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Research Paper

Reverse Iontophoresis of Amino Acids: Identification and Separation of Stratum Corneum and Subdermal Sources *In Vitro*

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Purpose. To differentiate the stratum corneum (SC) and subdermal sources of amino acids (AAs) extracted by reverse iontophoresis.
Methods. 13 zwitterionic AAs were quantified in this *in vitro* study. Repetitive tape-stripping permitted the distribution of the analytes to be determined in the SC. Iontophoresis experiments were performed in which the subdermal chamber contained either phosphate-buffered saline (PBS) only, or a mixture of the 13 AAs in PBS.
Results. AAs were homogeneously distributed across the SC and broadly divided into three groups (high, medium, low) in terms of total amount present. As expected, extraction to the cathode for the essentially neutral analytes involved was more efficient. Initial samples obtained during the first hour of iontophoresis primarily extracted AAs from the SC. The fluxes observed in the latter half of the 6-h experiment, on the other hand, correlated well with the corresponding subdermal concentrations.
Conclusion. A relatively short extraction period (~1 h) by reverse iontophoresis can be used to evaluate the content of AAs in the SC. Once this 'reservoir' has been depleted, reverse iontophoresis can then monitor the subdermal concentrations of the AAs. The latter appears most useful for compounds which are present at lower levels in the SC.

KEY WORDS: amino acids; non-invasive monitoring; reverse iontophoresis; skin reservoir; stratum corneum; tape-stripping.

INTRODUCTION

Amino acids (AAs) are the constituents of myriad proteins responsible for a diverse array of vital functions essential to life (1). Genetic defects affecting the metabolism of AAs, such as phenylketonuria, can result in severe problems if undetected and/or left untreated. In neonates, therefore, the levels of AAs are often measured in a blood sample to screen for such hereditary disorders (1,2).

Reverse iontophoresis enhances the extraction of (charged and polar) substances through the skin by the application of a small electrical current (<0.5 mA/cm^2) (3). The method has been successfully employed in the GlucoWatch® Biographer (4) to continuously monitor blood glucose. The flux of a solute extracted by reverse iontophoresis is the sum of the contributions of passive diffusion, electromigration and electroosmosis. Electromigration results from the direct interaction of a charged solute with the electrical field. Electroosmosis arises from the fact that the skin is negatively charged under normal physiological conditions; application of an electrical field therefore induces a net solvent (water) flow in the anode-to-cathode direction (5). The solute flux due to electroosmosis (J_{EO}) is proportional, at least to a first approximation, to its subdermal concentration (C_s):

$$J_s^{EO} = \upsilon \cdot C_s \tag{1}$$

where υ is the solvent volume flow (6). The third, passive contribution is typically negligible relative to electromigration and electroosmosis. The application of reverse iontophoresis to the skin provokes molecular transport towards the cathode by all three mechanisms. In the case of zwitterionic amino acids and glucose, the first molecules to reach the skin surface originate from the outermost layer of the membrane, the stratum corneum (SC).

AAs are excellent candidates for non-invasive monitoring via reverse iontophoresis due to their small molecular weight and polar nature, and both *in vitro* and *in vivo* studies exploring the concept for phenylalanine (7,8) and endogenous AAs (9,10) have been reported. The latter studies strongly inferred the presence of an important "skin reservoir" of AAs (9,10). Indeed, the initial period of iontophoresis yielded higher extraction fluxes of the AAs relative to those observed after longer extraction periods. Furthermore, measurable amounts of the AAs were extracted in the *in vitro* study even

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ABBREVIATIONS: AA, amino acid; IC-IPAD, ion chromatography with integrated pulsed-amperometric detection; NMF, natural moisturizing factor; SC, stratum corneum.

when the subdermal compartment contained no AAs. This "skin reservoir" of the AAs needs to be depleted, therefore, before iontophoresis can interrogate the subdermal compartment (as is the case with glucose). For some AAs, particularly *in vivo*, the "skin reservoir" was so large that monitoring their systemic fluctuations is not feasible. The SC's natural moisturizing factor (NMF) was suggested as a contributor to the important "skin reservoir."

Indeed, AAs form an important part (>50%) of the SC's natural moisturizing factor (NMF), a complex mixture of low molecular weight humectants key to the control of skin hydration (11–13). More precisely, NMF is composed primarily of free amino acids which are derived from filaggrin (14). Originally, profilaggrin, the precursor of filaggrin, is expressed in the keratinocytes of the granular layer (15). As these cells differentiate into corneocytes in the SC, profilaggrin undergoes proteolysis to filaggrin. The latter is ultimately degraded into free AAs, some of which are further transformed into pyrrolidone carboxylic acid, urocanic acid, citrulline and urea (16,17).

When NMF is compromised, the skin becomes dry and scaly and transepidermal water loss increases significantly. Equally, ichthyosis vulgaris and psoriasis vulgaris are skin disorders characterised by alteration of profilaggrin and filaggrin expression, and involve clear changes in the quality of barrier function (18,19). Correct and early diagnosis of filaggrin-related disorders is not straightforward, and it is interesting to ask whether the monitoring of AAs in the SC might be helpful in this regard (20,21). Similarly, from a simply cosmetic viewpoint, a non-invasive tool with which to evaluate skin hydration and the overall "health" of the barrier would be valuable.

