

Interaction between Tachykinins and CGRP in Human Skin

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Substance P (SP), neurokinin A (NKA) and calcitonin gene-related peptide (CGRP) coexist in nerve fibres in the skin. CGRP causes erythema upon intracutaneous injection. The erythema is independent of axon reflexes and release of mast cell histamine. SP is known to produce a flare reaction that is dependent on axon reflexes and release of mast cell histamine. The flare reaction to NKA is known to depend predominantly on axon reflexes. The purpose of the present study was to investigate possible cooperation between SP and CGRP. SP was found to shorten the duration of the reddening induced by CGRP, injected concomitantly. NKA did not shorten the duration of the CGRP response. Local elimination of mast cells in the skin by treatment with compound 48/80 had the effect that SP lost its ability to shorten CGRP-evoked erythema. These observations support the suggestion that an SP-evoked release of proteolytic enzymes from mast cells could lead to accelerated degradation of CGRP.

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Bayliss (1) showed a century ago that nerve-mediated vasodilatation (flare) in the skin could be induced by mechanical, thermal and chemical stimuli. The response to such stimuli involves antidromic impulse transmission in thin, unmyelinated sensory nerve fibres that have their cell bodies in the dorsal root ganglia. Since then, axon reflexes in the so-called C-fibres have been implicated in vascular dilatation in response to local injury (2).

An anatomical and functional coupling between C-fibres and mast cells has been proposed (3–6), but the precise mechanism behind the generation of the flare is still a matter of discussion (7). C-fibres contain neuropeptides, such as substance P (SP), neurokinin A (NKA) and calcitonin gene-related peptide (CGRP) (8, 9). These peptides fulfil many of the criteria for being neurotransmitters (10). When injected into the skin, SP induces a rapidly developing and rather short-lasting flare and wheal response, resembling that induced by histamine. The responses to NKA are similar but weaker. The flare reaction generated by SP (and by NKA) can be eliminated by local anaesthetics (11). The SP-induced flare can be eliminated also by local depletion of mast-cell histamine (by compound 48/80). The flare induced by NKA, on the other hand, is suppressed but not eliminated by this pretreatment (11). The wheal response induced by SP can be inhibited in part by local anaesthetics and by depletion of the mast-cell histamine store, while the NKA-induced wheal is unresponsive to these pretreatments (11). In agreement with these findings, SP has been found to be effective in releasing histamine from mast cells, while NKA is considerably less effective in this

respect (12). Hence, it seems that unlike NKA, SP acts by releasing histamine from mast cells, and the histamine released excites local C-fibres, generating both an orthodromic and an antidromic impulse flow. The dermal effects of CGRP differ from those evoked by the tachykinins. CGRP induces a slowly developing, widespread reddening with an irregular outline. The response is quite long-lasting (13, 14) and is unresponsive to pretreatment with local anaesthetics and/or histamine depletion (11).

Thus, SP, NKA and CGRP coexist in C-fibres but their dermal effects differ. SP and NKA induce short-lasting flare and wheal while CGRP induces a quite long-lasting erythema. The response to CGRP seems to be independent of mast cells and nerve blockade. The responses to SP are highly dependent on both C-fibres and mast cells, while the responses to NKA appear to be dependent on C-fibres but not mast cells.

The present report describes an attempt to examine the interactions in human skin between SP, NKA and histamine in relation to CGRP.

MATERIAL AND METHODS

CGRP, SP and NKA were purchased from Peninsula Europe, Merseyside, UK, and histamine dihydrochloride from Sigma, St Louis, MO, USA. All experiments were carried out using drugs dissolved in 0.9% saline. Solutions were prepared immediately before use. All injections were made intracutaneously (i.c.) in a randomized manner in a volume of 0.05 ml into the volar aspect of the upper portion of the lower arm. Eight subjects (aged 25–60 years, 3 females) participated in the study, which was approved by the Ethics Committee of Lund University.

Combined injections of two peptides or of a peptide and histamine were given. The effects were compared with those induced by injection of each agent alone. Saline alone produced a very faint and very short-lasting (<5 min) flare. Mast cells were depleted locally by pretreatment on 3 consecutive days with compound 48/80 (10 µg) (given in combination with 10 µg mepyramine in order to suppress the strong local reaction due to histamine release). The last dose failed to produce a flare reaction, indicating successful depletion of local histamine. Neuropeptides were injected on the day following the third injection of compound 48/80. Doses were SP 10 or 25 pmol and CGRP 5 or 10 pmol, histamine 250 pmol and NKA 10 pmol. Wilcoxon's rank sum test was used for statistical evaluation. The responses (area of redness) were outlined and traced on transparent plastic film 5 min after the injection (unless otherwise stated). The outlined areas were cut out and measured.

