Plenary lecture

Mast cells, neuropeptides and inflammation

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Introduction

Many urticarial reactions are sensitive to therapy with histamine H₁-receptor antagonists suggesting that this amine has an important role in their pathogenesis. Histamine, which is a potent mediator of vasodilatation and increased capillary permeability following its interaction with H₁- and H₂-receptors on blood vessels [1], has been associated with cutaneous inflammation since the first description of its ability to cause a weal and flare response when injected intradermally [2]. In the skin, histamine is localized to the mast cells of the dermis [3, 4] where it is stored within the granules ionically bound to the carboxylic residues of the heparin proteoglycan [5]. Although research into mast cell-mediated diseases has concentrated on the importance of cross-linkage of membrane-bound immunoglobulin E (IgE) by allergen as the primary initiating stimulus for mast cell mediator release, there are many allergy-like conditions where there is no obvious link with a known extrinsic allergen. Such diseases in the skin include chronic idiopathic urticaria, heat-induced urticaria, cold-induced urticaria and cholinergic urticaria.

The basis of a role for neuropeptides in cutaneous inflammation was laid by four classic papers more than 60 years ago. These described the presence of vasodilator fibres within peripheral nerves [6], the existence of an axon-reflex [7], the observation that antidromic stimulation of peripheral nerves could induce vasodilatation [8] and the proposal that the flare response to injected histamine was mediated by a neurogenic mechanism probably involving axon reflexes in primary afferent neurones [2]. More recent studies have shown that these fibres are unmyelinated C fibres [9] whose polymodal nociceptors respond to many stimuli including pressure, heat and the local injection of histamine [10–12]. Evidence that neuropeptides act as transmitters in these nerves has been obtained from three sources. The first is the immunocytochemical evidence that neuropeptides, synthesized within the cell body and transported down the axon [13], are concentrated in sensory nerve terminals and are released following antidromic stimulation [14, 15]. These peptides include substance P, somatostatin, neurokinin A and calcitonin gene related peptide (CGRP) [16–19]. The second is the ability of substance P, somatostatin and vasoactive intestinal polypeptide (VIP) to induce a weal and flare reaction when injected into human skin, substance P being approximately 100 times more potent than histamine [20–22]. In contrast, CGRP causes a local persistent erythema and neurokinin A a local vasodilatation neither of which are accompanied by flare reactions [23, 24]. The third derives from the use of capsaicin to deplete the neuropeptides from primary sensory afferent neurones [25], a procedure which prevents the neurogenic flare response following intradermal injection of histamine [21, 22] and attenuates both heat- and cold-
induced urticaria in man [26] and heat-induced oedema in rats [27].

The association of neuropeptides with mast cell histamine release stems from experiments in which substance P and related neuropeptides have been reported to release histamine from rat mast cells [28, 29] and from observations that provocation challenge of patients with either cold- or heat-induced or cholinergic urticaria leads to an elevation in the amounts of histamine in the venous blood draining the challenged but not the control arm [30-32]. In this review we present direct evidence of the ability of neuropeptides to release mediators from human mast cells, particularly the skin mast cell.

**Dispersion and purification of human skin mast cells**

Although studies with skin slices have yielded valuable information about the responsiveness of cutaneous mast cells to secretory stimuli, there are many practical problems associated with their use such as the variable distribution of mast cells [33], the presence of diffusional barriers to secretagogues and mediators and the inability to identify the cellular source of released mediators. To overcome these problems we have developed techniques to disperse and purify human skin mast cells [34].

In this procedure, samples of foreskin from young children (1-12 years) are chopped finely with scissors into fragments of 0.5 to 2.0 mm. In experiments to investigate histamine release induced by anti-IgE, skin slices are passively sensitised by incubation for 2 hours at 37°C in minimum essential medium (MEM) containing 5% foetal calf serum (FCS) and 10% serum from an atopic donor. To disperse mast cells, tissue is incubated for 60 minutes at 37°C in MEM (1 g tissue/10 ml buffer) containing collagenase, 1.5 mg/ml, hyaluronidase, 0.75 mg/ml and bovine serum albumin (BSA), 35 mg/ml. Dispersed cells are separated from undisassociated tissue by filtration whilst the remaining undigested tissue is subjected to a second identical digestion. The cells from both digests are then pooled and washed thoroughly before use in activation studies. Samples of the suspension are removed for determination of mast cell numbers by light microscopy following metachromatic staining with Kimura stain [35]. This technique has been shown to yield $1.16 \pm 0.10 \times 10^6$ mast cells/g weight of tissue ($n = 41$) in a purity of $4.73 \pm 0.27\%$ ($n = 41$). Trypan blue exclusion tests indicate a viability of $93.3 \pm 0.9\%$ ($n = 16$) [34].

Mast cells are purified as shown in Fig. 1. Discontinuous density gradients are prepared by overlaying successive 2 ml bands of 80%, 70%, 60%, 50% and 40% isotonic Percoll containing 0.1 mg/ml deoxyribonuclease to reduce cell aggregation. These bands correspond to densities of 1.100, 1.090, 1.076, 1.063 and 1.051 g/ml respectively. One millilitre aliquots of a suspension of freshly dispersed skin cells containing 20 to $70 \times 10^6$ nucleated cells/ml are carefully layered over the gradients which are then centrifuged at $500 \times g$ for 10 minutes at 20°C. Cells which collected at the interfaces of the different density layers are harvested and washed thoroughly before cell counting or activation for mediator release. Use of this technique [34], has enabled mast cells to be enriched to more than 80% purity for experiments to determine the cellular source of histamine and eicosanoids released into the supernatant following cell activation.