It follows that reverse iontophoresis has the potential to be used as a non-invasive tool with which to evaluate the overall health of the skin as well as systemic levels. The goal of this work, therefore, was to differentiate the SC and subdermal sources of the extracted AAs and thus provide guidance on the manner in which reverse iontophoresis might be used to probe these different compartments. The working hypothesis was that a relatively short (~1h) reverse iontophoresis extraction period would sample primarily the AAs present in NMF, and that information about the subdermal content would be obtained with longer times of current passage. *In vitro* experiments, using porcine skin as a model, were carried out to verify this hypothesis. First, the AAs levels in the SC were measured by extraction and analysis of consecutive tape-strips of the barrier. These values were then compared with the amount of AAs extracted by reverse iontophoresis and by passive diffusion from skin beneath which the subdermal compartment contained only phosphate-buffered saline. Finally, simulations of clinical monitoring reverse iontophoresis experiments, in which the subdermal compartment contained 0.1 to 0.5 mM of the AAs, were conducted. Concurrent extraction of glucose provided a positive control.

MATERIALS AND METHODS

Chemicals

Sodium chloride, sodium phosphate (dibasic), potassium phosphate (monobasic) and phosphoric acid (85%) were from Acros (Geel, Belgium). Sodium hydroxide (50%, ion chromatography eluent grade) was from Fluka (Buchs, Switzerland). Sodium azide, D-glucose, silver wire (>99.99% purity) and silver chloride (99.999%) were purchased from Sigma-Aldrich Co. (Gillingham, UK). The 20 primary AAs in powder form and an AA standard solution (containing 17 primary amino acids in 0.1 N HCl) were from Sigma (Gillingham, UK). Sodium acetate (anhydrous, electrochemical grade) was from Dionex (Sunnyvale, CA) and glacial acetic acid (HPLC grade) from Fisher Scientific (Loughborough, UK). All chemicals were at least of reagent grade unless stated otherwise and all aqueous solutions were prepared using high-purity deionized water (18.2 MΩ·cm, Barnstead Nanopure Diamond™, Dubuque, IA).

Skin Preparation

Pig skin, from the ear or abdomen, was gently washed under cold running water post-sacrifice at a local abattoir. The skin was then dermatomed to a nominal thickness of 750 μm (Zimmer™ Electric Dermatome, Dover, OH). The tissue samples obtained (~9 cm^2) were wrapped individually in Parafilm™ and stored for no more than three months at −20°C until use.

Tape-Stripping

Layers of SC were progressively removed by consecutively applying and removing 20 adhesive tapes (Scotch Book Tape, 3M, St. Paul, MN). To ensure that SC was removed from the same location, a 2 cm diameter template was used. Each tape (2.5×2.5 cm) was weighed before and after stripping on a 0.1-μg precision balance (Sartorius SE2-F, Epsom, UK), and the thickness of SC on each tape was then determined from its mass, the area stripped, and the known density of the tissue (1 g/cm^3) (22,23). Finally, each tape was extracted by shaking overnight in 1 ml of an aqueous solution of sodium azide (20 mg/l).

Reverse Iontophoresis

Experiments were performed in vertical cells, described elsewhere (24), having the two electrode chambers on the epidermal side of the skin. The area of skin exposed in each chamber was 0.8 cm^2. The subdermal compartment volume was ~7 ml. A constant current (Yokogawa 7651 Programmable DC source, Woodburn Green, UK) of 0.3 mA (0.38 mA/cm^2) was applied via Ag/AgCl electrodes. The extraction solution was 20 mM NaCl in 10 mM phosphate buffer at pH 7.4.

In a first experiment designed to extract the AAs and glucose from the skin, the magnetically stirred subdermal compartment contained only phosphate-buffered saline (PBS, 137 mM NaCl and 18 mM phosphate). The anodal and cathodal chambers were filled with 0.8 ml of the extraction solution and current was applied for 6 h in total. At 0.25, 0.5, 1, 2, 3, 4, 5, and 6 h, the current was stopped, and the cathodal and anodal solutions were removed for analysis. The two chambers were then replenished with fresh buffer and the current was restarted. In the second set of experiments the subdermal compartment contained the 20 primary AAs at concentrations of 0.1, 0.25 or 0.5 mM and glucose at 1, 2 or

5 mM in at pH 7.4. Every hour, the entire contents of the electrode compartments were removed for analysis and replenished with fresh solution.

Passive Diffusion

Identical experiments to those involving reverse iontophoresis, where the subdermal compartment contained only PBS, were performed except that no current was applied, and Franz cells (2 cm^2 surface area) were used.

Analytical Method

All sample solutions (from the electrode chambers, passive diffusion experiments, and the tape-strips) were passed through nylon syringe filters prior to analysis (0.45 μm, Nalgene, Hereford, UK) by ion chromatography with integrated pulsed-amperometric detection (IC-IPAD) for amino acids and glucose. The IC-IPAD system comprised three modules: a GP50 gradient pump, an AS50 autosampler, and an ED50 electrochemical detector (Dionex, Sunnyvale, CA). AAA-certified™ disposable gold working electrodes (Dionex, Sunnyvale, CA) were used for amino acid and glucose detection, and data acquisition was performed with Chromeleon software (Dionex). An improved gradient method for the separation of amino acids and carbohydrates was followed (25). Briefly, a complex gradient of four solutions (10 mM NaOH, 250 mM NaOH, 1 mM sodium acetate in 10 mM NaOH, and 100 mM acetic acid) was pumped at a constant flow rate of 0.25 ml/min, and separation was performed on an anion exchange column (AminoPac PA10, 2×250 mm analytical column and 2×50 mm guard column, Dionex) at 33°C. The run time for one sample was 92 min (including column washing and equilibration for subsequent sample) and included an 8 min isocratic period at 10 mM NaOH at the beginning of each run. A complex potential waveform (AAA-direct™ waveform, Dionex) *versus* a combination pH/Ag/AgCl reference electrode was applied at the detector to measure current at the surface of the electrode.