RESULTS

CGRP produced reddening upon intracutaneous injection. SP 25 pmol produced a strong flare and shortened the duration of the reddening induced by CGRP 5 pmol ($p < 0.005$) (Fig. 1). SP 10 pmol also shortened the duration of CGRP 10 pmol ($p < 0.05$). Pretreatment with compound 48/80 greatly reduced the response to SP + CGRP, and under these circumstances SP failed to shorten the duration of the CGRP-evoked erythema

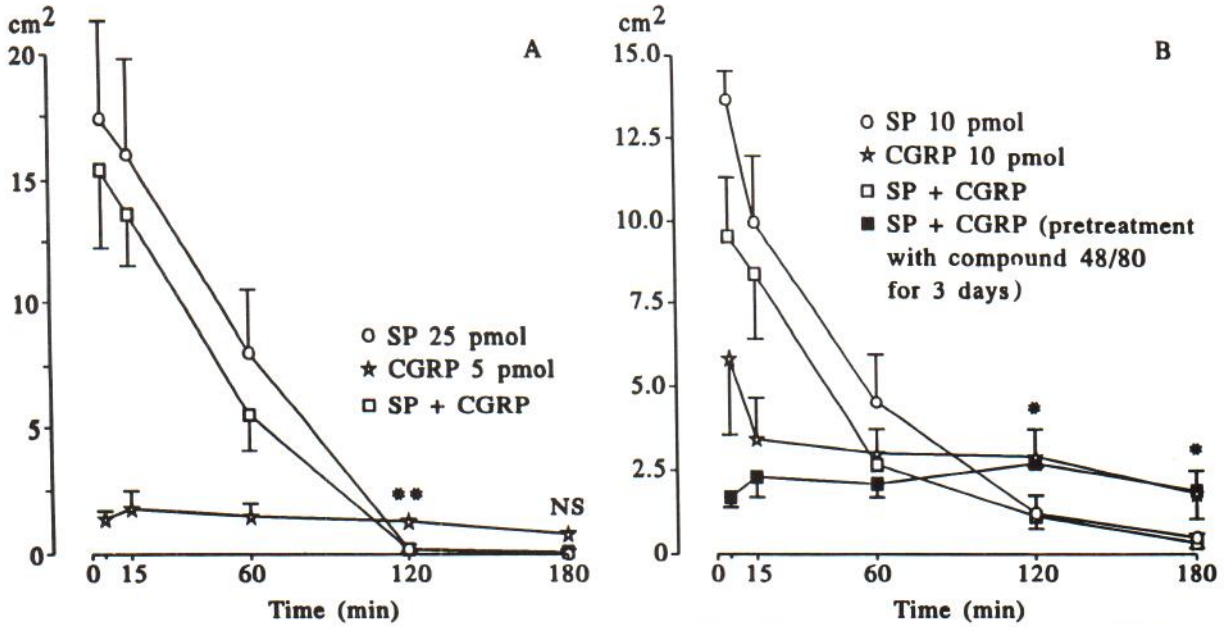


Fig. 1. Area of reddening of the skin in response to intracutaneous injection of SP, CGRP or the combination of SP and CGRP. SP shortens the response to CGRP. Mean \pm S.E.M. (vertical bars). Each value is the mean of 5–10 experiments. Statistical evaluation by Wilcoxon's rank sum test.

(Fig. 1). The combination of histamine 250 pmol and CGRP 10 pmol did not produce a response that was different from that of histamine alone, except for the late erythema caused by CGRP (Fig. 2A). Thus, histamine did not shorten the duration of the CGRP-evoked response. NKA was less effective than SP in inducing flare. When NKA 10 pmol was injected together with CGRP (10 pmol) there was an additive effect. NKA did not shorten the duration of the CGRP response (Fig. 2B).

DISCUSSION

It is to be expected that the neuropeptides SP, NKA and CGRP should cooperate, since they coexist in the same neurons (6, 15). Some evidence for the cooperation of tachykinins and CGRP in the skin has been presented (16–19).

