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High salt diet causes osmotic gradients and hyperosmolality in skin without affecting interstitial fluid and lymph

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Abstract

The common notion is that the body Na^+ is maintained within narrow limits for fluid and blood pressure homeostasis. Several studies have, however, shown that considerable amounts of Na^+ can be retained or removed from the body without commensurate water loss, and that the skin can serve as a major salt reservoir. Our own data from rats have suggested that the skin is hypertonic compared with plasma upon salt storage, and that this also applies to skin interstitial fluid. Even small electrolyte gradients between plasma and interstitial fluid would represent strong edema-generating forces. Since the water accumulation has been shown to be modest, we decided to re-examine with alternative methods in rats whether interstitial fluid is hypertonic during salt accumulation induced by high salt diet (8% NaCl and 1% saline to drink) or deoxycorticosterone pellet implantation. These treatments both resulted in increased systemic blood pressure, skin salt and water accumulation and skin hyperosmolality. Interstitial fluid isolated from implanted wicks and lymph draining the skin was, however, isosmotic, and Na^+ concentration in fluid isolated by centrifugation and in lymph was not different from plasma. Interestingly, by eluting layers of the skin we could show that there was an osmolality and urea gradient from epidermis to dermis. Collectively, our data suggest that fluid leaving the skin as lymph is isosmotic to plasma, but also that the skin can differentially control its own electrolyte microenvironment by creating local gradients that may be functionally important.

Key words: blood pressure; body water; extracellular fluid; extracellular matrix; lymph, sodium.

Introduction

A commonly accepted core mechanism in fluid volume and blood pressure regulation is the parallel relationship between body Na^+ and extracellular fluid content.[1] It is assumed that Na^+ readily equilibrates between the intravascular and interstitial compartments that together constitute the extracellular fluid, and that Na^+ concentrations are not remarkably different between the interstitial and intravascular volume. This idea is based on the relatively simple physico-chemical concept of passive body fluid equilibrium in closed systems.[2] To maintain blood pressure homeostasis, body fluid volume and thereby body Na^+ content has to be maintained within very narrow limits.

In long-term observations in humans, however, several studies have shown that considerable amounts of Na^+ are retained or removed from the subjects' bodies without commensurate water retention or loss.[3-6] This finding suggests that Na^+ could be stored somewhere in the body without commensurate water retention and thereby be "inactive" from a fluid balance viewpoint, e.g.[7]. Previously unidentified extrarenal, tissue-specific regulatory mechanisms that control the release and storage of Na^+ from a kidney-independent reservoir are a requirement in this scenario, thereby questioning the usual notion of a two-compartment model.[2] Later studies have indicated that skin might serve as major Na^+ reservoir, e.g.[8-10]. An implication of these observations might be that there is not a strict isotonicity of all body fluids and that skin electrolyte concentrations do not necessarily equilibrate with blood electrolytes.

One consequence of such electrolyte accumulation in excess of water would be that it might cause local hypertonicity. Indeed, using vapor pressure

osmometry we recently demonstrated that Na^+ accumulation in skin as a consequence of feeding the rats a high salt diet (HSD) results in a tissue that is hyperosmotic relative to plasma.[11] Supporting this notion, interstitial fluid (IF) sampled by microdialysis was found to be ~10 mosmol/kg and the Na^+ concentration in tissue regions presumed to be lymphatics ~20 mmol/l higher than plasma.[11]

Such electrolyte gradients would, even with a low capillary reflection coefficient, represent formidable transcapillary forces that would favor massive edema formation. Since the fluid accumulation in rats where salt accumulation has been induced by HSD and deoxycorticosterone acetate (DOCA) combined with 1% saline drinking water has been shown to be modest, e.g.[10, 11], we asked whether the gradients reported by us were representative, and decided to isolate interstitial fluid using alternative approaches. The present results suggest that there is no osmotic gradient between interstitial fluid in deeper dermis and plasma, but rather local gradients in skin such that the most superficial layer next to the keratinocytes contains more osmolytes than the deeper dermis. Herein we thereby underpin the recently proposed hypothesis that the skin may act as a functional counter current system and thus capable of differentially controlling its own microenvironment.

Concise Methods

Detailed Methods are available in the online only Data Supplement.

Animal protocols.

