

R. Borici-Mazi
S. Kouridakis
K. Kontou-Fili

Cutaneous responses to substance P and calcitonin gene-related peptide in chronic urticaria: the effect of cetirizine and dimethindene

Authors' affiliations:

R. Borici-Mazi, S. Kouridakis, K. Kontou-Fili,
Department of Allergology, Division of Internal
Medicine, Laikon General District Hospital of
Athens, Athens, Greece

Correspondence to:

K. Kontou-Fili, MD, PhD
Head, Department of Allergology
Division of Internal Medicine
Laikon General District Hospital of Athens
Athens 11527
Greece

Key words: calcitonin gene-related peptide (CGRP); cetirizine; chronic urticaria; cutaneous responses; dimethindene; H₁-antagonists; idiopathic urticaria; pressure urticaria; substance P.

Background: Neuropeptides appear to participate in the pathogenesis of chronic urticaria. The purpose of this study was to investigate the cutaneous responses to substance P (SP) and calcitonin gene-related peptide (CGRP) in delayed pressure urticaria patients (DPUpt) and chronic idiopathic urticaria patients (CIUpt) compared to healthy adults (HA), and also to evaluate the effect of H₁-antagonists on these responses.

Methods: Wheal (W) and flare (F) reactions to intradermally (ID) injected SP, CGRP, histamine (H), and diluent controls were evaluated in nine CIUpt, nine DPUpt, and nine HA at 3, 7, 15, 30, 60, and 120 min after injection. Maximal W and F, area under curve (AUC), and time of W and F disappearance were calculated at baseline and 4 h after a single dose of 20 mg cetirizine (Ce), 4 mg dimethindene (Di), or placebo (P), in a double-blind, placebo-controlled (DBPC), crossover, randomized study.

Results: CIUpt exhibited enhanced and longer lasting W reactions to SP and CGRP (AUC: $P<0.05$) than HA; SP- and CGRP-induced F (at maximal concentration) were larger and longer lasting in CIUpt than in HA ($P<0.003$ and $P=0.004$, respectively). In the DPU group, SP-induced W and F responses were intermediate in magnitude compared to CIUpt and HA. In HA, SP-induced flares were significantly suppressed only by Ce ($P<0.020$), while both Ce and Di affected SP-induced W and F in the two patient groups ($P<0.05$). CGRP-induced flare inhibition by the two H₁-antagonists was also greater in the urticaria patient groups than in HA.

Conclusions: CIUpt and, to a lesser extent, DPUpt showed enhanced SP- and CGRP-induced W and F reactions. CGRP elicited an immediate W and F response, followed by prolonged erythema. H₁-antagonists partially affected W and F reactions to

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SP and only the F response induced by CGRP; this effect was more pronounced in the urticaria patient groups than in HA. Overall, W and F cutaneous responses to SP were suppressed to a greater extent by Ce than Di.

The role of the nervous system and its neurotransmitters in the pathogenesis of allergy and inflammation has been investigated in recent years (1, 2). Human skin mast cells are closely associated with sensory nerve endings, which release neuropeptides (NPs) upon antidromic stimulation by physical or chemical stimuli and stress (3). Substance P (SP) and calcitonin gene-related peptide (CGRP) appear to be stored in peripheral and central terminals of the same sensory neurons; they are probably released together (4, 5). Intradermal (ID) injection of SP in normal human skin induces typical wheal (W) and flare (F) reactions (6–9) and local histamine release (10); in contrast, ID injection of CGRP causes delayed persistent erythema, but there is controversy about the initial W and F response reported by several investigators (6, 8, 11). Incubated with rat peritoneal and human dispersed mast cells, SP elicited significant histamine release, whereas CGRP was found to be a weak histamine releaser (6, 11, 12). In chronic idiopathic urticaria (CIU), there is evidence of enhanced cutaneous responses to vasoactive agents, including some NPs; as a consequence, NPs might contribute to lesion induction in this disorder (13–17). There are no published data on the cutaneous effects of NPs in delayed pressure urticaria (DPU). The first generation of antihistamines partially suppress the skin responses to SP and CGRP in healthy adults (HA) (7, 11). Cetirizine (Ce), a peripheral H₁-antagonist, is effective in controlling the symptoms of CIU (18) and possesses relevant therapeutic efficacy in the treatment of DPU (19, 20); furthermore, it has been shown to affect the cutaneous responses to SP in healthy volunteers (21). Several reports have also confirmed the efficacy of dimethindene (Di) in the treatment of CIU (22). The purpose of the present study was to investigate the cutaneous responses to SP and CGRP in DPU and CIU patients, compared to HA. The efficacy of two H₁-antagonists, Ce and Di, in suppressing W and F responses to NPs in each subject group was evaluated in the second part of this double-blind, placebo-controlled (DBPC) crossover study.

Material and methods

Subjects

Nine patients suffering from DPU and nine from CIU for at least 8 weeks were enrolled in the study, after giving

written, informed consent; an equal number of age-matched healthy volunteers served as the control group. The CIU study group consisted of patients reporting recurrent urticarial lesions; there was no history compatible with pressure-induced lesions, and the weight-challenge test was negative in all CIU patients (CIUpt). The diagnosis of DPU was confirmed by a relevant history and a positive delayed response (usually 6–9 h) to the weight test; this static pressure challenge was performed with a 7-kg weight suspended from the shoulder and/or forearm for 15 min. The exclusion criteria were as follows: age below 18 years; pregnancy or lactation, urticaria due to specific food, drug, or other known agent (except for pressure); symptomatic dermatographism, known allergy to Ce, Di, or cornstarch; cardiac, hepatic, or renal impairment; diabetes; other chronic diseases; and chronic corticotherapy. Drug consumption had to be discontinued as follows:

- 1) H₁-antagonists: ketotifen, 3 weeks; astemizole, 6 weeks; loratadine, terfenadine, Ce, and Di, 1 week
- 2) steroids, sedatives, hypnotics, antidepressants, muscle relaxants, Ca channel blockers, β -blockers, ACE inhibitors, contraceptives, and H₂-antagonists: 3 weeks.

