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Histamine is released from skin by substance P but does not act as the final vasodilator in the axon reflex

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1 We have explored in man the hypothesis that histamine released from dermal mast cells by neurotransmitters from afferent nerves contributes to vasodilatation of the axon reflex.

2 The ability of substance P to release histamine from human skin *in vivo*, and the effects of a histamine H_1-receptor antagonist on capsaicin-induced axon reflex flares were studied.

3 Intradermal injections of substance P (50 pmol) produced a weal and flare response which was associated with increased histamine concentration in blood draining the site (mean plasma histamine concentration before injection 0.17 ± 0.02 ng ml^{-1} ($\pm$ s.e.mean), concentration one minute after injection 1.26 ± 0.28 ng ml^{-1}, $n = 6$).

4 Terfenadine, an H_1-receptor antagonist, had no effect on the flare response to intradermal injection of capsaicin at a dose which inhibited by more than 60% the flare response to exogenous histamine and to histamine released from dermal mast cells by substance P.

5 Substance P releases histamine from human skin *in vivo*. However, whatever the nature of the neurotransmitter released from afferent nerves during the axon reflex, it does not produce vasodilatation through release of histamine from dermal mast cells. Histamine may still contribute to the flare by initiation of the reflex.

Introduction

Acute cutaneous inflammation, including that produced by chemical, thermal or mechanical injury, is associated with the development of a vasodilator response spreading several centimetres beyond the initial point of injury. Lewis (1927a) showed that formation of this 'flare' is via a local neurogenic mechanism. Vasodilatation can be produced by antidromic stimulation of afferent nerves (Bayliss, 1901; Langley, 1923) and spread of the flare is considered to be due to a local axon reflex mechanism involving antidromic conduction of impulses through terminal aborisations of sensory afferent nerves. Substance P, an undecapeptide, has been suggested as the vasoactive humoral agent released from sensory nerves within the area of flare (Henry, 1977; Burnstock, 1977). A recent hypothesis proposes that, during the axon reflex, neuronally released substance P degranulates dermal mast cells and histamine, released within the area of flare, is responsible for the final vasodilatation (Arvier *et al.*, 1977; Lembeck & Holzer, 1979; Foreman *et al.*, 1983).

We have recently described a method using intradermal injection and local venous sampling to demonstrate *in vivo* histamine release from human skin following antigen challenge (Heavey *et al.*, 1984). Using this method we have investigated the ability of substance P to release histamine in man. Capsaicin (8-methyl-N-vanillyl-6-nonenamide), the pungent extract of peppers, produces a flare response via a neurogenic mechanism (Jancso *et al.*, 1968). The contribution of histamine to capsaicin-induced axon reflex flares was examined using an antihistamine.

Methods

Studies were approved by the Ethics Committee of the Royal Postgraduate Medical School and Hammersmith Hospital. All volunteers gave written informed consent.

Intradermal injections were made in a volume of 50 µl with a 27 gauge needle. Substance P (Cambridge Research Biochemicals, England) was supplied as a solid and stored in mM aliquots (in 17 mM acetic acid

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in isotonic saline) at −80°C. Final dilutions of substance P were made in sterile, isotonic saline immediately before use. The equivalent vehicle for 50 pmol injections of substance P was 17 μM acetic acid in isotonic saline. Capsaicin (Sigma, England) was stored as a mM solution in ethanol at −20°C and diluted in isotonic saline immediately before use. Equivalent vehicle for 500 pmol injection of capsaicin was 180 mM ethanol in isotonic saline. Histamine was injected as the acid phosphate (British Pharmacopoeia), diluted in isotonic saline.

Measurement of plasma histamine concentration

A double isotope radioenzymatic method was used as previously described (Brown *et al.*, 1982). In brief, 5 ml of blood was collected into polypropylene tubes containing 50 μl 0.5 M ethylenediaminetetraacetate, pH 7.4. Samples were centrifuged at 2000 *g* for 10 min, the upper 1 ml of plasma aspirated and stored at −80°C. Duplicate 50 μl aliquots of the thawed sample were incubated with reduced glutathione (Sigma, England), histamine-N-methyl transferase (extracted from rat kidney), and [^{3}H]-S-adenosyl methionine (Amersham plc, England). A third aliquot was incubated in the same way but with [^{14}C]-S-adenosyl methionine (Amersham plc, England) and an excess of histamine. At the end of incubation, aliquots from the ^{14}C tubes were added to the ^{3}H tubes and served as an internal standard through the subsequent ether extraction, back extraction, thin layer chromatography, and extraction into toluene for liquid scintillation counting. Concentrations were calculated using the ratio of ^{3}H to ^{14}C in the samples compared to those in external standards which had been run in parallel. Concentrations are expressed as ng ml^{-1}; to convert to nmol l^{-1}, multiply by 9.01.

