl delivered at the hairpin turn [33].

The effects of calcitonin and dDAVP were quite different. They did not increase the Na and Cl deliveries but rather tended to decrease them. On the other hand, unlike glucagon, they both increased the TF/Pin, indicating a decrease in the water delivery rate (Fig. 3).

It is known that ADH induces water removal along the descending limb by a mechanism involving urea (M. Knepper and F. Roch–Ramel, this issue). According to the theory of passive concentration in the inner medulla [37] ADH, thanks to its effect on the water permeability of the distal and collecting tubules, delivers to the collecting tubules in the inner medulla a fluid of a sufficient urea concentration for this solute to diffuse into the interstitium by reason of its transepithelial concentration gradient. The interstitial hyperosmolality thus created in turn induces an osmotic water removal along the descending limb and increases the rate of urea entry in this segment [15]. Calcitonin, held to induce the same physiological effects as ADH on the cortical collecting tubules of the rat, should thus bring about water removal along the descending limb by a process similar to that induced by ADH, although to a lesser degree since calcitonin has no action on the medullary collecting duct in this species.

The main findings of these studies on the JM nephrons and their most probable interpretation are summarized in Figure 3. In *hormone–deprived* rats, as already mentioned, as fluid flows along the descending limb, it is concentrated by urea addition, since the Na concentration decreases without concomitant transepithelial water movement. This decrease in Na concentration therefore indicates that some Na is reabsorbed along the descending limb. It is nevertheless possible that some Na is also added to the tubular fluid, since in hormone–deprived rats the diluting capacity of the TALH persists and some Na reabsorbed from the TALH can be added to the descending limb, as discussed below.

Glucagon. Glucagon stimulates NaCl transport in the medullary thick ascending limb. The sodium chloride delivered to the interstitium would create a gradient favorable to its return by diffusion into the descending limb. The glucagon–induced increase of the salt delivery at the tip of the loops could not result from a direct hormonal effect, since in the rat no glucagon receptors have been found in the nephron segments located upstream to the thick ascending limb [8, 38]. The TALH, on the other hand, has an adenyl-cyclase system very sensitive to this hormone, and it is conceivable that the NaCl entering the interstitium because of the glucagon effect may reenter the lumen of the thin descending limb. Such NaCl recycling was already proposed as part of the concentrating mechanism along the descending limb of a desert rodent, *Psammonys obsesus* [39–41]. This recycling would be facilitated by the structure of the thin descending limbs of the juxtamedullary nephrons. In the inner stripe of the outer medulla, the thin descending limbs of these nephrons are very near to the medullary part of the thick ascending limbs [15], and their epithelium is much more sodium permeable than that of the thin descending limbs of the superficial nephrons [42]. Nevertheless, only a fraction of the salt thus added to the lumen by the recycling process would be present at the tip of the loop if some sodium were later to escape from the descending limb. Such an escape might occur if the transepithelial gradient of sodium concentration reversed somewhere along this segment, as already suggested by the data obtained here from the hormone–deprived animals.

Calcitonin and dDAVP. Calcitonin and dDAVP both induce water removal along the descending limb. We propose that this water removal increases the escape of NaCl at some point along the descending limb. This NaCl removal would counterbalance the upstream NaCl addition induced by hormone action, masking the hormonal stimulation of NaCl recycling. The Ca and Mg data support this view. Calcitonin and dDAVP both reduced by 30% the delivery of Mg and Ca at the hairpin turn whereas PTH and glucagon, which did not induce any water movement along the descending limb, had no effect on these deliveries. Osmotic water withdrawal would therefore cause (Mg, Ca) or accentuate (Na, Cl) electrolyte reabsorption out of the descending limb. An additional argument supports the hypothesis we propose. In intact Brattleboro rats, Jamison et al [35] found the fractional delivery of Na at the tip of Henle's loops of juxtamedullary nephrons to be positively correlated with the osmolality ($r = 0.87$, $P < 0.01$). Administering ADH to these animals increased the osmolality of the tubular fluid (by about 70%), mainly by water removal while the fractional delivery rate of Na, on the contrary, tended to decrease from a mean value of 56 ± 5 % during water diuresis, to 45 ± 5 % during ADH perfusion (the difference being however not significant), and there was no longer any correlation between fractional Na delivery rate and osmolality. One may therefore predict that NaCl recycling, that is an increase in NaCl delivery at the hairpin turn resulting from a stimulation of NaCl transport out of the thick ascending limb, can be unmasked only if net water withdrawal out of the descending limb is sufficiently limited so as not to accentuate a

