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Dose-dependent competitive block by topical acetylsalicylic and salicylic acid of low pH-induced cutaneous pain

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Summary In a human acid pain model, which uses continuous intradermal pressure infusion of a phosphate-buffered solution (pH 5.2) to induce localized non-adapting pain, the flow was adjusted to result in constant pain ratings of about 20% or 50% on a visual analog scale (VAS). Six volunteers in each group participated in 4 different placebo-controlled double-blind cross-over studies to measure rapidly evolving cutaneous analgesia from topically applied new ointment formulations of acetylsalicylic acid (ASA) and salicylic acid (SA) as well as of commercial ibuprofen and benzocain creams. Similar, log-linear dose–response curves were found for both ASA and SA, significant in effect at 3 g/kg and higher drug contents and reaching saturation level at 15 or 30 g/kg, respectively, which, 20 min after application, caused a mean pain suppression of 95% using ASA and 80% using SA. Half-maximal effects were achieved using 3 g/kg ASA or 15 g/kg SA. The SA action was also clearly slower to develop. With an increased flow of the acidic buffer, producing lower effective tissue pH and more intense pain, the effect of ASA and SA decreased to 73% pain suppression. A competitive mechanism of both drug effects was suggested by the fact that, with 15 g/kg ASA and SA, pain reduction could be reversed by increasing the buffer flow by a factor of 1.75, on average. Commercial ibuprofen (50 g/kg) and benzocain creams (100 g/kg) were comparably as effective as ASA and SA, but the local anesthetic caused a loss of all cutaneous sensations while the touch threshold (von Frey) under the specific analgesics was the same as under the placebo ointment. Thus, topical applications of non-steroidal anti-inflammatory drugs (NSAIDs) dissolved in different ointment formulations have proven dose-dependently effective and specific in suppressing experimental acidotic pain by a local and competitive mechanism.

Key words: Nociception; Proton; Transdermal; Analgesia; Ibuprofen; Benzocain; Aspirin

Introduction

Topical drug applications

Topical administrations of drugs, in the form of plaster applications which are increasingly being used, generally seem to be directed toward systemic effects but also toward local actions in the skin. Growing numbers of clinical reports indicate that topically applied non-steroidal analgesics are effective in pain therapy of acute and chronic inflammatory skin diseases such as acute and post-herpetic neuralgia (De Benedittis et al. 1992; King 1993). Very recently, in an experimental pain model, the effectiveness and mechanisms of topically applied acetylsalicylic acid (ASA), salicylic acid (SA) and indomethacin (dissolved in diethylether) were investigated. Pain was achieved by an intradermal low-pH buffer infusion into the palmar forearm. The study demonstrated that saturated solutions of these drugs applied to the painful skin area led to potent pain suppression of up to 96% within 20 min. During the observation time of up to 3 h, no significant plasma concentrations could be determined, thus suggesting a purely local mechanism. Deep analgesia was not accompanied by a loss of tactile sensation but was competitively counteracted by increasing the infusion rate of the painful buffer solution (Steen et al. 1995a).

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In the present psychophysical studies, the same experimental approach was used to compare dose–response mechanisms of partly commercial, partly new ointment formulations of widely used NSAIDs.

Protons as a mediator of pain states

High hydrogen ion concentrations in tissue have been found in inflammatory and ischemic diseases. It has thus been suggested that hydrogen ions play a major role in inflammatory pain (Lindahl 1961). In electrophysiological experiments, a low pH value induced sustained nociceptor excitation and sensitisation to mechanical stimulation (Steen et al. 1992). As a possible cellular correlate for the transduction mechanism of the excitatory action of protons, a sustained and slowly inactivating cation inward current, demonstrated in small dorsal root ganglion (DRG) cells, has been suggested (Bevan and Yeats 1991). A specific antagonist of low pH-induced nociceptor excitation has not yet been identified. However, SA was shown to antagonize competitively a low pH-induced decrease in membrane potential and conductance due to a reduction in K^+ and an increase in Cl^- conductance (Barker and Leviton 1972). The latter antagonism, and a similar action assumed for aspirin (ASA), might possibly be involved in the drug effects investigated in this study.

Protons in experimental pain

An experimental model for the cross-over assessment of pain reduction by transdermally applied analgesics has to provide a controllable method of producing graded pain which is sustained over the period of investigation and shows neither adaptation nor habituation. The pain model used in our study fulfilled these requirements (Steen and Reeh 1993). Since pain vanishes within seconds when the intracutaneous phosphate-buffered acid infusion is stopped by switching off the syringe pump, the procedure was regarded as harmless.

NSAIDs dissolved in vaseline / paraffin for pain treatment

Vaseline (*V. petrolatum*) is widely used as an ointment base for pharmaceuticals and cosmetics. Paraffin, the inert semi-solid substance, is a popular vehicle in clinical use as it is white to transparent, practically odorless, tasteless and of a consistency which is easy to apply. In these studies, ASA and SA were applied in a mixed ointment of *vaselinum album* and *paraffinum liquidum*. To our knowledge, no data is yet available whether salicylic drugs would act on pain when topically applied in a non-etheric or non-alcoholic vehicle. Furthermore we aimed at determining a dose–response relationship of ASA and SA drug effects and a direct comparison of the actions of ASA and its metabolite SA on cutaneous pain. The quantitative comparison was to be extended to a commercial ibuprofen cream and a topically applied local anesthetic, as positive controls, and to a placebo ointment as negative control.

Some of the results have previously been published in abstract form (Steen et al. 1994).

Materials and methods

Continuous intradermal infusion of phosphate-buffered solution (pH 5.2) into the palmar forearm of human subjects provided sustained ongoing pain, graded with different flow rates of a motorized syringe pump but constant over the observation periods if the flow was properly adjusted and maintained. The method has previously been validated and published in detail (Steen and Reeh 1993).

Study conditions and subjects

Twenty-five women and 5 men participated in this study. The subjects were 18–57 years of age, with a median age of 25 years. At the time of the experiments none of the subjects was suffering from an infection or gastrointestinal disease. Also volunteers were asked whether a prolonged bleeding time was likely or whether they suffered from any allergies. The subjects stated that they had not used drugs of any kind within 72 h prior to the experiment. None of them stated that they used pain medication on a regular basis. All the female volunteers declared that they were not pregnant. Before each experiment the subjects were informed about the procedure to the extent that they would get a cream applied to the skin that may or may not influence the pain and that they were free to withdraw from the experiment at any time. All subjects gave their written consent. This study was approved by the Ethics Committee of the Medical Faculty of the University of Bonn and conducted in accordance with the Declaration of Helsinki.

