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REPLY

The complexity of substance P-mediated mast cell activation

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We thank Azimi and Lerner for their Correspondence (Implications of MRGPRX2 in human and experimental cardiometabolic diseases. *Nat. Rev. Cardiol.* http://dx.doi.org/10.1038/nrcardio.2016.212; 2017)[1] on our Review (Mast cells in human and experimental cardiometabolic diseases. *Nat. Rev. Cardiol.* **12**, 643–658; 2015)[2].

Ligand–receptor interactions are the subject of research in a number of diseases, including those in the cardiometabolic field, and are also of interest in the drug discovery area. Precise knowledge about the selectivity and affinity of a ligand for a specific receptor is of high importance, not only for mechanistic insights into various disease processes, but also for successful pharmacotherapeutic use of a ligand. Inhibitors of receptors that induce mast cell activation in cardiovascular diseases, such as atherosclerosis, might provide novel therapeutics, potentially limiting the incidence of acute cardiovascular syndromes.

In the perivascular tissue of human coronary arteries, mast cells connect with sensory nerve fibres containing two neuropeptides — substance P and calcitonin gene-related peptide[3] — and the number of perivascular mast cells in human atherosclerotic coronary arteries correlates with the amount of neurofilament-positive nerve fibres in that area[4]. Neural release of substance P and the binding of the released substance P to its receptors on the mast cell surface is one of the mechanisms of mast cell activation.

Evidence from experimental models suggests that substance P can activate mast cells in a substance P receptor (SPR; also known as neurokinin 1 receptor or tachykinin receptor 1)-dependent manner[2]. Therefore, in collar-induced carotid artery atherosclerotic lesions in *Apoe*$^{-/-}$ mice, locally administered substance P increases mast cell number and activation, which can be inhibited by the SPR antagonist spantide I, which also reduces substance P-induced intraplaque haemorrhage[4]. A number of additional experimental studies have provided evidence for an interaction between substance P and SPR. For example, in a rat skin fracture model of complex regional pain syndrome, intraplantar substance P injection increased mast cell activation and postfracture pain, which the SPR antagonist LY303870 reduced[5]. In a mouse model of stress-induced hair growth inhibition, stress-dependent activation of skin mast cell activation was blocked if the mice were SPR-deficient (*Tacr1*$^{-/-}$)[6], and intradermal injection of substance P resulted in oedema in wild-type mice, but not in *Tacr1*$^{-/-}$ mice[7]. Together, the above data suggest that substance P can induce mast cell activation in an SPR-dependent fashion.

In their Correspondence[1], Azimi and Lerner state that substance P exerts mast cell-activating effects primarily by signalling via the Mas-related G-protein coupled receptor MRGPRX2. Indeed, substance P might signal via MRGPRX2 to activate mast cells in humans, given that short hairpin RNA-mediated inhibition of MRGPRX2 reduces substance P-induced activation of human cultured mast cells[8]. This hypothesis explains the observation that a tripeptide SPR antagonist QWF, which targets substance P binding to both human MRGPRX2 and mouse MRGPRB2, blocked mast cell activation[9]. Substance P might, therefore, signal via different receptors to activate mast cells in humans versus rodents. Importantly, however, using *Mrgprb2*$^{-/-}$ mice and mast cells derived from these mice, McNeil *et al.* demonstrated that not only substance P, but also other endogenous small basic peptides, cationic peptide drugs, and derivatives of the common mast cell activator compound 48/80, activate mouse mast cells via MRGPRB2 (REF. 10). Similarly, MRGPRX2 antagonists in humans might inhibit not only substance P-mediated receptor activation, but also MRGPRX2 activation by other ligands, a possibility that should be critically studied. In addition, no direct evidence exists to exclude the substance P–SPR pathway of mast cell activation in humans.

Taken together, substance P-mediated mast cell activation might be more complicated than currently understood. The myriad of new studies cited above illustrate the need to characterize the affinity of an (ant)agonist for a specific receptor in different species before it can be labelled as being relevant as a drug candidate, for example, to treat mast cell-dependent inflammatory conditions. In addition, in view of the bigger picture, other neuropeptides and their receptors might also participate in mast cell activation. Neuropeptide Y, calcitonin gene-related peptide, vasoactive intestinal polypeptide, and others are released from neurons in response to a stressor, and have been associated with mast cell activation[11,12]. Acute stress in mice by means of a 120-min restraint stress protocol can induce perivascular mast cell activation leading to increased intraplaque haemorrhage and plaque destabilization[13]. Acute stress leads to sudden release of multiple neuropeptides, each binding to its specific receptor, or to receptors of which we are currently unaware, which might reduce the effectiveness of single-target therapy. In cardiometabolic diseases, with stress as a contributing risk factor[14], it might therefore be therapeutically more relevant to target acute stress as a whole, instead of targeting a single receptor.

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doi:10.1038/nrcardio.2016.213
Published online 5 Jan 2017

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Competing interests statement
The authors declare no competing interests.