Electrically evoked neuropeptide release and neurogenic inflammation differ between rat and human skin

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1. Protein extravasation and vasodilatation can be induced by neuropeptides released from nociceptive afferents (neurogenic inflammation). We measured electrically evoked neuropeptide release and concomitant protein extravasation in human and rat skin using intradermal microdialysis.

2. Plasmapheresis capillaries were inserted intradermally at a length of 1·5 cm in the volar forearm of human subjects or abdominal skin of rats. Capillaries were perfused with Ringer solution at a flow rate of 2·5 or 1·6 μl min^{-1}. After a baseline period of 60 min capillaries were stimulated electrically (1 Hz, 80 mA, 0·5 ms or 4 Hz, 30 mA, 0·5 ms) for 30 min using a surface electrode directly above the capillaries and a stainless-steel wire inserted in the capillaries. Total protein concentration was assessed photometrically and calcitonin gene-related peptide (CGRP) and substance P (SP) concentrations were measured by enzyme-linked immunosorbent assay (ELISA).

3. In rat skin, electrical stimulation increased CGRP and total protein concentration in the dialysate. SP measurements showed a larger variance but only for the 1 Hz stimulation was the increased release significant.

4. In human skin, electrical stimulation provoked a large flare reaction and at a frequency of 4 Hz both CGRP and SP concentrations increased significantly. In spite of the large flare reactions no protein extravasation was induced, which suggests major species differences.

5. It will be of interest to investigate whether the lack of neurogenic protein extravasation is also valid under pathophysiological conditions.

Upon activation nociceptors release neuropeptides from their terminals in the central nervous system and in the periphery. In rodent skin neuropeptides induce a combination of vasodilatation and protein extravasation, which has been termed 'neurogenic inflammation' (Jancso *et al.* 1967). Neurogenic inflammation has been hypothesized to play an important pathophysiological role in diseases like migraine and asthma (Moskowitz, 1993). There is evidence from rat experiments that substance P (SP) is mainly active in inducing protein extravasation by activation of NK_1 receptors, whereas calcitonin gene-related peptide (CGRP) is responsible for neurogenic vasodilatation (Holzer, 1998). This assumption has been partly confirmed in human skin where exogenous SP-induced protein extravasation (Hägermark *et al.* 1978; Jorizzo *et al.* 1983; Devillier *et al.* 1986) and CGRP provoked lasting and pronounced vasodilatation (Brain *et al.* 1985). However, release of endogenous SP by capsaicin does not induce protein extravasation (Schmelz *et al.* 1997*a*) or indirect release of histamine from mast cells (Tausk & Undem, 1995; Huttunen *et al.* 1996; Petersen *et al.* 1997*b*).

Neuropeptide concentrations in human skin are problematic to assess in humans *in vivo* (Hargreaves *et al.* 1994; Petersen *et al.* 1997*b*). In contrast, release models in rat skin have been successfully used to assess chemically or electrically induced neuropeptide release *in vitro* (Hua & Yaksh, 1992; Kilo *et al.* 1997; Kress *et al.* 1999).

The aim of this study was to enable direct comparison of the pattern of electrically evoked neurogenic inflammation by the use of experimental conditions as similar as possible to human and rat skin. For this purpose, we used identical dermal microdialysis systems for stimulation and measurement of protein and neuropeptides. Microdialysis is a minimally invasive technique that was originally developed for applications in the central nervous system (Ungerstedt & Hallstrom, 1987), but has also been adapted for dermal use (Anderson *et al.* 1991). For the purpose of this study a microdialysis membrane with a high cut-off of 3000 kDa was chosen to analyse protein extravasation (Schmelz *et al.* 1997*a*). Simultaneously, nociceptor activation can be assessed indirectly by conventional psychophysical methods

and by analysis of the extent of the axon reflex erythema around the stimulated skin site.

