Absorption of an Oxytocin Antagonist (Antocin) and a Vasopressin Analogue (dDAVP) Through a Standardized Skin Erosion in Volunteers

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Purpose. Transdermal administration of the peptides [Mpa[1], D-Tyr (Ethyl)[2], Thr[4], Orn[8]]-oxytocin (antocin) and [Mpa[1], D-Arg[8]]-vasopressin (dDAVP) was studied in healthy volunteers.

Methods. A standardized skin erosion was formed preliminary by suctioning. The peptides were administered in plastic reservoirs through a 5 mm erosion and the absorption was followed for a six-day period with plasma concentration determinations on days 1, 3 and 6 with refilling the reservoirs daily with 15 μm and 10 mM solutions of dDAVP and antocin, respectively. Fourteen healthy non-smoking volunteers divided equally between the sexes, participated in the study. Plasma concentrations were measured using specific radioimmunoassays. Reservoir concentrations and metabolic stability of the peptides were determined using reverse-phase HPLC.

Results. Both antocin and dDAVP were absorbed across the skin erosion. The absorption pattern was biphasic with a high initial absorption during days 1 and 2 followed by a lower absorption on days 3 and 6. The absorption on day 1, which was estimated at more than 50% for both peptides during a 24 h period, corresponded to a simultaneous decrease in peptide concentration in the reservoirs. The extent of absorption for antocin on days 3 and 6 was 1/3 to 1/6, respectively, of that observed on day 1. Antocin was minimally degraded in the skin reservoir while dDAVP was intact. However, accumulation of cellular material appeared in the antocin reservoirs. The absorption of antocin was reduced by exposure to intact skin surrounding the skin erosion. No pain was experienced and no scar formation was observed.

Conclusions. The observed biphasic absorption may be a consequence of the mild inflammatory response occurring subsequent to eroding the skin. The standardized skin erosion may provide a route for the short-term delivery of otherwise poorly absorbable peptide and protein drugs.

KEY WORDS: peptides; drug administration; skin; absorption; oxytocin; vasopressin; drug absorption.

INTRODUCTION

The delivery of peptides and proteins across body barriers is a major problem in therapy. The amount of peptide drug absorbed after nasal and oral administration is usually below 10 and 1%, respectively (1) and it is therefore important to explore alternative routes of delivery. Transdermal delivery may offer an alternative with obvious advantages. However, the size and hydrophilic properties of peptides and proteins prevents their penetration of the lipophilic epidermal barrier. Under special circumstances transdermal passage can be accomplished aided by chemical enhancers or iontophoresis (2) although the safety and efficiency of these methods has not yet been proved. Transdermal patch administration is being explored but so far only a small number of lipophilic drugs can be made to pass passively across the epidermal barrier. A promising method for transdermal delivery of poorly-absorbed drugs was recently presented (3). The drug is administered via a standardized 5 mm skin erosion formed preliminarily by suctioning. We used a patch applied over a single skin erosion in volunteers, loaded it daily with the vasopressin analogue [Mpa[1], D-Arg[8]]-vasopressin (dDAVP, desmopressin) and found that plasma concentrations of dDAVP were measurable for at least 4 days. Another study showed that morphine was efficiently absorbed (75%) and elicited less side-effects (non-analgesic) than after intravenous administration (4).

The purpose of the present study was to further evaluate the performance of this transdermal method of drug administration by comparing in more detail the absorption of dDAVP with that of another potentially important peptide drug, [Mpa[1], D-Tyr(Ethyl)[2],Thr[4],Orn[8]]-oxytocin (antocin). Antocin has shown promising results as a tocolytic agent in the treatment of pre-term labour (5) and its usefulness might be increased if other routes of delivery than intravenous infusion could be utilized. In this study, antocin and dDAVP were administered at doses currently used in intravenous therapy and their transdermal absorption was followed for a six-day period. We further wanted information on the interaction of drug with intact skin and whether any degradation of the peptides occurred in the skin drug reservoirs.

