JPET Fast Forward. Published on January 23, 2008 as DOI: 10.1124/jpet.107.133868



**Title Page**

# $CGRP_{8-37}$ antagonizes capsaicin-induced vasodilation in the skin: evaluation of a human *in vivo* pharmacodynamic model.

B. J. Van der Schueren, A. Rogiers, F.H. Vanmolkot, A. Van Hecken, M. Depré, S. A. Kane, I. De Lepeleire, S.R. Sinclair, J.N. de Hoon

Center for Clinical Pharmacology, University Hospital Gasthuisberg (K.U. Leuven), Leuven, Belgium (B.J.V.D.S., A.R., F.H.V., A.V.H., M.D., J.N.d.H); Department of Pain Research, Merck Research Laboratories, West Point, Pennsylvania,USA (S.A.K); Department of Clinical Pharmacology, MSD (Europe) Inc., Brussels, Belgium (I.D.L.); Department of Clinical Pharmacology, Merck Research Laboratories, Upper Gwynedd, Pennsylvania, USA (S.R.S)

JPET Fast Forward. Published on January 23, 2008 as DOI: 10.1124/jpet.107.133868
This article has not been copyedited and formatted. The final version may differ from this version.



**Running Title Page**

**Running title:** Mediators of capsaicin-induced dermal vasodilation

**Address for correspondence:**
Bart Van der Schueren
Center for Clinical Pharmacology
University Hospital Gasthuisberg
Herestraat 49
3000 Leuven
Belgium
Tel : +32 (0)16/342027
Fax : +32 (0)16/342050
E-mail: bart.vanderschueren@uz.kuleuven.be

**Counts:**

- Text pages : 26
- Tables : 2
- Figures: 3
- References: 38
- Abstract word count: 177
- Introduction word count: 680
- Discussion word count: 1485

**Abbreviations:** AU, arbitrary perfusion units (AU); CGRP, calcitonin gene-related peptide; DBF, dermal blood flow; L-NMMA, $N^G$-monomethyl-L-arginine; NO, nitric oxide; SP, Substance P

**Recommended section assignment: Neuropharmacology**

JPET Fast Forward. Published on January 23, 2008 as DOI: 10.1124/jpet.107.133868
This article has not been copyedited and formatted. The final version may differ from this version.



**Abstract**

**Objectives** 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 CGRP receptor antagonists. **Methods** 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 $CGRP_{8-37}$ (1,200 $ng.min^{-1}.dL^{-1}$forearm), indomethacin (5 $\mu g.min^{-1}.dL^{-1}$forearm), $N^G$-monomethyl-L-arginine (L-NMMA, 0.2 $mg.min^{-1}.dL^{-1}$forerarm) 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. **Results** $CGRP_{8-37}$ inhibited the capsaicin-induced DBF increase: 217(145, 290)% in infused versus 370(254,486)% in the non-infused arm (mean (95% CI); p=0.004). In contrast, indomethacin, L-NMMA, aprepitant and the time of assessment did not effect the DBF response to capsaicin. **Conclusions** 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*.

JPET Fast Forward. Published on January 23, 2008 as DOI: 10.1124/jpet.107.133868
This article has not been copyedited and formatted. The final version may differ from this version.



**Introduction**

Neurogenic inflammation results from the release of bioactive substances from a subpopulation of primary sensory neurons consisting of Aδ- and C-fibre 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-fibres originating from the trigeminal ganglion, which contains 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 believed 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 holds promising prospects (Doods et al., 2000; Doods et al., 2007; Salvatore et al., 2007).

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 chilli peppers and activates the transient receptor potential vanilloid type 1 receptor on Aδ- and C-fibre nociceptors (Caterina et al., 1997). Binding of capsaicin to the vanilloid type 1 receptor provokes neurogenic inflammation through depolarisation of neurons leading to the release of bioactive mediators. CGRP, SP, Neurokinin A, nitric oxide (NO) and prostaglandins are amongst the mediators believed 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 makes 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

JPET Fast Forward. Published on January 23, 2008 as DOI: 10.1124/jpet.107.133868
This article has not been copyedited and formatted. The final version may differ from this version.



CGRP-receptor antagonists, $CGRP_{8-37,}$ 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). $CGRP_{8-37}$ has limited potency and cannot be administered systemically in humans to inhibit CGRP-induced vasodilation. This is circumvented when $CGRP_{8-37}$ 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 contra-lateral arm or non-infused arm is not treated and serves as the control arm. The effect of the cyclo-oxygenase inhibitor indomethacin and the non-selective nitric oxide synthase inhibitor $N^G$-monomethyl-L-arginine monoacetate (L-NMMA) on capsaicin-induced dermal vasodilation were also assessed by infusing dosages in the human forearm which have previously been shown to be effective in antagonising vasodilating prostaglandins and NO, respectively (Smits et al., 1995; de Hoon et al., 2003). As there is no infusible formulation of a SP antagonists available, the potent SP antagonist aprepitant was given orally and capsaicin-induced vasodilation was measured on the left arm pre-aprepitant and on the right arm at 4 hours post-aprepitant ($T_{max}$) (Brands et al., 2003; Patel and Lindley, 2003). As 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 hours later, in the afternoon, on the right arm.

JPET Fast Forward. Published on January 23, 2008 as DOI: 10.1124/jpet.107.133868
This article has not been copyedited and formatted. The final version may differ from this version.