secondary NaCl removal from this nephron semgment. In very recent experiments devoted to the mechanism of natriuresis elicited by pharmacolgical doses of ADH (750 pg/min · 100 g body wt) Pillai and Kokko [43] reported that ADH increased the fractional delivery of chloride to the tip of descending thin limb of Munich Wistar rats without altering the TF/P_{In}. These effects on the descending limb were similar to those we observed with physiological doses of glucagon [33]. They confirm our hypothesis that NaCl addition along the descending limb can be evidenced in the rat when removal of water along the descending limb is limited. This conclusion is also in agreement with that reached by Imai et al (contribution in this issue) who simulated the water and solute movements along the hamster thin descending limb of the long loop nephrons. From its permeability coefficients to water, Na and urea measured in vitro, they predicted that absorption of water along the descending limb, by causing an increase of Na concentration in the lumen, might lead to outward diffusion of this ion. Due to its high reflection coefficient for urea, when the cortico-papillary urea concentration gradient is increased, the water movements would be also increased, enhancing Na removal. On the other hand, net Na addition in the descending limb might occur in the outer medulla when the urea gradient is lowered.

Taken together, our data indicate the existence of sodium addition and/or, according to the circumstances, sodium removal along the descending limb. Because of our lack of knowledge of the precise NaCl and urea concentrations in the outer–medullary interstitium, we are not in a position to decide if these two processes take place along a different part of the descending limb (addition in outer–medulla, removal in inner–medulla) or if they both occur in the outer–medulla.

As already mentioned, *PTH* induced a small rise in TF/P_{Osm} and a small, but not significant, rise in TF/P_{In} leaving the total solute and NaCl fractional deliveries unchanged. Under these conditions, we are unable to draw any conclusion concerning the mechanism of the PTH effect on the osmolality at the hairpin turn of JM nephrons.

If the physiological significance of the hormonal effect on NaCl medullary recycling is easy to understand, such is not the case for NaCl removal from the descending limb. Medullary recycling occuring in the outer medulla would increase the delivery of NaCl into the inner medulla via the thin descending limb. It is possible that NaCl escape from the descending limb (if it occurs in the inner medulla) is a necessity for limiting the diluting effects on the interstitial osmolality of free water addition from both the medullary collecting ducts and the thin descending limbs. According to the "central core" model [44], the mass balance equations indicate that, among several factors, net solute transport out of the descending limb should enhance the concentration gradient in the inner medulla, while pure medullary recycling of solutes should decrease it.