Data Analysis and Statistics

Extraction fluxes were determined for each sampling interval. The equivalent volume flow (υ) was calculated from the flux and the subdermal concentration using Eq. 1. Data manipulation, linear regressions and statistics were performed using Graph Pad Prism V.4.00 (Graph Pad Software Inc., San Diego, CA). Linear regressions were tested for significance by ANOVA. When data were compared, the level of statistical significance was fixed at $p<0.05$. Unless stated otherwise, all values are expressed as mean ± standard deviation (SD) of six replicates obtained using skin (from the abdomen or ears) from two pigs.

RESULTS AND DISCUSSION

Analysis of AAs by IC-IPAD

Of the 20 primary AAs and glucose, 14 molecules were successfully quantified: glucose, asparagine (Asn), alanine (Ala), threonine (Thr), glycine (Gly), valine (Val), serine (Ser), proline (Pro), isoleucine (Ile), leucine (Leu), histidine (His), phenylalanine (Phe), tyrosine (Tyr) and tryptophan (Trp). Arginine, glutamine and cysteine coeluted with other substances and could not be assessed, while lysine, methionine, aspartate and glutamate were detected with insufficient precision to be quantified. All compounds measured, therefore, were essentially neutral (zwitterionic in the case of the AAs) at pH 7.4 and transported to the skin surface principally by electroosmosis and passive diffusion.

Distribution of AAs Across the SC Determined by Tape-Stripping

The average concentrations and the total amounts of the 13 AAs determined from the SC tape-stripping experiments are collected in Table I. The results can be divided into three clear groups: (1) AAs present at high levels (ranging from 23±6 to 40±11 mmol/kg): Ser, Gly, Ala and His; (2) those at

Table I. Quantities of AAs and Glucose in Skin Determined by Tape-Stripping, Passive Extraction and Reverse Iontophoresis (Mean ± SD; $n=6$)

AA	[AA] in SC from tape-stripping (mmol/kg)	AA removed from SC by tape-stripping (nmol/cm^2)	AA extracted		
			at cathode (nmol/cm^2)	at anode (nmol/cm^2)	by passive diffusion (nmol/cm^2)
Ser	40±11	37±9	68±15	45±12	47±12
Gly	25±6	23±6	53±16	38±14	34±7
Ala	24±8	22±4	39±8	25±8	26±6
His	23±6	21±4	37±5	10±4	23±6
Thr	10±3	10±2	16±4	10±3	10±3
Pro	7.9±2.3	7.3±1.5	14±3	8.5±2.1	8.5±2.7
Leu	7.7±1.8	7.3±2.1	12±3	7.2±2.6	9.4±2.8
Val	3.5±0.7	3.4±1.0	7.3±2.2	3.6±1.2	4.7±1.2
Ile	3.2±0.6	3.0±0.8	5.8±1.7	3.4±1.0	4.5±1.5
Tyr	3.4±0.5	3.2±0.6	4.0±1.2	2.5±0.6	3.0±0.9
Phe	2.2±0.4	2.1±0.5	3.1±1.3	1.9±0.4	2.3±0.8
Asn	1.9±0.5	1.8±0.6	2.9±1.2	1.8±0.8	2.4±1.0
Trp	0.6±0.1	0.5±0.2	0.6±0.3	0.3±0.1	0.4±0.2
Glucose	1.7±1.4	1.4±0.7	3.6±3.0	1.4±0.8	1.0±0.8

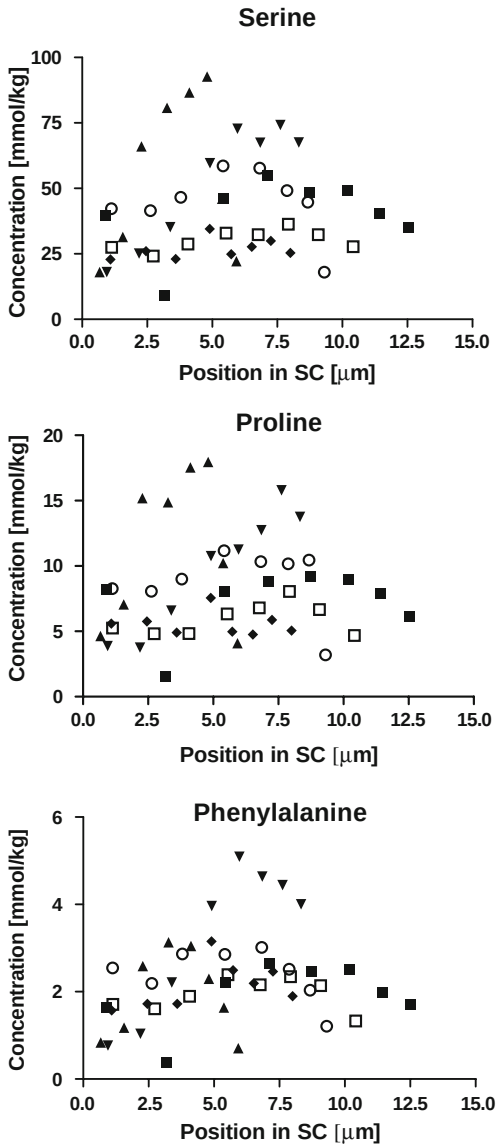

Fig. 1. Concentration profiles of Ser, Pro, and Phe across the SC, illustrating the behaviours of the three groups of AAs (high, medium and low concentrations) present in the barrier. Each symbol corresponds to a different piece of skin.

moderate concentrations (ranging from 7.7±1.8 to 10± 3 mmol/kg): Thr, Pro and Leu; and (3) the rest which were the least abundant (ranging from 0.6±0.1 to 3.5±0.7 mmol/kg): Val, Ile, Tyr, Phe, Asn and Trp. Representative distribution profiles of an illustrative AA from each group are shown in Fig. 1. All of the substances measured were homogenously distributed across the SC, with no significant difference between the levels found at the outer and inner surfaces of the barrier.