**Methods**

*Subjects*

After approval by the ethics committee of the University Hospital of Leuven, 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, non-smoking, healthy males between 18 and 45 years old.

*Study design*

Subjects were instructed to abstain from any drugs during three days and from chocolate-, alcohol- and caffeine-containing beverages and food during 12 hours preceding the screening visit and each study period. All measurements were performed while the subjects rested in a supine position on a comfortable bed in a quiet, temperature controlled room (ambient temperature of 24°C ± 1 °C). During each visit (screening and study periods) 10 mm rubber O-rings (McMaster-Carr, New Brunswick, US; 8 mm inner diameter) were placed at 4 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, the distal ring (i.e. closest to the wrist crease) 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. Subsequently, 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 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 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).

JPET Fast Forward. Published on January 23, 2008 as DOI: 10.1124/jpet.107.133868
This article has not been copyedited and formatted. The final version may differ from this version.



*Study 1: Effect of intra-arterial infusion of $CGRP_{8-37}$, L-NMMA and indomethacin on capsaicin-induced dermal vasodilation*

Twenty-one subjects were screened for this study. During the screening visit subjects were evaluated as being responders or non-responders. To that end all subjects received on both forearms a topical dose of 1000 µg capsaicin per 20 µl vehicle at the two proximal sites and 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 minute 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 non-dominant arm and, respecting the 1 minute time interval between applications, to all remaining sites. Subsequently, Laser Doppler scans were performed at precisely 10, 20 and 30 minutes post-capsaicin or placebo application at each site starting from site 1 on the dominant arm to site 4 of the non-dominant arm respecting the same time-intervals as during application. Scanning of all sites thus required approximately 8 minutes 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 out of the 21 screened subjects.

The 11 included subjects participated in a randomised, single-blind, 3-way, cross-over study *(flow chart, figure 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 non-dominant arm, first 0.9% saline (B Braun, Melsungen, Germany) was infused at 100 $\mu L.min^{-1}.dL^{-1}$ forearm using automated infusion pumps (Ivac® P1000, Ivac Medical Systems, Brussels, Belgium) for about 20 minutes 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 non-infused arm) as described above. Two minutes prior to capsaicin application on the non-dominant (i.e. infused arm) infusion with saline 0.9% was substituted by one of the following antagonists:

1) $CGRP_{8-37}$ at a 1,200 $ng.min^{-1}.dL^{-1}$ forearm dose which has previously been shown to inhibit CGRP-induced vasodilation in the human forearm (Vanmolkot et al., 2006).
2) Indomethacin at a 5 $\mu g.min^{-1}.dL^{-1}$ forearm dose known to suppress tromboxane B2 formation, a marker for cyclo-oxygenase activity, when infused in the human forearm (Patrono et al., 1980; de Hoon et al., 2003).

JPET Fast Forward. Published on January 23, 2008 as DOI: 10.1124/jpet.107.133868
This article has not been copyedited and formatted. The final version may differ from this version.



3) L-NMMA at 0.2 $mg.min^{-1}.dL^{-1}$ forearm as a non-selective nitric oxide synthase inhibitor. Together with L-NMMA, sodium nitroprusside (exogenous NO-donor, 0.2 $\mu g.min^{-1}.dL^{-1}$ forearm) was co-infused 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) in order to keep the rate of all intra-arterial infusions constant at 100 $\mu L.min^{-1}.dL^{-1}$ forearm. The total duration of antagonist infusion was 37 minutes as it was started after placebo application at site 4 of the dominant arm and prior to capsaicin application to site 1 of the non-dominant arm and was stopped when the last laser Doppler measurement at site 4 of the non-dominant 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 minutes of acclimatisation, 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 minutes post-capsaicin or placebo application. After this evaluation, 11 responders (defined as above) were included for participation in a randomised, open-label, 2-way, cross-over study *(flow chart, figure 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 supra-therapeutic dose of 375 mg aprepitant after the morning session ($T_{max}$=4h) (Patel and Lindley, 2003; Majumdar et al., 2006) which is known to achieve a level of ≥90% neurokinin-1 receptor occupancy in the central nervous system of humans (Patel and Lindley, 2003; Bergstrom 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 hours post-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.

JPET Fast Forward. Published on January 23, 2008 as DOI: 10.1124/jpet.107.133868
This article has not been copyedited and formatted. The final version may differ from this version.



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 wash-out 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 previously described (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 (α) of 0.05, a sample size of 11 subjects provides 80% power for detecting a difference in DBF increase following 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 percent change from baseline. This percent change was compared with the percent change of DBF at the placebo sites in all three treatment periods and both arms using ANOVA for repeated measures. In addition, the area under the curve of the percent change from baseline up to 30 minutes after capsaicin application ($AUC_{0\text{-}30}$) was calculated as a summary measure. At each time-point the mean of observations with the 95% two-sided confidence interval (95% CI) are given. The normality of the distribution of the data was assessed and Wilcoxon's matched-pairs signed-rank tests or paired Student's t-tests were performed accordingly to compare the DBF percent change at the 30 minutes time-point ($t_{30}$) and the $AUC_{0\text{-}30}$ 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 hours post aprepitant 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.

JPET Fast Forward. Published on January 23, 2008 as DOI: 10.1124/jpet.107.133868
This article has not been copyedited and formatted. The final version may differ from this version.



