Calcitonin Gene-Related Peptide\textsubscript{8-37} Antagonizes Capsaicin-Induced Vasodilation in the Skin: Evaluation of a Human in Vivo Pharmacodynamic Model


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ABSTRACT

The purpose of this study was to identify the mediators involved in capsaicin-induced vasodilation in the human skin and to evaluate a pharmacodynamic model for the early clinical evaluation of calcitonin gene-related peptide (CGRP) receptor antagonists. Dermal blood flow (DBF) response of the forearm skin to topically applied capsaicin was measured using laser Doppler perfusion imaging in 22 subjects. The effect of intra-arterially administered CGRP\textsubscript{8-37} (1200 ng \cdot min\textsuperscript{-1} \cdot \text{dl}\textsuperscript{-1} forearm), indomethacin (5 \mu g \cdot min\textsuperscript{-1} \cdot \text{dl}\textsuperscript{-1} forearm), and \textit{N}\textsuperscript{\textit{G}}\textsuperscript{-monomethyl}-l-arginine (l-NMMA; 0.2 mg \cdot min\textsuperscript{-1} \cdot \text{dl}\textsuperscript{-1} forearm), and orally administered aprepitant (375 mg) on capsaicin-induced dermal vasodilation was assessed. Furthermore, the diurnal variation of the DBF response to capsaicin was studied. CGRP\textsubscript{8-37} inhibited the capsaicin-induced DBF increase: 217(145, 290)% in infused versus 370 (254, 486)% in the noninfused arm [mean (95% CI); \( p = 0.004 \)]. In contrast, indomethacin, l-NMMA, aprepitant, and the time of assessment did not affect the DBF response to capsaicin. Thus, capsaicin-induced vasodilation in the human forearm skin is largely mediated by CGRP, but not by vasodilating prostaglandins, nitric oxide, or substance P. The response to capsaicin does not display a circadian rhythm. A pharmacodynamic model is proposed to evaluate CGRP receptor antagonists in humans in vivo.

Neurogenic inflammation results from the release of bioactive substances from a subpopulation of primary sensory neurons consisting of A\textsubscript{\textit{\textup{\textgamma}}} and C-fiber nociceptors. It is a known phenomenon within the skin, joints, gut, urinary, and respiratory tracts (Barnes, 2001; Bjorling et al., 2003; Levine et al., 2006; Zegarska et al., 2006). In addition, there is growing evidence that in the pathophysiology of migraine, headache develops at least in part as a result of sterile neurogenic inflammation of large intracranial blood vessels (Buzzi et al., 1995). These blood vessels are innervated by a dense supply of sensory C-fibers originating from the trigeminal ganglion, which contain several neuropeptides, including substance P (SP), neurokinin A, and calcitonin gene-related peptide (CGRP) (Quartu et al., 1992). The release of these vasoactive neuropeptides is thought to result in a sustained neurogenic inflammation within the cephalic tissue, which causes pain through the activation of nociceptors. The efficacy of the CGRP-receptor antagonists BIBN4096BS and MK-0974 in the treatment of acute migraine confirmed that compounds interfering with the mediators of neurogenic inflammation hold promising prospects (Doeds et al., 2000, 2007; Salvatore et al., 2008).

We previously studied dermal vasodilation elicited by the topical application of capsaicin to the forearm skin of healthy male subjects (Van der Schueren et al., 2007b). Capsaicin is the pungent ingredient in hot chili peppers, and it activates the pungent ingredient in hot chili peppers, and it activates