Passive and Reverse Iontophoretic Extraction of AAs from the SC

The quantities of AAs extracted passively and during reverse iontophoresis, when the subdermal compartment contained only PBS, were measured as a function of time. The total amounts recovered for each AA are summarized in Table I. The rates of extraction (i.e., the instantaneous fluxes) for the same three illustrative analytes are in Fig. 2. The pattern of behaviours observed was independent of the absolute level of AA in the SC. Initial fluxes were high but then settled to lower values at longer times. At 15 min, no

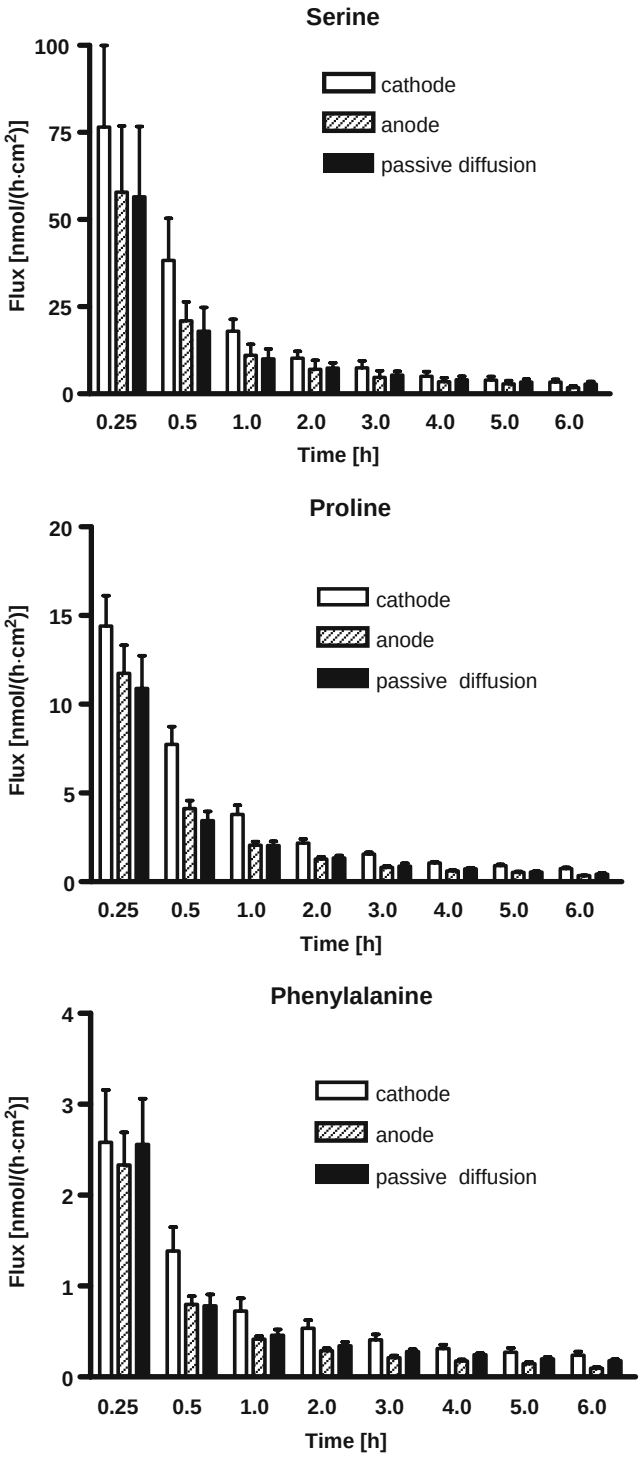

Fig. 2. Passive and reverse iontophoretic (cathode and anode) extraction fluxes as a function of time for Ser, Pro and Phe, illustrating the behaviours of the three groups of AAs (high, medium and low concentrations) present in the barrier. The subdermal solution contained no AAs in these experiments.

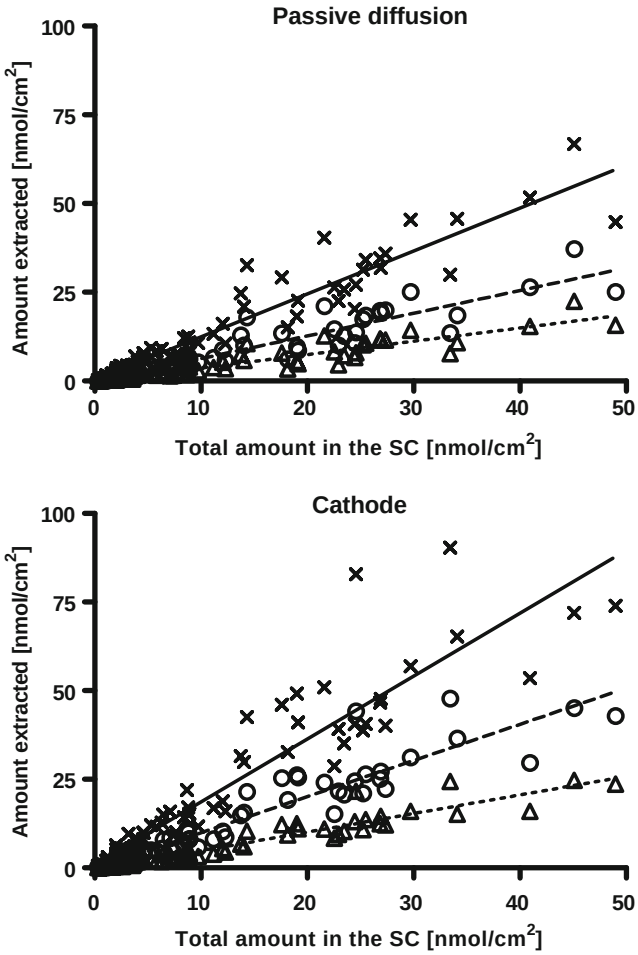

Fig. 3. Correlation between the amounts of AAs extracted passively, and by reverse iontophoresis to the cathode, after 15 min (*triangles*), 1 h (*circles*), and 6 h (*crosses*), with the quantities removed in the SC tape-stripping experiments. Linear regressions through the data are shown. The subdermal solution contained no AAs in the extraction experiments.

differences (except for histidine—see below) between the quantities extracted iontophoretically (cathode and anode) or passively were found. With increasing time, however, for all AAs, extraction to the cathode eventually and significantly exceeded that at the anode and that removed passively.