METHODS

Subjects

Thirteen healthy volunteers aged 23–34 years (7 male; 6 female) participated in the experiments after having given written informed consent. All subjects were familiar with the principles of the method and the general intention of the study, but were unaware of the specific experimental goals. They could withdraw from the experiment at any time. The study was approved by the local ethics committee and was conducted according to the Declaration of Helsinki. The volunteers were comfortably seated on a reclining chair and their left arm was placed in a vacuum cushion with the volar side up. The arm was fixed to keep it in the same relaxed position during the whole experiment. Experimental sessions lasted about 3 h.

Animals

Experiments were performed under protocols approved by the local ethics committee (Ansbach, Germany). Thirty-four male Wistar rats (mean weight, 386 ± 11 g) were anaesthetized with an intraperitoneal injection of thiopental (120 mg kg^{-1}). The abdomen was shaved with an electrical shaver. Skin temperature was held constant at 32 °C by use of an infrared bulb that was feedback-controlled from a thermocouple attached to the abdomen (Physitemp, Clifton, NJ, USA). Adequate depth of anaesthesia was checked at regular intervals throughout the experimental procedure by firmly squeezing the tail. In case of the induction of nocifensive reflexes, an additional 10 mg thiopental was applied I.P. in a volume of 0·5 ml. Animals were killed by intracardial injection of 1 ml lidocaine (2%) immediately after the end of the experimental procedure.

Microdialysis

The effects of electrical stimulation were analysed in humans and rats. Care was taken to use identical stimulation parameters and similar settings as far as possible. Four (1 Hz protocol) or six (4 Hz protocol) single plasmapheresis hollow fibres (Asashi, Japan; 0·4 mm diameter, cut-off 3000 kDa) were inserted intracutaneously for a length of 1·5 cm by a 25 gauge cannula in the abdominal skin of the rats or in the volunteers' left volar forearm. The insertion of the thin needles was well tolerated by all volunteers without local anaesthesia. The insertion depth was measured by ultrasound (Dermascan C, Cortex Technologies, Denmark) to be 0·65 mm on average (range, 0·4–0·9 mm; $n = 19$). All hollow fibres were orientated transversally to the axis of the rat's body or the human forearm. They were inserted in groups of two (1 Hz protocol) or three (4 Hz protocol) at a distance of 1–2 mm apart. The two or three groups of fibres were located 7 (human) or 4 cm (rat) apart to prevent mutual influence. The fibres were perfused with Ringer solution (Ringerlösung Fresenius, Germany) via Tygon tubing (Novodirect, Germany) and a microdialysis pump (Pump 22, Havard Apparatus, USA), on which four or six syringes (1 ml; Dispomed, Gelnhausen, Germany) were mounted and capped with 26 gauge hypodermic needles (Neoject, Hungary). For the 1 Hz protocol a flow rate of 2·5 μl min^{-1} was used. Because at this flow rate no CGRP increase was detected, the flow was reduced to 1·6 μl min^{-1} in the 4 Hz protocol to increase relative recovery. Increasing the number of capillaries per group to three instead of two ensured a sufficient amount of eluate. After passing through the skin the fibres were inserted into glass capillaries (150 μl; 1·0 mm inner diameter; Servoprax R, GLW, Germany) to collect the dialysate. Tilting the capillaries to an angle of 5 deg minimized outflow resistance. The air-exposed length of the microdialysis fibres was less than 3 mm on either the inflow or outflow side. The dialysate from each group of capillaries was collected in one common vial. Samples were taken every 30 min for a total period of 150 min and stored in polyethylene cups. They were immediately put on ice and separated for the different analyses.

Electrical stimulation

The plasmapheresis fibres, which were used for the electrical stimulation, were equipped with a stainless-steel wire (diameter 0·2 mm) in their lumen which acted as the cathode. They were electrically stimulated using a surface anode directly above the fibres (Fig. 1). To reduce the skin resistance electrode paste (electrode adhesive paste, Beckman, USA) was used. After 60 min of baseline measurement, constant current stimuli (0·5 ms, 80 mA, 1 Hz or 30 mA, 4 Hz) were applied for 30 min in rat or human skin from constant current stimulators (Digitimer Model DS7, UK). Each group of fibres was connected to a different stimulator. In each experiment one group of fibres was stimulated electrically. Control values were derived simultaneously from the non-stimulated group(s) of fibres. The dialysate from each group (stimulated and non-stimulated) was sampled separately. The stimulation was followed by a washout period of 60 min.

