Br J Clin Pharmacol 1995; **40**: 98–100

Intradermal actions of hypertonic saline involve neural and vascular mechanisms

A. M. SHARARA, M. A. HIGHAM, M. J. IREDALE & P. W. IND
Department of Medicine, Respiratory Division, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN

The aim of this study was to investigate whether the wheal and flare responses to intradermal injection of hypertonic (4.5%) saline (HTS) were inhibited by local injection of 1% lignocaine. Eight normal subjects were studied on one occasion. Lignocaine (0.125 ml) was infiltrated at four sites on one forearm and normal saline on the other. Five minutes later, duplicate intradermal injections of 30 μl of histamine (22.5 nmol ml^{-1}), substance P (1 nmol ml^{-1}), HTS and normal saline were given coded and in random order, one of each pair to each forearm. Lignocaine inhibited flare responses to histamine, substance P and HTS by 56% ($P < 0.01$), 78% ($P < 0.01$) and 77% ($P < 0.05$) respectively suggesting similar involvement of an axon reflex. Wheal to histamine was inhibited by 31% ($P < 0.02$) and to substance P by 33% ($P < 0.05$) but not to HTS. This suggests that the mechanism of wheal response to HTS differs from that of histamine and substance P.

Keywords hypertonic saline lignocaine wheal flare

Introduction

Cutaneous injury produces characteristic responses: an initial flare (erythema due to vasodilatation of skin arterioles) followed by a wheal (local oedema). The spread of flare was shown to be neurogenic by Lewis [1], who suggested the axon reflex. Stimulation of cutaneous nerves leads to antidromic impulses in the terminal arborizations of the sensory nerve [2]. Substance P release by antidromic impulses may stimulate mast cells to release histamine [3]. We hypothesised that intradermal injection of hypertonic saline (HTS), which is increasingly used to investigate mechanisms of bronchoconstriction in asthma [4], would produce a wheal and flare response with an axon reflex component with or without histamine release. We investigated the effect of locally injected lignocaine, which would be expected to inhibit axon reflex effects. We compared observed effects with those with injected histamine and substance P.

This paper was accepted in abstract form by the European Respiratory Society, Nice, 1994.

Methods

The study was approved by the Research Ethics Committee of the Royal Postgraduate Medical School and Hammersmith Hospital. All volunteers gave written informed consent.

Substance P (Sigma, Poole, UK) was dissolved and stored in mM aliquots (in 17 mM acetic acid in isotonic saline) at −80° C [5]. Final dilutions of substance P were made in sterile isotonic saline immediately before use [5]. Histamine was injected as the acid phosphate diluted in normal saline (NS).

Eight normal subjects were studied on one occasion. 0.125 ml of 1% lignocaine was infiltrated at each of four sites on one forearm and NS was similarly applied to the other arm. NS, HTS, histamine (22.5 nmol ml^{-1}) and substance P (1 nmol ml^{-1}) were injected intradermally in duplicate 30 μl volumes using a 29 gauge needle, one of each pair at a lignocaine site and one at a saline site. They were administered coded and in random order, with the subject and observer recording the results both blind. Flare area was outlined with a ballpoint pen at 5 min and wheal area at 15 min. A permanent record was made by transferring the outline on to 1 mm graph paper using transparent adhesive tape (Sellotape).

Results were expressed as mean ± 95% confidence interval (CI). Paired *t*-tests were used to compare changes in flare and wheal area after normal saline (control) or lignocaine treatments. Significance was taken as $P < 0.05$.

Correspondence: Dr P. W. Ind, Department of Medicine, Respiratory Division, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN

Results

All the intradermal injections caused minor discomfort lasting 10–15 s. Hypertonic saline was painful in five subjects. Histamine caused a sensation of itching in most subjects.

Lignocaine significantly inhibited flare responses to histamine, substance P and HTS by 56% ($P < 0.01$), 78% ($P < 0.01$) and 77% ($P < 0.05$) respectively (Figure 1a) and wheal responses to histamine and substance P by 31% ($P < 0.02$) and 33% ($P < 0.05$) (Figure 1b).