Under the conditions of the reverse iontophoretic experiments performed (pH7.4), the AAs studied would have been essentially (>94%) zwitterionic. Preferential extraction to the cathode via electroosmosis was anticipated, therefore, and was clearly seen when the amounts recovered at 6 h in the cathode and anode chambers were compared (Table I). Histidine is a weakly basic AA (pK_a of side chain = 6.0 (1)) and could be partially present in the cationic form inside the SC, at least at the beginning of the experiment, due to the slightly acidic nature of the membrane (26). Indeed, for this AA, the 6-h ratio of cathodal to anodal extraction was, on average, nearly 4, whereas for all other species, the ratio never exceeded 2.

Passive extraction of AAs from the SC was quite efficient, and no different from that to the anode when current was applied (with the exception of His), suggesting that the compounds were able to diffuse readily from the SC when the surface was contacted with an aqueous solution. This would suggest that the AAs 'released' from the SC were already present in the intercellular lipid domains (which constitute the predominant route of passive transport across the barrier (27)), as has been implied previously (11).

Perhaps not surprisingly, the total amounts of AAs extracted passively correlated well with the quantities recovered from the tape-stripping experiments (Fig. 3). With increasing time of extraction, the slope of the regression with the passive data approached a value of 1, suggesting that passive diffusion had depleted the SC reservoir of AAs. Cathodal extraction proved to be more efficient in extracting AAs from the skin (Table I), implying that reverse iontophoresis may be sampling regions of the skin deeper than the SC. This becomes more evident when the extraction fluxes from 3 to 6 h are compared, (Table II) in that for 11 out of 13 AAs, the ratio of average cathodal to passive fluxes significantly exceeded unity. The extent to which this finding might be exploited further is addressed below.

Finally, it is also appropriate to comment on the glucose data from these experiments. The presence of glucose in the SC has been demonstrated (28), and the need to deplete this reservoir for the application of non-invasive monitoring of blood sugar by reverse iontophoresis has been recognized in the development of the GlucoWatch® Biographer. The results in Tables I and II confirm the glucose store in the SC and the efficiency of iontophoresis to then pull this analyte from a deeper compartment is very obvious: the cathodal flux from 3 to 6 h surpassed the corresponding passive transport rate by nearly 7-fold.

Separating SC and Subdermal Sources of AAs

The next logical step was to study the iontophoretic extraction of AAs when the analytes were present in the subdermal compartment. The extraction fluxes of three AAs (Ser, Pro and Phe, representing, respectively, those present in the SC at high, medium and low levels), as a function of time and of subdermal concentration, are shown in Fig. 4. For all

Table II. Comparison of Cathodal Extraction Fluxes and Passive Diffusion Between 3 and 6 h (Mean ± SD, n=6)

AA	Flux to cathode [nmol/(h·cm^2)]	Passive flux [nmol/(h·cm^2)]	Ratio of cathodal to passive fluxes
Ser	4.1±1.2	3.4±1.0	1.2
Gly	3.7±1.2	2.3±0.6	1.6
Ala	2.7±0.6	1.7±0.5	1.6
His	1.8±0.4	1.8±0.5	1.0
Thr	1.1±0.3	0.8±0.2	1.4
Pro	0.9±0.2	0.6±0.2	1.5
Leu	1.1±0.3	0.8±0.3	1.4
Val	0.6±0.3	0.4±0.1	1.5
Ile	0.5±0.2	0.4±0.1	1.3
Tyr	0.4±0.1	0.3±0.1	1.3
Phe	0.3±0.1	0.2±0.1	1.5
Asn	0.2±0.1	0.2±0.1	1.0
Trp	0.05±0.03	0.03±0.01	1.7
Glucose	0.4±0.3	0.06±0.05	6.7