All animal experiments were conducted in accordance with the regulations of the Norwegian State Commission for Laboratory Animals, and with approval from the AAALAC International accredited Animal Care and Use Program at University of Bergen. Male Sprague-Dawley rats (n=61), 8-10 weeks old and with a body weight of 250-300g were randomly assigned to either low salt (LSD) (<0.1% NaCl and tap water) (n=20) or high salt diet (HSD), (8% NaCl and 1% saline water to drink) for 2 weeks (n=21). Another group received deoxycorticosterone acetate (DOCA) (50 or 100 mg/pellet, Innovative Research of America) (n=20) implanted subcutaneously and were given 1% saline water to drink for 3 consecutive weeks. Rats were anesthetized with 2% isoflurane in combination with O_2, or with pentobarbital sodium, 50 mg/kg body weight, given intraperitoneally, in terminal experiments.

Blood pressure was measured noninvasively with a tail cuff system (CODA-6, Kent Scientific, Torrington, CT).

Isolation of interstitial fluid and lymph

Interstitial fluid was collected by implantation of multifilamentous nylon wicks[12] presoaked in mock interstitial fluid (50% plasma diluted in Ringer's acetate) for 90 min or by centrifugation of skin at 100*g*.[13] Lymph (10-15 μl) was sampled after cannulating a collecting lymphatic of ~200 μm running laterally on the tail.

Analytical procedures

Osmolality in IF, lymph, skin samples and plasma was measured in a vapor pressure osmometer as described in detail previously.[11] The size

distribution of macromolecules in plasma and lymph was determined by size exclusion chromatography, and tissue electrolytes and water content was analyzed as described previously.[11] Tissues were ashed, and Na^+ and K^+ concentration measured by atomic adsorption spectrometry (Model 3100, Perkin Elmer).

From skin discs made with a cork bore we cut samples containing epidermis-upper dermis and lower dermis that were eluted overnight to isolate osmolytes that were assessed with the vapor pressure osmometer. Urea was measured in plasma and skin discs using a colorimetric assay kit (BioVision, Milpitas, CA, USA) and the protocol described by the manufacturer.

Data analysis and statistics. Data and statistical analysis was performed using GraphPad PRISM Version 6.0. Results were compared using two-tailed t-tests. Differences between groups were assessed by analysis of variance (ANOVA) followed by Tukey's multiple comparisons test unless otherwise specified. Values are given as mean ± SD, and $p < 0,05$ % was considered statistically significant.

Results

Establishment of models of salt sensitive hypertension

A major question that we wanted to address was whether there were electrolyte gradients between interstitial fluid and plasma in situations where salt accumulated in the tissue. We therefore decided to use the same models as those used previously to demonstrate salt accumulation, namely feeding a high salt diet (HSD)[10] and the DOCA-salt model without uni-nephrectomy[14].

Blood pressure was measured using the tail cuff method at the initiation of HSD or DOCA treatment (day 0), and was not different between rats assigned to LSD, HSD and DOCA-salt groups. As shown in Figure 1A, HSD as well as DOCA-salt resulted in an increase in mean blood pressure that averaged 18 and 51 mm Hg compared with LSD, respectively, in line with what have been reported previously where blood pressure was measured with a catheter in awake animals.[10, 14]

To investigate whether the HSD and DOCA-salt treatment resulted in salt accumulation we measured the skin water content by drying the skin until stable weight, and found that there was a modest increase in water content of 7 % in HSD compared with LSD, a difference that was more pronounced, averaging 18 % in the DOCA salt rats (Fig 1B). The HSD and DOCA salt treatment also resulted in an increase in Na^+ content relative to water (Fig. 1C) of 17 and 25 %, respectively, and to Na^+ content relative to dry weight of 37 and 43 % (Fig. 1D). The more pronounced Na^+ than water accumulation in HSD and DOCA-salt than in LSD rats suggests that some is bound as "inactive" in tissue glycosaminoglycans (e.g.[10]). Collectively, the blood pressure increase and salt and water accumulation in skin corresponds well to what has been found in previous experiments with these models.[10, 14]

Tissue osmolality

We recently demonstrated that HSD results in skin hyperosmolality relative to plasma[11], and wanted to verify this finding in the present series. Whereas the osmolality in dermis was not different from plasma in LSD rats (Fig. 2), the average osmolality of dermis was 12 and 13 mosmol/kg higher than that

in plasma in HSD rats and DOCA salt rats (Fig. 2), respectively, thereby verifying that skin salt accumulation leads to tissue hyperosmolality.

