Advances in Pain Research and Therapy, Vol. 20,
edited by Federigo Sicuteri, et al.,
Raven Press, Ltd., New York © 1992.

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Physiological Factors Influence Substance P-Evoked Flare in Human Skin

Joanna Wallengren, *Rolf Håkanson, and †Åke Andrén-Sandberg

*Departments of Dermatology, *Pharmacology, and †Surgery, University of Lund, S-223 62 Lund, Sweden*

REACTIONS TO SUBSTANCE P

The skin is rich in neuropeptides (1). Substance P (SP) is of particular interest since it probably acts as a transmitter, together with calcitonin gene-related peptide, in a population of sensory nerve fibers (C-fibers) that play an important role in the mediation of inflammation (2). When injected into the skin, SP induces wheal locally and rapidly spreading vasodilation (flare) (3–7). The flare is dependent on axon reflexes and hence on an intact sensory nerve supply. The wheal, which reflects plasma extravasation, occurs by a direct mechanism distinct from that involved in the flare reaction (2). Mast-cell histamine plays an important role in the SP-evoked flare reaction (3–7). Mast cells and sensory nerve fibers often display an intimate topographic relationship (8), suggestive of a close functional interaction. In fact, SP mobilizes histamine from mast cells (4,5,9,10) and histamine stimulates C-fibers to release SP (11).

The present study is concerned with the influence of sex, age, stress, body regions, environmental temperature, and diurnal variation on neurogenic and direct vascular responses to SP in the skin.

MATERIALS AND METHODS

The protocol was approved by the Ethics Committee of the University Hospital at Malmö. All adult participants and the parents of the children studied gave their consent after receiving information on how the study was to be conducted and why.

Substance P was purchased from Peninsula Europe Ltd., St. Helens, Merseyside, UK.

Forty-three healthy volunteers (17 females and 26 males) took part in the investigation. Four were around 10 years old. The rest included five young people 18–20 years old, 20 medical students (23–30 years), and 6 staff members (30–60 years).

In addition, we solicited the participation of 10 competitive athletes (18–23 years old, males, first division handball players).

Substance P was injected intradermally in a dose of 50 pmol (if not otherwise stated) in a volume of 0.05 ml. Injections were usually made on the volar side of the forearm, except in one study when several different skin regions were studied on all participants (n = 7): volar and dorsal part of the forearm and upper arm, nape of the neck, thorax, abdomen, trochanter of the hip, upper part of the leg, lower part of the leg, and upper aspect of the foot. The flare reactions were outlined and traced on transparent plastic film 5 min after the injection. Afterward, the profiles were cut out and weighed and the area of the flare was calculated. Also, the wheal response was assessed in the same way, the outline was confirmed by palpation. The intensity of the itch was based on the subjective sensations of the volunteers and recorded as intense, moderate, or slight.

All experiments were performed between 8:30 a.m. and 4 p.m. if not otherwise stated.

In order to investigate if and how stress influences the response to SP, athletes were studied before (at rest) and immediately after a competitive endurance test (racing for 20 min at room temperature). It was competitive in the sense that the results were decisive for who would be selected for the national team. The athletes participated in this study only.

The influence of temperature and time of day was studied on seven medical students. For the temperature study the room was maintained at 17, 22, or 28°C. The subjects were permitted to acclimatize to the room temperature for about 45 min before the test; they were wearing light dress (trousers and a shirt with long sleeves). No physical activity was involved. For the time study the temperature was maintained at 22°C. The first injection of SP was made at 11 a.m. in one arm and the second injection was made at 11 p.m. in the other arm.

The results were analyzed statistically by Student's *t*-test for paired observations and by Wilcoxon's test.

RESULTS

Different regions of the skin responded differently to SP. This study was performed on students, 24–28 years old. The largest flare was seen on the upper thorax (Fig. 1A); the largest wheal was seen on the upper aspect of the foot and the flexor surface of the upper arm (Fig. 1B). The most intense itch (subjective assessment) was reported from the nape of the neck and from the upper aspect of the foot. Thus the intensity of the itch was not proportional to the flare response. The room temperature did not affect the dermal response to SP (Fig. 2). The sex of the subjects did not influence the flare or wheal (Fig. 2).