SUBJECTS AND METHODS

Subjects

Healthy volunteers were studied in experiments that took place over a three-month period. There were equal numbers of males and females. Informed consent was obtained from each person. The study was approved by the Ethical Committee of the University of Lund. During the course of the study the subjects abstained from smoking, alcohol and medication. The subjects were divided into two groups (mean age weight: 1) 27.3 ± 6.9 years 66.5 ± 4.4 kg (n = 14), 2) 32.3 ± 7.6 years 64.5 ± 9.7 kg (n = 6).

Drugs

Antocin (10 mM) and dDAVP (Octostim^{R} 15 μM) were produced by Ferring AB, Malmö, Sweden. Both drug formulations contained chlorobutanol hemihydrate and were dissolved in isotonic saline.

Preparation of Delivery Site

The volar side of one forearm was cleansed with cycloheximide solution and the suctioning device was applied to

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the skin. A suction cup (diameter 5 mm) was sealed to the skin and a relative vacuum of 200 mmHg below atmospheric pressure was applied and maintained by using a membrane pump. The vacuum exposure split the epidermis and distended it with clear transudate within 2–3 h. The erosion was completed by removing the distended layer. The drop of transudate was absorbed in a piece of medical gauze. A sterile cylindrical polymetylmetacrylate reservoir (diameter 2.5 mm, volume 0.8 ml) with a lid at one end and a circular perforation at the other was sealed to the adjacent skin at its perforated end. The reservoir was immobilized by applying an outer adhesive dressing. Normal physical activities including sports and showering were encouraged.

Experimental Series

1A. Antocin and 1B. dDAVP absorption; group 1 volunteers (see above). In both series the reservoir was applied in such a way that its circular perforation matched the underlying 5 mm erosion as exactly as possible, avoiding exposure of intact skin inside the reservoir. 2. Antocin absorption; group 2 volunteers (see above). The size of the reservoir perforation was increased in such a way that an area of intact skin was included within the reservoir. This area was approximately 40 times larger than that of the 5 mm deepithelialized spot.

In all experiments the reservoir was filled with 0.5 ml of the drug (time 0, either at 08.00 h or at 12.00 h). The concentration of the antocin and dDAVP solutions were 10 mM and 15 μM, respectively. Each day during the following six days the reservoir was emptied and refilled with the drug.

Venous heparinized blood samples were drawn from a short indwelling catheter in the contralateral arm directly before drug administration and 5, 10, 20, 30, 60, 90, 120, 180, 240 min and 24 h after administration. On days three and six the blood sampling procedure was repeated.

The erosions and adjacent skin were inspected on a daily basis.

Peptide Analyses

dDAVP and antocin were measured in plasma using specific RIA methods. Both the assay for dDAVP (6) and for antocin (7) were previously described. The inter- and intraassay variations were 5.75 and 4.06%, respectively, at 20 fmol of antocin/assay tube.

The analyses of peptides in the reservoir fluid were performed by means of high-performance liquid chromatography (HPLC). Two systems were used. For antocin a Kromasil C_8 precolumn and a KR 100-5 C_8 1572 (100 × 2.1 mm) column, Hichrom, Scantec were used. The flow rate was 0.2 ml/min with UV detection at 190 nm. The peptide was eluted with a mobile phase consisting of A) Acetonitrile: H_2O: TEAP (2: 97.9: 0.1 w/v%) and B) Acetonitrile: H_2O: TEAP (90: 9.9: 0.1%). A and B were mixed yielding a final content of 27% acetonitrile at isocratic elution condition. The reservoir fluid was pretreated by extraction with acetone and petroleumether followed by centrifugation and evaporation. dDAVP was analyzed using the HPLC system previously described (8). The reservoir fluid was boiled for 5 min, followed by centrifugation at 3000 × g for 10 min. The recovery was 90–100%.