There was minor nonsignificant inhibition of the wheal response to NS but no effect on HTS. Lignocaine also inhibited flare to NS by 68% but this did not achieve statistical significance, due to interindividual variability.

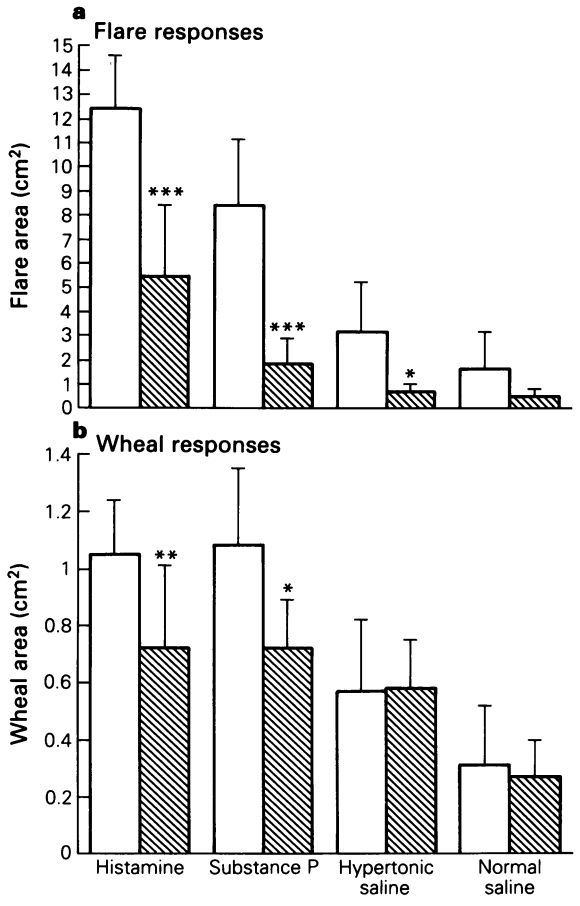

Figure 1 Comparison of the effect of lignocaine (1%) and control (normal saline) administered 5 min before intradermal injection of histamine, substance P, hypertonic saline, and normal saline in eight normal subjects. Flare (a) and wheal (b) responses are shown after normal saline infiltration (open) and after 1% lignocaine infiltration (hatched). Lignocaine and control treatments were compared by paired *t*-test. $*P < 0.05$, $**P < 0.02$, $***P < 0.01$. Results are expressed as mean ± 95% CI.

Discussion

We showed that preinjection of lignocaine had no effect on wheal induced by hypertonic saline whereas wheal to histamine and substance P in the same subjects was significantly inhibited. Furthermore, the flare response to these agents was inhibited by lignocaine to similar extents. This suggests comparable involvement of the axon reflex [6] mediated by primary afferent C fibres, with or without mast cell activation by sensory neuropeptides. This is supported by peripheral nerve section [7] and topical capsaicin experiments [8]. Histamine may act indirectly, as well as directly, by stimulating sensory nerves [9].

In preliminary studies [10] we demonstrated a dose-response effect with increasing tonicity of saline as previously shown by Djukanovic when increasing concentrations of hypertonic saline were used as a control for hyperosmolarity [11]. In our study the wheal and flare responses to hypertonic saline were comparable though significantly smaller than those to the chosen doses of histamine and substance P. Ideally, dose-responses to each agent would have been constructed. However, it is unlikely that this difference of magnitude accounts for the difference in effect of lignocaine on wheal as the flare response to the different agents was inhibited to similar extents despite comparable differences in magnitude of initial stimulus.

The failure of lignocaine to inhibit wheal to HTS, unlike responses to histamine and substance P [6], suggests that wheal response to hypertonic saline does not involve neurogenic mechanisms. This is in agreement with the finding that the wheal response to hypertonic saline differs from the other two agents in being unaffected by histamine H_1-receptor blockade [11].