Isolation of interstitial fluid using wicks

Having shown that the diet induced salt accumulation and tissue hyperosmolality, we asked whether the increased tissue salt was reflected in the IF, and implanted nylon wicks[15] that had been primed by "mock IF", i.e. a 1:1 mixture of rat plasma and Ringer's, subcutaneously for IF isolation. The wick technique was chosen as an alternative to microdialysis applied in a previous study.[11] In an attempt to verify that the wick fluid would be capable of reflecting changes in surrounding IF, we in preliminary experiments used a "cross-over method"[16] where wicks were soaked in solutions that were made hyper-osmolal (340 mosmol/kg) by adding NaCl or hypo-osmolal (250 mosmol/kg) by diluting the Ringer's with distilled water. The rationale for this method is that the hyperosmotic wick fluid will be diluted and the hypo-osmotic will be concentrated, and that the true IF concentration is found where the line connecting the points cross the identity line for observed and primer solution values. We observed that after 90 min of implantation, the osmolality in fluids isolated from wicks presoaked in hypo- and hypertonic priming solutions was identical, and thus that this was a sufficient equilibration period and that the wick fluid would be able to reflect the surrounding IF.

We then implanted wicks subcutaneously that had been presoaked in plasma and Ringer's with an osmolality averaging that of plasma (306 mosmol/kg). As shown in Supplemental Figure S1, the osmolality in wick fluid

was similar to that of plasma in LSD as well as HSD rats, suggesting that there is no gradient between IF and plasma even in HSD rats with hyperosmolal skin.

Isolation of IF by skin centrifugation

We wanted to use an alternative method for IF isolation and chose tissue centrifugation that have been shown to yield samples representative for IF, at least with respect to proteins.[13] In an attempt to reduce the potential contamination from intracellular fluid we applied a lower g-force (100 g) to isolate fluid than in the initial method evaluation study. In contrast to wick fluid, the mean osmolality in IF isolated by centrifugation was 13 mosmol/kg higher than in plasma (Figure 3A) in LSD rats. A slightly, but not significantly, higher difference between centrifugate and plasma of 17 mosmol/kg was found in HSD and DOCA salt rats, the latter having an even higher salt accumulation in skin than the HSD rats.

Since we suspected that the higher osmolality in skin IF than in plasma irrespective of salt intake was due to leakage of intracellular ions (notably K^+) during centrifugation, we measured the K^+ and Na^+ concentration in both fluids. We found that the K^+ concentration in IF was about 20 mmol/l higher in HSD as well as LSD than in plasma (Fig. 3B). In contrast, IF Na^+ concentration was 10-15 mmol/l lower than plasma (Fig. 3C), suggesting that the IF isolation procedure led to diffusion of K^+ down a steep concentration gradient combined with a slight extrusion of intracellular fluid rich in K^+ diluting the extracellular Na^+. This observation explains the observed difference in osmolality electrolyte concentration and suggest that the method is unsuitable for IF electrolyte studies.

Osmolality in lymph is similar to plasma

It is generally agreed that in a steady-state situation, lymph is representative for IF[17] and can thus serve as a reference fluid in our context. After some trial and error, we managed to cannulate collecting lymphatics draining the tail skin, and were with intermittent gentle massage of the tail able to isolate 10-15 μl in 10-20 min. All the included samples were clear and without any blood contamination when inspected in situ with the stereomicroscope used during cannulation.

In preliminary experiments we found that there may be evaporation during the sampling period as shown by an increase in osmolality of up to 5% of plasma contained in capillaries exposed to conditions similar to the harvesting situation. Harvesting into mineral oil filled capillaries might have reduced this problem, but was not feasible due to loss of capillary force necessary to sample lymph. In each experiment we therefore corrected for evaporation during harvesting by the increase in osmolality in an equal volume of plasma isolated from a distal cut of the tail vein. The duration of the exposure and the conditions were identical to those prevailing during lymph sampling.

The osmolality of lymph and corresponding tail plasma is shown in Figure 4. We observe that there was no difference in osmolality between lymph and tail plasma (used as reference in this series) neither in LSD, HSD nor in DOCA salt rats (Fig. 4). In this series we also measured Na^+ concentration in lymph recovered from the osmometer after measurement, and even though we had rather high scatter in the data, we found no difference in concentration between lymph and plasma for any of the groups (data not shown).