Pharmacokinetic Calculations

Areas under curves (AUC) based on plasma concentrations of the peptides were calculated using the trapezoidal rule. The extent of transdermal absorption (F) on the first day was determined by the relationship.

$$F\,(\%) = \frac{Clp \cdot AUC_{td}}{Dose_{td}} \times 100 \qquad (1)$$

where Clp is the plasma clearance rate obtained after intravenous administration (8, 9), AUC_{td} was determined for the 0–4h period but also approximated for 0–24 h. Results are expressed as means ± S.D. or individually. One-way ANOVA is used for statistical comparisons.

RESULTS

The plasma time-concentration profiles for antocin on days 1, 3 and 6 (experiment 1A) are shown in Fig. 1. Absorption was substantial on day 0 and a stable plasma level was established in approximately 100 min. By day 3 the level was reduced to 1/3 of the original. Between days 3 and 6 the absorption stabilized and only a small further decrease was noted. The corresponding time-concentration profiles for dDAVP (experiment 1B) showed higher plasma concentrations on day 1 after which they remained stable at a lower level between days 3 and 6 (Fig. 2). Exposure to epidermis reduced antocin absorption (experiment 2) and the antocin plasma concentrations were low on day 3 and almost undectable by day 6 (Fig. 3).

Calculated values for areas under the curves (AUC) are shown in Table I. Clp values of 23.5 ± 7.6 (antocin) and 17.5 ± 2.01 × h^{-1} (dDAVP) were used in the calculations. The AUC-values for the two peptides were in the same range during the first 0–4 h after drug administration on day 0. The extent of absorption for the first 24 h period was also calculated and was found to be 57.3 ± 12.3 and 52.7 ± 15.6% for antocin and dDAVP, respectively. At 24 h the concentration

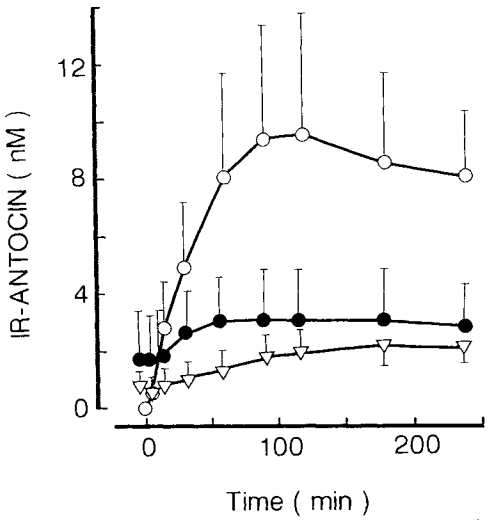

Fig. 1. Plasma time concentration curves of immunoreactive (IR) antocin in blood on days 0 (○), 3 (●), and 6 (▽) after transdermal administration of 0,5 ml of a 10 mM solution in isotonic saline. Values are given as means ± S.D. (N = 6).

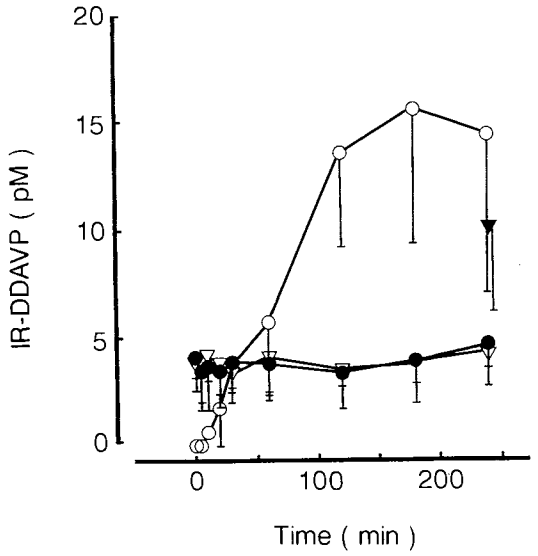

Fig. 2. Plasma time-concentration curves of IR-dDAVP on days 0 (○), 1 (▼), 3 (●) and 6 (▽) after transdermal administration (0,5 ml) of a 15 μM solution in isotonic saline. Values are given as means ± S.D. (N = 8). Note that blood sampling on day 1 only took place at t = 240 min.