Local anaesthetics may have vasoconstrictor properties *in vivo* but it is unlikely that this accounts for the inhibitory effects observed. First, intradermal injection of lignocaine has a mixed vasodilator/vasoconstrictor effect: the act of injection may cause neurogenic vasodilatation in addition to the effect of blockade of sympathetic vasoconstrictor tone while the effect on vascular smooth muscle may be constrictor [12] or, when interacting with histamine, dilator [13]. Second, if vasoconstriction was responsible for inhibition of wheal to histamine and substance P then pharmacological antagonism should have resulted in inhibition of hypertonic saline rather than a differential effect. Third, no pallor was observed.

In conclusion, we have demonstrated that intradermal injection of hypertonic saline produces an axon reflex flare response, which is inhibited by lignocaine comparably to its effects on histamine and substance P. However, wheal response to hypertonic saline differs from that to histamine and substance P in being unaffected by lignocaine, suggesting a greater local effect e.g. perhaps on shrinkage of capillary endothelial cells causing leakage of fluid and protein. The relevance of these findings to the effects of inhaled hypertonic saline in inducing bronchoconstriction is unclear.

References

1 Lewis T. *The blood vessels of the human skin and their responses*. Shaw and Sons, London 1927.

2 Kiernan JA. The involvement of mast cells in vasodilatation due to axon reflexes in injured skin. *Q J Exp Physiol* 1972; **57**: 311–317.

3 Fewtrell CMS, Foremay JC, Jordan CC, Oehme P, Renner H, Stewart JM. The effects of substance P on histamine and 5-hydroxytryptamine release in rat. *J Physiol Lond* 1982; **330**: 393–411.

4 Himender K, Makker HK, Holgate ST. The contribution of neurogenic reflexes to hypertonic saline-induced bronchoconstriction in asthma. *J Allergy Clin Immunol* 1993; **92**: 82–88.

5 Barnes PJ, Brown MJ, Dollery CT, Fuller RW, Heavey DJ, Ind PW. Histamine is released from skin by substance P but does not act as the final vasodilator in the axon reflex. *Br J Pharmacol* 1986; **88**: 741–745.

6 Forman J, Jordan C. Histamine release and vascular changes induced by neuropeptides. *Agents Actions* 1983; **13**: 105–116.

7 Jessell TM, Tsunoo A, Kanazawa I, Otsuka M. Substance P: depletion in the dorsal horn of rat spinal cord after section of the peripheral process of primary sensory neurones. *Brain Res* 1979; **168**: 247–259.

8 Bernstein JE, Swift RM, Soltani K, Lorincz AL. Inhibition of axon reflex vasodilatation by topically applied capsaicin. *J Invest Dermatol* 1981; **76**: 394–395.

9 Torebjork HE, Hallin RG. Identification of afferent C units in human skin nerves. *Brain Res* 1974; **67**: 387–403.

10 Sharara AM, Higham MA, Iredale MJ, Ind PW. Hypertonic saline injected intradermally causes flare and wheal by neural and vascular mechanisms. *Thorax* 1994; **49**: 1064P.

11 Djukanovic R, Finnerty JP, Holgate ST. Wheal-and-flare responses to intradermally injected adenosine 5'-monophosphate, hypertonic saline, and histamine: Comparison of atopic and nonatopic subjects. *J Allergy Clin Immunol* 1989; **84**: 373–378.

12 Fruhstorfer H, Nolte H, Ziegenhagel U. Hautdurchblutung nach subkutaner Lidocain-Infiltration mit und ohne Zustav von Adrenalin oder Ornipressin. *Regional Anaesthesie* 1990; **13**: 97–100.

13 Fruhstorfer H, Wagener G. Effects of intradermal lignocaine and mepivacaine on human cutaneous circulation in areas with histamine-induced neurogenic inflammation. *Br J Anaesth* 1993; **70**: 167–172.

(*Received 30 September 1994, accepted 29 March 1995*)