The study was approved by the ethics subcommittee of the Laikon General District Hospital Scientific Committee (Greece).

Neuropeptides and reagents

Lyophilized SP and CGRP were provided by UCB Pharma SA, Braine l'Alleud, Belgium. Stock solutions of each neuropeptide (1 mmol/l in 0.1% acetic acid) were prepared in individual vials and stored at –70°C. Each study day, frozen aliquots were thawed and diluted with phosphate-buffered saline (pH 7.4) to the following concentrations: SP 0.25, 0.5, and 1.0 μ mol/l; CGRP 0.5, 1.0, and 2.0 μ mol/l. Sterile histamine solution (10 μ g/ml) and phosphate-buffered saline were used as positive and negative controls, respectively. Ce 20 mg, Di 4 mg, and placebo were prepared in identical capsules and coded by a physician not involved in the study.

Cutaneous tests

Sequential injections (25 μ l) of each NP (in three concentrations) and the two control solutions were applied on the back of each subject at 3-min intervals. Cutaneous injections were administered ID with tuberculin syringes (1 ml)

bearing 27-gauge needles. On the first study day, the NPs were always applied on the left half of the back, 5 cm apart. On subsequent study days, the ID injections were applied by alternating each half of the back and using the same order of injections. The intracutaneous tests were always performed at exactly the same hour of the day for each subject. The content of each syringe was unknown to the investigator performing the tests. Further to ensure the reproducibility and comparability of dermal reactions, all tests were performed by the same investigator. Cutaneous W and F responses were evaluated 3, 7, 15, 30, 60, and 120 min after injection. The W and F areas were outlined with a soft, fine-point marker, copied on transparent tape, and transferred to the patient's data sheet; they were subsequently measured with a handheld scanner connected to a personal computer.

Study design

This was a randomized, DBPC, three-period crossover study. Each subject attended the outpatient clinic on 5 different days over a mean period of 21.52 consecutive days. On day 1, the "selection" visit, the demographic data and medical history were recorded; complete physical examination and weight-challenge tests were also performed. On day 2, the "experimental" visit, baseline skin tests were performed for half of the subjects at 12 a.m. and for the rest at 2 p.m. At visits 3, 4, and 5, the study agent (Ce, Di, or placebo [P]) was administered in a single dose, 4 h before repeating the cutaneous tests (at either 8 a.m. or 10 a.m., according to the schedule). A 4-day washout period followed before the same process was repeated with the second and third ingested substance. At the beginning of each study day, the investigator recorded any unusual sensations or adverse effects reported to have occurred in the interval following the administration of H₁-antagonist or placebo; the evaluation of effects was based upon the subject's report and the investigator's questionnaire and/or observation.

Statistical analysis

The evaluation of cutaneous responses at baseline and after treatment was performed separately for the W and F, for each concentration of every agonist, and separately for the three groups of subjects. Three variables were calculated:

- 1) the maximal W and F area (Mx)
- 2) the W or F response-time area under curve (AUC)
- 3) the time of complete W and F disappearance (T).

The immediate F and the prolonged erythema were included in the AUC of CGRP flare. The median, mean, standard error of the mean (SEM), minimal, and maximal values were calculated for all variables. Statistical analysis was performed by parametric (ANOVA) and nonparametric (Kruskal-Wallis and Friedman) tests. A global comparison of treatment performed effects (GTE) was first obtained. In case of a global significance, two-by-two comparisons of the three treatments were performed by the same test. Lastly, a descriptive analysis of the nature, severity, frequency, and duration of all adverse events reported by the subjects after each treatment was obtained. All statistical tests were carried out two-sided at the 5 % level of significance.

Results

The demographic data on all subjects involved in the study are shown in Table 1; the two patient groups were sex-matched, but, for technical reasons, more men were included in the group of HA.

Cutaneous responses to SP and CGRP at baseline

By ID injection, both NPs induced W and F responses in all subjects involved in the study. The cutaneous responses to SP and CGRP in the lower two concentrations at baseline are presented in Table 2. The time-course curves of W and F responses for the maximal concentration of both SP and CGRP are illustrated in Fig. 1. CIUpt exhibited greater SP-induced W responses (mean AUC) than HA in all concentrations ($P=0.007^*$, 0.038^{**} , 0.019^{***})†. Compared to DPUpt, CIUpt showed a trend toward greater W responses only in the lower concentrations ($P=0.058^*$, 0.085^{**})†. SP-induced W responses consistently lasted longer (T) in CIUpt than in HA, but significant differences were observed in two concentra-

Table 1. Demographic characteristics of three subject groups

	HA	DPU	CIU	All
Age (years) ¹	40.30	37.22	33.42	36.98
Weight (kg) ¹	77.22	72.28	74.44	74.65
Height (cm) ¹	172.10	168.56	171.70	170.80
Body mass ¹ (kg/m ²)	25.92	25.21	25.18	25.44
Sex				
M	7 (78%)	4 (44%)	4 (44%)	15 (56%)
F	2 (22%)	5 (56%)	5 (56%)	12 (44%)

¹Data are presented in mean values.

Table 2. NP-induced cutaneous responses in three subject groups at baseline

NP $\mu\text{mol/l}$	Parameter	Wheal			Flare		
		HA	DPU	CIU	HA	DPU	CIU
SP 0.25	Mx	45.1	68.4**¶	113.9†¶	309.1	554.0	583.3
	AUC	23.5#	44.9#¶	86.0†¶	34.8	122.5*	250.9†
	T	120	>120	>120	15	60*	120‡
SP 0.5	Mx	57.8¶	68.9¶	108.7¶	545.5	491.8	622.4
	AUC	35.4	48.1¶	85.4†¶	96.1	127.2	216.9†
	T	120	>120	>120	30	60	120†
CGRP 0.5	Mx	68.1	74.5	94.9	373.2	376.3	339.5
	AUC	15.2¶	36.2	58.8¶	97.3	169.4	188.8
	T	60¶#	120#	120¶	>120	>120	120
CGRP 1.0	Mx	55.0	65.0	125.4†	377.9	404.5	503.7
	AUC	17.5	39.5¶	90.6†¶	124.1	211.7	323.1
	T	120¶#	120#	>120¶	>120	>120	>120