Recording and stimulation procedures

Each subject was comfortably seated in a dentist's chair. Randomly the left or right arm was placed on a cushion and a 27-ga cannula was inserted intracutaneously in the palmar side of the lower forearm. We established continuous pressure infusion of a phosphate-buffered solution (pH 5.2), joined upstream by a sterile filter, into the intact skin, using a conventional motorized syringe pump that provided various flows rated in fixed steps. This resulted in a localized burning pain sensation which was sustained as long as a constant flow was maintained. The intensity of the pain sensation could be varied by changing the flow rate and, in this study, flow rates between 12 and 40 ml/h (mean: 24.8 ml/h) were needed to achieve individual pain ratings of around 20% and between 20 and 60 ml/h (mean: 39.5 ml/h) for 50% ratings on a visual analog scale (VAS) ranging from 'pain threshold' to 'intolerable pain' (0–100% VAS).

Data processing

Every 10 sec the subjects were asked, by means of an acoustic signal, to assess their current pain intensity by moving a lever that controlled a horizontally displayed electronic VAS consisting of 90 rectangular LEDs in an unbroken line. The signal was passed as an analog voltage signal through an AD converter (DCI-12; Decision Computer International, Taiwan) and, for quantitative analysis, data was stored in a 486 AT type computer. The recording software was a self-designed data aquisition program ('pmess') which performs online graphical presentation of the recorded data. The subjects confirmed each single rating by pressing a button, and a second beep tone acknowledged acquisition of the single rating signal. The lever was moved back to zero after each rating by the subject.

Drug application protocol

The infusion solution was made from an isotonic solution of essential salts and sugars, which was buffered by appropriate proportions of NaH_2PO_4 and Na_2HPO_4 (Sigma) to produce the buffered pH level of 5.2 (see Steen et al. 1992 for details). This solution is referred to as 'phosphate-buffered solution' in the following text. Before starting the pain recording, a preliminary run of low-pH infusion for 5–10 min was used to adjust the flow in order to reach individual pain ratings of 20% on the VAS (studies 1–3) and 50% VAS (study 4). The initial 5 min of stable flow rates and VAS recordings were defined as 'baseline' which was followed by covering the painful skin area with 10 ml of the different ointment formulations. This was performed by the investigator (wearing plastic gloves), who distributed about a 1 mm layer of the ointments within 5 sec widely overlapping the infiltration in size (> 2 cm) on the surface of the palmar forearm skin of the subjects. This was followed by immediately covering the arm with a Moltex® plastic dressing to reduce cooling effects. Observation and recording times were 60 min in study 1, 30 min in studies 2 and 4 and up to 60 min in study 3. Studies 1 and 4 were placebo-controlled. All 4 studies were generally designed as double-blind, randomized and cross-over.

Study 1

Six volunteers were asked to rate their pain estimations after randomized application of ASA, ibuprofen, benzocain and placebo. Each drug was applied to each volunteer at an interval of at least 3 days to the painful (VAS 20%) left or right forearm. Ten milliliters of ASA (3 g) in *paraffinum subliquidum* (1 g) and *vaselinum album* (ad 100 g) were used. As a placebo, lactose in the same formulation was applied. Commercial ibuprofen (50 g/kg = 5%, Dolgit® cream, Dolorgiet, Bonn, Germany) and benzocain cream (100 g/kg = 10%, Anaesthesin® cream, Ritsert, Eberbach, Germany), were employed for comparison with the self-designed ASA formulation (30 g/kg = 3%).

Study 2

A dose–response relation was determined by applying ASA in vaseline/paraffin in different concentrations (0.3–50 g/kg = 0.03–5%) (see Figs. 4 and 5) to the skin of 6 volunteers at intervals of at least 3 days, in a double-blind randomized order. In another 6 volunteers, SA in the same concentrations and vehicle were topically applied.

Study 3

After 5 min of stable VAS ratings of 20%, submaximal concentrations of 1.5% ASA or SA, respectively, were applied to the skin. These experiments were conducted cross-over double-blind on separate days for 6 individual subjects. If the ensuing pain suppression settled at rating values stable for 5 min, the effect was antagonized by increasing the acid pH buffer flow rate in steps (beginning with an increase of 20 ml/h). If, in so doing, the initial 20% VAS rating was not reached with stable ratings of 5 min, the flow rate was further increased in 10 ml/h steps until the aim was achieved. The amount of flow rate increase required was recorded.

Study 4

In this study, pain ratings of 50% on the VAS were achieved by adjusting the flow rate appropriately. This was followed by application of either ASA, SA (3%) or placebo in cross-over mode. The pain suppression was compared with the effects of the topically applied drugs on lower ongoing pain intensities as in study 2.

Graphical presentations and statistical analysis

The 'Sigmaplot' software package was employed for off-line graphic representation of the data (Jandel, Berkeley, CA). For statistical analyses the 'Superior Performing Software System' (SPSS Software, Munich, Germany) was used; the Wilcoxon matched-pairs test for comparing the ratings before and after application of the drugs and the Mann-Whitney U test for comparison of active drugs with placebo were employed. These non-parametric tests were performed by entering summated rating values (n = 30) across the last 5 min (maximum effect period) of each individual record; the same pooling was done with the baseline values across the 5 min before drug application. P values lower than 0.05 were regarded as significant.

Results

Vehicle effects

Analgesia due to the cooling effect of topical vehicles is well known and has to be separated from the drug effect. Immediate and mild pain reduction (16% on average), after applying the vaseline/paraffin preparation, was rapidly overcome within 2 min 49 sec, on average (0–7: 50 min (n = 60)). Transient pain relief due to cooling and analgesic actions due to the drugs could be clearly separated owing to the different time courses.