Intradermal injection with local venous sampling

Intradermal injection of 50 pmol substance P was made on the volar aspect of one forearm and blood for measurement of plasma histamine concentration sampled from an indwelling catheter (19 or 21 gauge winged needle, Abbott, U.K.) in an ipsilateral antecubital fossa vein. This technique allows simultaneous assessment of weal and flare response and of histamine release. Six atopic volunteers were studied and were selected on the basis of a previously demonstrated ability to produce detectable histamine release following intradermal injection of antigen (*D. pteronyssinus* or Mixed Grass Pollens B2; Bencard, England) at the same site used in the present study. Three volunteers also received substance P vehicle. Blood samples were drawn at intervals before and after intradermal injection and plasma histamine concentration measured as described above. Flare areas were measured at intervals after injection, being traced by an independent observer onto clear cellophane placed over the skin. Areas of the tracings were measured using a digitising pad and calculated according to the trapezoid rule. Weal areas were measured 15 min after injection by marking the skin directly with a ballpoint pen and at the end of the study transferring the impression to plain paper using clear adhesive tape.

Effect of an antihistamine

This study was performed in a double-blind, placebo-controlled, crossover manner with placebo and antihistamine given in randomized order to eight subjects. Terfenadine 60 mg (Merrell-Dow, France) or matching placebo was given 4 h before intradermal injections of histamine (10.9 nmol), substance P (25 pmol), capsaicin (500 pmol and 250 pmol) and capsaicin vehicle in the back. The site of injection of each agonist was randomized among individuals but consistent within an individual for each part of the study. Weal areas were measured at 5 and 15 min and the maximum recorded. Flare areas were recorded at 3 and 10 min.

Statistical methods

Results are expressed as mean ± s.e.mean. Paired two-tailed *t* test was used to compare changes in weal areas, flare areas and plasma histamine concentrations. Significance is taken as $P < 0.05$.

Results

Release of histamine by substance P

Intradermal injection of substance P (50 pmol) produced a weal and flare response with a weal area at 15 min of 1.3 ± 0.07 cm^2. Five subjects developed itching, beginning between 15 and 45 s after injection and lasting approximately five minutes. Plasma histamine concentrations following substance P injection are shown in Figure 1a. Mean resting histamine concentration was 0.17 ng ml^{-1} (range 0.12 to 0.22). In all cases there was a rise above the basal concentration following injection of substance P, generally to >1 ng ml^{-1}. Peak concentration occurred at one minute in 5 subjects and at three minutes in the sixth. Concentrations remained elevated above basal for 20 min and had returned to resting values by 40 min. Injection of substance P vehicle in 3 subjects, all of whom had peak concentrations after substance P of >1.35 ng ml^{-1}, produced no detectable rise in venous plasma histamine concentration.

Figure 1b shows the time course of the flare response. It closely follows that of histamine release, peak area occurring early and declining over 20 min.