Results are expressed in mean (Mx, AUC) and median (T) values. Signed values show statistical significance ($P<0.05$).
 ‡CIU>HA and DPU.
 †CIU>HA.
 *DPU>HA.
 #DPU>HA ($P=\text{trend}$).
 ¶CIU>DPU and/or HA ($P=\text{trend}$).

tions ($P=0.006^*$, 0.022^{***})†. SP-induced W responses, at $1.0 \mu\text{mol/l}$, also lasted longer in DPUpt than HA ($P=0.019$). SP-induced W size reached maximal values at 15–30 min in HA and CIUpt; in DPUpt, the maximal W-induced size was obtained at 30 min after injection. SP-induced F responses were consistently larger (AUC: $P<0.003$) and longer lasting (T: $P<0.006$) in CIUpt than in HA. As shown in Fig. 1a, DPUpt showed more persistent F than HA ($P=0.089$ trend); this F was smaller (AUC: $P=0.085$ trend) and less persistent than in the CIU group (T: $P=0.082$ trend). F size was maximum at 3 min after ID application of SP in all study groups; the time course of SP-induced F in CIUpt exhibited a late peak at 60 min, when the responses of the other two study groups were approaching zero value (Fig. 1Aa).

ID application of all concentrations of CGRP induced consistently biphasic erythematous reactions in all subjects involved in the study; the initial W and F responses were similar to those of SP, while the prolonged CGRP-induced erythema lasted several hours after ID application of the NP. The CGRP-induced W size was maximal at 15 min. In HA, the CGRP-induced W responses disappeared at 120 min; in contrast, they extended beyond the observation period in the two patient groups. CGRP-induced W responses (AUC) were significantly greater in CIUpt than HA, in the two higher

concentrations ($P=0.019^{**}$, 0.007^{***})‡, and a trend was observed in the lowest one ($P=0.055^*$)‡. The W response also lasted longer in DPUpt and CIUpt than HA (0.055^{***} and $P=0.004^{***}$)‡. However, the W pattern exhibited interpatient variability. There were no significant differences in F responses between the study groups, except for the maximal concentration of CGRP, which caused significantly greater F responses in CIUpt than HA ($P=0.004$). The maximum size of the initial F was observed 3 min after ID injection of CGRP. The time-course curves of F reaction in the two patient groups were characterized by a second peak at 60 min, and this corresponded to the maximal value of the observed prolonged erythema (Fig. 1Bb).

Fig. 2 shows the W and F reactions elicited by equivalent concentrations of SP and CGRP ($1 \mu\text{mol/l}$) as compared to H and buffer control. SP-induced and CGRP-induced W and F responses were different from those of the diluent control ($P<0.05$). CGRP-induced W reactions were three times smaller than SP-induced W in HA; the ratio of CGRP/SP wheal sizes was greater in DPUpt and CIUpt (0.57 and 0.91, respectively). CGRP-induced F (including the prolonged erythema) was larger than SP-induced F in HA and DPUpt; the ratio CGRP/SP was 0.83 in CIUpt.

The effect of H_1 -antagonists on cutaneous responses to NPs: time-course results

The effect of Ce and Di on NP-induced skin reactions was evaluated by comparing the W and F areas caused by ID-injected SP and CGRP after each treatment. In HA, the F responses induced by the two higher concentrations of SP were significantly suppressed by Ce ($P=0.020^{**}$, 0.003^{***} Ce vs P), while a trend was also observed in the lowest concentration (GTE, $P=0.062$). W responses to SP were not significantly reduced by H_1 -antagonists. The initial F reaction induced by ID-injected CGRP was inhibited by the two active treatments (GTE, $P=0.062$ trend*, $P=0.034$ Di vs P***); in contrast, the prolonged erythema was not affected by either antihistamine (Tables 3 and 4, Fig. 3). In the DPU group, F responses to SP were affected by both drugs: Ce consistently caused greater inhibition of AUC than P ($P=0.003^*$, 0.003^{**} , 0.020^{***})†, while both drugs reduced the time of complete flare disappearance ($P<0.020$, Ce vs P; $P=0.020$, Di vs P) in all concentrations. W responses to all concentrations of SP were reduced by both drugs, even though this effect was more pronounced by Ce; a trend was observed in GTE ($P<0.07$). CGRP-induced F responses were effectively suppressed by both treatments, compared to P; Ce inhibited the F induced by $0.5 \mu\text{mol/l}$ of CGRP ($P=0.034$,

† 0.25 ; $^{**}0.5$; $^{***}1.0$, $\mu\text{mol/l}$ of SP.