Study 1: topical NSAID effects compared with a local anesthetic

The effect of ASA in a vaseline/paraffin ointment formulation (3%) on cutaneous experimental pain was compared with a commercial ibuprofen (5%) and benzocain cream (10%). In the placebo trace of the original recording (Fig. 1) constant pain ratings throughout the observation period (60 min) can be seen; placebo (lactose in vaseline/paraffin ointment) was applied to a wide area on and around the painful skin after 5 min of baseline recording and remained ineffective. In contrast, following ASA treatment, the pain ratings decreased and then completely vanished after 28 min in this particular subject. This effect of ASA did not have a local anesthetic nature, since the von Frey threshold in all subjects remained the same before and after application of the drugs (or placebo) in vaseline. Also, the subjects did not report altered sensation in the treated skin. Ibuprofen and benzocain were comparably as effective as ASA, but the local anesthetic caused a loss of all cutaneous sensation. A summary of the results of 6 volunteers treated with the topical drugs is given in Fig. 2. In the first 5 min after application of ASA, ibuprofen, and benzocain, there was already a significant pain suppression ($P < 0.05$ and $P < 0.03$, respectively; Wilcoxon) compared to the baseline, and the ratings further decreased until after about 30 min of the protocol. With the placebo, pain ratings were not significantly reduced during the observation period of 55 min after application. Comparing the verum to placebo data in averaged 5 min samples, a significant difference was observed with ASA from 15 min after

application ($P < 0.03$–0.004; U test); with ibuprofen and benzocain, this was the case from 20 min after application ($P < 0.04$–0.004). With all drugs the maximum pain suppression was observed in the 50–55 min time segment after application of the drug, which was the last 5 min of the protocols. At that time, pain was reduced, compared to initial (baseline) values, due to ASA application by 95%, ibuprofen by 94% and benzocain by 93% (all $P < 0.03$). With the placebo, there was an insignificant overall reduction in VAS ratings of 34%, on average. Fig. 3 compares the magnitude of the pain ratings in the last 5 min of the recording for each drug and demonstrates the large differences between the active drugs and placebo which were all significant ($P < 0.004$). An apparent reduction of pain ratings in the placebo data did not reach significance as compared to the baseline (before drug application). Differences between the active drugs at that time period were negligible and insignificant.

Study 2: dose-dependent suppression of pain

The concentration of ASA normally used in our studies (3%) seemed to be supramaximal with respect to pain suppression. Thus, in the 2nd study (part A) with 6 volunteers, we investigated ASA in different concentrations (0.03%–5%), to determine a dose–response relationship. The active metabolite of ASA, salicylic acid, was included in this study (part B) and applied in identical formulation and concentrations to the acidotic skin of 6 different subjects.

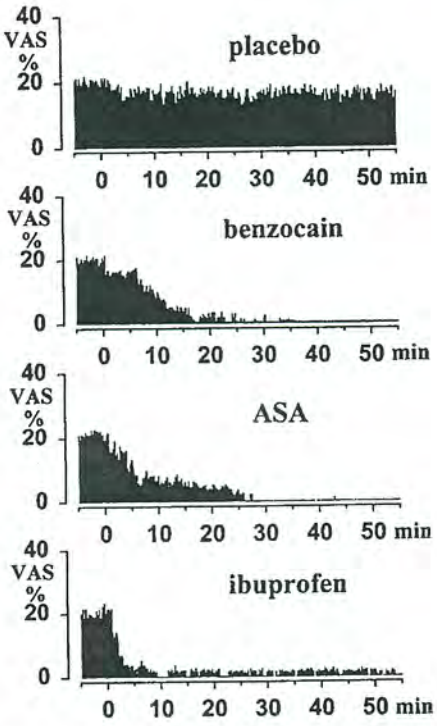

Fig. 1. The figure shows 1 subject's pain intensity ratings via VAS recorded during constant infusions of low pH buffer (pH 5.2) into the forearm skin for 1 h. The infusion rate was adjusted so as to produce 20% VAS ratings. After 5 min of constant ratings different drugs were applied topically to the skin in a vaseline/paraffin oil preparation (ASA 3%) or as commercial ointments at time zero. The drug sessions were randomized, double-blind and spaced by at least a 3-day interval between cross-over.

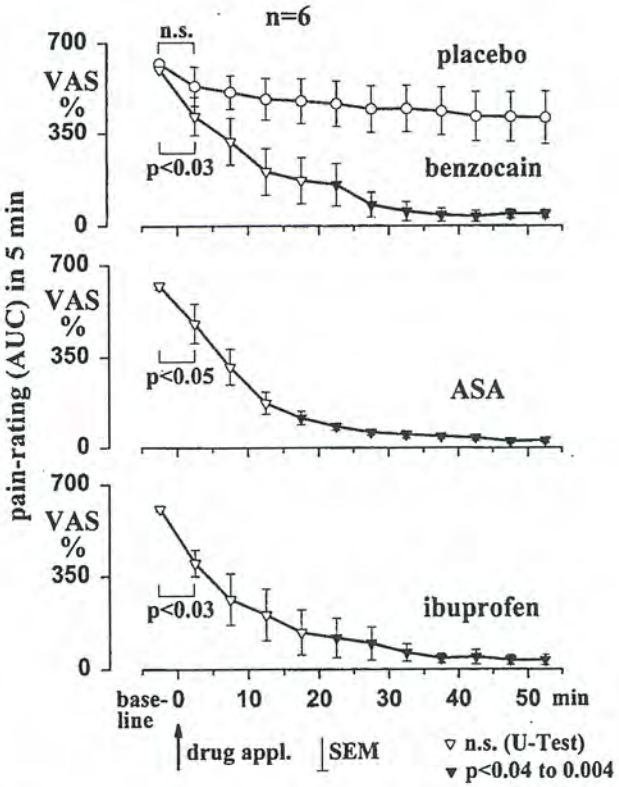

Fig. 2. Summary of topical drug effects on pain from experimental tissue acidosis in human skin. Sixty minutes of VAS recording during intradermal infusion of acid buffer were subdivided into 5-min segmentals comprising 30 discrete ratings at 10-sec intervals; these were summed up, and the individual sums were averaged across the 6 subjects involved in the study. The results (data symbols) provide a measure of the segmental area under the curve (AUC) of pain ratings. Empty triangles represent insignificant, filled triangles significant, differences between active drug and placebo sessions (Mann-Whitney U test). The baseline value was significantly different from the first and from all further segmental 5-min values following active drugs, but not following placebo application (multiple Wilcoxon tests).