ABBREVIATIONS: SP, substance P; CGRP, calcitonin gene-related peptide; MK-0974, N-[G(3R,8S)-6-(2,3-difluorophenyl)-2-oxo-1-(2,2,2-trifluoroethyl)azepan-3-yl]-4-[2-oxo-2-(1H-imidazo[4,5-b]pyridin-1-yl)piperidine-1-carboxamide; l-NMMA, \textit{N}\textsuperscript{\textit{G}}\textsuperscript{-monomethyl}-l-arginine; DBF, dermal blood flow; AU, arbitrary perfusion units; DSB, dermal blood flow; CI, confidence interval; NIA, noninfused arm; IA, infused arm; AUC, area under the curve; NO, nitric-oxide; BIBN4096BS, 1-Piperidinocarboxamide, N-[2-[[5-amino-1-[[4-(4-pyridinyl)-1-piperazinyl][carbonyl][penty]lamino]-1-[[3,5-dibromo-4-hydroxyphenyl][methyl]-2-oxoethyl]-4-(1,4-dihydro-2-oxo-3(2H)-quinazolinonyl)]-1-[[3,5-dibromo-4-hydroxyphenyl][methyl]-2-oxoethyl]-4-(1,4-dihydro-2-oxo-3(2H)-quinazolinonyl)]-1-[[3,5-dibromo-4-hydroxyphenyl][methyl]-2-oxoethyl]-4-(1,4-dihydro-2-oxo-3(2H)-quinazolinonyl)]-]; [R-\textit{R'},\textit{S'}].
the transient receptor potential vanilloid type 1 receptor on Aδ- and C-fiber nociceptors (Caterina et al., 1997). Binding of capsaicin to the vanilloid type 1 receptor provokes neurogenic inflammation through depolarization of neurons leading to the release of bioactive mediators. CGRP, SP, neurokinin A, NO, and prostaglandins are among the mediators thought to play a role in neurogenic inflammation in healthy human skin (Wallengren, 1997). Although dermal vasodilation is only one component of capsaicin-induced neurogenic inflammation, the easy and objective assessment of it by laser Doppler perfusion imaging makes it an attractive parameter for evaluating neurogenic inflammation (Van der Schueren et al., 2007b). Furthermore, most putative mediators of neurogenic inflammation have vasodilatory properties, which make it reasonable to assume that, if these mediators are major contributors to capsaicin-induced neurogenic inflammation, antagonizing them will affect the dermal blood flow response.

The aims of this study were 1) to characterize and identify the mediators involved in the dermal vasodilation induced by capsaicin application to the human skin and 2) to evaluate a human in vivo pharmacodynamic model for its usefulness in the early clinical development of CGRP antagonists. As CGRP-receptor antagonist, CGRP8-37, a C-terminal fragment of the CGRP peptide, which has been shown to block CGRP-induced vasodilation in a competitive manner in both animal and human studies, was used (Chiba et al., 1989; Vanmolkot et al., 2006). CGRP8-37 has limited potency, and it cannot be administered systemically in humans to inhibit CGRP-induced vasodilation. This is circumvented when CGRP8-37 is administered into the brachial artery, resulting in sufficiently high local concentrations within the forearm without causing any systemic effects (Vanmolkot et al., 2006). In this way, the contralateral arm or noninfused arm is not treated, and it serves as the control arm. The effect of the cyclooxygenase inhibitor indomethacin and the nonselective nitric-oxide (NO) synthase inhibitor Nω-monomethyl-L-arginine monooacetate (L-NMMA) on capsaicin-induced dermal vasodilation were also assessed by infusing dosages in the human forearm that have previously been shown to be effective in antagonizing vasodilating prostaglandins and NO, respectively (Smits et al., 1995; de Hoon et al., 2003). Because there is no infusable formulation of a SP antagonist available, the potent SP antagonist apreptin was given orally, and capsaicin-induced vasodilation was measured on the left arm preapreptin and on the right arm 4 h after dosing apreptin (Tmax) (Brands et al., 2003; Patel and Lindley, 2003). Because in the latter part of the study protocol measurements of dermal perfusion on both arms were not simultaneously performed, the diurnal reproducibility of the capsaicin-induced DBF response was evaluated by adding a control period in which no treatment was given, but only capsaicin was applied once in the morning on the left arm and 4 h later, in the afternoon, on the right arm.

Materials and Methods

Subjects. After approval by the ethics committee of the University Hospital of Leuven (Leuven, Belgium), written informed consent was obtained from all subjects during a screening visit. In total, 44 subjects were recruited for participation in at least one part of the study. All subjects were white, nonsmoking, healthy males between 18 and 45 years old.