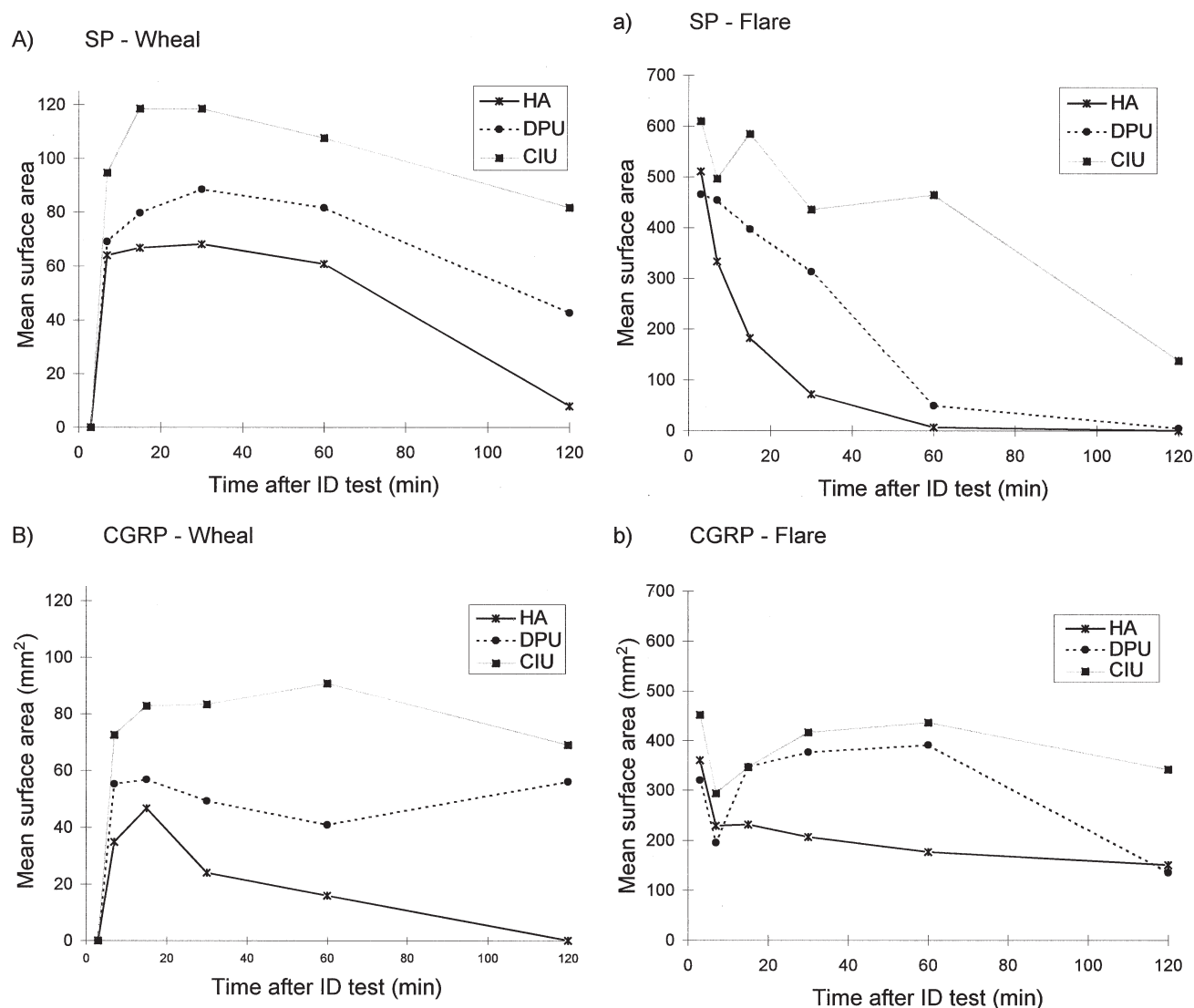


Figure 1. Time course of mean wheal and flare surface areas for 120 min after ID injection of Aa) SP (1.0 µmol/l) and Bb) CGRP (2.0 µmol/l) in nine HA, nine DPUpt, and nine CIUpt in baseline cutaneous tests.

Ce vs P), while both drugs suppressed the F reactions of the highest CGRP concentration ($P=0.034$). The W responses to CGRP were not significantly affected by either drug, and a trend (GTE: $P=0.072$) was observed toward a reduction in the AUC (only in the maximal NP concentration) by both treatments (Tables 3 and 4, Fig. 4).

In CIUpt, SP-induced F responses (AUC) were consistently suppressed by both drugs (Ce and Di) compared to P ($P<0.020^{**}$, ***); the two H_1 -antagonists also reduced significantly the time of F disappearance in the two higher concentrations (GTE, $P<0.05$). The AUC of the SP-induced W response was inhibited by both treatments compared to P with no differences between active drugs (GTE, $P<0.05$).

‡ *0.5; **1.0; ***2.0, µmol/l of CGRP.

Both antihistamines consistently reduced the CGRP-induced F responses ($P=0.030$ Ce and Di vs P), but they did not affect the W responses (Tables 3 and 4, Fig. 5).

Adverse events were reported by three subjects (11%) after placebo treatment (fatigue, somnolence, exacerbation of urticaria), by six (22%) subjects after Ce treatment (somnolence, exacerbation of urticaria), and by 20 subjects (74%) after Di treatment (somnolence, headache, dizziness, fatigue, anorexia, pallor).

Discussion

The clinical response of chronic urticaria to antihistamines (18, 22) and the observed elevated histamine levels in

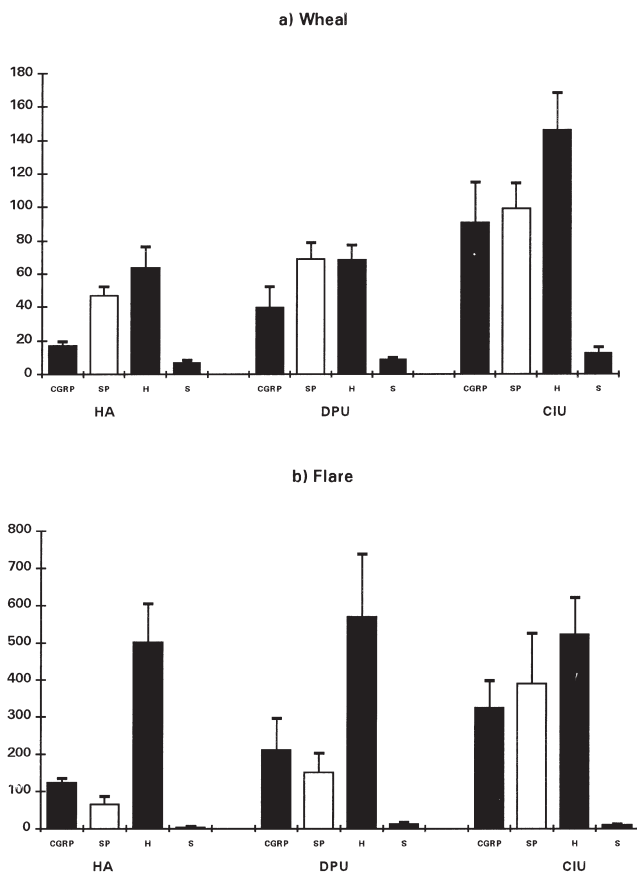


Figure 2. Wheal (a) and flare (b) reactions to CGRP and SP, at equivalent concentrations (1.0 $\mu\text{mol/l}$), histamine 10 $\mu\text{g/ml}$, and buffer control in three study groups. Results are expressed in mean AUC $\pm$ SEM over 2 h (mm^2).