Although in individual subjects an increase in pain suppression from 1.5–3% and 5% ASA seemed to be present (Fig. 4), the average data from 6 subjects did not yield significant differences between 1.5% ASA and the effects of higher ASA concentrations (Fig. 5). This is also demonstrated by the mean pain reduction achieved during the 20–25 min period after application as compared to baseline, which was 12% in the placebo trace, 18% using 0.03% ASA, 45% with 0.3% ASA, 90% with 1.5% ASA, 95% with 3.0% ASA and 91% with 5% ASA (all significant on a $P < 0.03$ level; Wilcoxon test). This dose–response relation is illus-

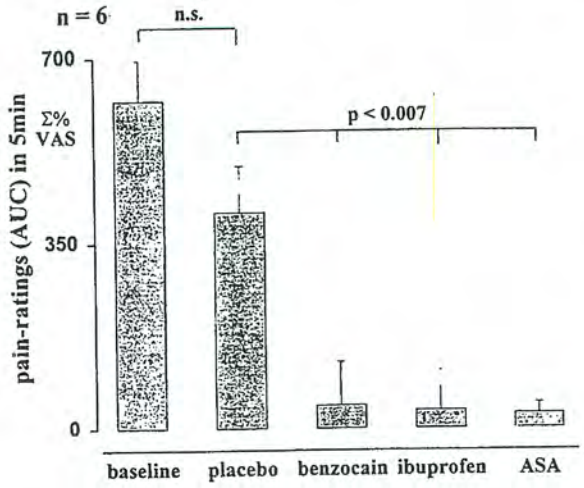

Fig. 3. Summary of maximal effects achieved with topical ASA, ibuprofen, benzocain and placebo. Rating values (n = 30) out of the first (baseline) and the last 5 min (ASA, ibuprofen, benzocain and placebo) were summated which provided an integrated measure, an area under the curve (AUC) of successive pain ratings. These values were averaged (n = 6) across subjects and used for statistical processing. Drug effects were statistically compared to placebo using the Mann-Whitney *U* test and to the individual baseline values using the Wilcoxon test.

trated in Fig. 6 where the differences between active drug and placebo rating values during the 25 min following application are shown. In fact, saturation of the analgesic effect was achieved with 1.5% ASA. A threshold concentration below 0.3% ASA can be extrapolated and a half-maximal effect was achieved using 0.3% ASA (Fig. 6). The differences in VAS ratings between the active drug and placebo treatments show, using the Mann-Whitney *U* test, that the 0.03% concentration was not significantly effective at any segmental (5 min) time period following application. Using 0.3%, the averaged difference to placebo was significant from 15 min after application ($P < 0.01$ up to 0.007). Even faster, with 1.5% and higher concentrations, the rating difference was significant 5 min following application ($P < 0.03$ up to 0.004).

The action of SA on the experimental pain followed a linear dose–response relationship as well and only with 2 of the 5 doses was SA significantly weaker than ASA. During the final 5-min periods of recording compared to the baseline pain reductions of 18% following placebo application, 16% following 0.03% SA, 36% following 0.3% SA, 71% using 1.5% SA, 79% using 3.0% SA and 80% using the 5.0% SA formulation were achieved. Pain suppression was already significant versus baseline with all doses 5 min following application ($P < 0.03$, Wilcoxon) (Fig. 5). The statistical analysis of the analgesic effects, i.e., the different active drugs compared to placebo data during the 20–25 min period, demonstrates that the 0.03% concentration was not significantly different to placebo; using 0.3% concentration the difference was significant with $P = 0.01$; using higher concentrations, however, the differences were clearly significant (all $P < 0.004$; *U* test). The 1.5% SA concentration was significantly different to placebo after 10–15 min ($P < 0.04$), whereas the 3% and 5% concentrations were significantly different already 5 min after application ($P <$ 0.007). Thus, the threshold concentration had to be estimated above 0.03% and the half-maximal effect at 1.5% SA concentration. A saturation can be assumed at 3% SA dose, since the 3% and 5% concentrations were equally effective ($P = 1.0$). The 1.5–3.0% and 5.0% differences were not significant ($P < 0.6$ and 0.3, respectively) but all doses were significantly different from the 0.3% concentration (Fig. 6).

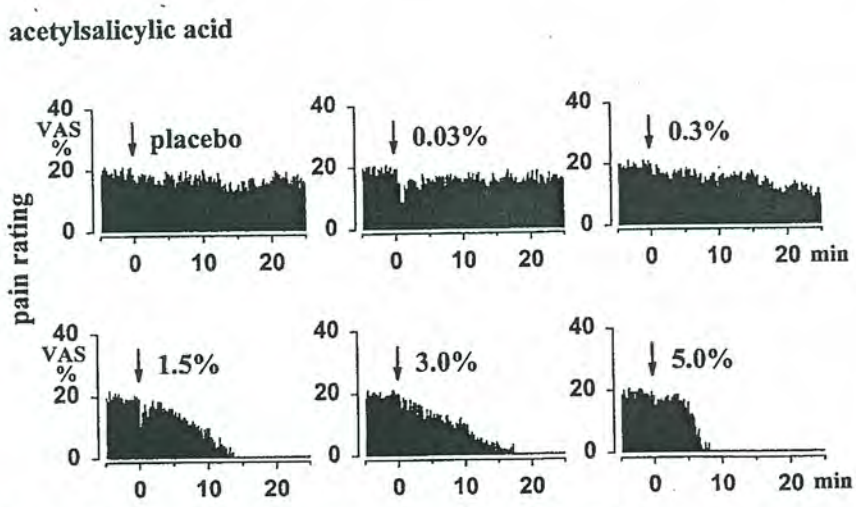

Fig. 4. A specimen recording of 1 volunteer showing the dose-dependent suppression by ASA of pain from cutaneous acidosis. Either placebo or ASA in different concentrations (0.03–5%) were applied (arrows) in experimental sessions at intervals of 3 days.

Study 3: counteracting analgesic action by intensifying the pain stimulation

In a 3rd study with 6 volunteers, we tried to overcome the analgesic action of ASA and SA by increasing the buffer flow. Both analgesics were topically used at (for better comparison) an identical concentration (1.5%) which, at least for SA, was a submaximal dose (see above). The specimen in Fig. 7 shows individual pain ratings of 1 subject undergoing treatment with 1.5% ASA and SA, respectively, and demonstrates that a single step in flow rate was sufficient to reach the original pain level following SA, but not ASA, treatment, in which case another 10 ml/h was needed to reach 20% VAS pain ratings again. Taking average data from 6 subjects as a basis, the drug effect was fully developed with 1.5% ASA between 9 and 19 min (mean: 13 min) and with 1.5% SA between 13 and 19 min (mean: 15 min). The initial 20% rating was approximately re-achieved by increasing the buffer flow by a factor of 1.69 (SA range: 1.33–1.83) and 1.81 (ASA range: 1.25–2.43), on average.