**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 2-6 hours 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 ± SD (range) for age, weight and height of the 11 included subjects was 25 ± 5 (20-37) years, 81 ± 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 post capsaicin or placebo are given in table 2.

DBF at baseline did not differ between arms (Table 2) and increased following capsaicin application when compared to placebo in all three treatment periods and both arms ($P < 0.0001$, ANOVA for repeated measures; Figure 2). The capsaicin-induced DBF increase was partially blocked in the $CGRP_{8-37}$-infused arm when compared with the non-infused arm (Table 2 and Figure 2A). Indomethacin and L-NMMA did not effect the capsaicin-induced increase in DBF (Table 2 and Figures 2B and 2C).

The DBF changes at the placebo sites did not differ between the infused and non-infused arm during $CGRP_{8-37}$ and indomethacin infusion. In contrast, $AUC_{0-30}$ of the DBF percent change from baseline at the placebo sites was smaller in the L-NMMA-infused than the non-infused arm (Table 2).

*Study 2: Effect of oral administration of aprepitant on capsaicin-induced dermal vasodilation*

Mean ± SD (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, 046) (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 5,551 (3,084; 8,017) %.min in the morning and 4,829 (2,051; 7,608) %.min in the afternoon (Figure 3A).

JPET Fast Forward. Published on January 23, 2008 as DOI: 10.1124/jpet.107.133868
This article has not been copyedited and formatted. The final version may differ from this version.



In the treatment period, baseline DBF averaged 0.41 (0.37, 0.45) AU in the morning (i.e. before aprepitant administration). Four hours post-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 (Figure 3B): expressed as $t_{30}$ the increase in DBF averaged 319 (233, 405) % pre-aprepitant versus 305 (210, 400) % 4 hours post-dose; $AUC_{0-30}$ averaged 4,201 (2,465; 5937) %.min pre- and 4,403 (2,812; 5995) %.min post-aprepitant.

JPET Fast Forward. Published on January 23, 2008 as DOI: 10.1124/jpet.107.133868
This article has not been copyedited and formatted. The final version may differ from this version.



**Discussion**

This study demonstrates in healthy male volunteers *in vivo* that DBF response following capsaicin application to the skin is to a large extend 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 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 non-peptide CGRP receptor antagonist BIBN4096BS (Verheggen et al., 2002). When the dose of $CGRP_{8-37}$ used in the present study, was intra-arterially co-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%) (Vanmolkot 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 about 50% (Hayes et al., 1993). Recently, the potent oral CGRP antagonist MK-0974 was shown to inhibit the dermal vasodilation following 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). We do realize however that the present study set-up 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 $CGRP_{8-37}$ 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 $CGRP_{8-37}$ infusion had no effect on resting DBF, which is in agreement with our previous observation that $CGRP_{8-37}$ 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 not affect tissue perfusion under resting conditions.

JPET Fast Forward. Published on January 23, 2008 as DOI: 10.1124/jpet.107.133868
This article has not been copyedited and formatted. The final version may differ from this version.



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 neurokinin-1 receptors are functionally blocked. In contrast, also in mice, $CGRP_{8-37}$ is only able to inhibit capsaicin-induced vasodilation in combination with a SP antagonist (Grant et al., 2002). Thus an interaction between functional neurokinin-1 and CGRP receptors is suggested. Our results demonstrate that in humans no such interaction seems to exist as aprepitant does neither increase nor inhibit capsaicin-induced vasodilation, whereas $CGRP_{8-37}$ clearly decreases the response to capsaicin. This confirms findings by Petersen et al., who could not detect SP nor histamine release of the skin following intradermal capsaicin administration to humans (Petersen et al., 1997). We therefore suggest that, in humans, SP has no major role in capsaicin-induced vasodilation. Although, it can not be excluded that in case of blockade of CGRP receptors, SP may in part take over to compensate for inhibition of the CGRP-dependent vasodilation, this hypothesis was not tested because of the recent observation that over 80% of capsaicin-induced dermal vasodilation was inhibited by MK-0974 (Van der Schueren et al., 2007a). Taking in account the reproducibility of capsaicin-induced vasodilation, it would require an unfeasibly large number of subjects to detect any additional inhibition by aprepitant (Van der Schueren et al., 2007b). In view of Petersens and our findings, we conclude that, under physiological conditions, SP has no major role in capsaicin-induced vasodilation in the human skin.

As 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 non-infused (control) arm. As the DBF response to capsaicin does not demonstrate a circadian rhythm, this non-simultaneous assessment is valid.

The inability of indomethacin to inhibit the increase of capsaicin-induced DBF indicates that potent cyclo-oxygenase inhibition does not affect this response. We propose therefore, 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 by conflicting findings in different research settings. On the one hand, cyclo-oxygenase 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

JPET Fast Forward. Published on January 23, 2008 as DOI: 10.1124/jpet.107.133868
This article has not been copyedited and formatted. The final version may differ from this version.



cyclo-oxygenase-2 (Southall et al., 2003). On the other hand Herbert et al. demonstrated that neither acetylsalicylic acid nor indomethacin affected capsaicin-induced increase in DBF in human skin (Herbert et al., 1993), though they did find a significantly lower increase of DBF with indomethacin pre-treatment 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 set-up the inflamed area underlying capsaicin-soaked plasters should be distinguished from proper neurogenic inflammation.