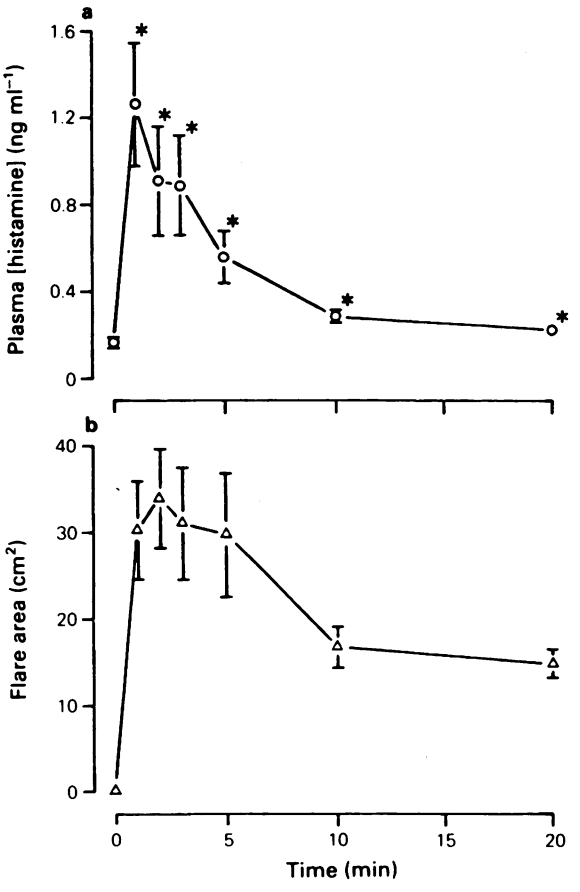

Figure 1 (a) Release of histamine into venous blood and (b) flare production following intradermal injection of substance P in 6 subjects. Substance P (50 pmol) was injected at time zero on the ventral aspect of one forearm and blood sampled from an ipsilateral antecubital fossa vein approximately 10 cm proximal. Shown are means, with vertical lines representing s.e. mean. Plasma histamine concentrations after injection were compared with those before injection using paired t test. $^{*}P<0.05$.

Effect of an antihistamine

The effect of terfenadine on flare area (at 3 and 10 min) and maximum weal area is shown in Figure 2. Flare responses to histamine and substance P were inhibited at both time points. At 3 min, mean inhibition of histamine flare was 63% and substance P flare 62%. Histamine-induced weal was reduced by 45% in the presence of an H_1-receptor blocker, but there was no effect on substance P-induced weal. Capsaicin, at two different doses, produced a flare which was similar in size to that produced by substance P but was not

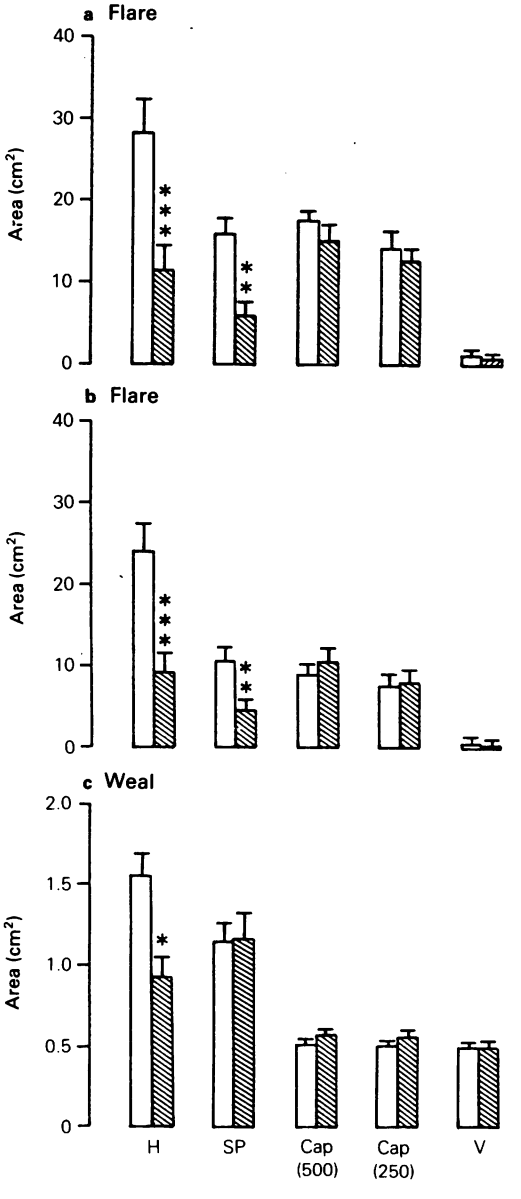

Figure 2 Effect of an antihistamine on weal and flare responses to histamine (H, 10.9 nmol), substance P (SP, 25 pmol), capsaicin (Cap, 250 and 500 pmol), and capsaicin vehicle (V, 180 mM ethanol in isotonic saline) in 8 subjects. Flare areas were recorded at (a) 3 and (b) 10 min after injection. (c) Weal areas were measured at 5 and 15 min and the maximum recorded. Each column represents the mean response, with vertical lines indicating s.e.mean, after administration of an antihistamine (terfenadine 60 mg by mouth 4 h previously, hatched columns) or placebo (open columns). Active (terfenadine) and placebo treatments were compared using paired t test. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

affected by terfenadine. No clear wealing was seen after capsaicin injection.