Because the tail lymph originated from skin that is different from that of the back, we measured Na^+ concentration in tail skin after ashing. As for back skin, we found a modest increase in water content in DOCA salt rats (Supplemental Fig. S2A), and a marked increase in skin Na^+ relative to water (Supplemental Figure S2B) and dry weight (Supplemental Fig. S2C). Collectively, these data suggest that the tail skin behaved as back skin, and that there is no electrolyte gradient between plasma and IF, even in situations where the tissue osmolality is increased.

No difference in capillary sieving properties

Having lymph available enabled us to assess the sieving properties of the capillary wall that may be affected if the high salt load results in a low grade inflammation[14, 18] by size exclusion chromatography (Figure 5). We first assessed the concentration of albumin in plasma and lymph in all groups and found that plasma albumin was 33.0 and 28.0 mg/ml in the LSD and HSD groups, respectively ($p>0.05$), with corresponding albumin L/P ratios of 0.31 and 0.29 ($p>0.05$) (Fig. 5A). In DOCA salt, plasma albumin averaged 18.8 mg/ml ($p<0.01$ and $p=0.056$ when compared with LSD and HSD, respectively), with a L/P of 0.31 ($p<0.05$ for plasmas for both of the other groups) (Fig. 5A).

In the chromatograms we were able to separate protein peaks that we have earlier identified as $\alpha 2$ macroglobulin, fibrinogen, murinoglobulin, haptoglobin, IgG and $\alpha 2$ antitrypsin by mass spectrometry[19]. We related their respective UV signals to that of albumin in lymph as well as plasma to investigate whether there were increased L/P ratios relative to their respective albumin concentration (Fig. 5B). There were no indications of such reduced size

selectivity since none of the respective relative concentrations differed significantly between the groups (Fig. 5B). Collectively, the unchanged sieving properties suggest that there was no difference in capillary size selectivity during high salt conditions.

Osmolyte and electrolyte gradients in skin

Since the lymph osmolality and Na^+ concentration in lymph were not different from plasma we asked whether there could be electrolyte gradients within the skin as suggested in a recent paper.[20] We therefore eluted, in distilled H_2O, skin samples separated into epidermis-upper dermis (upper 0.5 mm) and lower dermis (subsequent 0.5 mm) with the tissue slicer as described for the punch biopsies. That the latter sample type did not include subcutaneous fat was shown by regular hematoxylin-eosin stained histological sections of back skin from two rats.

In samples reconstituted from eluate that had been evaporated to dryness, the osmolality per gram dry weight was 24, 36 and 111% higher in epidermis-dermis than in dermis for LSD ($p<0.01$), HSD ($p<0.01$) and DOCA salt ($p<0.0001$) (Figure 6A). Moreover, in these samples the osmolality in epidermis-dermis was significantly higher in HSD and DOCA salt than LSD rats ($p<0.05$ and $p<0.01$, respectively). These experiments suggest that there is an osmolyte gradient from epidermis to dermis in skin, and that this gradient is increased during salt accumulation.

To determine if the elutable fraction reflected the salt retained in the skin we eluted tissue slices of skin in distilled H_2O. By ashing the tissue after elution, this fraction was measured by relating the mass of Na^+/K^+ in eluate to that

remaining in eluted skin. We found that 21 ± 7 and 14±3% of Na^+, and 24 ± 4 and 20 ± 4% of K^+ (n=6 for all groups) remained in the tissue after elution. Although there was a tendency to a higher eluted fraction of Na^+ in HSD, none of these fractions differed significantly between HSD and LSD diets. Elution should thereby reflect a given fraction of the tissue level of these electrolytes.

When searching for osmolytes that contributed to the observed gradient we assessed Na^+ and K^+ in reconstituted eluate, and found no concentration differences in these electrolytes in LSD skin (Fig. 6B and C). There was, however, a Na^+ concentration gradient opposite that for osmolality. Na^+ in dermis exceeded the concentration in epidermis-dermis with 16 ($p<0.05$) and 27% ($p<0.01$) in HSD and DOCA salt rats, respectively (Fig. 6B). For K^+, a concentration gradient following that of osmolytes was found for DOCA salt only (Fig. 6C). These experiments suggested that Na and K+ could not explain the observed osmolyte gradient.