Study 4: topical analgesic effects on more intense experimental pain

In a 4th study the flow rate of the acid buffer was adjusted, at the beginning, to yield pain ratings of about 50% on the VAS. Fig. 8 gives individual pain ratings of 1 subject; a pain suppression to 50% of the initial level was achieved using SA (3%), within 15 min after application, and after 5 min following topical ASA treatment (3%) ratings below 20% were constant in this particular subject. In the placebo trace, the VAS rating was totally unaffected by application of the ointment. The average data from 6 volunteers in this study showed, taking the last 5-min period of the recording in comparison to baseline, a pain suppres-

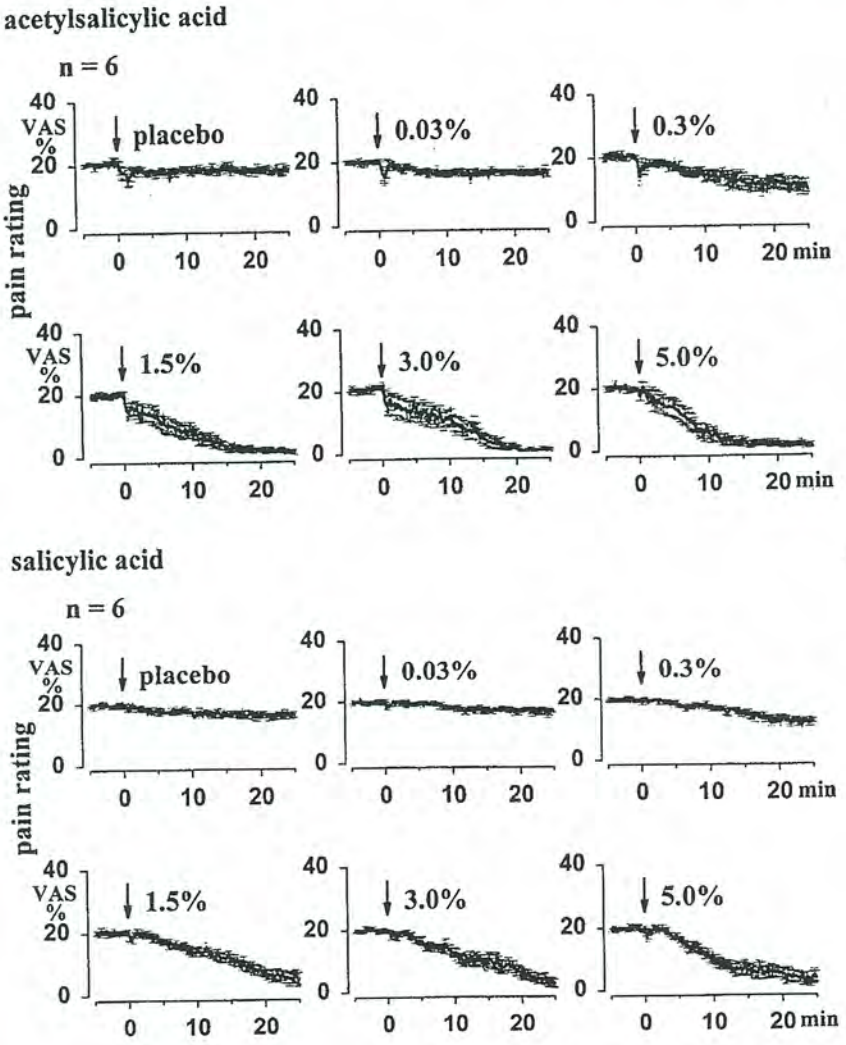

Fig. 5. Averaged pain rating curves from 2 × 6 volunteers: at intervals of at least 3 days between cross-over, either placebo or ASA in 5 different concentrations (0.03–5%) were applied to 6 subjects (arrows); 6 other subjects received placebo or different concentrations of SA in the same random order and double-blind. A dose-dependent suppression of pain from local tissue acidosis in human skin is demonstrated. The black curves represent the mean, the grey shadows indicate the SEM range.

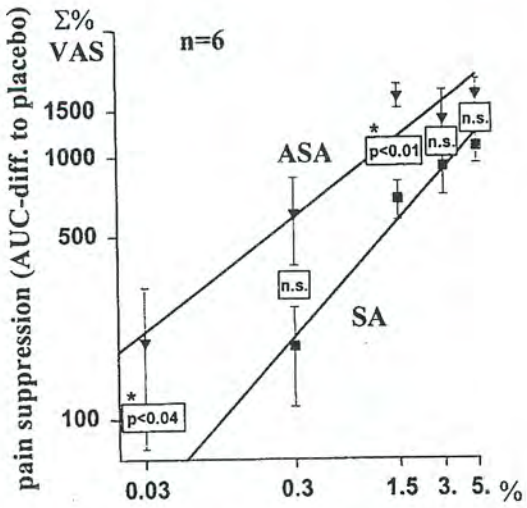

Fig. 6. Log-log presentation of the linear dose–response relation found for topically applied ASA and SA. The pain response magnitude was assessed as the total area of difference between the pain rating curves under placebo and under active medications. Approximately half-maximal analgesic effects were achieved using 0.3% ASA or 1.5% SA in vaseline/paraffin. ASA was significantly more effective than SA at 2 drug concentrations (*).

sion of 72% was achieved from SA and 73% from ASA, on average. This was significant on a $P < 0.03$ level (Wilcoxon) from 5 min (ASA) and 10 min (SA) following application. A reduction of 10% was seen in the placebo trace, which became significant 15 min after application. The different drug/placebo ratings were significant after the 5–10-min period following application (and all segmental time periods later) with SA and ASA ($P < 0.01$ up to $P < 0.004$; U test) (Fig.