Study Design. Subjects were instructed to abstain from any drugs during 3 days and from chocolate-, alcohol-, and caffeine-containing beverages and food during the 12 h preceding the screening visit and each study visit. All measurements were performed while the subjects rested in a supine position on a comfortable bed in a quiet, temperature-controlled room (ambient temperature 24 ± 1°C). During each visit (screening and study periods), 10-mm rubber O-rings (8-mm inner diameter; McMaster-Carr, New Brunswick, NJ) were placed at four equally spaced sites on the volar surface of the forearms. The rings were positioned so that their distal edges were 10, 14, 18, and 22 cm proximal to the wrist crease, within approximately 1 cm of the midline and avoiding visible veins. The proximal ring (i.e., closest to the antecubital crease) is referred to as site 1, and the distal ring (i.e., closest to the wrist crease) is referred to as site 4. After placement of the O-rings, a laser Doppler perfusion imager (HR-LDPI system, Periscan PIMII; Perimed, Järfälla, Sweden) was used to obtain baseline scans of the DBF of the areas defined by the rings. In subsequent experiments, these O-rings served as reservoirs to contain the topically applied 20-μl capsaicin or placebo solutions. Capsaicin powder was obtained from Sigma-Aldrich N.V. (Bornem, Belgium), and it was dissolved in a 3:3:4 mixture of ethanol 100%, Tween 20, and distilled water. Capsaicin was diluted so that 20 μl of the mixture contained 1000 μg of capsaicin. The placebo solution corresponded to the same 3:3:4 mixture of ethanol 100%, Tween 20, and distilled water without capsaicin. We fully described the methodology previously (Van der Schueren et al., 2007b).

Study 1: Effect of Intra-Arterial Infusion of CGRP8-37, L-NMMA, and Indomethacin on Capsaicin-Induced Dermal Vasodilation. Twenty-one subjects were screened for this study. During the screening visits, subjects were evaluated as being responders or nonresponders. To that end, all subjects received on both forearms a topical dose of 1000 μg of capsaicin per 20 μl of vehicle at the two proximal sites, and they received placebo (i.e., 20 μl of vehicle) at the two distal sites. The application of the capsaicin solution always started at site 1 of the dominant arm. Capsaicin or placebo solutions were then applied with 1-min intervals to the remaining sites of the dominant arm. Two minutes after the last application on the dominant arm, application started at site 1 of the nondominant arm, and, respecting the 1-min time interval between applications, to all remaining sites. In subsequent experiments, laser Doppler scans were performed at precisely 10, 20, and 30 min postcapsaicin or placebo application at each site starting from site 1 on the dominant arm to site 4 of the nondominant arm, respecting the same time intervals as during application. Scanning of all sites thus required approximately 8 min at each time point. Only responders, defined as subjects with a capsaicin-induced increase in DBF of ≥100% in both proximal sites of both arms were included. This criterion was fulfilled by 11 of the 21 screened subjects.

The 11 included subjects participated in a randomized, single-blind, three-way, crossover study (flow chart in Fig. 1A). In each of the treatment periods, after insertion of a 27-gauge mounted needle (Sterican; B. Braun, Melsungen, Germany) into the brachial artery of the nondominant arm, first 0.9% saline (B. Braun) was infused at 100 μl · min⁻¹ · dl⁻¹ forearm using automated infusion pumps (Ivac P1000; Ivac Medical Systems, Brussels, Belgium) for approximately 20 min for equilibration. Subsequently, baseline DBF was measured at both arms. Thereafter, capsaicin and placebo were applied to the skin of the dominant arm (i.e., the noninfused arm) as described above. Two minutes before capsaicin application on the nondominant (i.e., infused arm), infusion with saline 0.9% was substituted by one of the following antagonists:

1. CGRP8-37 at a 1200 ng · min⁻¹ · dl⁻¹ forearm dose that has previously been shown to inhibit CGRP-induced vasodilation in the human forearm (Van der Schueren et al., 2006).
2. Indomethacin at a 5 μg · min⁻¹ · dl⁻¹ forearm dose known to suppress tromboxane B2 formation, a marker for cyclooxygenase

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activity, when infused in the human forearm (Patrono et al., 1980; de Hoon et al., 2003).

3. L-NMMA at a 0.2 mg·min⁻¹·dl⁻¹ forearm dose as a nonselective nitric-oxide synthase inhibitor. Together with L-NMMA, sodium nitroprusside (exogenous NO donor; 0.2 μg·min⁻¹·dl⁻¹ forearm) was coinfused intra-arterially to correct for L-NMMA-induced vasoconstriction, which results from inhibition of basal endothelial NO release (i.e., “NO-clamp” technique) (Smits et al., 1995).