Analysis

Each sample was analysed for CGRP, substance P and total protein. The neuropeptides were measured using the enzyme-linked immunosorbent assay (ELISA) technique (Cayman, USA; Spibio, France). Cross-reactivity of the CGRP ELISA to amylin was $< 0{\cdot}01$ % and cross-reactivity of the SP ELISA to neurokinin A

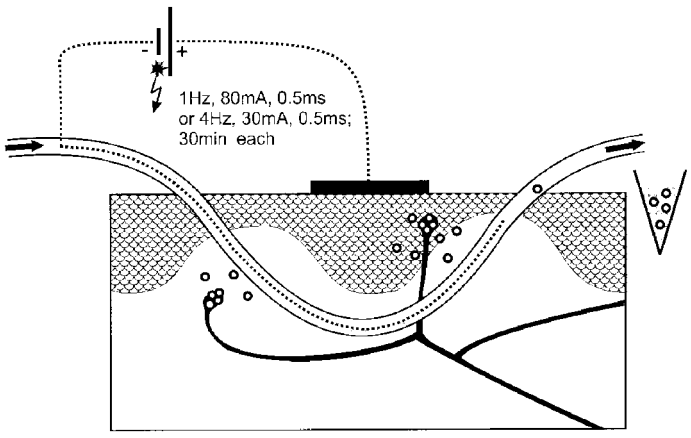

Figure 1. Schematic diagram of the experimental set-up

Note that two or three microdialysis fibres were inserted in parallel to increase relative recovery of the microdialysate.

was 2·7 %. The protein extravasation (total protein content) was determined photometrically (MRX reader, Dynatech, Germany) using Coomassie Blue dye (Bradford, 1976) for the analysis and bovine serum albumin as a standard.

Psychophysics

In the human subjects, maximum pain sensations evoked by the electrical stimulation were assessed on a numeric scale from 0 to 10, in which the value of 0 indicated no sensation and 10 the maximum pain the subject could imagine.

Flare areas

The extent of the electrically evoked visible axon reflex erythema was marked on the forearm skin at its maximum size immediately after the stimulation period. At the end of the experiment the borders were traced on a transparent acetate sheet, digitized and the areas of the flare reaction were evaluated planimetrically using specialized software (Nischik & Forster, 1997).

Statistics

For statistical evaluation an ANOVA for repeated measures was calculated using stimulation frequency and treatment (stimulated/non-stimulated) as independent variables. Scheffé's *post hoc* test was used to identify significant differences. Pain ratings and flare sizes were compared using the non-parametric Mann-Whitney *U* test. *P* values less than 0·05 were considered significant. All values are given as means ± S.E.M. or median with quartiles given in parentheses as appropriate.

RESULTS

In humans, insertion of the fibres caused a small flare reaction (4·6 ± 1·1 cm^2, $n = 13$) lasting ~40 min. This was a slightly less intense reaction than that described previously by Anderson *et al.* (1994), which can be explained by the smaller diameter of the cannulae used in this study. At the end of the baseline period no visible erythema was left. Electrical stimulation induced a large visible axon reflex flare of 29·5 ± 3·1 cm^2 at 1 Hz ($n = 5$) and 35·3 ± 2·1 cm^2 at 4 Hz ($n = 8$). No significant difference could be determined between the flare sizes of the two frequencies.

The intensity of pain sensations did not differ significantly between the two stimulation frequencies. Maximum pain ratings were 7 (6, 7) for 1 Hz ($n = 5$) and 7 (6·5, 7) for 4 Hz ($n = 8$). Although being not far from the tolerance limit, all subjects completed the whole stimulation period of 30 min. The character of the sensation was described as purely painful by all subjects and had a sharp stinging and a burning component.