of dDAVP had declined to 4.1 ± 0.5 pM and that of antocin to 1.8 ± 1.1 nM, about four times lower than the maximum level. On day 3 and 6 the AUC-values for dDAVP were reduced to 1/3 of the value on day 0, for antocin to 1/3 (day 3) and 1/6 (day 6) of the day 0 value (Table I). The 666 times higher dose of antocin resulted in a correspondingly higher plasma level of antocin (Figs. 1–2).

The concentrations of antocin and dDAVP in the reservoirs increased with number of days (Fig. 4) as would be expected from the decreased blood concentration. An additional, a more lipophilic peak in the reservoir containing antocin was present after HPLC analysis of the reservoir fluid

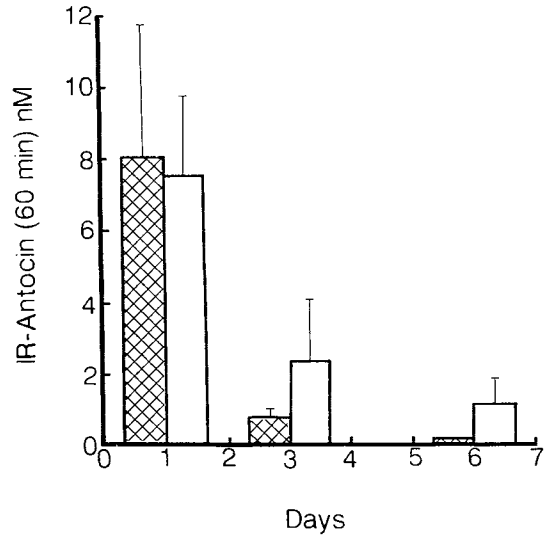

Fig. 3. Comparison of IR-antocin plasma concentrations at t = 60 min after transdermal administration (10 μM) between drug with (hatched bars) and without (open bars) contact with skin surrounding the erosion. Values are given as means ± S.D. (N = 6).

(Fig. 5). This peak was barely detectable on day 1 but was observed in all reservoirs on day 6. The fraction of remaining intact antocin in relation to total peak area is shown in Fig. 6. dDAVP in the reservoirs was chromatographically intact during the experimental period.

In the experiments with dDAVP the erosions appeared red and without accumulation of cellular material in all volunteers and the adjacent skin looked normal. As late as day 6 there were no signs of reepithelialization. However, by days 9–10 an epithelial cover, reflecting light in a characteristic way, was noted in all subjects. In the experiments with antocin some cellular material accumulated successively on the erosions although findings were otherwise similar to those described for dDAVP. An erythema, which faded away, was noted on the skin sites after the experiments. There was no pain reported in these experiments and subsequent side-effects have not been observed. Three months after these experiments were performed there were no signs of the skin erosion and no scars had formed.

DISCUSSION

This study showed that therapeutic transdermal delivery of potent peptide drugs through a standardized skin erosion may be feasible for at least 6 days. The results are all the more interesting since a simple aqueous formulation intended for intravenous administration was contained in the adhesively applied reservoirs, with no effort made to optimize the qualities of the formulation relative to tissue compatibility or absorption.

Suctioning produces a skin erosion whose properties are reproducible and therefore may be called "standardized". Irrespective of the epidermal thickness the split always occurs through the lamina lucida part of the basal lamina, leaving the underlying connective tissue mat of lamina densa relatively intact (10, 11). Except for solitary micropetecciae the dermal microcirculation remains intact and functional (12). Measurements by vital microscopy has shown that the total length of visible functional capillaries in a 6 mm lesion is 1600 μm (12) which makes the total capillary wall area approximately 1 mm^2. The rich sub-papillary plexus of capillaries and the venules remain unaccounted for in the calculation. Laser Doppler assessments of the total dermal microvasculature underneath erosions kept occluded by plastic film have shown that a pronounced and stable hyperaemia prevails through day 3 in one study (13) and through day 4 in two other studies (14, 15). After days 3 and 4, blood flow diminishes gradually.