All antagonists and sodium nitroprusside were dissolved in 0.9% saline immediately before each experiment, and doses were normalized to forearm volume (measured by water displacement) to keep the rate of all intra-arterial infusions constant at 100 μl·min⁻¹·dl⁻¹ forearm. The total duration of antagonist infusion was 37 min, as it was started after placebo application at site 4 of the dominant arm and before capsaicin application to site 1 of the nondominant arm, and it was stopped when the last laser Doppler measurement at site 4 of the nondominant arm was performed.

Study 2: Effect of Oral Administration of Aprepitant on Capsaicin-Induced Dermal Vasodilation. For this study, another 23 subjects were screened during a morning visit. After 20 min of acclimatization, baseline DBF was measured in the left arm. Subsequently, capsaicin and placebo were applied to the skin of the left forearm as described above, and laser Doppler scans were performed at precisely 10, 20, and 30 min after capsaicin or placebo application. After this evaluation, 11 responders (defined as above) were included for participation in a randomized, open-label, two-way crossover study (flow chart in Fig. 1B).

In each of the two study periods, the capsaicin-induced DBF response was evaluated twice: once in the morning at the left arm and once in the afternoon at the right arm. During one study period (i.e., the treatment period), subjects received a supratherapeutic dose of 375 mg of aprepitant after the morning session ($T_{max} = 4$ h) (Patel and Lindley, 2003; Majumdar et al., 2006), which is known to achieve a level of $\geq 90\%$ neurokinin-1 receptor occupancy in the central nervous system of humans (Patel and Lindley, 2003; Bergström et al., 2004; Majumdar et al., 2006). During the other period (i.e., control period), no treatment was given. The DBF responses to capsaicin on the right forearm were evaluated at either 4 h after aprepitant during the treatment period or at the corresponding time during the control period. By comparing the morning responses to capsaicin with the afternoon responses, the contribution of SP to capsaicin-induced vasodilation was evaluated during the treatment period. During the control period, a diurnal variation in capsaicin-induced vasodilation was excluded.

In both studies, the sequence in which the treatments were administered over the course of periods was randomly allocated. In each case, study periods were separated by washout periods of at least 1 week.

Measurements. Supine systolic blood pressure, diastolic blood pressure, and heart rate were measured in the dominant arm with a validated semi-automated oscillometric device (Omron HEM-705CP; Omron Healthcare, Hamburg, Germany). DBF was determined as described previously (Van der Schueren et al., 2007b).

Data Analysis and Statistics. Based on the within-subject standard deviation observed in the reproducibility study and given a type I error probability ($\alpha$) of 0.05, a sample size of 11 subjects provides 80% power for detecting a difference in DBF increase after capsaicin application of 20% between arms (Van der Schueren et al., 2007b). The baseline DBF was expressed in arbitrary perfusion units (AU) (Fullerton et al., 2002). The change in DBF in response to capsaicin was expressed as the percentage change from baseline. The percentage change was compared with the percentage change of DBF at the placebo sites in all three treatment periods and both arms using analysis of variance for repeated measures. In addition, the area under the curve of the percentage change from baseline up to 30 min after capsaicin application ($AUC_{0-30}$) was calculated as a summary.
measure. At each time point, the mean of observations with the 95% two-sided confidence interval (CI) are given. The normality of the distribution of the data were assessed and Wilcoxon’s matched pairs signed rank tests or paired Student’s t tests were performed accordingly to compare the DBF percentage change at the 30-min time point \(t_{30}\) and the AUC\(_{0-30}\) between the subjects forearms within the same study period.

Blood pressure and heart rate were compared between baseline and the end of the infusion (study 1) and between pre- and 4-h postaprepitant administration (study 2) by Wilcoxon’s matched pairs signed rank test.

Unless stated otherwise, data are expressed as mean ± 95% CI. \(P < 0.05\) was considered statistically significant.

Results

Capsaicin application was well tolerated by all subjects, and no adverse events of note were reported. In most subjects, capsaicin provoked a local flare and stinging sensation that disappeared within 2 to 6 h after application. All included subjects completed the study.