Neuropeptides

In the rat experiments the control values of CGRP remained at a constant baseline level of ~21 pg ml^{-1} in the 1 Hz ($n = 15$) and 24 pg ml^{-1} in the 4 Hz protocol ($n = 17$) during the whole observation time. During the 1 Hz stimulation the CGRP release increased significantly by ~50 % ($n = 15$; $P < 0·01$), whereas the corresponding control values remained constant. Electrical stimulation at 4 Hz increased the CGRP concentration in the dialysate by ~250 % ($n = 17$; $P < 0·001$) compared with the controls in which the CGRP levels declined slightly. The CGRP increase provoked by the 4 Hz stimulation was significantly higher compared with the 1 Hz stimulation ($P < 0·001$). The CGRP concentration following the 4 Hz stimulation period remained at a significantly higher level ($P < 0·05$) 30 min after the end of stimulation (Fig. 2*A*).

In human skin electrical stimulation at 1 Hz ($n = 5$) did not provoke a significant increase in CGRP release, whereas the 4 Hz stimulation ($n = 7$) increased the CGRP concentration by ~100 % ($P < 0·01$). The corresponding control values decreased slightly. During the whole experiment non-

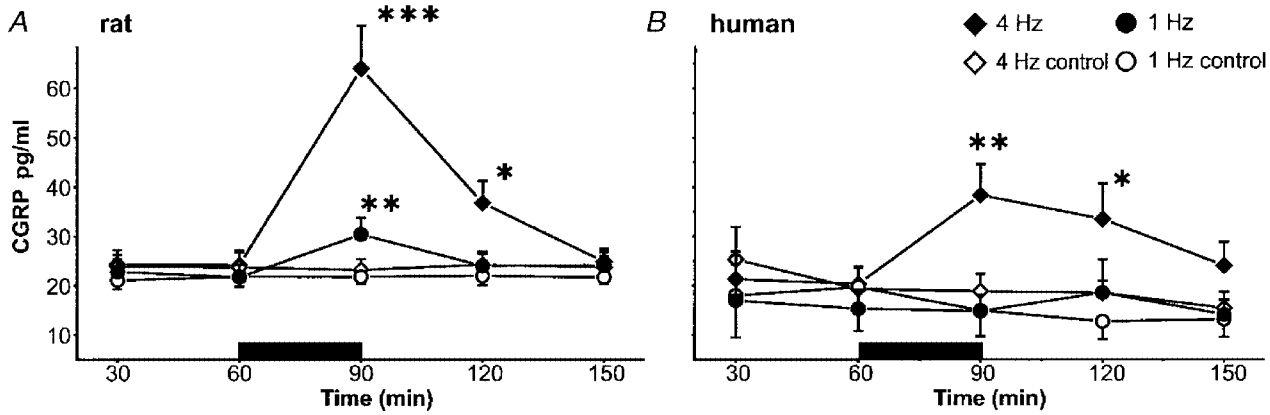

Figure 2. Time courses of CGRP concentration in the dialysate of intradermal microdialysis fibres after stimulating (filled bar) at 4 Hz ($n = 17$ rats; $n = 7$ humans) and 1 Hz ($n = 15$ rats; $n = 5$ humans) in comparison to the control values in rat and human skin

Because of the lower flow rate (1·6 and 2·5 μl min^{-1}) CGRP levels at the 4 Hz stimulation are significantly higher for both species ($P < 0·05$). In rat (*A*), CGRP increase following the 4 Hz stimulation significantly exceeded that following the stimulation at 1 Hz and outlasted the stimulation period. In human skin (*B*), the stimulation at 1 Hz did not induce any increase in CGRP, whereas the stimulation at 4 Hz provoked a significant release of CGRP, which remained at a higher level during the whole observation time. Control values remained at a constant baseline level. At both frequencies CGRP levels in rat skin are significantly above those in human skin ($P < 0·05$). (*** $P < 0·001$, ** $P < 0·01$ and * $P < 0·05$; Scheffé's *post hoc* test.)

stimulated values were measured at levels of ~19 pg ml^{-1} ($n = 7$). Following the electrical stimulation at 4 Hz, the CGRP concentration remained elevated during the first washout interval ($P < 0{\cdot}05$) compared with control ($n = 7$) (Fig. 2*B*).