Discussion

It has been known for more than fifty years that the flare spreading from a site of cutaneous injury is effected through a neurological mechanism, but neither the neurotransmitter nor the final vasodilator agent is known. Substance P has been proposed to act as both the transmitter and vasodilator. It is present within sensory nerves (Hokfelt *et al.*, 1975; Cuello *et al.*, 1978) and can be released on antidromic stimulation (Olgart *et al.*, 1977; Bill *et al.*, 1979). Intra-arterial infusion in man causes vasodilatation (Eklund *et al.*, 1977) and intradermal injection produces a weal and flare response (Hagermark *et al.*, 1978). More recently it has been postulated that, during the axon reflex, substance P released from afferent nerves within the area of flare does not effect vasodilatation directly but wholly, or in part, through the release of histamine from dermal mast cells (Arvier *et al.*, 1977; Lembeck & Holzer, 1979; Foreman *et al.*, 1983). Thus, substance P can release histamine from mast cells *in vitro* (Johnson & Erdos, 1973; Erjavee *et al.*, 1981; Fewtrell *et al.*, 1982) and the flare it produces on intradermal injection in man can be inhibited by antihistamines (Coutts *et al.*, 1981; Foreman *et al.*, 1983). Structural analogues of substance P show a close association between flare-producing ability and ability to release histamine from mast cells *in vitro* (Foreman *et al.*, 1983). Lewis (1927b) recognised the ability of histamine to produce a flare response and also suggested that endogenous 'H-substance', defined only as histamine-like, might cause vasodilatation within the area of neurogenic flare.

Our initial study showed that substance P can release histamine from human skin. The release was accompanied by itching and showed a close temporal relationship to flare production. This, together with the inhibition of flare by a histamine H_1-receptor antagonist provides strong evidence that histamine contributes significantly to substance P-induced flare. These results, therefore, are compatible with the hypothesis that substance P released during the axon reflex produces vasodilatation through histamine release. In the second study we investigated whether afferent nerve transmitters released during the axon reflex could release significant amounts of histamine. The effect of an antihistamine on capsaicin-induced axon reflex flares was examined. Capsaicin was chosen since the flare response it produces is known to be dependent on local neurogenic mechanisms (Jancso *et al.*, 1968). Interpretation of the effects of antihistamines on reflex vasodilatation is difficult since histamine may act at two sites. Firstly, any histamine released at the site of the injury can initiate the reflex. Secondly, and according to the hypothesis of Lembeck and Foreman, histamine release by afferent nerve transmitters may act as the final vasodilator within the area of flare. Inhibition of flare responses by an antihistamine cannot therefore be used as evidence to support the Lembeck/Foreman hypothesis, since it may solely be blocking histamine released at the site of injury. Conversely, lack of an effect of antihistamines on reflex flare would argue against the Lembeck/Foreman hypothesis but may simply mean that the antihistamine was not present at a concentration sufficient to block endogenously released histamine. In our study with terfenadine, substance P was included as a control to release endogenous histamine. Terfenadine, an H_1-receptor antagonist, was used as the antihistamine since vasodilatation by histamine is mediated mainly by H_1-receptors (Owen, 1977). Flare responses to both exogenous and endogenous histamine were inhibited by more than 60% by terfenadine, whereas the flare response to capsaicin was unaltered. Histamine, therefore, does not act as the final vasodilator in the axon reflex. This conclusion applies whatever the nature of the transmitter released from afferent nerves, since an intact reflex was examined. Mast cell products other than histamine may be involved since Kiernan (1972, 1975) has shown an increased number of degranulated mast cells in the area of flare surrounding mechanical injury in the rabbit ear and following antidromic stimulation of sensory nerves in the rat.

Our results appear to contradict those of Lembeck & Holzer (1979) who found that antidromic stimulation of the saphenous nerve in anaesthetized rats caused vasodilatation and an increase in capillary permeability which was inhibited by pretreatment with antihistamines. Although artificial stimulation of nerve trunks does lead to release of significant amounts of histamine, our conclusion is based on observations on local spread of flare following a cutaneous stimulus in man.

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