Study 1: Effect of Intra-Arterial Infusion of CGRP\(_{8-37}\), L-NMMA, and Indomethacin on Capsaicin-Induced Dermal Vasodilation. Mean ± S.D. (range) for age, weight, and height of the 11 included subjects was 25 ± 5 (20–37) years, 78 ± 7 (69–91) kg, and 182 ± 5 (174–192) cm, respectively. Compared with baseline, a small increase (\(P < 0.05\)) in diastolic blood pressure was seen at the end of all three treatment periods (Table 1). DBF at baseline and after capsaicin or placebo are given in Table 2.

DBF at baseline did not differ between arms (Table 2), and it increased after capsaicin application compared with placebo in all three treatment periods and both arms (\(P < 0.0001\), analysis of variance for repeated measures; Fig. 2). The capsaicin-induced DBF increase was partially blocked in the CGRP\(_{8-37}\)-infused arm compared with the noninfused arm (Table 2; Fig. 2A). Indomethacin and L-NMMA did not affect the capsaicin-induced increase in DBF (Table 2; Fig. 2, B and C).

The DBF changes at the placebo sites did not differ between the infused and noninfused arm during CGRP\(_{8-37}\) and indomethacin infusion. In contrast, AUC\(_{0-30}\) of the DBF percentage change from baseline at the placebo sites was smaller in the L-NMMA-infused than the noninfused arm (Table 2).

Study 2: Effect of Oral Administration of Aprepitant on Capsaicin-Induced Dermal Vasodilation. Mean ± S.D. (range) for age, weight, and height of the 11 included subjects was 26 ± 5 (19–41) years, 72 ± 12 (55–94) kg, and 180 ± 7 (165–192) cm, respectively. No changes in systolic and diastolic blood pressure or heart rate were observed during the experiments.

In the control period, before capsaicin application, baseline DBF was similar in morning and afternoon at 0.39 (0.37, 0.42) AU (i.e., left arm) and 0.42 (0.38, 0.46) AU (i.e., right arm), respectively. The response to capsaicin showed no diurnal variation. In the morning, DBF response expressed as \(t_{30}\) averaged 402 (258, 546)% versus 340 (170, 510)% in the afternoon. This corresponds to an AUC\(_{0-30}\) of 5551 (3084, 8017)%·min in the morning and 4829 (2051, 7608)%·min in the afternoon (Fig. 3A).

In the treatment period, baseline DBF averaged 0.41 (0.37, 0.45) AU in the morning (i.e., before aprepitant administration). Four hours after aprepitant, DBF before capsaicin administration was slightly higher at 0.49 (0.43, 0.51) AU (\(P < 0.05\)). Aprepitant did not affect capsaicin-induced increase in DBF (Fig. 3B): expressed as \(t_{30}\), the increase in DBF averaged 319 (233, 405)% preaprepitant versus 305 (210, 400)% 4 h postdose; AUC\(_{0-30}\) averaged 4201 (2465, 5937)%·min pre- and 4403 (2812, 5995)%·min postaprepitant.

Discussion

This study demonstrates in healthy male volunteers in vivo that DBF response after capsaicin application to the skin is to a large extent antagonized by a CGRP receptor antagonist, whereas inhibition of SP, NO, and prostaglandins had no substantial effect.

The central role of CGRP in the DBF response to capsaicin was largely expected based on both animal and human studies (Hughes and Brain, 1991; Hershey et al., 2005). The observation that the inhibition of DBF increase by CGRP\(_{8-37}\) is incomplete does not discard CGRP as an important mediator of the vasodilatory response to capsaicin, but it is probably due to the limited potency of CGRP\(_{8-37}\). CGRP\(_{8-37}\) is known to have a 2000 time lower affinity to the CGRP receptor of human arteries than the nonpeptide CGRP receptor antagonist BIBN4096BS (Verheggen et al., 2002). When the dose of CGRP\(_{8-37}\) used in the present study was intra-arterially infused with a 120-fold lower dose of CGRP into the brachial artery of the human forearm, the degree of inhibition of the CGRP-induced vasodilation was comparable with the inhibition of the capsaicin-induced DBF response observed in the present study (i.e., around 50%) (Vannmolkot et al., 2006). Furthermore, when CGRP\(_{8-37}\) and CGRP are simultaneously injected into the volar forearm of healthy volunteers, the ability of CGRP to increase DBF is again only inhibited by approximately 50% (Hayes et al., 1993). Recently, the potent oral CGRP antagonist MK-0974 was shown to inhibit the dermal vasodilation after capsaicin application by approximately 80%, which further substantiates CGRP as an important mediator of capsaicin-induced vasodilation in the skin (Van der Schueren et al., 2007a). However, we do