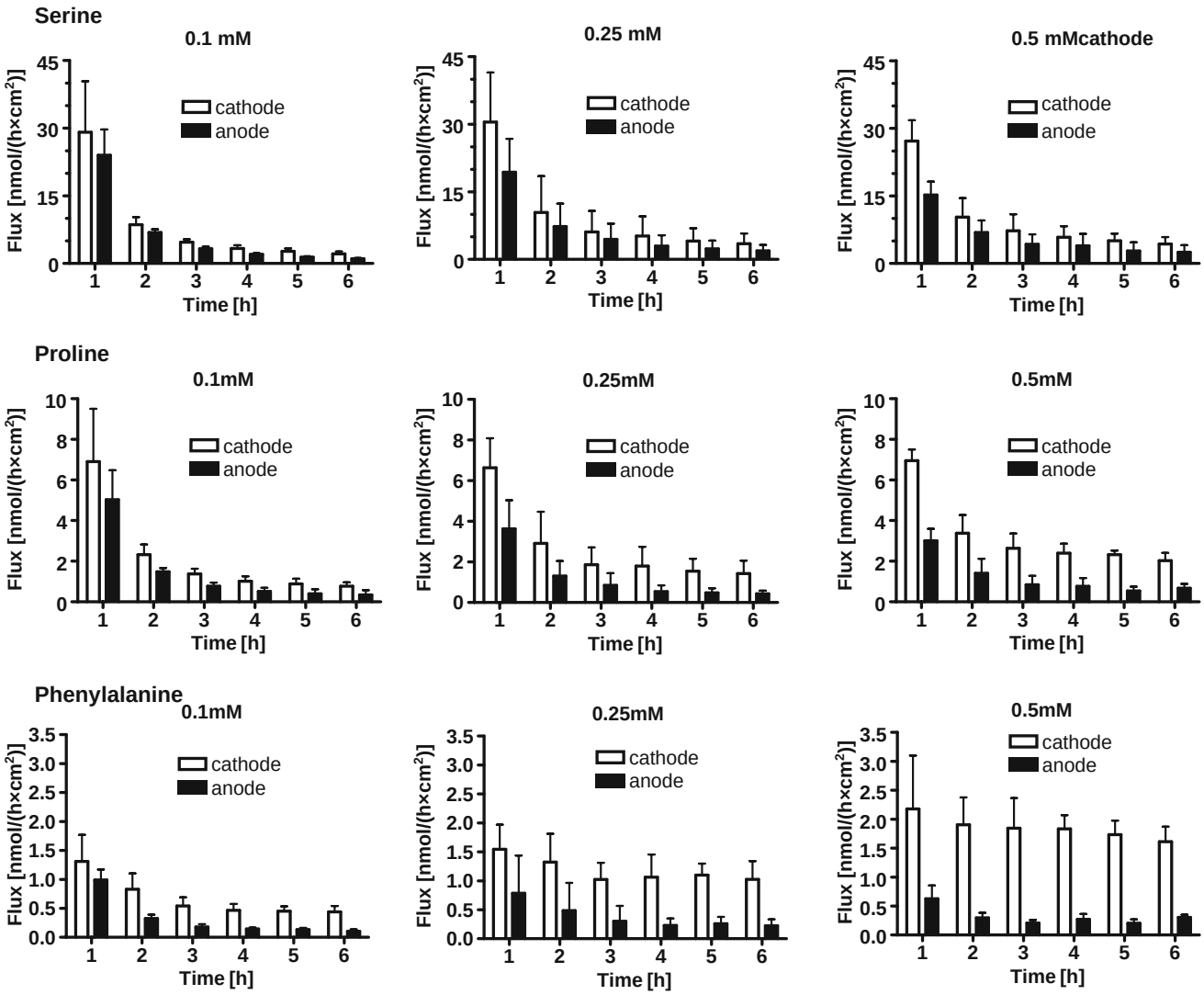

Fig. 4. Iontophoretic extraction fluxes of Ser, Pro and Phe at the cathode and anode as a function of time and subdermal concentrations.

AAs, and for glucose, extraction to the cathode was significantly higher than that to the anode (paired *t*-test, $p<0.05$).

For the AAs, which are most abundant in the SC (Ser, Gly, Ala, His), the extracted fluxes were not very sensitive to the subdermal levels, suggesting that the 'reservoir' of these compounds, requires a significant period to be fully depleted. The extraction was initially very high and then decayed over time, consistent with classic release behaviour from a membrane initially loaded with solute (28).

In contrast, the AAs least present in the SC (Phe, Trp, Met, Asn, Tyr, Ile, Val) as well as glucose, revealed a clear relationship between extraction flux and subdermal concentration over the last 3 h of iontophoresis. In this case, the relatively small SC 'reservoir' of these compounds is quickly depleted, and reverse iontophoresis then samples the subdermal space. At the highest AA concentrations, there is no obvious drop-off in the extraction flux with increasing time of current passage, implying that even at the earliest times, the contribution of the SC source of AAs is negligible relative to that originating from the subdermal space.

Logically, the behaviour of the AAs present in the SC at intermediate levels (Pro, Thr, Leu) falls between the two extremes just described, with the extraction fluxes becoming more obviously sensitive to the subdermal levels at higher concentrations and longer times of current passage.

The evolution of the extraction process as a function of time for different examples of the AAs studied is illustrated in Fig. 5. When the amounts extracted at the cathode are compared with the amounts found in the SC by tape-stripping, it becomes apparent that the first hour of iontophoresis samples primarily the SC and is insensitive to the subdermal level. In the case of an AA which is abundantly present in the SC, such as Ser, and even Pro, 6 h of current passage are insufficient to unambiguously demonstrate that the SC 'depot' has been fully depleted. On the other hand, for AAs which are less concentrated in the SC, like phenylalanine and tryptophan, reverse iontophoresis effectively 'clears' the SC reservoir within a couple of hours of current passage and the extraction is then very sensitive to the presence (or not) of the AA in the subdermal compartment.

Fig. 6 confirms that the AAs extracted to the cathode in the first hour of iontophoresis correlated very well with the corresponding amounts measured in the SC tape-stripping experiment. Regardless of the subdermal concentration of the