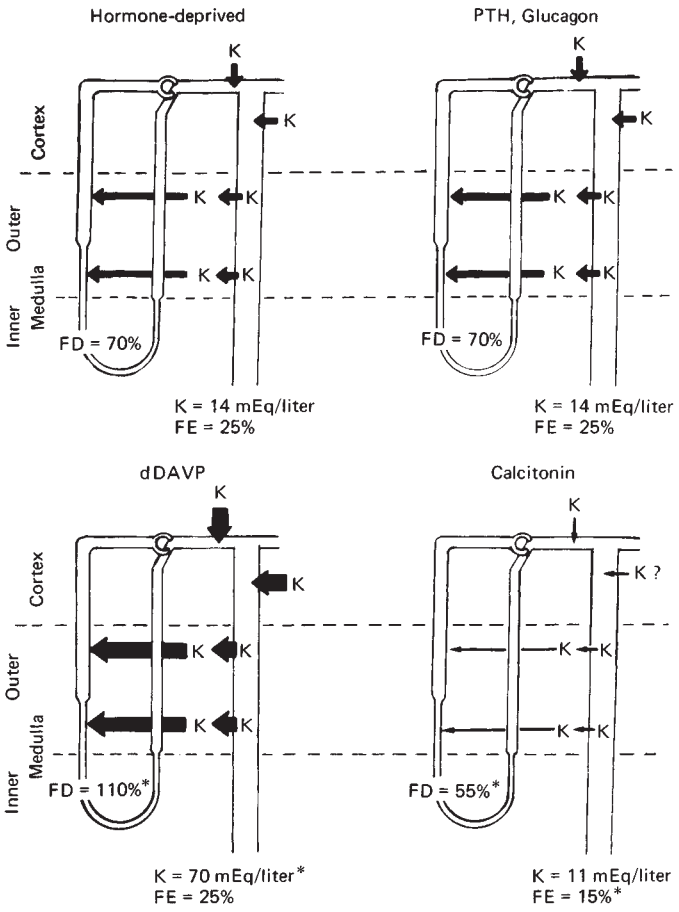

Fig. 4. *Effects of PTH, glucagon, dDAVP and calcitonin on K medullary recycling.* Experiments were performed in hormone–deprived (HD) rats, and peptides were administered at the doses indicated in the legend of Fig. 1. Abbreviations are: (FD) fractional delivery of K (as % of the filtered load); (K) potassium concentration in urine; (FE) urinary fractional excretion of K (as % of the filtered load). The thickness of the arrows illustrates the magnitude of the K fluxes. This figure shows that PTH and glucagon, which do not modify the fractional delivery of K to the collecting ducts [28] nor the concentration and fractional excretion of K in urine, also do not change the fractional delivery of K to the hairpin turn. The ADH analog dDAVP, by stimulating K secretion in the distal tubule [12, 26] and the cortical collecting ducts [45] and by increasing water reabsorption in these two segments enhances the luminal concentration of K in the medullary collecting ducts. All these three actions contribute to increase passive diffusion of K from the collecting ducts to the medullary interstitium and thus favor the recycling between the collecting ducts and the loop. In urine, whereas K concentration is increased, FE% K is unaltered by dDAVP. Calcitonin decreases K secretion in the distal tubule [27] (its effects on K secretion by the cortical collecting ducts have not been studied) and also reduces the fractional delivery of K to the hairpin turn; simultaneously the concentration of K in urine tends to diminish and the FE% K is significantly decreased. (*) Significantly different ($P < 0.05$) from HD (control) rats. Data in the figure from [32–34].

Effects of the four peptides on medullary K recycling

Considering the effects of the hormones on K secretion into the distal tubule, the K delivery rates at the hairpin turn of juxtamedullary nephron are exactly those expected from the theory (Fig. 4).

In the hormone–deprived rats there is still a net addition of K along the distal tubule, which explains why the fraction of filtered potassium remaining at the hairpin turn of juxtamedullary loops (70%) is significantly higher than the fraction of filtered sodium remaining at this site (36%). In addition, as expected from the theory [46], there is a significant correlation between the fraction of potassium remaining at the tip of the juxtamedullary loops and the urinary fractional excretion of potassium [32]. Extrapolation to zero excretion, that is under theoretical conditions on which net potassium secretion is

completely abolished, gives 33% as the fraction of potassium remaining in the loop, a figure not very different from the fraction of sodium remaining. Glugacon and PTH administered to these rats did not change the fractional deliveries of K^+ to the collecting ducts [28] and neither did they lead to modification of the fractional deliveries at the hairpin turn. Calcitonin and dDAVP exert opposite effects on both distal K secretion and K fractional deliveries of the hairpin turn to juxtamedullary nephrons.

The physiological significance of such hormonal control of medullary K recycling is unknown for the simple reason that the exact meaning of K recycling is in itself unclear. It is possible that this process may control the rate of net NaCl reabsorption by the TALH [47, 48], and hence influence the urinary concentrating mechanism, but it seems also likely that K recycling is part of a renal mechanism implicated in K homeostasis [46].

Effects of ADH, PTH calcitonin and glucagon on overall renal function

It is important to note that in all of the studies carried out in either adult or young hormone–deprived rats none of the hormones induced a natriuresis, indicating that undesirable pharmacological effects were avoided (as expected) since the doses used achieved variations in hormonal concentration in the plasma within physiological ranges. Each of the four hormones decreased urinary excretion of Mg and Ca, the stimulation of loop reabsorption being largely responsible for this effect. This effect was not surprising for PTH and calcitonin, but was unexpected for ADH and glucagon. This observation raises the question of the physiological role of the latter hormones in Mg and Ca balance. Such implications are possible since glucagon [49] and dDAVP [50] are capable of diminishing Mg, and dDAVP of diminishing Ca excretion when administered to intact animals. In adult hormone–deprived rats and at the doses employed, only dDAVP and salmon calcitonin induced an antidiuretic response, whereas human calcitonin, glucagon and PTH had no major effect on the urine flow rate and osmolality. In young hormone–deprived rats, or in adult hormone–deprived rats at higher doses, however, human calcitonin decreased urinary flow rate and increased urine osmolality. Potassium excretion was not altered by either PTH or glucagon. With dDAVP, in spite of stimulation by this peptide of K secretion in the terminal nephron segments, the urinary response was highly variable, the urinary excretion rate of K being either unaffected [12, 32] or even decreasing [3]. The variability of this response depended in part on the degree of antidiuresis. With calcitonin, a decrease is generally observed [10, 34].