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time</th>
<th>L-NMMA</th>
<th>CGRP(_{8-37})</th>
<th>Indomethacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>Baseline</td>
<td>123 (119, 127)</td>
<td>120 (116, 125)</td>
<td>126 (119, 132)</td>
</tr>
<tr>
<td></td>
<td>40 min</td>
<td>125 (120, 129)</td>
<td>123 (119, 126)</td>
<td>128 (120, 136)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>Baseline</td>
<td>67 (43, 92)</td>
<td>66 (42, 91)</td>
<td>69 (44, 93)</td>
</tr>
<tr>
<td></td>
<td>40 min</td>
<td>72 (46, 96)</td>
<td>71 (46, 95)</td>
<td>72 (48, 97)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>Baseline</td>
<td>60 (35, 84)</td>
<td>55 (30, 79)</td>
<td>58 (34, 83)</td>
</tr>
<tr>
<td></td>
<td>40 min</td>
<td>59 (35, 84)</td>
<td>57 (33, 82)</td>
<td>59 (34, 83)</td>
</tr>
</tbody>
</table>

\(^{a}P < 0.05\) versus baseline assessed by Wilcoxon matched pairs signed rank test.

TABLE 1
Study 1: hemodynamic parameters
Data are presented as mean ± 95% confidence interval; number of subjects = 11.
realize that the present study setup differs, which makes it hard to compare results across studies. For example, with MK-0974, inhibition of CGRP receptors within the central nervous system could account for a more robust inhibition of capsaicin-induced DBF responses, whereas brachial infusion of CGRP8-37 only inhibits CGRP released from peripheral nociceptive nerves in the skin of the forearm. On the whole, our findings indicate that CGRP is an important mediator of capsaicin-induced vasodilation in the skin, validating the use of the proposed pharmacodynamic model in the early clinical development of CGRP receptor antagonists. It is also interesting to note that CGRP8-37 infusion had no effect on resting DBF, which is in agreement with our previous observation that CGRP8-37 does not affect resting forearm blood flow (Vanmolkot et al., 2006). These data suggest that CGRP is not an important mediator of resting blood flow and that CGRP receptor antagonists are unlikely to affect tissue perfusion under resting conditions.

The absence of a substantial contribution of the other mediators (i.e., vasodilating prostaglandins, NO, and SP) to capsaicin-induced DBF increase may seem rather surprising, especially for SP. Studies on neurogenic inflammation in animal skin have pointed to SP as a major inflammatory mediator (Lembeck and Holzer, 1979; Grant et al., 2002). In mice, capsaicin-induced vasodilation increases when neurotransmitter (Lembeck and Holzer, 1979; Grant et al., 2002). In view of Petersen’s and our findings, we conclude that, under physiological conditions, SP has no major role in capsaicin-induced vasodilation in the human skin.

Because aprepitant could not be locally administered by arterial infusion, it was impossible to compare the DBF response simultaneously between the infused (treated) arm and the noninfused (control) arm. Because the DBF response to capsaicin does not demonstrate a circadian rhythm, this nontumultaneous assessment is valid.

The inability of indomethacin to inhibit the increase of capsaicin-induced DBF indicates that potent cyclooxygenase inhibition does not affect this response. Therefore, we propose that vasodilating prostaglandins are not responsible for the dermal vasodilation component of neurogenic inflammation in humans. The role of prostaglandins in neurogenic vasodilation has been debated because of conflicting findings in different research settings. On the one hand, cyclooxygenase inhibitors have been shown to inhibit CGRP release at the spinal level in rats (Southall et al., 1998; Seidel et al., 2003). Furthermore, treating human keratinocytes with capsaicin results in a dose-dependent expression of cyclooxygenase-2 (Southall et al., 2003). On the other hand, Herbert et al. (1993) demonstrated that neither acetylsalicylic acid nor indomethacin affected capsaicin-induced increase in DBF in human skin (Herbert et al., 1993), although they did find a significantly lower increase of DBF with indomethacin pretreatment in the area directly underlying the application site of capsaicin, but not in the area surrounding the application site. The latter confirms the contention that in their study setup, the inflamed area underlying capsaicin-soaked plasters should be distinguished from proper neurogenic inflammation.