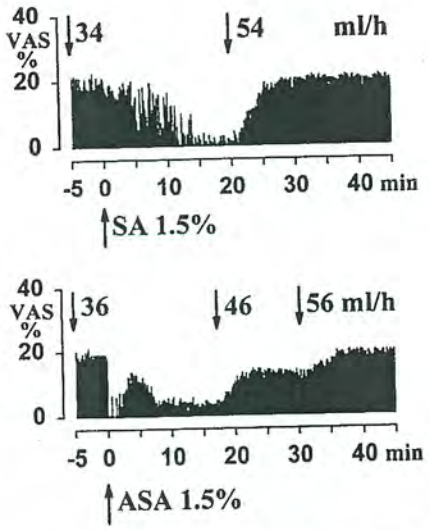

Fig. 7. Pain ratings from 1 subject under topical ASA and SA medication in (sub) maximal dose. In this study, the flow rate of the acidic buffer was increased when the drug effect had fully developed (constant low ratings for 5 min). It was recorded how much increase in flow was needed to re-achieve approximately 20% pain rating on the VAS.

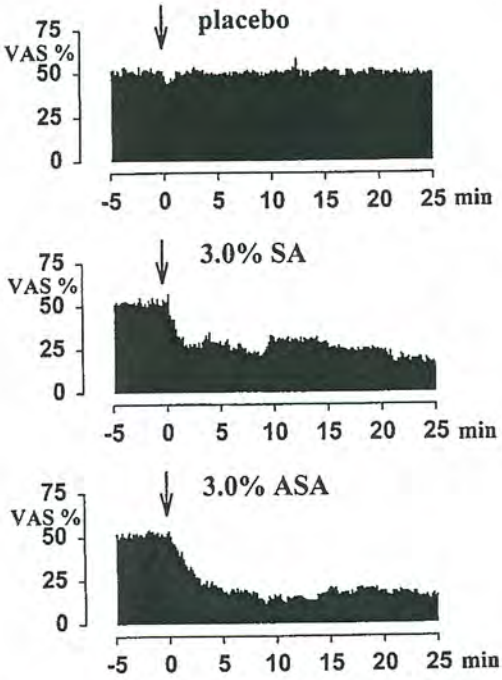

Fig. 8. Flow rate of the acid buffer was adjusted, in this study, so as to produce mean pain ratings of approximately 50% on the VAS. The figure shows specimen pain ratings of 1 subject before, and up to 25 min following, topical application of placebo, ASA or SA (in vaseline/paraffin ointment) to the skin.

9), whereas the effects of SA and ASA were different from each other only in the segmental time period of 10–15 min after application ($P < 0.03$), but were the same at all later time points ($P = 1$). The differences to placebo were about the same for both drugs when pooling all VAS ratings after application (Fig. 10).

Pain intensity and drug effects

Comparing the effects of the same dose of ASA on different intensities of pain, Fig. 11 demonstrates that the relative suppressive effect of ASA is more pronounced the weaker the initial pain was. Taking all ratings following drug application (25 min) as a basis, in the case of higher pain intensities (50% VAS) an average reduction of 46% was obtained from ASA (data from study 4). With the lower initial pain of 20%, a pain suppression by 62% (ASA) was achieved (data from study 2). Taking the last 5-min period of recording as a basis (20–25 min after application) this difference was even more prominent. Suppression of intense pain was 73% (ASA) in this segmental time period; with 20% initial pain, however, the ASA drug effect was a 95% pain reduction (Fig. 11).

The findings given above were about the same in the case of SA, although not as prominent. Twenty-five minutes after application, the 50% pain was suppressed by 50% and the 20% pain was reduced by 54%. In the last 5 min of recording 50% pain was suppressed by 72%, and with 20% pain SA was more effective with 79% reduction. This data supports a

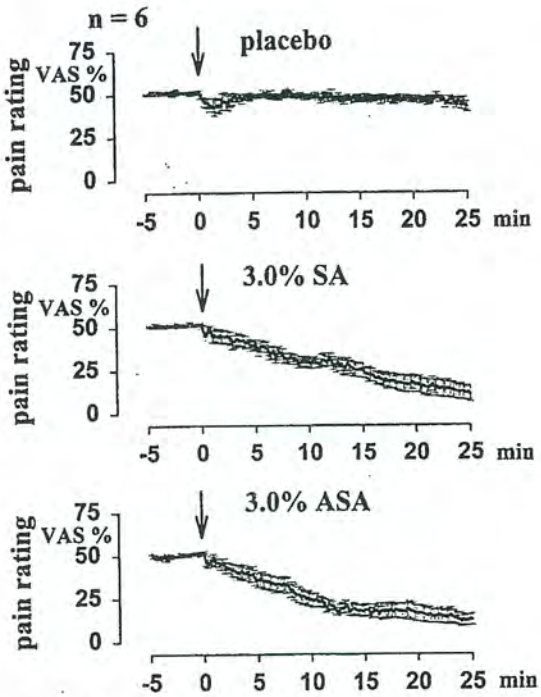

Fig. 9. Averaged data of VAS pain ratings of 6 subjects before, and up to 25 min after, topical application to the skin of placebo, ASA or SA (in vaseline/paraffin ointment). This resulted in a marked suppression of the intense pain (50% VAS ratings) that was induced by increased flow rates of acidic phosphate-buffered solution. The black curves represent the means, the grey shadows indicate the SEM range.

general trend of these drugs to be more effective with lower initial pain. In contrast, the placebo effects, taking longer or shorter sampling periods into account,

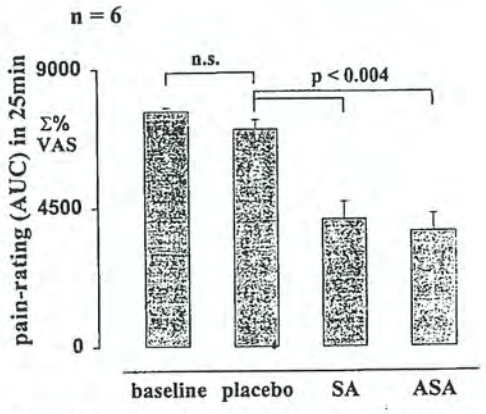

Fig. 10. Averaged data of VAS pain ratings of 6 subjects before (baseline) and up to 25 min after topical application to the skin of placebo, ASA or SA (in vaseline/paraffin ointment; data from Fig. 9). Flow rates of acidic phosphate-buffered solution were adjusted, in this study, as to induce intense pain (50% VAS ratings). Under these conditions, the topical drugs still reached a relative suppression of pain by almost 75%, on average. Drug effects were statistically compared to placebo using Mann-Whitney *U* test and to the individual baseline values Wilcoxon test.