Physiological significance of the multiple hormonal control of thick ascending limb function

The preceding series of experiments suggest that four peptide hormones control the urinary concentrating mechanism to different degrees. Three of the hormones, glucagon, calcitonin and ADH, are particularly well adapted to this function by virture of their ability to stimulate NaCl reabsorption in the loop of Henle. The question that arises at this point is the physiological significance of such multiple hormonal control of medullary function. To answer this question, it is necessary first to establish whether one hormone can exert its effect on the thick ascending limb, even in the presence of the other hormones. By allowing a precise control of the circulating levels of the hormones, the hormone–deprived model is well adapted to answering that question.

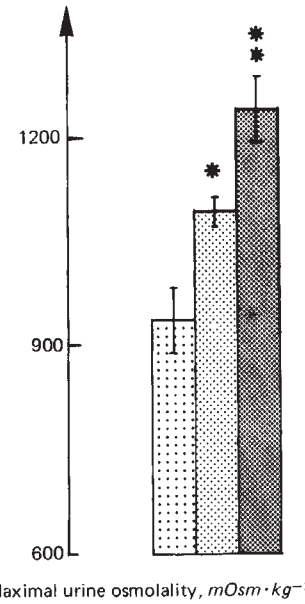

Fig. 5. *Glucagon–dependent increase in maximal urine osmolality.* Each column represents the mean of the maximal urine osmolality reached in each of the five different animals of each group. In all three groups AVP was given at 40 pg/min. Symbols are: (▨) AVP; (▩) AVP + Glu, 1 ng · min^{-1}; (■) AVP + Glu 10 ng · min^{-1}; (*) significantly different from rats that did not receive glucagon (P at least <0.05); (**) significantly different from the two other groups. Reproduced from [55] with permission.

We have seen that in hormone–deprived rats, glucagon and dDAVP stimulate Mg reabsorption by the loop of Henle. Investigations of the renal effects of glucagon in adult hormone–deprived rats, also infused with maximal doses of arginine vasopressin (AVP), show that when half maximal or supramaximal doses of glucagon were infused simultaneously with AVP, excretion of Mg was lower than when AVP or glucagon were administered alone [51] indicating additivity of effects on Mg renal reabsorption. These effects were observed without any alteration of the GFR or the filtered load of Mg. Since the hormone–mediated increase of Mg reabsorption resulted from changes in Mg reabsorption in the thick ascending limb [3, 6, 12], glucagon and AVP should excert additive effects in common target cells. It may be supposed, therefore, that maximal response of these cells requires the presence of more than one hormone. In addition, we have seen that in hormone–deprived rats, dDAVP and glucagon stimulate NaCl reabsorption by the loop of Henle and increase the corticomedullary NaCl concentration gradient. If they exert additive effects on the thick ascending limb, one should expect that the urine osmolality will be higher when AVP and glucagon are given simultaneously than when AVP is administered alone. This is the case, as shown in Figure 5. Thus it is possible that the effects of one hormone on its target cells persists in the presence of other hormones which act on the same cells.

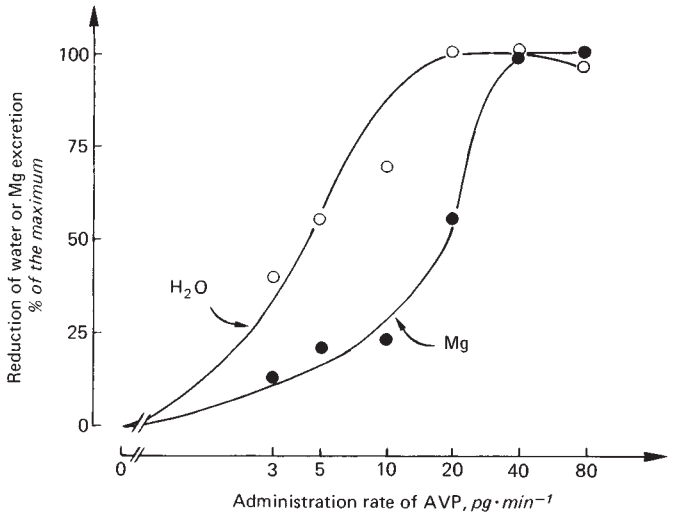

Fig. 6. *Dose–dependent effects of arginine vasopressin on the reduction of absolute water and Mg excretion rates by the kidney in hormone–deprived rats, expressed as % of the maximal reduction.* Each point is the mean of the means. Reproduced from [52].