We demonstrated that the arterial infusion of a nonselective NO synthase inhibitor L-NMMA does not affect dermal vasodilation after capsaicin application (Smits et al., 1995). This finding is particularly interesting because there is much debate surrounding the role of NO in the vasodilator mechanism of CGRP. If one considers endogenous CGRP release from capsaicin-sensitive nociceptors in the skin pivotal for the capsaicin-induced DBF response and one takes into account that the vasodilator effect of CGRP in humans is partly mediated by the release of NO (de Hoon et al., 2003), one

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Arm</th>
<th>CGRP8-37</th>
<th>Indomethacin</th>
<th>L-NMMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (all sites) DBF (AU)</td>
<td>NIA</td>
<td>0.44 (0.42, 0.46)</td>
<td>0.51 (0.48, 0.54)</td>
<td>0.48 (0.46, 0.49)</td>
</tr>
<tr>
<td>IA</td>
<td>0.44 (0.41, 0.46)</td>
<td>0.49 (0.46, 0.52)</td>
<td>0.49 (0.46, 0.51)</td>
<td></td>
</tr>
<tr>
<td>1000 µg of capsaicin t50 (%)</td>
<td>NIA</td>
<td>370 (254, 486)</td>
<td>337 (256, 418)</td>
<td>309 (243, 375)</td>
</tr>
<tr>
<td>IA</td>
<td>217 (145, 290)</td>
<td>319 (247, 392)</td>
<td>314 (252, 375)</td>
<td></td>
</tr>
<tr>
<td>AUCo-30 (% · min)</td>
<td>NIA</td>
<td>5093 (3211, 6974)</td>
<td>4999 (2899, 5956)</td>
<td>3717 (2569, 4866)</td>
</tr>
<tr>
<td>IA</td>
<td>2721 (1929, 3513)</td>
<td>3945 (2834, 5056)</td>
<td>3417 (2569, 4308)</td>
<td></td>
</tr>
<tr>
<td>Placebo t50 (%)</td>
<td>NIA</td>
<td>20 (7, 32)</td>
<td>10 (1, 19)</td>
<td>13 (2, 23)</td>
</tr>
<tr>
<td>IA</td>
<td>11 (0, 22)</td>
<td>10 (0, 20)</td>
<td>6 (0, 12)</td>
<td></td>
</tr>
<tr>
<td>AUCo-30 (% · min)</td>
<td>NIA</td>
<td>179 (12, 361)</td>
<td>141 (48, 230)</td>
<td>172 (3, 341)</td>
</tr>
<tr>
<td>IA</td>
<td>134 (71, 339)</td>
<td>65 (74, 209)</td>
<td>1 (71, 73)</td>
<td></td>
</tr>
</tbody>
</table>

Note: *P = 0.004 (Wilcoxon’s matched pairs signed rank test comparing IA with NIA).

b P = 0.004 (paired Student’s t test comparing IA with NIA).

c P = 0.01 (paired Student’s t test comparing IA with NIA).
would expect an inhibitory effect of L-NMMA. Hughes and Brain (1994) demonstrated that in the rabbit cutaneous microvasculature, NO synthase inhibition had no effect on the subjects after capsaicin/placebo per arm = 22) during intra-arterial infusion of indomethacin (5 μg · min⁻¹ · dl⁻¹ forearm) before placebo application was comparable between both arms. **, *P* = 0.004 at t₃₀ (Wilcoxon’s matched pairs signed rank test). ++, *P* = 0.004 for AUC₀₃₀ (paired Student’s t test). B, DBF response (mean ± 95% CI; number of observations after capsaicin/placebo per arm = 22) during intra-arterial infusion of L-NMMA (0.2 mg · min⁻¹ · dl⁻¹ forearm) in combination with sodium nitroprusside (0.2 μg · min⁻¹ · dl⁻¹ forearm). DBF increase was similar in the L-NMMA IA and the NIA after application of 1000 μg of capsaicin. Resting DBF (i.e., after placebo application) was unaffected by aprepitant. Resting DBF (i.e., after placebo application) was unaffected by aprepitant.