The epidermal splitting process induces a mild inflammatory reaction and initiates repair (11). The early response includes sequential migration of cells (16) and liberation of inflammatory mediators as well as proteolytic enzymes and growth factors. Of the series of events that are initiated, local hyperaemia and permeability increase as well as the ultimate restoration of the epidermal barrier, may in particular influence drug absorption. The vascular endothelium has a small population of large variable, pores whose dimensions are subject to direct physiological regulation in addition to a large population of static small pores with fixed hydraulic conductivities (17). Under basal conditions the variable pores are mostly closed, restricting the passage of large mac-

Table I. Areas Under the Curves (AUC_{td}) During 4 h for Antocin and dDAVP After Transdermal Administration[a]

$Dose_{td}$	Day 1	Day 3	Day 6
	AUC_{td} (0–4 h)		
Antocin (5 μmol)	29280 ± 8029	8966 ± 6472	4794 ± 4769[b] (pmol · l^{-1} · h)
dDAVP (7.5 nmol)	42.192 ± 12.777	15.020 ± 5.828	14.939 ± 3.004 (pmol · l^{-1} · h)

[a] N = 6–8. Values are shown as means ± S.D.
[b] The change of AUC_{td} between days 3 and 6 was considered non-significant ($p > 0.05$).

romolecules. In the prevailing situation the pores would open in response to inflammatory mediators.

Vasopressin analogues such as antocin and dDAVP may change the dermal microvascular tone (18) and might, thus, indirectly affect absorption. However, dDAVP induces only small changes in the microvasculature of eroded skin (3). Data on antocin in this respect is lacking.

A biphasic absorption pattern was demonstrated for antocin, characterized by an initial phase of high absorption on day 0, followed by a stable phase between day 3 and 6 when the absorption was reduced. The absorption pattern for dDAVP resembled that for antocin. Due to the relatively short half-lives of antocin and dDAVP (1 h) maximal concentration should be reached within four hours. Increased vascular permeability produced by inflammatory mediators may enhance the absorption particularly during the initial phase (19, 20). The presence of drug solution on the tissue surface may prolong the permeability increase beyond the few hours described in classical reactions to minor injury. If this is correct the absorption in the late phase may be increased by adding a suitable factor to the preparation (21). Earlier we have shown that the dermal hyperaemia was definitely present on days 3 and 4 when absorption had already been reduced. This makes it likely that volume flow is above the threshold where it would become a limitation to drug absorption. However, data on the relation between dermal blood flow and absorption is lacking. As the effect of inflammatory mediators abate, an increased sensitivity of the dermal microcirculation to tone-increase induced by dDAVP and antocin cannot be excluded. Even on day 6 the dDAVP plasma concentrations remained well within the therapeutic range normally required for treatment of diabetes insipidus. To achieve therapeutic plasma concentrations for antocin the dose was increased 666 times relative to dDAVP. For antocin this resulted in correspondingly higher plasma concentrations. Even so, the antocin concentrations fell below the therapeutic levels used for pre-term labour on days 3 and 6. It remains to be seen whether the biphasic absorption pattern observed for antocin and dDAVP also applies to other molecular species. Nasal delivery of dDAVP as a spray leads to a bioavailability of 3% at 4 h and about 5% at 24 h (9). Thus, a substantial improvement (F = 52.7%) was obtained by using transdermal administration. However, at this stage we do not want to see the erosion technique used in permanent therapy as would be required for diabetes insipidus and while the antocin application requires further investigation it may well become a useful therapeutic option, as may other vasopressin analogues.