lesional and normal skin of CIUpt (23) or in the fluid of suction blisters (24) underline the role of mast-cell-derived histamine in the pathogenesis of this disorder. Increased histamine levels in the venous blood draining challenged cutaneous sites several hours after weight application have also been demonstrated in DPUpt (24). Barlow et al. have shown that mast-cell degranulation occurs in pressure-induced wheals 6 h after weight application in DPUpt (25). However, additional proinflammatory mediators, including NPs, appear to be involved in the lesion induction of many forms of chronic urticaria.

The cutaneous responses to SP in the two chronic urticaria patient groups were found to be larger and longer lasting than in HA. Two mechanisms appear to be involved in the W and F production: a direct effect of SP on the microcirculation, causing vasodilation and plasma extravasation (26, 27), and/or an indirect one, through histamine release from skin mast cells (10). This should obviously have

† *0.25; **0.5; ***1.0 $\mu\text{mol/l}$ of SP and 0.5; **1.0; ***2.0, $\mu\text{mol/l}$ of CGRP.

Table 3. Effect of H_1 -antagonist treatment on cutaneous wheal and flare responses to SP

	SP 0.25 ¹		SP 0.5 ¹	
	Wheal	Flare	Wheal	Flare
HA				
P	30.3 $\pm$ 4.1	27.7 $\pm$ 4.4	39.7 $\pm$ 6.5	32.0 $\pm$ 5.8
Di	20.1 $\pm$ 2.9	20.0 $\pm$ 11.2#	29.5 $\pm$ 3.5	23.1 $\pm$ 7.0
Ce	24.6 $\pm$ 3.6	14.4 $\pm$ 2.3#	30.1 $\pm$ 3.1	22.5 $\pm$ 8.1†
DPU				
P	39.9 $\pm$ 5.7	110.8 $\pm$ 19.0	49.9 $\pm$ 8.1	204.1 $\pm$ 93.3
Di	36.3 $\pm$ 6.0#	57.1 $\pm$ 18.7	41.3 $\pm$ 5.2#	61.4 $\pm$ 20.9
Ce	26.7 $\pm$ 4.0#	35.8 $\pm$ 10.8‡	35.2 $\pm$ 6.5#	31.0 $\pm$ 10.2‡
CIU				
P	69.8 $\pm$ 13.5	116.0 $\pm$ 22.9	97.0 $\pm$ 18.1	221.8 $\pm$ 71.6
Di	54.3 $\pm$ 9.9‡	63.0 $\pm$ 18.4#	61.9 $\pm$ 13.8*	79.8 $\pm$ 22.3*
Ce	44.8 $\pm$ 8.1†	45.9 $\pm$ 9.5#	51.2 $\pm$ 6.7†	60.8 $\pm$ 19.5†

¹Concentrations are expressed in $\mu\text{mol/l}$. Mean AUC $\pm$ SEM (over 2 h) values (mm^2) of SP-induced W and F areas after each treatment are shown. Signed values represent significant differences in two-by-two comparisons of treatment effect.
*Di greater efficacy than P ($P < 0.05$).
†Ce greater efficacy than P ($P < 0.05$); ‡ $P < 0.005$.
#Ce or Di better ($P = \text{trend}$) than P.

occurred in all three subject groups of the present study; however, increased mast-cell releasability in CIUpt (28), heightened sensitivity of CIUpt microvasculature to vaso-

Table 4. Effect of H_1 -antagonist treatment on cutaneous wheal and flare responses to CGRP

	CGRP 0.5 ¹		CGRP 1.0 ¹	
	Wheal	Flare	Wheal	Flare
HA				
P	19.9 $\pm$ 5.0	109.8 $\pm$ 33.5	19.6 $\pm$ 3.5	119.0 $\pm$ 28.6
Di	14.3 $\pm$ 4.4	58.9 $\pm$ 19.5#	16.4 $\pm$ 4.1	98.1 $\pm$ 24.5#
Ce	19.1 $\pm$ 4.0	59.9 $\pm$ 8.8#	20.9 $\pm$ 5.1	84.0 $\pm$ 9.6#
DPU				
P	40.6 $\pm$ 11.9	176.1 $\pm$ 45.6	43.0 $\pm$ 9.0	224.0 $\pm$ 64.2
Di	15.7 $\pm$ 3.5	113.4 $\pm$ 36.3	26.0 $\pm$ 5.0	133.4 $\pm$ 20.2#
Ce	20.3 $\pm$ 3.6	91.4 $\pm$ 9.7†	27.5 $\pm$ 4.8	131.4 $\pm$ 24.2#
CIU				
P	73.5 $\pm$ 24.1	312.4 $\pm$ 150.3	66.0 $\pm$ 17.8	258.2 $\pm$ 48.6
Di	33.8 $\pm$ 9.6	93.2 $\pm$ 13.3*	46.5 $\pm$ 9.5	144.1 $\pm$ 20.6*
Ce	30.3 $\pm$ 7.7	81.1 $\pm$ 17.2†	43.9 $\pm$ 9.1	129.5 $\pm$ 24.5†

¹Concentrations are expressed in $\mu\text{mol/l}$. Mean AUC $\pm$ SEM (over 2 h) values (mm^2) of CGRP-induced W and F areas after each treatment are presented. Signed values represent significant differences in two-by-two comparisons of treatment effect.
*Di greater efficacy than P ($P < 0.05$).
†Ce greater efficacy than P ($P < 0.05$).
#Ce or Di better ($P = \text{trend}$) than P.