CGRP levels in rat skin were significantly higher than in human skin following both stimulation frequencies ($P < 0{\cdot}05$). According to the lower flow rate in the 4 Hz stimulation protocol (1·6 *vs.* 2·5 μl min^{-1}) the CGRP concentrations were significantly higher compared with the 1 Hz protocol in rat and human (rat and human: $P < 0{\cdot}05$).

In rat, electrical stimulation at 1 Hz evoked a slight, but significant release of substance P ($n = 15$; $P < 0{\cdot}01$), whereas no increase was observed in the controls ($n = 15$). The non-stimulated substance P values remained stable at 16 pg ml^{-1} during the whole experimental time. The stimulation at 4 Hz evoked a similar increase in substance P ($n = 13$). However, because of higher variability this increase did not reach a significant level. In the controls substance P remained constant during the stimulation period ($n = 13$; Fig. 3*A*).

Similar to the CGRP results, no release of substance P was observed in human skin at a stimulation frequency of 1 Hz ($n = 5$). However, stimulation at the higher frequency (4 Hz; $n = 7$) significantly increased substance P levels in the dialysate ($P < 0{\cdot}05$), whereas control levels declined slightly (Fig. 3*B*).

Substance P levels following electrical stimulation were found to be significantly higher in rat compared with human skin ($P < 0{\cdot}05$). According to the lower flow rate in the 4 Hz stimulation protocol (1·6 *vs.* 2·5 μl min^{-1}) the substance P concentrations were significantly higher compared with the 1 Hz protocol in rat and human (rat and human: $P < 0{\cdot}001$).

Protein extravasation

Total protein content in rat and human skin increased with reduced flow as expected. Significantly higher levels were measured in the 4 Hz protocols (1·6 μl min^{-1}) compared with the 1 Hz protocols (2·5 μl min^{-1}) (rat, $P < 0{\cdot}001$; human, $P < 0{\cdot}05$).

In rat, the total protein concentration in the dialysate in the stimulated and the non-stimulated control capillaries decreased exponentially during the first hour. In the 1 Hz protocol it declined from ~0·75 to 0·45 mg ml^{-1} 60 min later ($n = 15$). In the 4 Hz protocol the protein content dropped from ~1·0 to 0·6 mg ml^{-1} ($n = 17$). While the total protein concentration in the control area steadily declined, electrical stimulation evoked a significant increase in total protein at the stimulation site. Total protein significantly increased during stimulation at 1 Hz ($n = 15$; $P < 0{\cdot}001$) and 4 Hz ($n = 17$; $P < 0{\cdot}001$). The total protein levels remained significantly elevated at 30 min ($P < 0{\cdot}01$) and 60 min ($P < 0{\cdot}05$) after the end of the 4 Hz stimulation. In the 1 Hz experiments only the first washout value was significantly elevated compared with the control value ($P < 0{\cdot}01$) (Fig. 4*A*).

In human skin the protein extravasation exponentially decreased in the dialysate collected from the stimulated and non-stimulated capillaries at both frequencies. Following electrical stimulation at 1 Hz total protein declined from ~0·65 to 0·45 mg ml^{-1} ($n = 5$), and no significant differences were observed between stimulated and non-stimulated sites. A similar pattern was observed at both 4 Hz stimulation ($n = 8$) and the subsequent washout periods (Fig. 4*B*).