In a previous study we found no potentiation of the flare and wheal response by the concomitant injection of SP and a threshold dose of CGRP (9). The human skin seems to be richer in CGRP than in SP (9) and hence, larger concentrations of CGRP than of SP may be released upon C-fibre

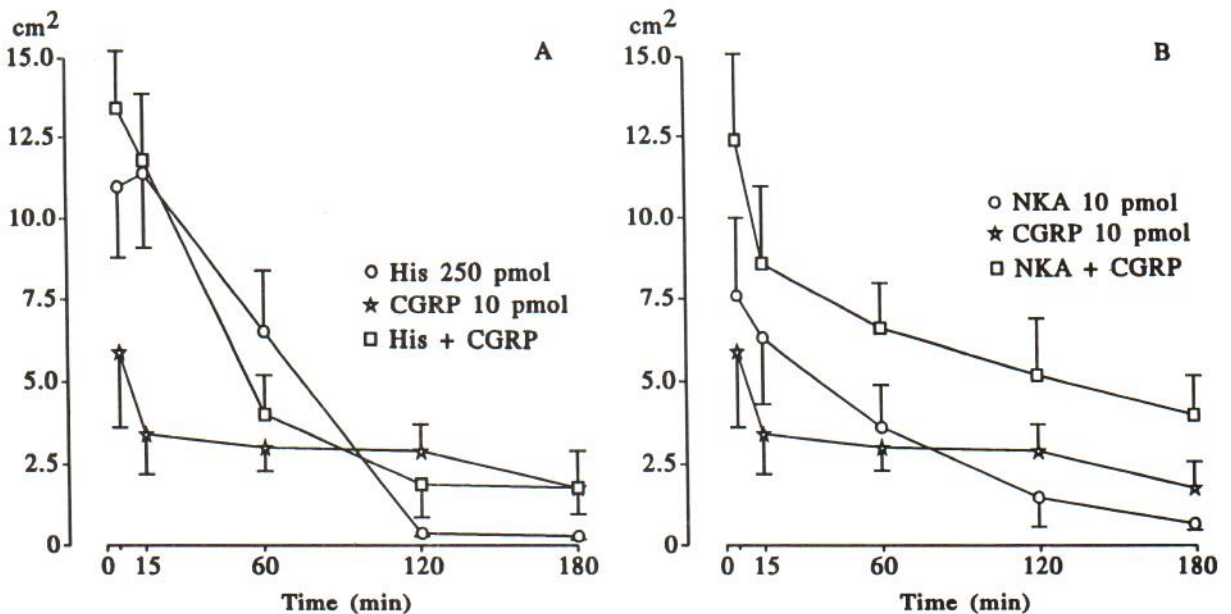


Fig. 2. Area of reddening of the skin in response to intracutaneous injections of histamine (250 pmol), NKA (10 pmol), CGRP (10 pmol) and the combination of histamine and CGRP or of NKA and CGRP. Neither histamine nor NKA seemed to shorten the response to CGRP. Mean \pm S.E.M. Each value is the mean of 5–10 experiments. Statistical evaluation by Wilcoxon's rank sum test.

stimulation. We repeated the experiments of Brain & Williams (16) and could confirm that equimolar amounts (10 pmol) of SP and CGRP injected concomitantly produced a smaller area of reddening 2–3 h after the injection than did CGRP alone. Also, 25 pmol of SP shortened the duration of the reddening induced by 5 pmol of CGRP. As first suggested by Brain & Williams (16), an SP-evoked release of proteolytic enzymes from local mast cells could lead to accelerated degradation of CGRP, thereby shortening the duration of the response to CGRP. This interpretation is in agreement with the observation that combined treatment with SP and CGRP produced as long-lasting an erythema as did CGRP alone in the absence of mast cells (following local pretreatment with compound 48/80). This view was supported also by the observation that NKA, which is less effective than SP in causing mast cells to discharge (12), failed to shorten the duration of the CGRP-evoked erythema.

Injection of CGRP together with a substance that produces increased vascular permeability could be expected to cause local dilution of CGRP. However, such a dilution cannot by itself explain why SP shortens the CGRP response upon concomitant injection, since exogenous histamine and NKA, which both increase vascular permeability, did not shorten the duration of the CGRP-evoked response. The flare response to exogenous histamine is partly dependent on endogenous histamine, since it can be suppressed by pretreatment with compound 48/80 (11). Alternatively, the suppressed response could reflect tachyphylaxis to histamine induced by the release of endogenous histamine during pretreatment with compound 48/80. However, unlike SP, histamine did not shorten the duration of the CGRP-evoked response, perhaps suggesting that histamine is less effective than SP in stimulating mast cell discharge.

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