We demonstrated that the arterial infusion of a non-selective NO synthase inhibitor L-NMMA does not affect the dermal vasodilation following capsaicin application (Smits et al., 1995). This finding is particularly interesting as 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 would expect an inhibitory effect of L-NMMA. Hughes et al. demonstrated that in the rabbit cutaneous microvasculature, NO synthase inhibition had no effect on the CGRP-induced vasodilation, whereas it did reduce capsaicin-induced vasodilation (Hughes and Brain, 1994). 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, Goldsmith et al. were able to inhibit CGRP-induced DBF increase by injecting L-NMMA into skin of the human forearm (Goldsmith et al., 1996). Our findings indicate that, in the human skin, NO is neither involved in the release of CGRP nor in its vasodilator mechanism. As in our model we compare the capsaicin-induced dermal vasodilation between infused and non-infused arm, it could be argued that “spillover” of L-NMMA from the infused arm to the non-infused arm would prevent us from detecting an inhibitory effect of L-NMMA. However, spillover of L-NMMA to the non-infused arm seems very unlikely. First, L-NMMA has been shown to quickly disappear from plasma (Mayer et al., 1999). Secondly, the capsaicin-induced increase in DBF in the non-infused arm did not differ from the DBF responses in the non-infused arm 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 as it was also seen in the other treatment periods and reported by other authors 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 inflammation. It should be noted that L-NMMA slightly decreased resting DBF in the infused arm when compared to the non-infused arm. This reflects the limitations of the “NO clamp” technique in which the co-infusion of nitroprusside as NO donor to compensate for the inhibition of basal endothelial NO release in incomplete.

Our findings validate the pharmacodynamic model using capsaicin-induced vasodilation to assess CGRP antagonists in vivo in humans (Van der Schueren et al., 2007b). We thus successfully translated the pharmacodynamic assay of Hershey et al. into the human species (Hershey et al., 2005). This is valuable, as our study also clearly illustrates the variability of mediators involved in neurogenic inflammation between species.

In summary as capsaicin-induced vasodilation in the human skin is to a large extend antagonized by $CGRP_{8-37}$, but not by L-NMMA, aprepitant or indomethacin, CGRP seems to be a major mediator involved in this response. Therefore, the approach presented in this study is proposed as a human in vivo pharmacodynamic model which can be used in the early clinical development of CGRP receptor antagonists. Moreover, we demonstrated that the capsaicin-induced vasodilation does not display a circadian rhythm.

JPET Fast Forward. Published on January 23, 2008 as DOI: 10.1124/jpet.107.133868
This article has not been copyedited and formatted. The final version may differ from this version.



**Acknowledgements**

The authors also gratefully acknowledge Jo Van Effen and Marc Oeyen for their assistance during the laser Doppler imaging and Ayline Yudhira for her help with the data analysis.

**References**

Barnes PJ (2001) Neurogenic inflammation in the airways. *Respir Physiol* **125**:145-154.

Bergstrom M, Hargreaves RJ, Burns HD, Goldberg MR, Sciberras D, Reines SA, Petty KJ, Ogren M, Antoni G, Langstrom B, Eskola O, Scheinin M, Solin O, Majumdar AK, Constanzer ML, Battisti WP, Bradstreet TE, Gargano C and Hietala J (2004) Human positron emission tomography studies of brain neurokinin 1 receptor occupancy by aprepitant. *Biol Psychiatry* **55**:1007-1012.

Bjorling DE, Beckman M and Saban R (2003) Neurogenic inflammation of the bladder. *Adv Exp Med Biol* **539**:551-583.

Brands KM, Payack JF, Rosen JD, Nelson TD, Candelario A, Huffman MA, Zhao MM, Li J, Craig B, Song ZJ, Tschaen DM, Hansen K, Devine PN, Pye PJ, Rossen K, Dormer PG, Reamer RA, Welch CJ, Mathre DJ, Tsou NN, McNamara JM and Reider PJ (2003) Efficient synthesis of NK(1) receptor antagonist aprepitant using a crystallization-induced diastereoselective transformation. *J Am Chem Soc* **125**:2129-2135.

Buzzi MG, Bonamini M and Moskowitz MA (1995) Neurogenic model of migraine. *Cephalalgia* **15**:277 280.

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD and Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* **389**:816-824.

Chiba T, Yamaguchi A, Yamatani T, Nakamura A, Morishita T, Inui T, Fukase M, Noda T and Fujita T (1989) Calcitonin gene-related peptide receptor antagonist human CGRP-(8-37). *Am J Physiol* **256**:E331-335.

de Hoon JN, Pickkers P, Smits P, Struijker-Boudier HA and Van Bortel LM (2003) Calcitonin gene related peptide: exploring its vasodilating mechanism of action in humans. *Clin Pharmacol Ther* **73**:312-321.

Doods H, Arndt K, Rudolf K and Just S (2007) CGRP antagonists: unravelling the role of CGRP in migraine. *Trends Pharmacol Sci* **28**:580-587.

Doods H, Hallermayer G, Wu D, Entzeroth M, Rudolf K, Engel W and Eberlein W (2000) Pharmacological profile of BIBN4096BS, the first selective small molecule CGRP antagonist. *Br J Pharmacol* **129**:420-423.

Fullerton A, Stucker M, Wilhelm KP, Wardell K, Anderson C, Fischer T, Nilsson GE and Serup J (2002) Guidelines for visualization of cutaneous blood flow by laser Doppler perfusion imaging. A report from the Standardization Group of the European Society of Contact Dermatitis based upon the HIRELADO European community project. *Contact Dermatitis* **46**:129-140.