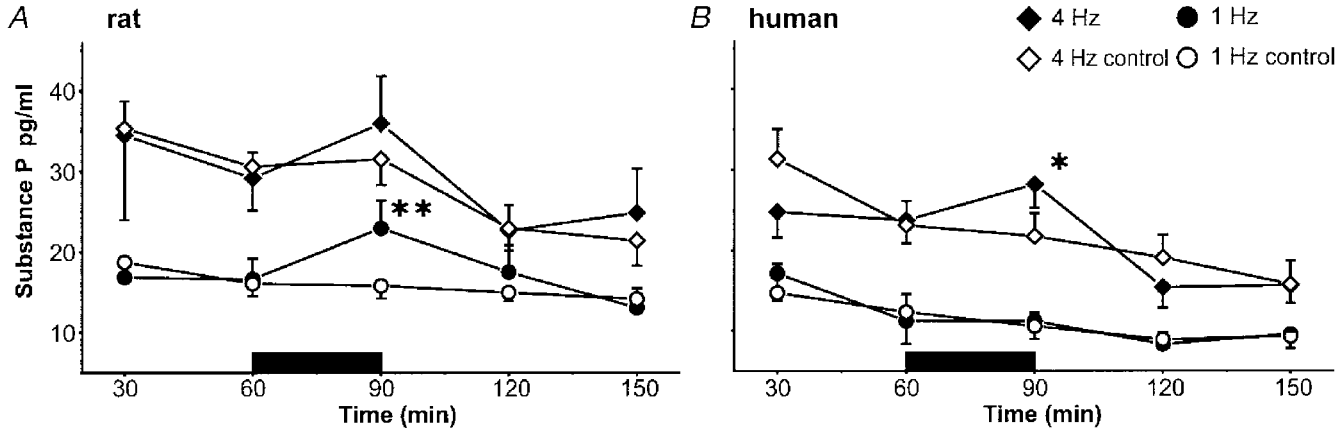

Figure 3. Time courses of substance P in rat (1 Hz, $n = 15$; 4 Hz, $n = 13$) and human (1 Hz, $n = 5$; 4 Hz, $n = 7$) skin

In rat skin significantly higher SP levels could be measured than in human skin ($P < 0{\cdot}05$). According to the lower flow rate neuropeptide concentrations in the 4 Hz protocol are significantly higher compared with the 1 Hz protocol in both species ($P < 0{\cdot}001$). In rat skin (*A*), a significant release of SP was observed during stimulation at 1 Hz, whereas the increase at the higher stimulation frequency did not reach a significant level. In contrast, in human skin (*B*) the stimulation at 1 Hz did not show any effects, whereas with the elevated stimulation frequency a significant SP release could be detected. Control values remained at an almost constant baseline level in both protocols. (** $P < 0{\cdot}01$ and * $P < 0{\cdot}05$; ANOVA, Scheffé's *post hoc* test.)

DISCUSSION

Electrically evoked neurogenic inflammation in rat skin

Antidromic stimulation of nociceptive afferent fibres classically provokes a combination of vasodilatation and protein extravasation (Jancso *et al.* 1967). For the assessment of protein extravasation following electrical stimulation of peripheral nerves or dorsal roots (Pinter & Szolcsanyi, 1995) the Evans Blue technique has traditionally been used. Our results on electrically induced protein extravasation confirm earlier studies showing effects at low stimulation frequencies using antidromic nerve stimulation (Szolcsanyi, 1996). Similarly, dose-dependent CGRP release upon chemical (Kilo *et al.* 1997) or electrical stimulation (Hua & Yaksh, 1992; Kress *et al.* 1999) has been reported before in *in vitro* systems.

Electrically evoked neurogenic inflammation in human skin

In pig skin mechano-insensitive nociceptors have been found to be responsible for the axon reflex erythema (Lynn *et al.* 1996). In human skin these mechano-insensitive, but chemosensitive, nociceptors have been implicated in the axon reflex flare (Schmelz *et al.* 2000*a*). Interestingly, thresholds for transcutaneous stimulation of these fibres have been found to be unexpectedly high (30–60 mA) when compared with conventional mechanoresponsive 'polymodal' nociceptors (< 10 mA) (Weidner *et al.* 1999). This fact might explain why in human subjects even painful electrical stimulation failed to elicit a visible flare reaction comparable to the large axon reflex erythema flare provoked by chemical stimulation, but the flare could be detected by laser Doppler techniques (Magerl *et al.* 1987; Westerman *et al.* 1987). In our study electrical stimulation at a very high intensity was applied. The resulting flare reaction was intense and of similar size to the axon reflex erythema provoked by intense chemical stimulation such as intradermal injection of 100 μg capsaicin (Simone *et al.* 1989) (32 cm^2) or 100 μg histamine (21 cm^2) (Simone *et al.* 1987). This result suggests that widely branched chemonociceptors were excited by the intense electrical stimulation. Thus, our results are consistent with the high electrical threshold, large innervation territories and sustained responses upon stimulation with capsaicin or histamine (Schmelz *et al.* 1997*b*; Schmelz *et al.* 2000*b*) of mechano-insensitive nociceptors.