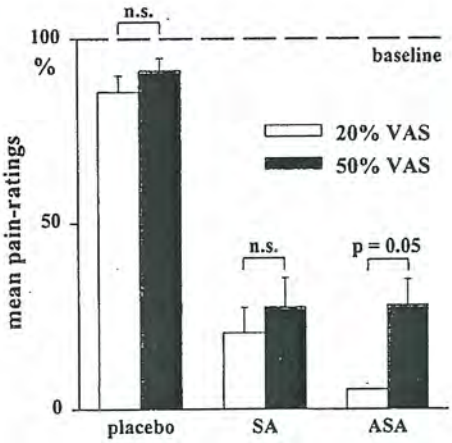

Fig. 11. Pain ratings (n = 30 from each of 6 subjects) during the fully developed drug or placebo effect, respectively (20–25 min, p.a.) were averaged taking data from Figs. 5 and 9 which represent different levels of ongoing experimental pain (20% vs. 50% VAS). Relative to baseline, ASA was more effective at lower pain intensity (*U* test). Both drugs, SA and ASA (3% in vaseline/paraffin), were significantly more effective than placebo ($P < 0.004$) in reducing both pain intensities but not different in effect from each other ($P = 0.2$ and 1, respectively).

were not prominently different at higher or lower initial VAS rating values with about 7–12% pain reduction (Fig. 11).

Discussion

With a new route of administration, a topical application using vaseline/paraffin as a vehicle, it was demonstrated that aspirin (ASA) and its metabolite, salicylic acid (SA), block cutaneous pain from low pH dose-dependently and almost equally effectively. The analgesic effects were comparable to a commercial, more highly concentrated, ibuprofen cream. As a positive control, a local anesthetic (commercial benzocain cream) and, as a negative control, a placebo cream (lactose in a vaseline/paraffin formulation) were used. In contrast to the NSAIDs, the local anesthetic left the area of application with a loss of all cutaneous sensations, whereas the touch threshold remained unchanged using the analgesics.

Pain reduction of topical analgesics and local anesthetics

The topical treatment of cutaneous pain from skin diseases has been tried before. The use of topical capsaicin has been recommended in treatment of post-herpetic neuralgia (Bernstein et al. 1987; Watson et al. 1988; Drake et al. 1990) as well as the application of local anesthetics (Rowbotham and Fields 1989; Stow et al. 1989). However, the most encouraging clinical reports of beneficial treatment of acute and chronic post-herpetic neuralgias have been published using

classical analgesics topically (De Benedittis et al. 1992; King 1993).

Choice of solvent

Sudden and complete pain relief, resulting from the cooling effects of evaporating etheric or alcoholic solvents in which the NSAIDs were topically applied, have regularly been reported (De Benedittis et al. 1992; King 1993; Steen et al. 1995a). For clinical purposes (e.g., treatment of post-zosteric neuralgia) the cooling effect of ether might, on the one hand, be beneficial with respect to pain treatment. On the other hand, acceptance of the potentially dangerous and irritating ether solution was rather poor. Thus, for clinical treatment of pain states, alternative solvents had to be found.

Handling of the vaseline/paraffin ointment was easy. However, action of the drugs investigated was slightly less effective than in the previous diethylether study (Steen et al. 1995a). In the study presented here, maximal pain suppression during intense stimulation (50% VAS pain) was 73% following ASA treatment, and 72% using SA. In the diethylether study a pain suppression of 90% and 88%, respectively, was achieved under the same conditions. With 20% initial pain ratings on the VAS, a difference between the studies was not obvious since both showed marked ASA effects on moderate pain (in diethylether: 96%; in vaseline: 95% suppression). SA, in both studies slower and slightly but significantly less effective than ASA, was again more effective in diethylether (92% pain suppression) than in vaseline (80% pain suppression).

Cooling effects interfere with assessment of the analgesic actions of topically applied NSAIDs to some extent, since cooling is known to stop nociceptor activity of any origin (Kunesch et al. 1987), and analgesic effects in the present model could also be achieved with a piece of ice (Steen and Reeh 1993). For example, using diethylether as a solvent the cooling effect lasted for 3–4 min and led to a temporary pain reduction of 64% (all ratings in the first minute after application) on average (Steen et al. 1995a). To study the time course and magnitude of pharmacological actions the spoiling effect of cooling could be minimized by using the vaseline/paraffin ointment formulation as a solvent; the remaining cooling effect led to a pain reduction of 16%, lasting less than 3 min on average.

Topical application – local or systemic action of the drugs

In most cases of transdermal administration of drugs the systemic actions (e.g., in the treatment of cardiac ischemia, hormonal dysregulation, motion sickness or platelet inhibition) have largely been investigated. The local effects are not well understood. Thus, it was still an open question whether analgesic creams and ointments were directed towards systemic effects or towards local analgesic actions.

ASA and SA in diethylether. In cooperation with the Department of Clinical Pharmacology of the University of Heidelberg, using high-performance liquid chromatography (detection level: 0.1 μg/ml) we tried, in an earlier stage of our studies, to determine plasma concentrations of ASA and SA but we failed in every single case at 11 time points up to 3 h after topical application in diethylether. These drugs were most effective in suppressing cutaneous pain at time points in which no detectable plasma concentration was apparent (Steen et al. 1995a). These data were in accordance with a study by Keimowitz et al. (1993) in which, after application for 15 min of ASA dissolved in isopropyl alcohol (in order to inhibit platelet cyclooxygenase systemically), no detectable plasma level was achieved before 3 h after application. Feldmann and Maibach (1970) demonstrated, detecting urine concentration, that ASA and SA (dissolved in acetone) were moderately good at penetrating the skin (ASA: 0.141% dose/h; SA 0.116% dose/h, in the first 12 h). Although no data is available with vaseline as a vehicle, there is no doubt that local analgesic, but not systemic, action was responsible for the deep analgesia reported in this study.

Ibuprofen cream. The absorption through, and distribution in, guinea pig skin of the ibuprofen formulation used in this study was investigated by Giese (1990). Radioactively-labeled ibuprofen was found enriched in skin and muscle tissue after a 10-min incubation period following topical application; whereas, in plasma, a concentration above detection level was only found 90–120 min after administration. The plasma concentration was up to 1000 times lower (120 min following application) compared to that in muscle tissue.