Goldsmith PC, Leslie TA, Hayes NA, Levell NJ, Dowd PM and Foreman JC (1996) Inhibitors of nitric oxide synthase in human skin. *J Invest Dermatol* **106**:113-118.

Grant AD, Gerard NP and Brain SD (2002) Evidence of a role for NK1 and CGRP receptors in mediating neurogenic vasodilatation in the mouse ear. *Br J Pharmacol* **135**:356-362.

Hayes NA, Bunker CB, Marshall I, Dowd PD and Foreman JC (1993) The action of a calcitonin gene-related peptide antagonist in human skin. *Agents Actions* **3**:C211-C214.

Herbert MK, Tafler R, Schmidt RF and Weis KH (1993) Cyclooxygenase inhibitors acetylsalicylic acid and indomethacin do not affect capsaicin-induced neurogenic inflammation in human skin. *Agents Actions* **38**:C25-27.

Hershey JC, Corcoran HA, Baskin EP, Salvatore CA, Mosser S, Williams TM, Koblan KS, Hargreaves RJ and Kane SA (2005) Investigation of the species selectivity of a nonpeptide CGRP receptor antagonist using a novel pharmacodynamic assay. *Regul Pept* **127**:71-77.

Hughes SR and Brain SD (1991) A calcitonin gene-related peptide (CGRP) antagonist (CGRP8-37) inhibits microvascular responses induced by CGRP and capsaicin in skin. *Br J Pharmacol* **104**:738-742.

Hughes SR and Brain SD (1994) Nitric oxide-dependent release of vasodilator quantities of calcitonin gene-related peptide from capsaicin-sensitive nerves in rabbit skin. *Br J Pharmacol* **111**:425-430.

Lembeck F and Holzer P (1979) Substance P as neurogenic mediator of antidromic vasodilation and neurogenic plasma extravasation. *Naunyn Schmiedebergs Arch Pharmacol* **310**:175-183.

Levine JD, Khasar SG and Green PG (2006) Neurogenic inflammation and arthritis. *Ann N Y Acad Sci* **1069**:155-167.

Majumdar AK, Howard L, Goldberg MR, Hickey L, Constanzer M, Rothenberg PL, Crumley TM, Panebianco D, Bradstreet TE, Bergman AJ, Waldman SA, Greenberg HE, Butler K, Knops A, De Lepeleire I, Michiels N and Petty KJ (2006) Pharmacokinetics of aprepitant after single and multiple oral doses in healthy volunteers. *J Clin Pharmacol* **46**:291-300.

Mayer BX, Mensik C, Krishnaswami S, Derendorf H, Eichler HG, Schmetterer L and Wolzt M (1999) Pharmacokinetic-pharmacodynamic profile of systemic nitric oxide-synthase inhibition with L-NMMA in humans. *Br J Clin Pharmacol* **47**:539-544.

Patel L and Lindley C (2003) Aprepitant--a novel NK1-receptor antagonist. *Expert Opin Pharmacother* **4**:2279-2296.

Patrono C, Ciabattoni G, Pinca E, Pugliese F, Castrucci G, De Salvo A, Satta MA and Peskar BA (1980) Low dose aspirin and inhibition of thromboxane B2 production in healthy subjects. *Thromb Res* **17**:317-327.

Petersen LJ, Winge K, Brodin E and Skov PS (1997) No release of histamine and substance P in capsaicin-induced neurogenic inflammation in intact human skin in vivo: a microdialysis study. *Clin*

*Exp Allergy* **27**:957-965.

Quartu M, Diaz G, Lai ML and Del Fiacco M (1992) Immunohistochemical localization of putative peptide neurotransmitters in the human trigeminal sensory system. *Ann N Y Acad Sci* **657**:469-472.

Salvatore C, Hershey J, Corcoran H, Fay J, Johnston V, Moore E, Mosser S, Burgey C, Paone D, Shaw A, Graham S, Vacca J, Williams T, Koblan K and Kane S (2007) Pharmacological Characterization of MK-0974, a Potent and Orally Active CGRP Receptor Antagonist for the Treatment of Migraine. *J Pharmacol Exp Ther*.

Seidel K, Hamza M, Ates M and Guhring H (2003) Flurbiprofen inhibits capsaicin induced calcitonin gene related peptide release from rat spinal cord via an endocannabinoid dependent mechanism. *Neurosci Lett* **338**:99-102.

Smits P, Williams SB, Lipson DE, Banitt P, Rongen GA and Creager MA (1995) Endothelial release of nitric oxide contributes to the vasodilator effect of adenosine in humans. *Circulation* **92**:2135-2141.

Southall MD, Li T, Gharibova LS, Pei Y, Nicol GD and Travers JB (2003) Activation of epidermal vanilloid receptor-1 induces release of proinflammatory mediators in human keratinocytes. *J Pharmacol Exp Ther* **304**:217-222.

Southall MD, Michael RL and Vasko MR (1998) Intrathecal NSAIDS attenuate inflammation-induced neuropeptide release from rat spinal cord slices. *Pain* **78**:39-48.