Electrical stimulation at both frequencies provoked a large axon reflex erythema but only at 4 Hz could a significant increase in CGRP be measured. This discrepancy might be explained best by the lower flow rate and higher number of microdialysis capillaries (3 instead of 2) used in the 4 Hz protocol. Lower flow rate and larger surface area will increase recovery and therefore the sensitivity of the method increases. The time course of CGRP increase reflects the longer half-life of this neuropeptide in the skin (Brain *et al.* 1985; McEwan *et al.* 1988).

Stimulated SP increases have been problematic to assess in non-neuronal tissues of humans (Schmelz *et al.* 1997*a*; Petersen *et al.* 1997*a*) and animals (Hua & Yaksh, 1992; Kress *et al.* 1999; Ebersberger *et al.* 1999). Using low flow rates and three parallel microdialysis capillaries we succeeded in measuring SP increases provoked by electrical stimulation. However, the increases were only moderate and showed greater variance than the CGRP data. These results might reflect the high prevalence of CGRP-positive dermal nerve fibres in human skin compared with SP-positive fibres

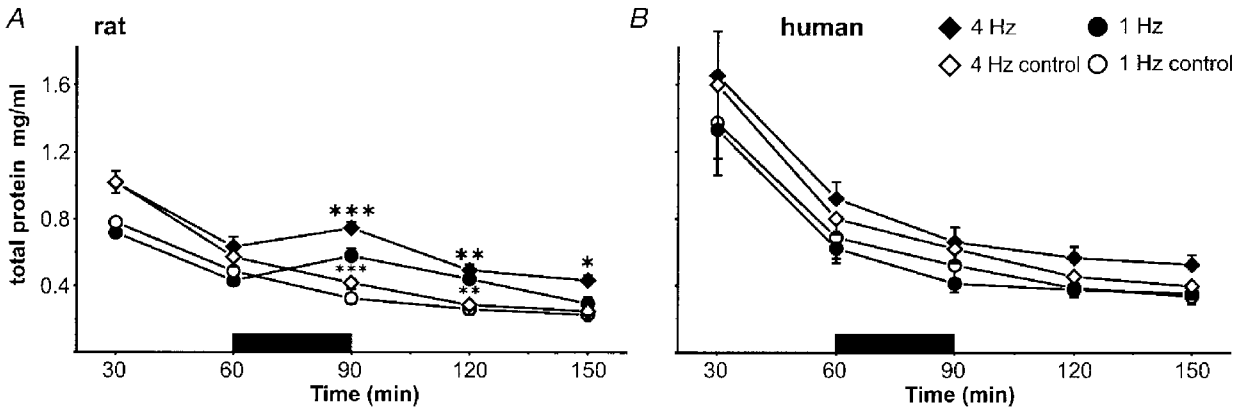

Figure 4. Protein extravasation in electrically stimulated and non-stimulated capillaries in rats (1 Hz, $n = 15$; 4 Hz, $n = 17$) and humans (1 Hz, $n = 5$; 4 Hz, $n = 8$)

In rat skin (*A*), total protein content exponentially declined after insertion of the microdialysis fibres to a baseline level, from which electrical stimulation evoked a significant increase of protein at both 1 and 4 Hz. In contrast, protein content in the dialysate derived from the non-stimulated capillaries continued to decrease gradually. Following 4 Hz stimulation protein extravasation was elevated during the complete sampling period, whereas at the 1 Hz stimulation only the first washout value was significantly increased. In human skin (*B*), plasma extravasation exponentially decreased in the stimulated and non-stimulated capillaries at both frequencies. In both species the 4 Hz protein values were significantly elevated compared with the 1 Hz values due to the lower perfusion rate (rat skin, $P < 0{\cdot}001$; human skin, $P < 0{\cdot}05$). (*** $P < 0{\cdot}001$, ** $P < 0{\cdot}01$ and * $P < 0{\cdot}05$, ANOVA, Scheffé's *post hoc* test.)