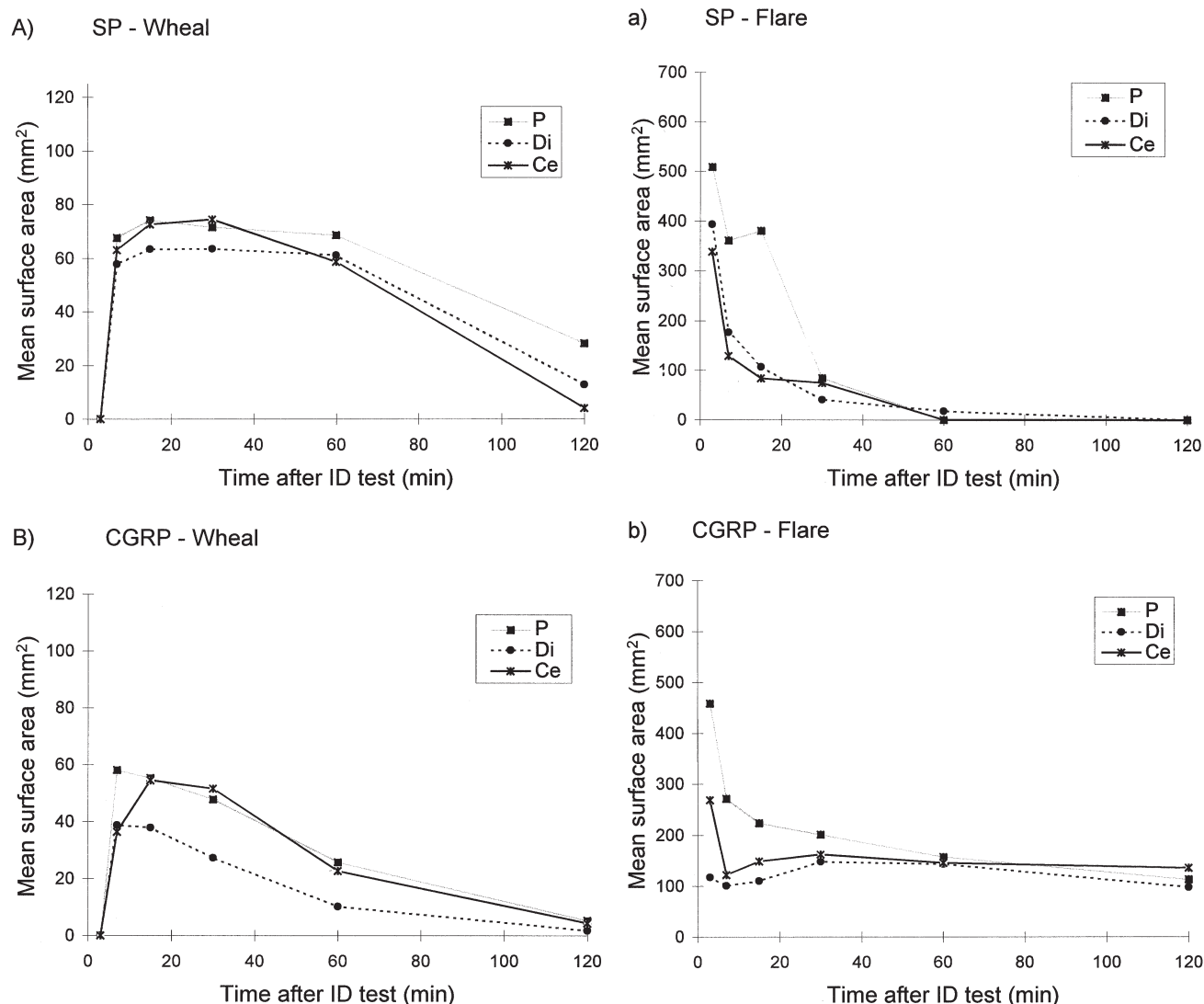


Figure 3. Time course curves of wheal and flare areas for Aa) SP (1.0 µmol/l) and Bb) CGRP (2.0 µmol/l) in healthy adults, 4 h after single dose of placebo, dimethindene, or cetirizine.

active agents (13–17), and enhanced granulocyte accumulation induced by SP itself (29) may explain the greater size and longer duration of W and F responses and also the second W peak, observed at 60 min in CIUpt. However, there is no report referring to either increased mast-cell releasability or enhanced cutaneous sensitivity to vasoactive agents in DPU. On the other hand, increased expression of E-selectin and ICAM-1, a higher number of T cells, and generalized eosinophil activation have been demonstrated in the unchallenged skin of DPUpt (25, 30, 31). Thus, a silent, proinflammatory microenvironment in DPU might contribute to enhanced W and F responses in this patient group, compared to HA. Furthermore, the eosinophil accumulation observed after injection of SP in normal human skin (29), may also be magnified in the DPU microenvironment, thus creating favorable conditions for greater W and F responses.

This latter view is supported by the observation that ID injection of VIP (vasoactive intestinal peptide), which does not cause eosinophil accumulation (29), elicited in the same group of DPU patients W and F reactions no different from those of HA (13).

ID injections of all concentrations of CGRP (at doses of 15, 30, and 60 pmol) induced biphasic cutaneous responses in all subjects involved in this study. In reviewing the relevant literature, we noted that Fuller et al. did not consistently observe W formation after ID injection of CGRP up to 250 pmol (8). An immediate W response to ID injected CGRP was a consistent finding in all 27 subjects of the present study, despite interpatient variability in the magnitude of response. Thus, our data agree with those published by Piotrowski & Foreman, who also observed initial W reactions to ID-injected CGRP at doses of 12.5–50 pmol