Van der Schueren BJ, de Hoon JN, Kane SA, Xiao A, Willson KJ, Xu Y, Hickey L, Palcza J, De Lepeleire I, Vanmolkot FH, Van Hecken A, Depré M, Murphy MG and Sinclair SR (2007a) MK-0974, an oral CGRP antagonist, inhibits capsaicin-induced vasodilation in the human skin. *Basic Clin Pharmacol Toxicol* **101**:33 (015).

Van der Schueren BJ, de Hoon JN, Vanmolkot FH, Van Hecken A, Depré M, Kane SA, De Lepeleire I and Sinclair SR (2007b) Reproducibility of the capsaicin-induced dermal blood flow response as assessed by laser Doppler perfusion imaging. *Br J Clin Pharmacol* **64**:580-590.

Vanmolkot FH and de Hoon JN (2005) Reproducibility of forearm vasodilator response to intra-arterial infusion of calcitonin gene-related peptide assessed by venous occlusion plethysmography. *Br J Clin Pharmacol* **59**:387-397.

Vanmolkot FH, Van der Schueren BJ and de Hoon JN (2006) Calcitonin gene-related peptide-induced vasodilation in the human forearm is antagonized by CGRP8-37: evaluation of a human in vivo pharmacodynamic model. *Clin Pharmacol Ther* **79**:263-273.

Verheggen R, Bumann K and Kaumann AJ (2002) BIBN4096BS is a potent competitive antagonist of the relaxant effects of alpha-CGRP on human temporal artery: comparison with CGRP(8-37). *Br J Pharmacol* **136**:120-126.

Wallengren J (1997) Vasoactive peptides in the skin. *J Investig Dermatol Symp Proc* **2**:49-55.

Zegarska B, Lelinska A and Tyrakowski T (2006) Clinical and experimental aspects of cutaneous neurogenic inflammation. *Pharmacol Rep* **58**:13-21.

**Footnotes**

*Financial support:*

This work was supported by grants from the Research Foundation-Flanders (F.W.O. –Vlaanderen) and the Merck Imaging group.

*Meeting abstracts*

B.J. Van der Schueren, F.H. Vanmolkot, **J.N. de Hoon.** $CGRP_{8-37}$ inhibits capsaicin-induced vasodilation in the human skin. *J Headache Pain* 2006; 7(S1): S 30-31.

*Reprint requests*

Bart Van der Schueren
Center for Clinical Pharmacology
University Hospital Gasthuisberg
Herestraat 49
3000 Leuven
Belgium
E-mail: bart.vanderschueren@uz.kuleuven.be

JPET Fast Forward. Published on January 23, 2008 as DOI: 10.1124/jpet.107.133868
This article has not been copyedited and formatted. The final version may differ from this version.



**Legends for Figures**

**Legend to figure 1**

A. Study 1: flow chart.

NIA, non-infused arm (i.e. dominant arm); IA, infused arm (i.e. non-dominant arm)

**B. Study 2: flow chart**

**Legend to figure 2**

A. Dermal Blood Flow (DBF) response (mean ± 95 % CI, number of subjects = 11, number of observations after capsaicin/placebo per arm = 22) during intra-arterial infusion of $CGRP_{8-37}$ ( 1,200 $ng.min^{-1}.dL^{-1}$ foreram). $CGRP_{8-37}$ inhibits DBF increase in the infused arm (IA) when compared to the non-infused arm (NIA) after application of 1000 µg capsaicin, whereas resting DBF (i.e. after placebo application) was comparable between both arms.

*p = 0.004 at $t_{30}$ (Wilcoxon's matched-pairs signed-rank test).

** p = 0.004 for $AUC_{0-30}$ (paired Student's t-test).

B. Dermal Blood Flow (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 $\mu g.min^{-1}.dL^{-1}$ forearm). DBF increase was similar in the indomethacin infused arm (IA) and the non-infused arm (NIA) after application of 1000 µg capsaicin. Resting DBF (i.e. after placebo application) was unaffected by indomethacin infusion.

C. Dermal Blood Flow (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 SNP (0.2 $\mu g.min^{-1}.dL^{-1}$ forearm). DBF increase was similar in the L-NMMA infused arm (IA) and the non-infused arm (NIA) after application of 1000 µg capsaicin. Resting DBF (i.e. after placebo application) was slightly lower in the L-NMMA infused arm (IA) compared to the non-infused arm (NIA) (p=0.01, paired Student's t-test comparing $AUC_{0-30}$)

JPET Fast Forward. Published on January 23, 2008 as DOI: 10.1124/jpet.107.133868
This article has not been copyedited and formatted. The final version may differ from this version.



**Legend to figure 3**

A. Dermal Blood Flow (DBF) response (mean ± 95 % CI, number of subjects = 11, number of observations after capsaicin/placebo per arm = 22) during morning and afternoon. DBF increase was similar in the left arm in the morning and the right arm in the afternoon after application of 1000 µg capsaicin. Resting DBF (i.e. after placebo application) was also similar during the morning and afternoon session.

B Dermal Blood Flow (DBF) response (mean ± 95 % CI, number of subjects = 11, number of observations after capsaicin/placebo per arm = 22) after aprepitant (375mg) administration. DBF increase was similar in the left arm before aprepitant administration and the right arm 4 hours after the intake of aprepitant. Resting DBF (i.e. after placebo application) was unaffected by aprepitant.

JPET Fast Forward. Published on January 23, 2008 as DOI: 10.1124/jpet.107.133868
This article has not been copyedited and formatted. The final version may differ from this version.