Benzocain cream. Plasma concentrations in short time courses following topical application of the local anesthetic are not, to the best of our knowledge, available as published data. Also, no data exist concerning drug effects on pain of the commercial benzocain cream used in this study. In response to an inquiry to the company (Ritsert, Eberbach, Germany), we were informed that, in an unpublished study with an ointment containing 20% benzocain, after 7 applications within 3 days to the lower back skin of the patient, a maximum plasma concentration of 40 ng/ml was measured (HPLC method) 2.5 h following the last application. This indicates that even with a more intensive and longer-lasting topical exposure to the drug than in the present study it was not possible to induce an effective plasma concentration.

Placebo effect

A placebo effect of 19% pain suppression, on average, was observed which was slightly less prominent

than in the previous diethylether study (24%; Steen et al. 1995a). This reduction in pain rating was not due to adaptation or habituation during pH stimulation, since this was not seen in control data of pH infusion without drug application (Steen and Reeh 1993) or in electrophysiological experiments with low pH-induced excitation of nociceptors which respond in a non-adapting, sustained manner (Steen et al. 1992). The placebo trace of Fig. 8 is from 1 of the subjects representing a group of individual ratings which did not decline at all during 30 min of pH infusion. Therefore, the pain reduction seen in other subjects is interpreted as a placebo effect which is a well-established phenomenon in algesimetric experiments (Anton et al. 1985).

Dose-dependent suppression of pain

The active metabolite of ASA, salicylic acid, well known from topical applications in dermatology because of its keratolytic action, had previously been shown to act almost as effectively on cutaneous pain as did ASA (Steen et al. 1995a) and, in the present studies, similar log-linear dose–response curves have been shown for both substances. These show that ASA and SA effects were saturated in the doses (3%) in which the substances have usually been applied to skin in the different studies of this investigation. It can be assumed that the commercial ibuprofen formulation and benzocain, which were used in even higher concentrations, were also supramaximal in dosage. Thus all 4 agents were similarly effective on the moderate pain induced in studies 1 and 2 (> 93% pain suppression).

Counteracting the analgesic action

We were able to counteract pain suppression by ASA and SA (1.5%) by increasing the painful flow of buffer. The slightly higher increase of flow rate needed to overcome ASA- in comparison to SA-induced pain suppression matches the fact that the dose used was maximal for ASA but submaximal for SA (see Fig. 6). The competitive mechanism and unaltered touch thresholds during topical analgesia suggest a specificity of the (acetyl) salicylic antinociception.

The effectiveness of topical analgesics in relation to pain intensity

Pain reduction with the drugs was relatively less complete with higher than with lower pain intensity in accordance with Beecher's observation in respect to 'weak' analgesics. His further report that placebos are more effective when pain is severe (Beecher 1956) does not agree with the placebo results of our studies in which placebo was slightly, but not significantly, more effective on the weaker pain condition.

Possible mechanisms of the antinociceptive drug effects

In these studies, ASA, SA and ibuprofen showed potent antinociceptive effects. In a previous study indomethacin exhibited the same specific effects. This seems to suggest that cyclooxygenase inhibition is a common action principle of these drugs. Since prostaglandins do not directly excite nociceptors (Mizumara et al. 1987; Lang et al. 1990; Rueff and Dray 1993), they cannot be the likely mediator of pH-induced nociceptor excitation. Prostaglandins (PG), however, could increase the sensitivity of nerve endings to low pH, as they sensitize nociceptors to bradykinin in some preparations, in vitro (Mizumara et al. 1987; Rueff and Dray 1993). Indeed, there is an indication that such a mechanism may be active in the cat cornea where topically applied PGE_2 (10^{-5} M) is reported to enhance nociceptor responses to 10 mM acetic acid (Belmonte et al. 1994). In the skin such an interaction has been studied using an ample combination of inflammatory mediators (bradykinin, serotonin, histamine and PGE_2, all 10^{-6} M) which greatly enhanced nociceptor responces to low pH in vitro, and the pain in humans induced by acid buffer infusion (Steen et al. 1995b,c). However, in a further study using a skin–nerve preparation, in vitro, the inflammatory mediator combination was applied at pH 6.1, producing vigorous and sustained nociceptor discharge, which was the same regardless of whether PGE_2 (10^{-5} M) was contained. This was also the case if the skin was deprived of cyclooxygenase products by superfusion with flurbiprofen which, in addition, prevented secondary PG release in response to bradykinin (Reeh and Brehm 1993). Thus, the sensitizing action of prostaglandin seems to undergo occlusion when other sensitizing agents occur, probably bradykinin in the first place. In this context, our present results could indicate that, in artificially acidotic but otherwise intact skin, in the absence of other mediators, prostaglandins exert a tonic sensitizing influence on nociceptors that can be interrupted by local cyclooxygenase blockade. In addition, tissue acidosis could provoke increased PG production – a hypothesis which can be tested.

Alternative to inhibition of prostaglandin synthesis, other 'peripheral' actions of, at least, ASA and SA have previously been reported in order to explain their analgesic effects. ASA reduces ongoing nociceptor activity and hypersensitivity to mechanical stimulation in a rat preparation of the inflamed ankle joint; PGE_2 injection is unable to restore the arthritic symptoms (Grubb et al. 1991). More specifically towards pH-induced excitation, in molluscan neurons, a decrease in membrane potential and conductance due to a reduction of K^+ and an increase in Cl^- conductance with lowered pH have been shown to be competitively antagonized by SA (Barker and Levitan 1972). Stimulated by these findings, we tried ASA and SA in our rat

skin–saphenous nerve preparation, in vitro, and found a dose-dependent reduction of pH-induced nociceptor excitation at concentrations of both drugs in the range of therapeutic plasma levels (Stefanidis et al. 1994). It has not yet been attempted to counteract this effect by substituting PGE_2. Local anesthetic effects, as reported for high SA concentrations ($> 10^{-3}$ M; Ricciopp Neto and Narahashi 1976), could, however, be excluded. They are also unlikely in the present study since the analgesia could be reversed by increasing the intensity of pain stimulation and tactile sensitivity remained unimpaired.

Further experiments in psychophysiological, as well as in neurobiological, studies should try to establish the links connecting prostaglandin synthesis to pH sensitivity. Apart from the open questions, however, classical antipyretic analgesics have proven most effective in suppressing acidotic pain by, most probably, a purely local effect. Clinical significance of these findings may be expected in cases of cutaneous pain, for example after burn injury, sunburn, cutaneous viral infections or vasculitic pain.

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