The flare response to SP was greater during the night (11 p.m.) than during the

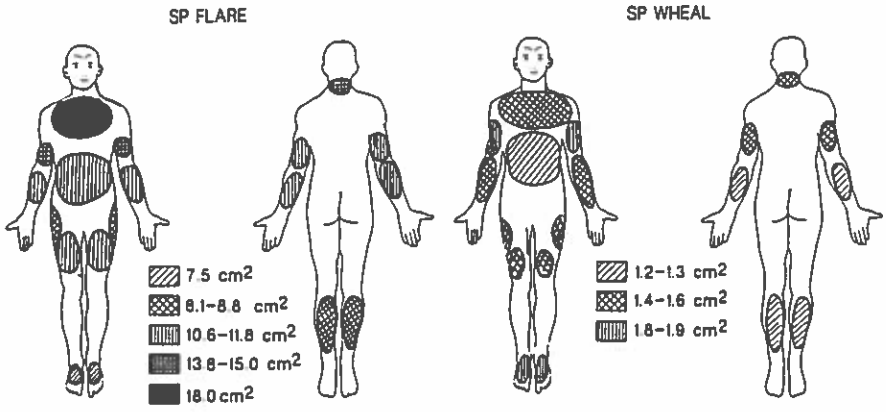

FIG. 1. The flare and wheal responses to SP (50 pmol) in different regions of the skin. The responses are expressed as the area (cm^2) engaged (n = 7, medical students, age 24–28 years).

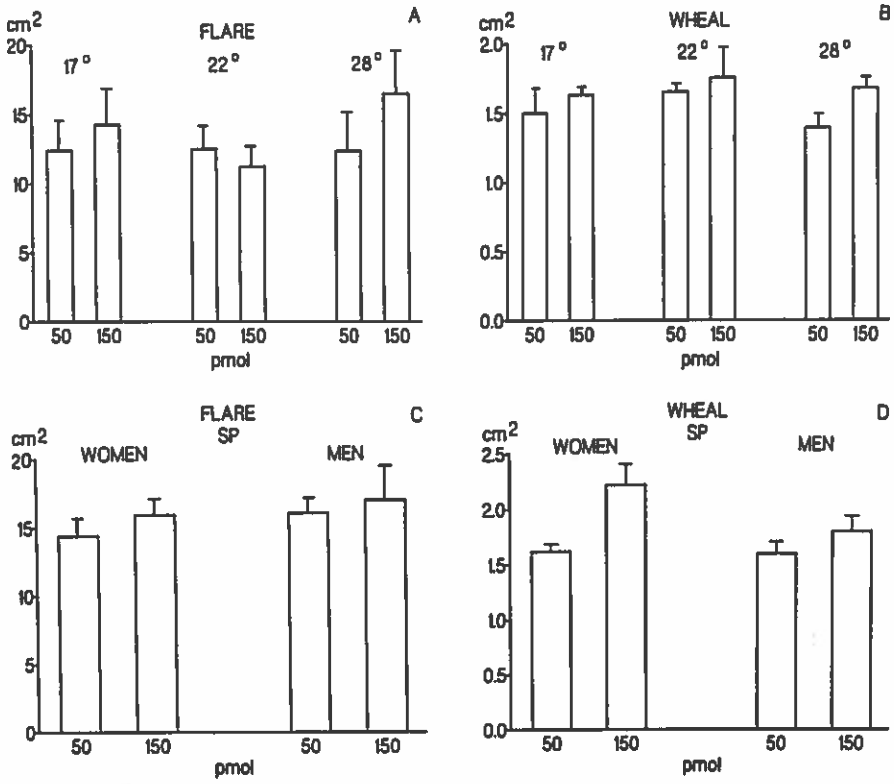

FIG. 2. The flare and wheal responses to SP (50 or 150 pmol) were not affected by the temperature of the environment (**A, B**) or the sex of the subjects (**C, D**). Seven subjects participated in the temperature study. Six males and six females participated in the comparison between the sexes. There were no statistically significant differences.

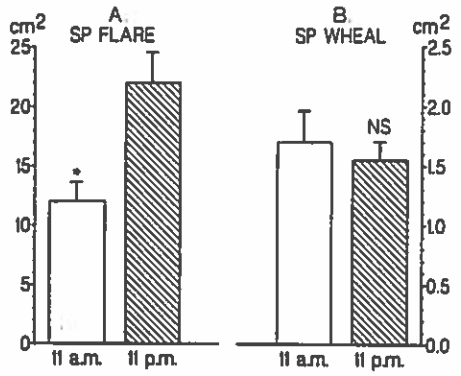

FIG. 3. The flare response but not the wheal response to SP (150 pmol) differs during the day (11 a.m.) and night (11 p.m.). Mean ± SEM (n = 17, age 23–30 years). *$p<0.05$, Student's *t*-test and Wilcoxon's test for paired data.

day (11 a.m.) (Fig. 3A); the wheal did not differ (Fig. 3B). We observed a marked decrease of the flare response to SP with increasing age (Fig. 4); the change in the wheal was not statistically significant.

Athletes had smaller SP-evoked flare under resting conditions than age-matched controls (compare Fig. 4A and Fig.5, Wilcoxon test for unpaired data, $p=0.037$), and the SP-evoked flare in the athletes was greatly reduced following a competitive endurance test (Fig. 5).