**Tables**

**Table 1**

**Study 1: haemodynamic parameters**

Data presented as mean ± 95% confidence interval; number of subjects = 11

SBP, DBP and HR: systolic blood pressure, diastolic blood pressure and heart rate.

| Parameter | Time | L-NMMA | $CGRP_{8-37}$ | Indomethacin |
|---|---|---|---|---|
| **SBP (mmHg)** | **Baseline** | 123 (119, 127) | 120 (116, 125) | 126 (119, 132) |
| | **40 min** | 125 (120, 129) | 123 (119, 126) | 128 (120, 136) |
| **DBP (mmHg)** | **Baseline** | 67 (43, 92) | 66 (42, 91) | 69 (44, 93) |
| | **40 min** | 72[a] (46, 96) | 71[a] (46, 95) | 72[a] (48, 97) |
| **HR (bpm)** | **Baseline** | 60 (35, 84) | 55 (30, 79) | 58 (34, 83) |
| | **40 min** | 59 (35, 84) | 57 (33, 82) | 59 (34, 83) |

[a] $p < 0.05$ versus baseline assessed by Wilcoxon matched-pairs signed-rank test.

**Table 2**

**Study 1: comparison of dermal blood flow responses between arms**

Data presented as mean ± 95% confidence interval; number of subjects = 11

$t_{30}$, DBF percent change 30 minutes post capsaicin or placebo; $AUC_{0\text{-}30}$, area under the curve of the DBF percent change up to 30 minutes post capsaicin or placebo. Number of observations at baseline, n = 44 for NIA and IA; number of observations after capsaicin/placebo, n = 22 for NIA and IA. IA, infused arm; NIA, non-infused arm

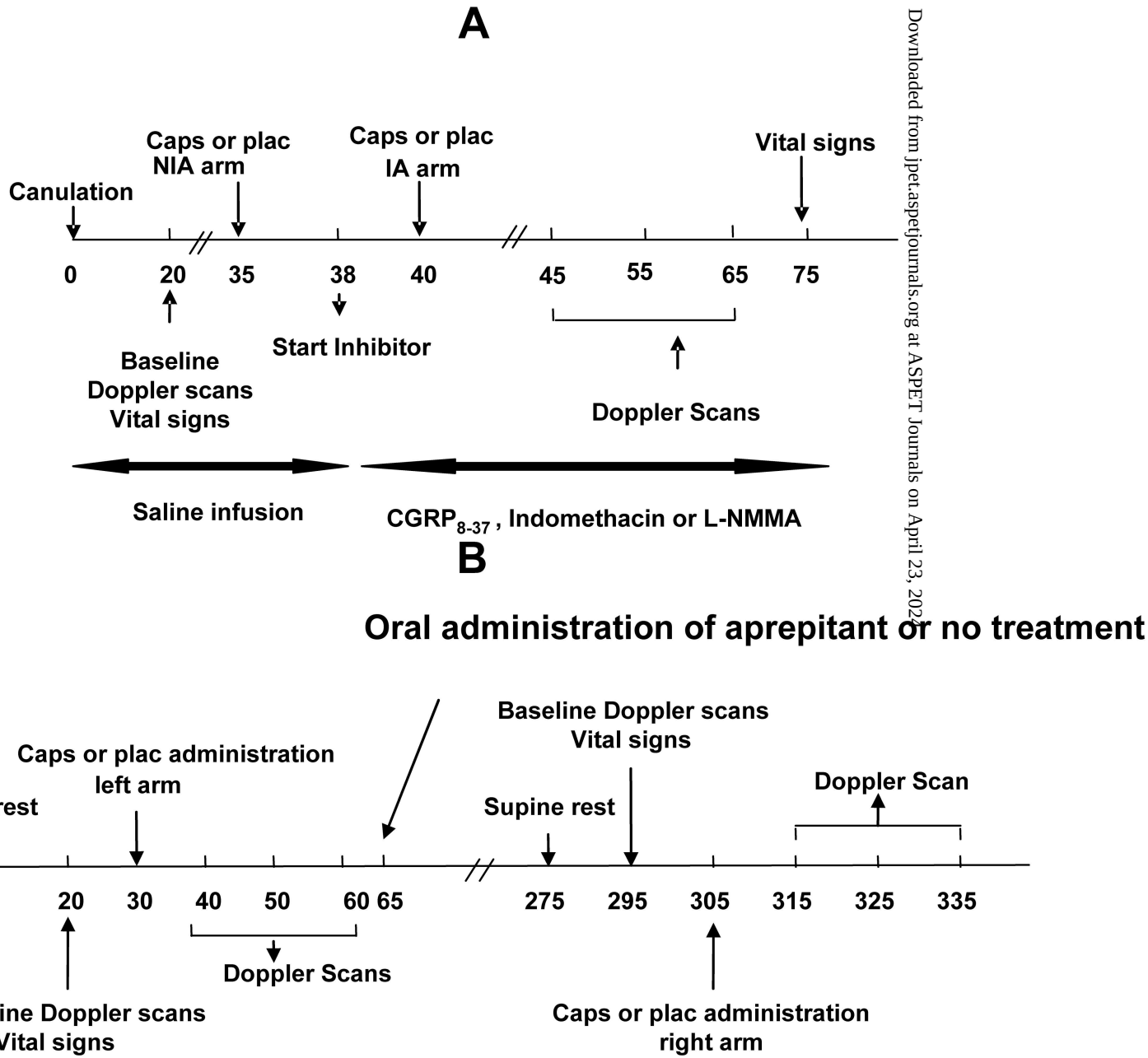
| | Parameter | Arm | $CGRP_{8\text{-}37}$ | Indomethacin | L-NMMA |
|---|---|---|---|---|---|
| **Baseline (all sites)** | **DBF (AU)** | **NIA** | 0.44 (0.42, 0.46) | 0.51 (0.48, 0.54) | 0.48 (0.46, 0.49) |
| | | **IA** | 0.44 (0.41, 0.46) | 0.49 (0.46, 0.52) | 0.49 (0.46, 0.51) |
| **1000 µg capsaicin** | **$t_{30}$ (%)** | **NIA** | 370 (254, 486) | 337 (256, 418) | 309 (243, 375) |
| | | **IA** | 217 (145, 290)[a] | 319 (247, 392) | 314 (252, 375) |
| | **$AUC_{0\text{-}30}$ (%.min)** | **NIA** | 5,093 (3,211; 6,974) | 4,392 (2829; 5,956) | 3,717 (2,569; 4,866) |
| | | **IA** | 2,721 (1,929; 3,513)[b] | 3,945 (2834; 5,056) | 3,417 (2,526; 4,308) |
| **placebo** | **$t_{30}$ (%)** | **NIA** | 20 (7, 32) | 10 (1, 19) | 13 (2, 23) |
| | | **IA** | 11 (0, 22) | 10 (0, 20) | 6 (0, 12) |
| | **$AUC_{0\text{-}30}$ (%.min)** | **NIA** | 179 (-2, 361) | 141 (-48, 330) | 172 (3, 341) |
| | | **IA** | 134 (-71, 339) | 68 (-74, 209) | 1 (-71, 73)[c] |