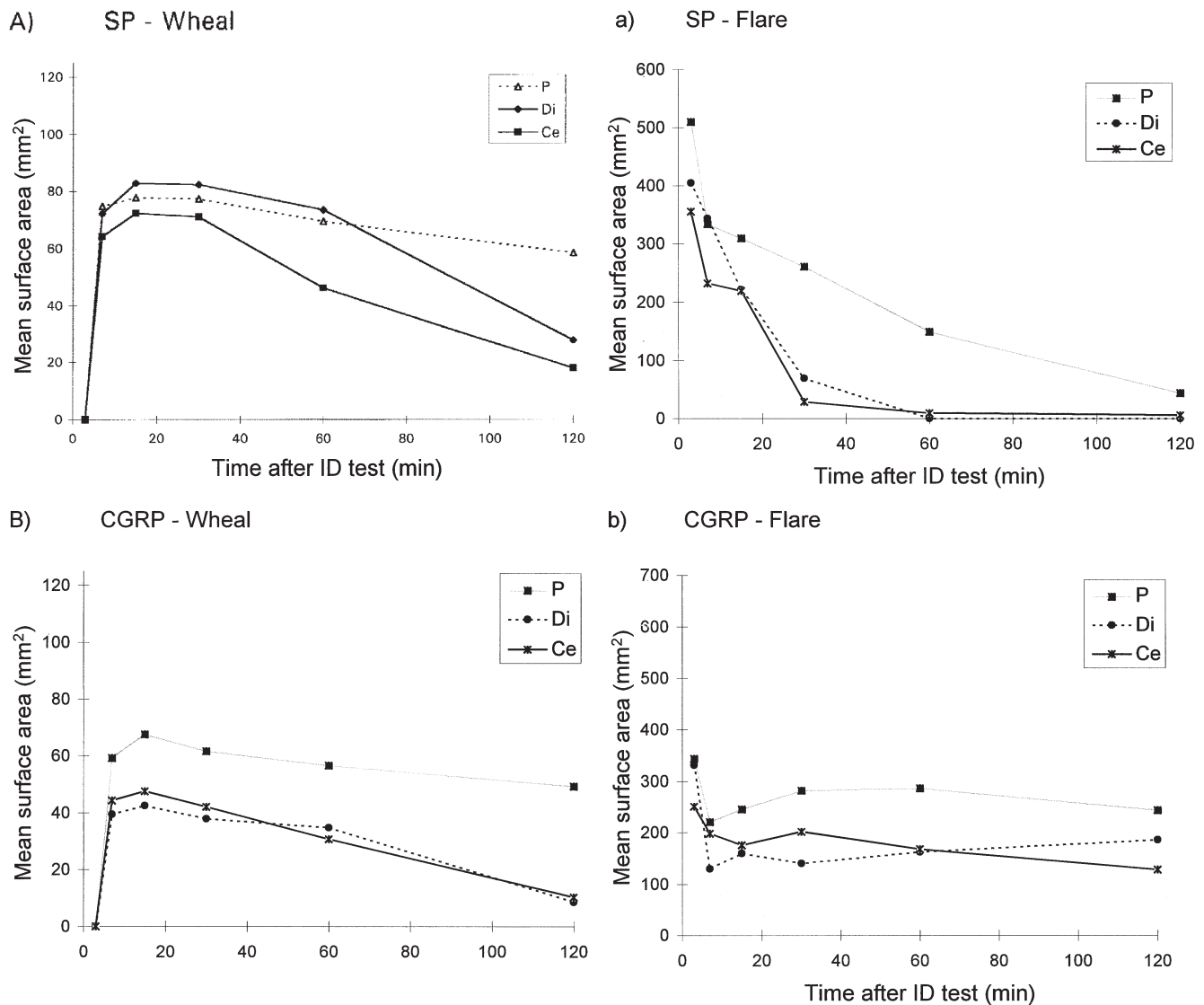


Figure 4. Time-course curves of wheal and flare areas for Aa) SP (1.0 $\mu\text{mol/l}$) and Bb) CGRP (2 $\mu\text{mol/l}$) in seven DPUpt, 4 h after placebo, dimethindene, or cetirizine intake.

(11). In Fuller et al.'s study, drug consumption had been discontinued for only 48 h before cutaneous challenges with CGRP; skin tests with NP were not always performed at the same time of the day; and, lastly, an earlier time (10 min) for recording of W reactions was chosen; these factors might account for the observed differences.

CGRP, a potent vasodilator of the major resistance vessels, probably acts directly on vascular smooth-muscle cells (26, 32, 33). Detectable histamine release from rat and human mast cells has been observed upon incubation with CGRP (6, 11, 12). ID injection of this NP induces neutrophil accumulation, synchronous with the prolonged erythema (11, 29). CGRP by itself has been considered to have little or no effect on vascular permeability, but the occurrence of synergism with other vasoactive mediators, such as hista-

mine, C₅a, leukotriene B₄, platelet-activating factor, bradykinin, etc., has been reported; therefore, CGRP appears to affect capillary permeability indirectly (34). The increased mast-cell releasability and subsequent abundant release of secondary mediators in CIUpt skin constitute a strong basis for this synergism and may explain the larger and longer-lasting W response in CIUpt than in HA; it may also explain the failure of H₁-antagonists to suppress W formation effectively. Synergism of vasoactive mediators might also contribute to the greater size and duration of the prolonged erythema response in these patients.

Several studies have evaluated the role of H₁-antagonists in suppressing the cutaneous responses to SP and CGRP in healthy volunteers (7, 8, 11, 21); the SP-induced F reduction by antihistamines has been considered to result from H₁-