(Wallengren *et al.* 1987; Chan *et al.* 1997; Schulze *et al.* 1997).

Although electrical stimulation provoked massive axon reflex erythema and clear neuropeptide release, no neurogenic protein extravasation was detected. Dermal microdialysis has been shown to be a very sensitive method of detecting protein extravasation and by far exceeds the sensitivity of visual wheal formation (Weidner *et al.* 2000). Moreover, in rat skin there was a pronounced protein extravasation that we assessed using the same technique. Therefore, the lack of neurogenic protein extravasation in human skin cannot be attributed to limitations of the microdialysis technique. Our findings corroborate studies showing unchanged protein extravasation following capsaicin stimulation (Schmelz *et al.* 1997*a*) in human and also pig skin (Pierau *et al.* 1994). There is also no evidence for an indirect mechanism of neurogenic protein extravasation in human skin via histamine release from SP-activated mast cells (Tausk & Undem, 1995; Huttunen *et al.* 1996; Petersen *et al.* 1997*a*; Schmelz *et al.* 1999).

Implication for species differences

The basic mechanism underlying the differences between neurogenic protein extravasation in rodents and in humans is, as yet, unclear. Theoretically, lower SP concentrations, faster SP breakdown, fewer NK_1 receptors and/or longer distance from the nociceptive nerve terminals to the vessels could be of importance. Exogenously applied SP is also capable of inducing protein extravasation in human skin. When applied via microdialysis capillaries vasodilatation and protein extravasation are elicited at concentrations of 10^{-8} M without concomitant release of histamine (Weidner *et al.* 2000) suggesting there is no lack of functional NK_1 receptors in human skin. There is also evidence that rat organs with lower SP content, such as muscle, do not exhibit neurogenic protein extravasation (McMahon *et al.* 1984). Significantly lower SP concentrations in human skin as found in our study could thus contribute to the lack of protein extravasation. In accordance with our data, the total SP concentration in human forearm skin was lower than in abdominal skin of adult rat (Eedy *et al.* 1991, 1994). In addition, CGRP-positive fibres around vessels in human skin were found to co-localize especially somatostatin, but not SP (Gibbins *et al.* 1987). Thus, for SP the diffusion distance to the vessels might be larger.

In summary, transcutaneous electrical stimulation provoked neuropeptide release and vasodilatation in rat and human skin, whereas neurogenic protein extravasation was only observed in rat skin. It can be speculated whether this difference may offer a reason for the failure of NK_1 antagonists in the treatment of migraine (Roon *et al.* 2000), although they potently inhibit electrically evoked protein extravasation in rat dura mater (Polley *et al.* 1997). In accordance with our results, no increase in fluorescein-marked retinal protein extravasation could be detected in acute migraine, whereas electrical stimulation of the trigeminal ganglion evoked massive retinal protein extravasation in rat (May *et al.* 1998).

However, the lack of neurogenic protein extravasation in healthy human skin or in the retina during a migraine attack does not exclude a role for neuropeptides under pathological conditions. Higher SP concentrations have been suggested to be a relevant pathophysiological factor for the vasodilatation, oedema and trophic disturbances seen in patients suffering from complex regional pain syndrome (M. Sudeck, reflex sympathetic dystrophy) (Blair *et al.* 1998). Indeed, transcutaneous electrical stimulation with identical parameters to those in the present study provoked enhanced protein extravasation in this patient group, which was also accompanied by an enhanced flare reaction (Weber *et al.* 2000). Thus, although neurogenic protein extravasation is absent in healthy human skin, it may well be an important factor under pathophysiological conditions.

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