A second important question is the physiological meaning of the dual actions of antidiuretic hormone, to increase NaCl transport by the TALH and water permeability of the collecting duct. It was therefore important to test the sensitivity to ADH of the two structures.

To characterize, in vivo, the sensitivity to vasopressin of the thick ascending limbs and collecting ducts of the rat, vasopressin was infused to hormone–deprived rats at rates between 3 and 80 pg · min^{-1}. The response of the collecting ducts was evaluated by changes in urine flow and the response of the thick ascending limb by changes in Mg excretion rate, since the AVP-mediated effects on Mg excretion are tightly linked to Mg reabsorption in the TALH [12]. Figure 6 shows that Mg excretion was maximally reduced when AVP was given at 40 pg · min^{-1} and was decreased by 50% at an AVP infusion rate of 20 pg · min^{-1}. The urinary flow rate exhibited a different response. The maximal antidiuretic effect was virtually obtained at 20 pg · min^{-1} and a half maximal reduction of diuresis was found at rates between 3 to 5 pg · min^{-1}. There was therefore a four– to six–fold difference between the doses giving the half maximal response of Mg and water reabsorption. In addition, the figure shows that AVP infusion rates of between 0 and 10 pg · min^{-1} caused the steepest reduction of water excretion by the kidney whereas the Mg excretion was scarcely affected. It was concluded from these results [52] that at low plasma concentrations of AVP (AVP infusion rates around 3 to 5 pg · min^{-1} leading to plasma concentrations close to 0.5 pg · ml^{-1} [53]) the antidiuretic response is confined to an action on the collecting duct whereas higher concentrations are required to stimulate NaCl reabsorption by the thick ascending limb and achieve maximal antidiuresis. These physiological data are in agreement with the cyclase responsiveness to AVP of these two segments [54].

During hydration, the plasma AVP concentration falls below 0.5 pg/ml [55]. With a normal water supply, therefore, low levels of circulating ADH control the water balance by modulating exclusively the water permability of the collecting ducts. During water deprivation, high levels of circulating ADH would permit an additional economy of water by increasing the cortico-medullary NaCl concentration gradient through its effect on NaCl reabsorption by the thick ascending limb.

That plasma ADH may fall within minutes to undetectable levels after water intake [55] emphasizes the importance of other hormones for maintaining the activity of the TALH. It is possible that glucagon or calcitonin is required under physiological conditions in which the circulating level of ADH is reduced, to prevent dissipation of the cortico-medullary osmotic gradient under all circumstances in which the urinary concentrating ability of the kidney is modulated only by the permeability to water of the collecting ducts. The time courses for dissipation and production of the cortico-medullary concentration gradient are very different [56]. It was shown with the hormone–deprived model that depletion of the four hormones acting on the cyclase system of the rat TALH almost completely suppresses the cortico-medullary concentration gradient within two hours, or even less. On the other hand, in some species several days are necessary for the urine to reach maximal hypertonicity [56]. It is therefore important that the gradient be preserved whenever ADH secretion is reduced, not only to allow short term regulation of water balance, but also to minimize the energetic cost of the elaboration of hypertonic urine during the shift from water diuresis to antidiuresis. A cyclase system sensitive to several hormones in the thick ascending limb should be of particular importance in those species, as in human beings [8], in which the TALH does not respond to ADH.

Conclusions

The use of the hormone–deprived model has made it possible to discover the potential effects of several peptide hormones on renal tubular function. Concerning the mechanism of urine concentration, these experiments show that NaCl undergoes medullary recycling, probably from the TALH to the descending limb of JM nephrons, even in the rat. One may predict that such a recycling will be evidenced only if net water withdrawal out of the descending limbs is sufficiently limited to not accentuate a secondary NaCl output, which may normally occur somewhere along the descending thin limb. Furthermore, two hormones other than ADH, calcitonin and glucagon, by the similarity to ADH of their action on NaCl transport in the loop of Henle and of their effects on the cortico-medullary concentration gradient, may participate in the control of the urinary concentrating mechanism. If the water supply is normal, low levels of circulating ADH would control the water balance mainly by modulating the water permability of the collecting duct, the TALH being weakly stimulated by the hormone, and glucagon and (or) calcitonin would maintain the activity of the medullary TALH to preserve the cortico-medullary concentration gradient from dissipation. Under conditions of water deprivation, high levels of circulating ADH would permit an additional economy of water by increasing the cortico-medullary concentration gradient through its effects, additive to those of glucagon and probably calcitonin, on NaCl transport by the TALH.

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