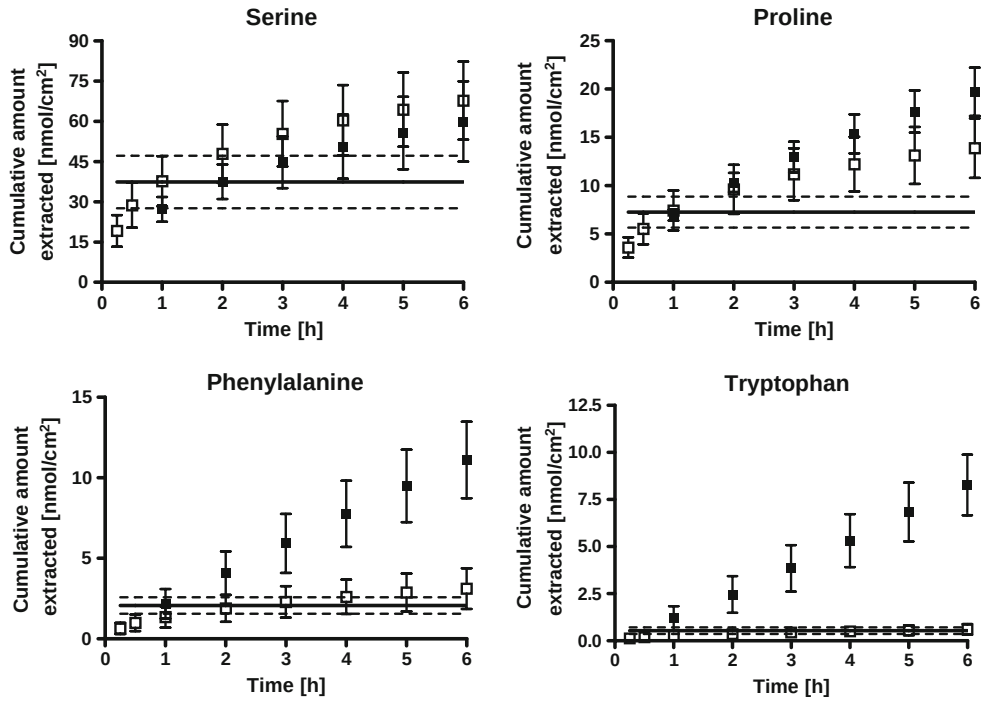

Fig. 5. Cumulative extraction of 4 AAs by reverse iontophoresis as a function of time when initially present in the subdermal compartment at either 0 (*open squares*) or 0.5 mM (*filled squares*). The solid and dashed lines represent, respectively, the mean amount and the ± SD of the AAs recovered in the SC by tape-stripping.

AAs, almost all the individual data points fell within the 95% confidence interval of the regression obtained (slope = 0.88± 0.05) when no AAs were introduced into the solution beneath the skin. This means that the ratio of the amounts extracted by reverse iontophoresis to those measured in the tape-stripping did not deviate appreciably from unity and demonstrates that the initial period of current passage is sampling, to all intents and purposes, only the SC. A relatively short reverse iontophoresis extraction period could therefore be used to non-invasively monitor aspects of skin "health"; for example, lower AA levels have been observed in dry and scaly SC compared to healthy skin (11–13,20) and, quite recently, loss of function mutations in the filaggrin gene (a major predisposing factor for atopic dermatitis) have been correlated with a reduced level of NMF in the SC (21). The contrast to glucose, in that case, is striking. When the subdermal concentrations of glucose are set at 1, 2.5 and 5 mM, respectively, the amounts extracted at the cathode in the first hour are ~4, 6 and 12 times greater than the quantity recovered from the SC by tape-stripping.

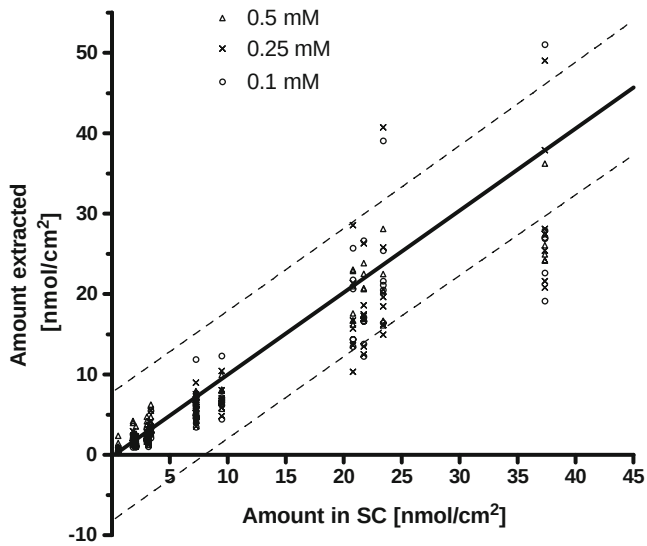

Fig. 6. AAs extracted at the cathode in the first hour of reverse iontophoresis (when their subdermal concentration was 0.1, 0.25 or 0.5 mM) plotted as a function of the corresponding levels in the SC determined by tape-stripping. The *solid* and *dashed lines* show the regression obtained (±95% confidence interval) when no AAs were added to the subdermal solution.

For all AAs, at the three subdermal concentrations tested, the extraction fluxes to the cathode during the 3–6 h period of current passage were relatively constant. When these values were normalized by the corresponding subdermal concentration, an apparent electroosmotic flow was determined in accord with equation 1. The results are collected in Fig. 7; also shown for comparison are the amounts of AAs determined in the SC by tape-stripping. The normalized fluxes were evaluated relative to those measured for glucose, the electroosmotic extraction of which after 3 h of iontophoresis is known to originate uniquely from the subdermal compartment (the skin depot having been completely cleared by this time).

Clearly, the AAs, which are most abundant in the SC, demonstrated normalized fluxes which were significantly higher than those for glucose, signalling that these extractions included an important contribution from the SC 'reservoir'.