Enzymatic degradation of peptide on the site of administration was nil for dDAVP and marginal for antocin. Thus, the dose of these peptides can be maintained within acceptable limits in a simple aqueous solution. In fact, the appearance of antocin and dDAVP in blood was followed by a concomitant decrease in drug concentration in the reservoirs.

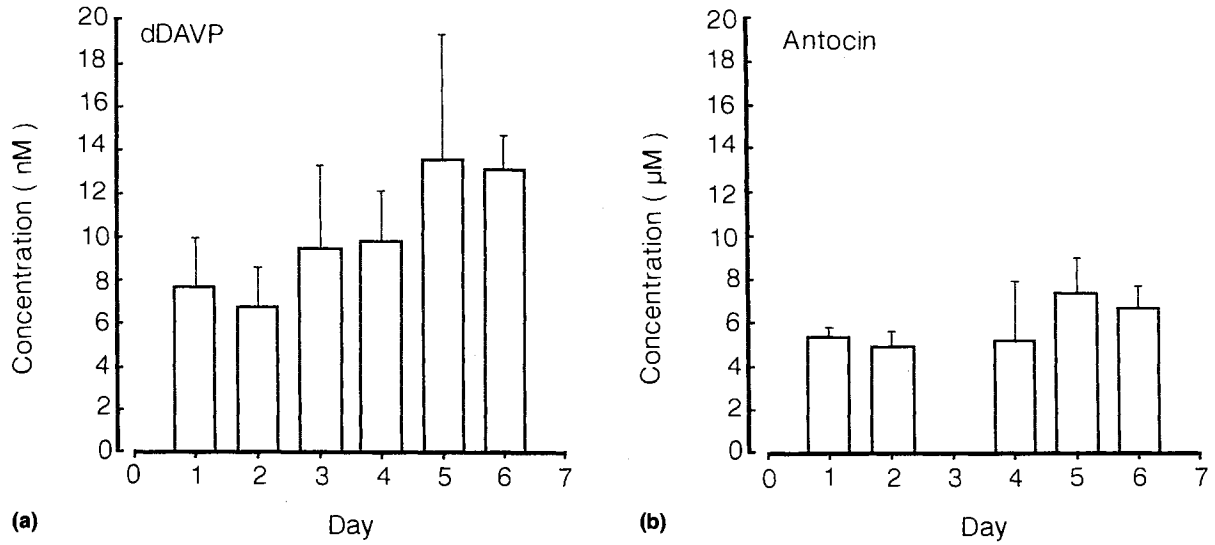

Fig. 4. Reservoir concentrations of a) dDAVP and b) Antocin as measured by HPLC during the six-day experimental period. Values are shown as means ± S.D. (N = 3–6).