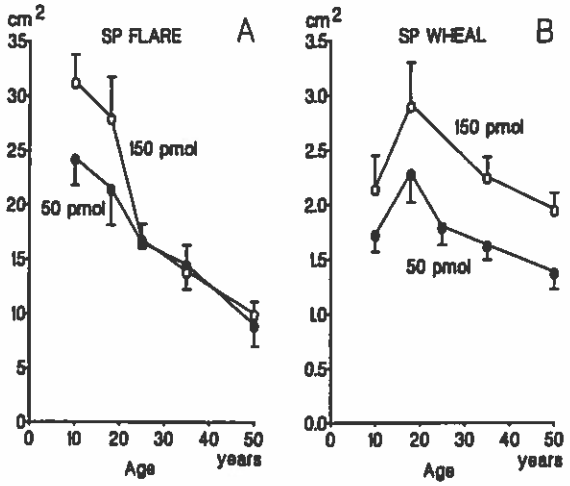

FIG. 4. The flare and wheal responses to SP (50 pmol) in individuals of different ages. Injections were made in the skin of the upper arm. Mean ± SEM, four or five individuals in each group. The athletes (Fig. 5) were not included in this study.

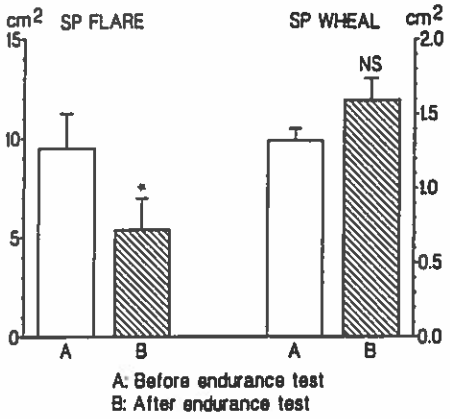

FIG. 5. The flare responses to SP (50 pmol) in 10 athletes (18–23 years old) at rest (A) and immediately after a competitive endurance test lasting 20 min (B). Mean ± SEM. *$p<0.05$, Student's *t*-test and Wilcoxon's test for paired data.

DISCUSSION

The concentration of SP is higher in densely innervated regions like fingers and toes than in less densely innervated regions (12). The concentration of SP in different areas of the skin may reflect the density of the sensory nerve supply. The flare response to injury is thought to reflect axon reflexes engaging SP-containing C-fibers. The greatest flare was observed on the thorax, in confirmation of the finding that flare is less marked in more distal locations (13). The greatest wheal was observed on the upper aspect of the foot and on the flexor surface of the upper arm. The itch was most pronounced on the foot and the nape of the neck, which agrees with previous reports that the itch sensation differs between different skin regions (14); moreover, it does not seem to be correlated to the flare response.

Magerl et al. (13) reported that the wheal response to histamine was greater in females than in males. We could not confirm this observation with SP as the challenging agent. Also the flare response to SP in our study was independent of sex. Environmental temperature did not influence the response to SP, which, however, differed during day and night, being greatest during the night.

The flare response to SP was markedly reduced with age. This is in agreement with previous reports that increasing age is associated with diminishing skin responses to capsaicin (15), histamine, and bradykinin (16). Helme and McKernan (15) reported that the concentration of SP in the skin is lower in old individuals than in young. They suggested that this might reflect a progressive loss of sensory nerve terminals with age, leading to a reduced collateral spread for axon reflexes and thereby affecting the size of the flare. However, the number of free nerve endings in the skin does not seem to change with age (17). Alternatively, the small SP responses in old individuals could reflect changes in the effector organ (i.e., vascular dysfunction) (18) or paucity of mast cells (19).

Athletes displayed a smaller flare response to SP than age-matched nonathletic controls, and the SP-evoked flare in athletes was reduced further following a com-

petitive endurance test. This suppression may reflect the stress-induced rise in circulating catecholamines (20) or endogenous opioids (21). Thus the adrenals and/or the sympathetic nervous system may act to suppress neurotransmission in sensory terminals. If this interpretation is correct, the small flare response seen in athletes at rest may reflect a generally higher sympathetic tone in athletes than in nonathletes. Whatever the interpretation, the results suggest that a test of the axon reflex (in this case the response to intradermal injection of SP) can provide information on C-fiber impulse traffic in an individual. It is to be expected, however, that factors other than the C-fiber impulse traffic will influence the microvasculature (e.g., circulating hormones and neurotransmitters) and hence the response to SP.

We conclude that the flare component of neurogenic inflammation diminishes with increasing age and in conditions of physical stress, and that the magnitude of the flare response depends on the time of day.

ACKNOWLEDGMENTS

This study was supported by Edvard Welander Foundation and the Swedish MRC (04X-1007). The statistical analysis was performed by Björn Edman, Ph.D.

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