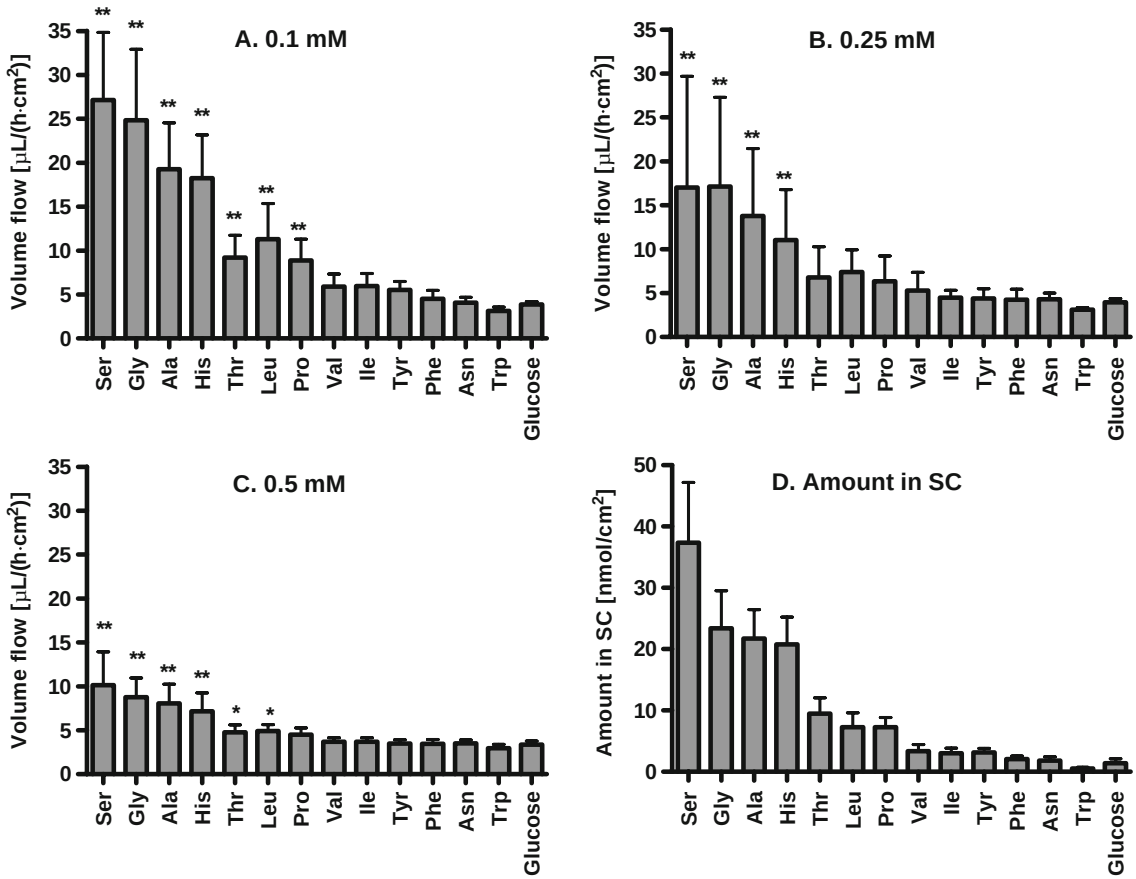

Fig. 7. Panels **A**, **B**, **C**—AA and glucose cathodal extraction fluxes (during the 3–6 h period) normalized by the subdermal concentration (mean ± SD) in PBS background electrolyte. Values significantly different from that for glucose (Anova followed by a Dunnett's test) are indicated with asterisks (** $p<0.01$, * $p<0.05$). Panel **D** displays the amounts of the AAs in the SC (mean ± SD) from tape-stripping experiments.

These relative contributions became smaller as the subdermal concentrations increased. For the least present AAs in the SC, on the other hand, their normalized fluxes were comparable to that of glucose, indicating that their extraction was originating exclusively from the subdermal space.

From a practical standpoint, the results imply that while reverse iontophoretic monitoring of systemic AA levels may be possible in theory, there are clearly some important limitations. First, for the most abundant AAs in the SC, the time necessary to deplete this depot is more than 6 h and the probability, therefore, that useful information can be obtained from the subdermal compartment is negligible. Second, for AAs which are present at low levels in the SC, although the approach looks more promising (in that it is possible to show a correlation between extraction flux and the subdermal concentration), the actual *in vivo* concentration ranges of these species in the systemic compartment are often less than 0.1 mM. There is an issue of assay sensitivity as a result, as well as a concern that even the low SC levels of these AAs may overwhelm the contribution to the extracted flux from the systemic source. The greatest potential value of the approach concerns AAs, which are at low concentrations in the SC and in the plasma, but which show large positive deviations in the latter compartment when a pathology exists. For example, in phenylketonuria, plasma concentrations can exceed 1 mM (29), a dramatic increase over the normal range of 35–80 μM (30). A monitoring system in children afflicted with this metabolic disease may represent, therefore, a practically useful device.

CONCLUSIONS

This investigation shows that AA sampling from the skin can be accomplished by tape-stripping, by passive extraction and by reverse iontophoresis. Tools to examine quantitatively the key components of NMF are implied. The advantage of tape-stripping is that it samples uniquely the SC and can produce a full concentration profile of the AAs across the barrier. Passive extraction also reveals the quantities of the analytes in the SC (but without the distribution profiles); however, 6 h are needed to withdraw all the AAs present in the SC. Reverse iontophoresis to the cathode is more efficient and can shorten the time needed to fully sample the SC. Indeed, a relatively short period of reverse iontophoresis (~1 h) is an effective, minimally-invasive method with which to determine the levels of many AAs within the skin. Furthermore, reverse iontophoresis can also "interrogate" deeper skin compartments. When the period of current passage is prolonged, the levels of AAs collected at the cathode are correlated with their subdermal concentrations for those compounds having relatively small SC reservoirs (e.g., phenylalanine). Fluctuations in the systemic concen-

trations of such AAs, as a result, for example, of a metabolic disorder, may therefore be usefully monitored by reverse iontophoresis. Further *in vivo* studies are underway to verify that the SC and subdermal sources of AAs can also be differentiated in reverse iontophoresis extractions in human.

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