Because urea is an important contributor to the osmotic gradient found in the kidney medulla, we determined urea in epidermis-dermis and lower dermis after homogenization and elution of excised skin. Interestingly, the urea concentration was higher in epidermis-dermis than in lower dermis in LSD, HSD and DOCA salt, being most pronounced in HSD (Fig. 6D), suggesting that urea apparently contributed to the observed osmolal gradient, and supporting the idea of counter-current exchange in the skin.[20]. Plasma urea was equivalent in LSD and HSD, both exceeding the concentration in DOCA salt rats ($p<0.01$ for both comparisons) (Fig. 6E).

Discussion

Several recent studies have suggested that the skin may serve as reservoir for Na^+ and thereby be involved in fluid homeostasis and blood pressure regulation. Herein we investigated whether Na^+ accumulation induced either by a high salt diet or DOCA combined with 1% saline to drink was reflected in skin interstitial fluid. Both experimental interventions resulted in increased blood pressure, and moreover in elevated skin tissue osmolality compared with plasma. The osmolality of skin interstitial fluid isolated from implanted wicks or from tail skin lymph was however similar to that of plasma, suggesting that fluid drained from the interstitium is isosmotic to plasma. Notwithstanding this finding, by elution we were able to demonstrate that there was an osmolality- and urea gradient from epidermis through dermis to subcutis. This gradient may reflect a proposed counter-current mechanism for electrolyte homeostasis,[20] and calls for a more refined analysis of osmolyte and electrolyte distribution in the various layers of skin.

Evaluation of methods and limitations

We used wick implantation, tissue centrifugation and lymph vessel cannulation to access the interstitial fluid in skin, all having their own limitations that have to be taken into consideration when interpreting the data. They have all been evaluated and shown to reflect protein concentration in IF (for review see[21]), not so with electrolytes. Clearly, as shown here, the centrifugation method is unsuitable for studies of interstitial fluid electrolytes, likely because of K^+ leakage from the cells during fluid isolation.

Wick implantation results in an acute inflammatory response and protein extravasation that subsides within 30 min.[22] To reduce the potential influence of inflammation that may affect ion homeostasis across cell membranes and thus wick fluid osmolality, we harvested wicks after 90 min implantation time. That ion gradients are equilibrated quickly between surrounding interstitium and wick fluid was shown by the "crossover" experiments with hypo- and hyperosmolar priming solutions that produced identical wick fluid osmolality after a 30 min equilibration period. Provided that 90 min implantation time made the influence of the inflammatory reaction minimal, wick fluid should reflect osmolality in the subcutaneous space. This assumption is supported by our finding of similar osmolality in wick fluid and lymph that may be considered as reference IF (see below).

Lymph can be harvested involving minimal trauma and represents the "gold standard" for interstitial fluid provided steady state conditions[21] that were prevailing in our experiments. Since the other major component of the tail, tendon, does not contain lymphatics,[23] the harvested tail lymph will be representative for skin. Moreover, tail skin accumulated salt to a similar extent as back skin and therefore representative for skin in general. Accordingly, the lymph as well as wick data suggest that interstitial fluid from skin is isosmotic with plasma in situations where the tissue is hyperosmotic as a result of salt accumulation. Another implication of this observation is that we by tissue osmometry are able to detect the "inactive sodium" stored in negatively charged glycosaminoglycans in the tissue[8] that is not reflected in the interstitial fluid leaving skin.

Comparison to previous lymph and IF data

The question whether there is an electrolyte gradient between IF and plasma has been addressed in several previous studies, and the results are diverging and inconclusive. Haljamäe developed a liquid-paraffin cavity technique where he could sample nanoliter samples from subcutis.[24] Together with collaborators he found higher concentrations of Na^+ and K^+ in subcutis than in plasma as well as in IF harvested from subcutaneously implanted capsules,[25] and ascribed the deviations from the expected Gibbs-Donnan distribution to charged anionic macromolecules in the extracellular matrix. In support of these data are the finding of ion gradients between interstitial fluid sampled by wicks as well as lymph from subcutis and plasma[26] In contrast, Gilanyi et al[27] sampled interstitial fluid with the liquid-paraffin as well as capsule technique and found no difference in Na^+, K^+ and Cl^- concentration between IF and plasma, in agreement with capsule data from subcutis obtained by Gullino et al[28]. The reason for this discrepancy is not evident, but as pointed out by Szabo and Magyar,[26] the small sample sizes make the IF samples prone to evaporation and other handling errors[27] that will increase the ion concentration.