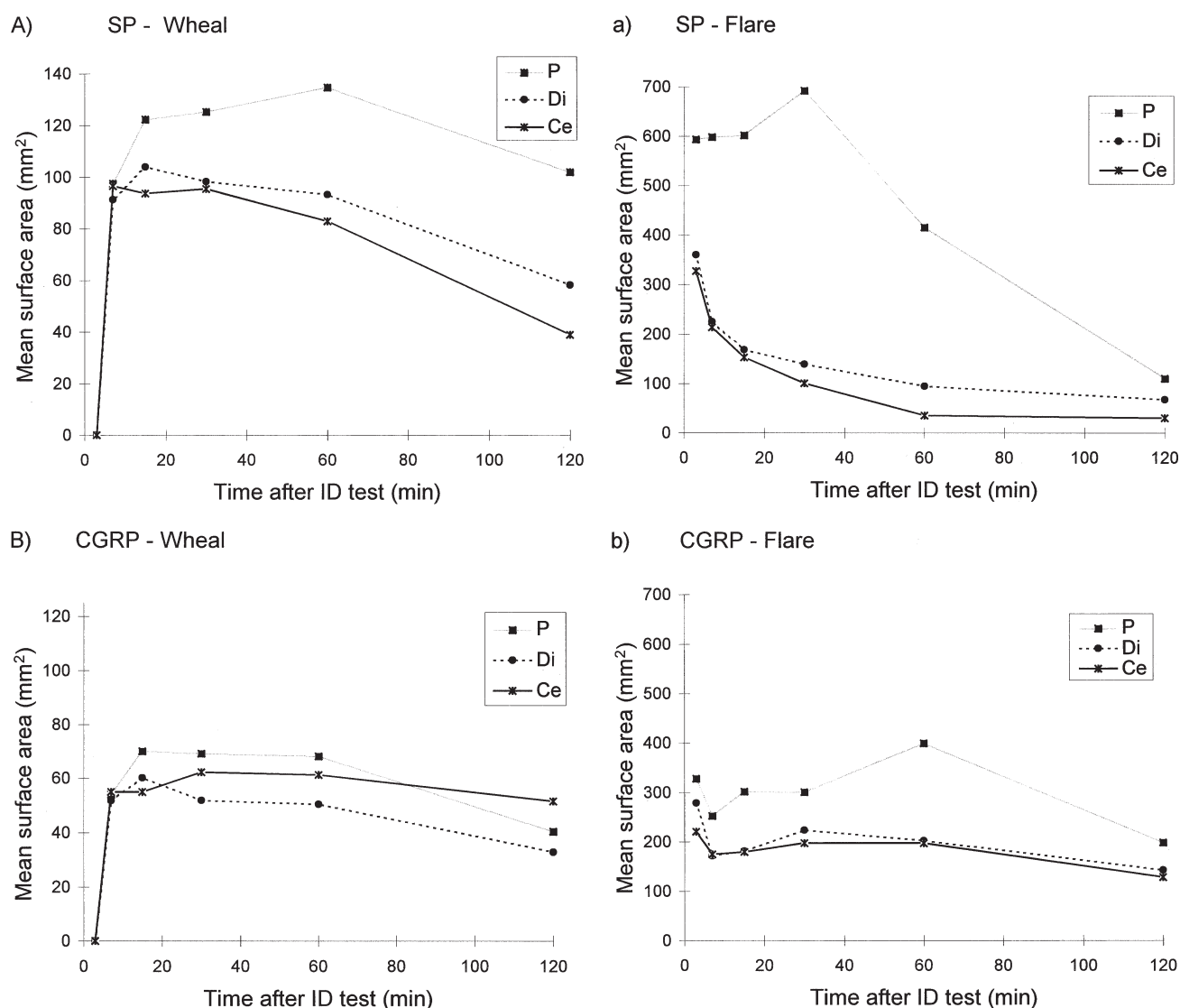


Figure 5. Time-course curves of wheal and flare areas for Aa) SP (1.0 $\mu\text{mol/l}$) and Bb) CGRP (2.0 $\mu\text{mol/l}$) in CIUpt, 4 h after placebo, dimethindene, or cetirizine ingestion.

receptor blockage. Overall, the F responses to SP were affected by H_1 -antagonists in our study groups; however, only Ce inhibited the F reactions in HA and DPUpT (AUC), while both drugs suppressed these responses in CIUpt. Even though SP is known to release H from mast cells, recent findings by the cutaneous microdialysis technique did not confirm H as the final mediator of the axon reflex-mediated F (35); thus, a direct antineuropeptide effect of H_1 -antagonists, particularly Ce, might be suggested. The reduction of SP-induced W by both antihistamines may also be explained by strict H_1 -blockage; furthermore, the anti-inflammatory properties of newer H_1 -antagonists, widely investigated for Ce (19, 20, 36), and a probable, direct antineuropeptide effect might also contribute to the inhibition of NP-induced W. Studies in rat mast cells have suggested that basic

secretagogues, such as SP, may interact directly with membrane-associated G proteins involved in the activation-secretion coupling, thus bypassing classical receptors (37). Ce appears to reduce the cutaneous responses to SP, probably by modulating the G proteins involved in SP-receptor activation (38); this mechanism might explain the greater efficacy of Ce than Di in reducing cutaneous responses to SP.

The two H_1 -antagonists reduced the initial (immediate) F reaction induced by CGRP in all study groups. These data might suggest that ID injection of CGRP initially causes histamine release from mast cells, resulting in the production of an axon reflex-mediated F; it is this response that is inhibited by antihistamines, in a manner analogous to that of SP-induced F reduction. However, the prolonged F was

partially suppressed by the two antihistamines only in the urticaria subjects. Taking into consideration the fact that the prolonged CGRP-induced F is of greater magnitude in the two patient groups than in HA and that it is partially suppressed by H₁-antagonists in these subjects, one might conclude as follows:

- 1) prolonged F is caused by a direct and indirect effect of CGRP on the microvasculature
- 2) H₁-antagonists suppress only the indirect (histamine-induced) component, as is more obvious in urticaria.

Unlike the F response, CGRP W was not affected by H₁-antagonists. This finding might suggest either that histamine is not involved in the W production or that higher than normally achieved tissue levels of antihistamines are required to suppress vascular permeability when mediator interactions appear to be involved (39). In addition, the observed interpatient variability in the pattern of W responses has probably contributed to the lack of statistical

significance in CGRP-induced W suppression by H₁-antagonists.

In conclusion, the W and F responses induced by SP and CGRP were larger and longer lasting in CIU and DPU than in HA. CGRP differed from SP in that it caused prolonged erythema and a variable W pattern, even though W were consistently elicited in all three study groups. The H₁-antagonists partially suppressed the cutaneous responses to SP and CGRP. CGRP W were not inhibited by either Ce or Di. Ce was more effective than Di in reducing SP cutaneous responses.

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