Fig. 2. A, DBF response (mean ± 95% CI; number of subjects = 11; number of observations after capsaicin/placebo per arm = 22) during intra-arterial infusion of CGRP₈₋₃₇ (1200 ng · min⁻¹ · dl⁻¹ forearm). CGRP₈₋₃₇ inhibits DBF increase in the IA compared with the NIA after application of 1000 μg of capsaicin, whereas resting DBF (i.e., after placebo application) was comparable between both arms. **, *P* = 0.004 at t₃₀ (Wilcoxon’s matched pairs signed rank test). ++, *P* = 0.004 for AUC₀₃₀ (paired Student’s t test). B, DBF response (mean ± 95% CI; number of subjects = 11; number of observations after capsaicin/placebo per arm = 22) during intra-arterial infusion of indomethacin (5 μg · min⁻¹ · dl⁻¹ forearm). DBF increase was similar in the indomethacin IA and the NIA after application of 1000 μg of capsaicin. Resting DBF (i.e., after placebo application) was unaffected by indomethacin infusion. C, DBF response (mean ± 95% CI; number of subjects = 11; number of observations after capsaicin/placebo per arm = 22) during intra-arterial infusion of L-NMMA (0.2 mg · min⁻¹ · dl⁻¹ forearm) after aprepitant (375 mg) administration. DBF increase was similar in the left arm before aprepitant administration and the right arm 4 h after the intake of aprepitant. Resting DBF (i.e., after placebo application) was unaffected by aprepitant.

Fig. 3. A, DBF response (mean ± 95% CI; number of subjects = 11; number of observations after capsaicin/placebo per arm = 22) during intra-arterial infusion of indomethacin (5 μg · min⁻¹ · dl⁻¹ forearm) after aprepitant (375 mg) administration. DBF increase was similar in the indomethacin IA and the NIA after application of 1000 μg of capsaicin. Resting DBF (i.e., after placebo application) was unaffected by indomethacin infusion. C, DBF response (mean ± 95% CI; number of subjects = 11; number of observations after capsaicin/placebo per arm = 22) during intra-arterial infusion of L-NMMA (0.2 mg · min⁻¹ · dl⁻¹ forearm) in combination with sodium nitroprusside (0.2 μg · min⁻¹ · dl⁻¹ forearm). DBF increase was similar in the L-NMMA IA and the NIA after application of 1000 μg of capsaicin. Resting DBF (i.e., after placebo application) was unaffected by aprepitant.
CGRP-induced vasodilation, whereas it did reduce capsaicin-
induced vasodilation. This would suggest that in rabbit skin
the vasodilator mechanism of CGRP is independent of NO,
whereas NO may play an important role in the release of
CGRP from capsaicin-sensitive nerves. In contrast, Gold-
smith et al. (1996) were able to inhibit CGRP-induced DBF
increase by injecting L-NMMA into skin of the human fore-
arm. Our findings indicate that, in the human skin, NO is
neither involved in the release of CGRP nor in its vasodilator
mechanism. Because in our model we compare the capsaicin-
induced dermal vasodilation between infused and noninfused
arm, it could be argued that “spillover” of L-NMMA from the
infused arm to the noninfused arm would prevent us from
detecting an inhibitory effect of L-NMMA. However, spillover
of L-NMMA to the noninfused arm seems very unlikely. First,
detecting an inhibitory effect of L-NMMA has been shown to quickly disappear from plasma
of L-NMMA to the noninfused arm. In contrast, Gold-
smith et al. (1996) were able to inhibit CGRP-induced neurogenic in-
duction of NO to capsaicin-induced neurogenic in-
arterial brachial infusion even when infusing vasodilators
other treatment periods and reported by others using intra-
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during the laser Doppler imaging and Ayline Yudhira for help with
the data analysis.
the data analysis.
during the other study
periods, including the screening period. Finally, the slight
increase in diastolic blood pressure after L-NMMA infusion is
most likely procedure-related, because it was also seen in the
other treatment periods and reported by others using intra-
arterial brachial infusion even when infusing vasodilators
(Vanmolkot and de Hoon, 2005). Therefore, we are confident
to conclude that there is no substantial contribution of de
novo synthesized NO to capsaicin-induced neurogenic in-
flammation. It should be noted that L-NMMA slightly
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