If we accept that there is no gradient between IF and lymph, both sampled from subcutis in control situation, then the question is whether there is a gradient when NaCl accumulates and the tissue gets hyperosmotic relative to plasma. We addressed this issue in a recent paper using energy-dispersive x-ray electron microprobe analysis on lymph vessels and microdialysis and found significantly higher Na^+ concentration with both techniques.[11] The microdialysis probes were placed intracutanously, and although the dialysate had higher Na^+ concentration and osmolality than arterial plasma, there was no difference

between LSD and HSD rats. The explanation for this finding is not readily evident, but these data might question the ability of the method to reflect IF osmolality shown to be similar in HSD and LSD, or tissue osmolality shown here to be increased in HSD and DOCA salt rat skin. The significantly higher Na^+ concentration in lymphatics than in plasma observed with the x-ray electron microprobe analysis may reflect intracutaneous electrolyte gradients discussed in more detail below.

Osmolyte and electrolyte gradients in skin and implications of the data

Even though our data suggest that IF in subcutis as well as lymph draining from the skin are isosmotic to plasma, the elution experiments clearly show that more osmolytes can be mobilized from superficial skin, including the epidermis, than deeper dermal parts. These observations agree well with previous x-ray dispersive studies, where a gradient was demonstrated from epidermis to subcutis in human[29] as well as in guinea pig skin.[30] In line with these observations, by application of 7 T $^{23}Na^+$ MRI it was shown that there was a substantial accumulation of Na^+ in or directly under epidermis.[31] Interestingly, this Na^+ accumulation was not associated with a significant volume increase in the same area as would have been expected if the Na^+ exerted at least some of its crystalloid osmotic effect. The apparent conundrum of skin Na^+ gradients and lack of corresponding fluid accumulation led Hofmeister et al[20] to suggest that there is a functional countercurrent mechanism that enables the skin to differentially control its own microenvironment. Our data showing an epidermal to dermal osmolyte gradient that also includes urea and is increased in salt accumulation supports the hypothesis. Apparently, the lower content of Na^+ in

epidermis-dermis than in the deeper dermis suggests that Na^+ does not contribute to this gradient. We should, however, bear in mind that Na^+ will predominantly distribute in the extracellular fluid space that will likely be higher in dermis than in the more cell rich epidermis. Na^+ concentration in the available space in epidermis may therefore be higher than in dermis and contribute to the osmotic gradient in vivo, calling for additional experiments where electrolyte gradients and distribution volumes are determined at higher resolution at the tissue level.

Perspectives

Here we have investigated whether salt accumulation in skin results in an interstitial fluid that is hypertonic relative to plasma. Wick fluid and lymph, that may both represent IF during steady state conditions and thus representative for IF returning to the general circulation, were isosmotic to plasma. Elution experiments of epidermis together with upper dermis and lower dermis suggested that there is an osmotic gradient from superficial to deeper layers of skin, again suggesting that the skin may differentially control its own microenvironment, and together with the kidney actively participate in fluid volume regulation. A more detailed assessment of this gradient where electrolyte gradients are determined in higher resolution may lead to a better understanding of the functional implications of this observation.

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Disclosures.

None.

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Novelty and Significance

What is New?

Through isolation of skin lymph from rats and tissue elution we could address whether there are osmotic gradients between interstitial fluid and plasma and within the skin during salt accumulation.

What Is Relevant?

Recently it has been shown that considerable amounts of Na^+ can be retained or removed from the body without commensurate water loss, and that the skin can serve as a major salt reservoir.

Summary

Skin lymph is isosmotic to plasma, and the skin can differentially control its own electrolyte microenvironment by creating local gradients.

Figure legends

Figure 1. A. Individual values of mean blood pressure measured with tail cuff plethysmography in awake rats in low salt diet (LSD) (n=20), high salt diet (HSD) (n=21) and DOCA salt diet (n=20). Also shown mean and SD. **: $p<0.01$ and ****: $p<0.0001$ when compared with LSD. B. Individual values of skin water content in

low salt diet (LSD) (n=11), high salt diet (HSD) (n=11) and DOCA salt diet (n=11). Also shown mean and SD. **: $p<0.01$ when compared with control. C. Individual values of skin Na^+ content relative to skin water in LSD, HSD and DOCA salt diet (n=11 for all diets). Also shown mean and SD. ****: $p<0.0001$ when compared with control. D. Individual values of skin Na^+ content relative to dry weight in LSD, HSD and DOCA salt diet (n=11 for all diets). Also shown mean and SD. ****: $p<0.0001$ when compared with control.