[a] p = 0.004 (Wilcoxon's matched-pairs signed-rank test comparing IA to NIA)

[b] p = 0.004 (paired Student's t-test comparing IA to NIA)

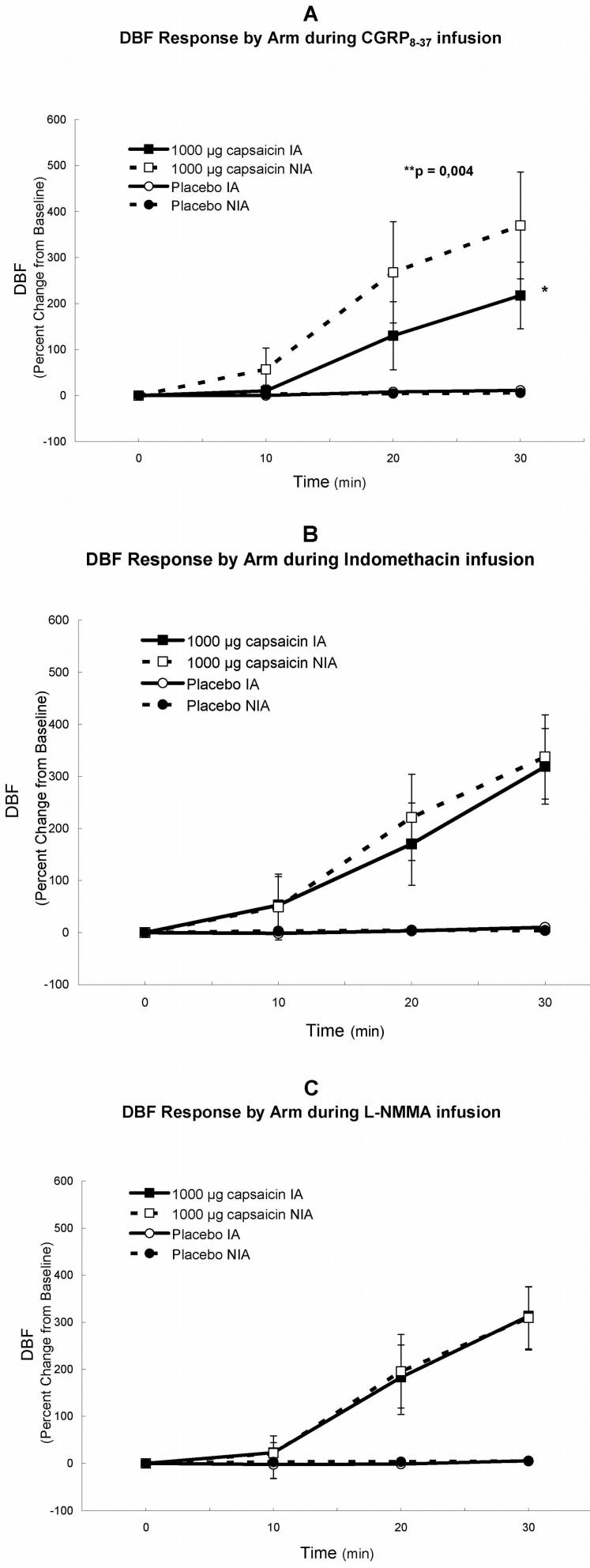
[c] p = 0.01 (paired Student's t-test comparing IA to NIA)

# Figure 1



JPET Fast Forward. Published on January 23, 2008 as DOI: 10.1124/jpet.107.133868


# Figure 2



JPET Fast Forward. Published on January 23, 2008 as DOI: 10.1124/jpet.107.133868


# Figure 3