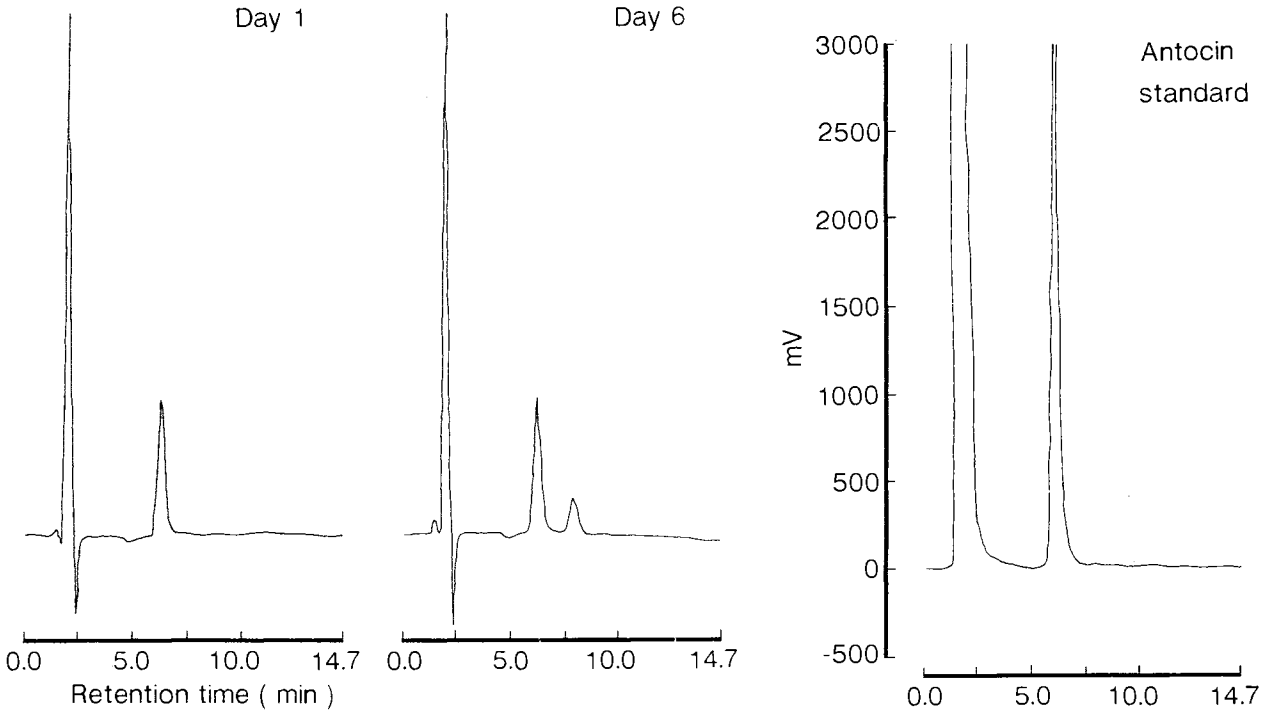

Fig. 5. Elution profiles of antocin on HPLC after exposure to the erosion for 24 h. An additional, more lipophilic peak appeared with increasing number of days (middle).

The ratios between the concentrations in the reservoir and the plasma was quite similar for antocin and dDAVP. This finding may be a consequence of the similarity in molecular size and plasma clearance rates. The antocin preparation elicited a cellular reaction which may be an effect of the higher drug concentration. Antocin became less efficiently absorbed when the wide collar of the epidermis was included in the reservoir.

In conclusion, we have shown that small peptides such as dDAVP and antocin are absorbed in significant amounts through the standardized skin erosions for at least 6 days.

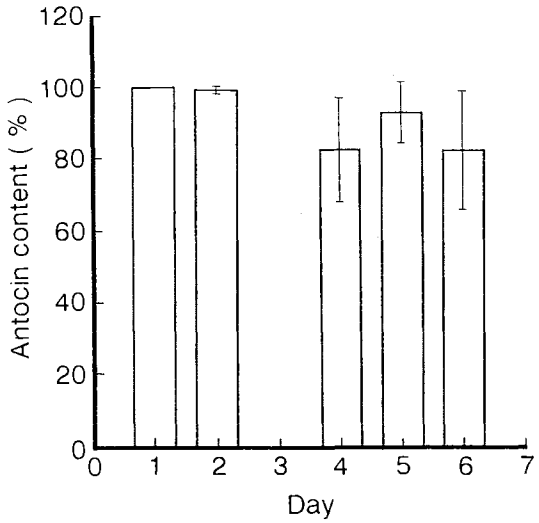

Fig. 6. The fraction of remaining intact antocin in the skin reservoirs as determined by HPLC with increasing number of days. Samples were taken after exposure to the erosion for 24 h.

The absorption pattern for these vasopressin analogues was bi-phasic and the permeation was most efficient during the early phase. Therapies limited in time to weeks or months would provide the candidates for this new therapeutic route. Potent peptides with fitting therapeutic windows are likely to be found. Future research concerning the effects of inflammatory mediators and repair on drug absorption may be useful for further increasing the delivery.

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