Figure 2. Individual osmolality values of skin (n=17) and plasma (n=17) in low salt diet (LSD), high salt diet (HSD) (n=18, skin and plasma) and DOCA salt diet (n=9 for skin and n=11 for plasma). Also shown mean and SD. ***: $p<0.001$ and ****: $p<0.0001$ when compared with plasma.

Figure 3. A. Individual osmolality values of skin interstitial fluid (IF) isolated by centrifugation (n=6) and plasma (n=10) in low salt diet (LSD), high salt diet (HSD) (n=6 for IF, n=8 for plasma) and DOCA salt diet (n=4 for IF and plasma). Also shown mean and SD. ***: $p<0.001$, ****: $p<0.0001$ when compared with respective plasma. B. Individual values of K^+ concentration in skin interstitial fluid (IF) (n=6) and plasma (n=5) in LSD and HSD (n=8 for IF and plasma). Also shown mean and SD. ****: $p<0.0001$ when compared with respective plasma. C. Individual values of Na^+ concentration in skin interstitial fluid (IF) and plasma in LSD (n=6 for IF and plasma) and HSD (n=8 for IF and plasma). Also shown mean and SD. ***: $p<0.001$, ****: $p<0.0001$ when compared with respective plasma.

Figure 4. Individual osmolality values of skin lymph and plasma in low salt diet (LSD) (n=6 for lymph and plasma) (panel A), high salt diet (HSD) (n=11 for lymph and plasma) (panel B) and DOCA salt (n=6 for lymph and plasma) (panel C). Also shown mean and SD. None of the lymph osmolality values were different from the corresponding plasma.

Figure 5. A. Individual values of concentration of rat serum albumin in plasma and lymph as determined by high performance liquid chromatography in low salt diet (LSD) (n=5 for lymph and plasma) (left panel), high salt diet (HSD) (n=5 for lymph and plasma) (middle panel) and DOCA salt (DOCA) (n=4 for lymph and plasma) (right panel). Also shown mean and SD. B. Individual values for sieving of plasma proteins relative to the corresponding sieving of albumin in lymph and plasma in LSD (n=4) (left panel), HSD (n=5) (middle panel) and DOCA salt (n=4) (right panel). Also shown mean and SD. a2M: α2 macroglobulin, Fib: fibrinogen, MUG: murinoglobulin, Hapt: haptoglobin, IgG: immunoglobulin G and a2a: α2 antitrypsin

Figure 6. Individual values of osmolal content after elution in distilled H_2O of epidermis and associated upper dermis (Epidermis) and corresponding lower dermis (Dermis) isolated from rats on low salt diet (LSD) (n=11), high salt diet (HSD) (n=9) and DOCA salt (n=6). (#: $p<0.05$ and ††: $p<0.01$ vs LSD Epidermis, ANOVA followed by Dunnett's test). B: Individual values of Na^+ content of epidermis and associated upper dermis (Epidermis) and corresponding lower dermis (Dermis) isolated from rats on low salt diet (LSD) (n=10), and high salt diet (HSD) (n=17) and DOCA salt (n=6). C: Individual values of K^+ content of

epidermis and associated upper dermis (Epidermis) and corresponding lower dermis (Dermis) isolated from rats on low salt diet (LSD) (n=11), and high salt diet (HSD) (n=16) and DOCA salt (n=6). D: Individual values of urea content of epidermis and associated upper dermis (Epidermis) and corresponding lower dermis (Dermis) isolated from rats on low salt diet (LSD) (n=10), and high salt diet (HSD) (n=9) and DOCA salt (n=5). E: Individual values of urea concentration in plasma isolated from rats on low salt diet (LSD) (n=6), high salt diet (HSD) (n=6) and DOCA salt (n=5). (**: $p<0.01$, ANOVA). All panels also show mean and SD. For A-D: *<$p<0.05$, **: $p<0.01$, ****: $p<0.0001$, when comparing Epidermis-dermis and Dermis with Student's t-tests in corresponding tissues.