



Letter to the Editor

## SUBSTANCE P OR PGE2 ALONE AND IN COMBINATION INDUCE IL-1 SECRETION AND INFLAMMATION IN THE RAT PAW

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### INTRODUCTION

Substance P (SP), discovered by von Euler in 1931, was described as a potent vessel depressor (1). Later, in 1970, Leeman et al. published the purified chemical structure of SP from bovine hypothalamic tissue (2). In this article, the authors identified SP as an undecapeptide present in numerous organs, tissues, and cells. Moreover, they later discovered the neurokinin (NK1), the receptor of SP.

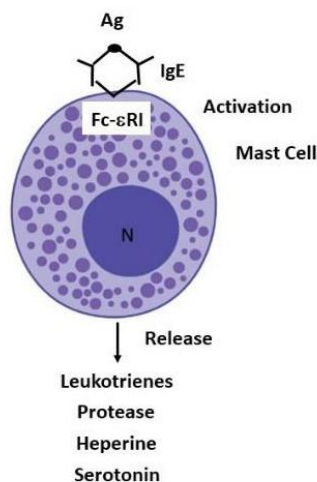
SP stimulates the turnover of cell membrane phospholipids through the activation of calcium receptors. It also stimulates mast cells (MCs), as reported by Chang and Leeman (2), participating in the inflammatory process. It has been reported that mast cell line (LAD2) secretes IL-1 $\beta$  when it is activated with SP for 24 hours (3). When activated by IgE through their Fc $\epsilon$ RI receptors, MCs immediately release inflammatory mediators (4) (Fig.1).

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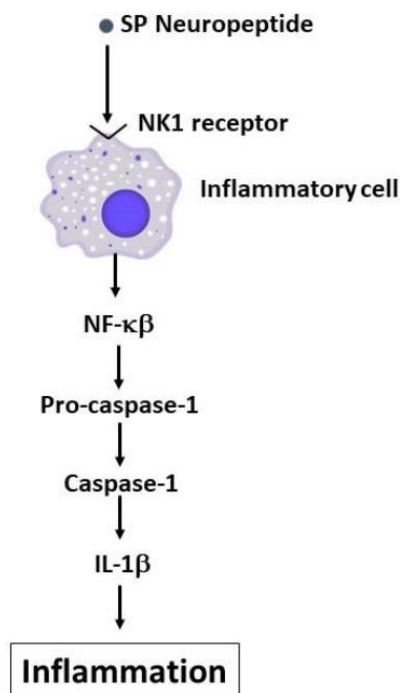
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**Fig. 1.** This figure depicts the antigen activation mast cell through IgE receptor, releasing leukotrienes, protease, heperine and serotonin.

In addition, IL-1 stimulation of MCs occurs through the production of caspase-1 and subsequent production of IL-1, which mediates the inflammatory process (5). In fact, in an interesting experiment, it was reported that when SP is simultaneously administered together with IL-33 (SP + IL-33), there is a strong secretion of IL-1 $\beta$  (10 times more than cells treated with IL-1 alone), an effect that further increases inflammation (3).

Moreover, SP administered in combination with IL-33 strongly stimulates the gene expression of tumor necrosis factor (TNF), a potent pro-inflammatory cytokine (6,7). This result demonstrates the cooperation between the SP NK-1 receptor and the IL-33 ST2 receptor in the inflammatory process. SP and IL-33 together markedly enhance TNF synthesis and secretion from human mast cells mediated by the interaction of their receptors (8). SP, binding its receptor NK-1, activates many cells, leading to the release of IL-1 $\beta$  and causing inflammation (Fig.2).



**Fig. 2.** Substance P neuropeptide binds its receptor NK1 and activates inflammatory cells to generate IL-1 and therefore, inflammation.

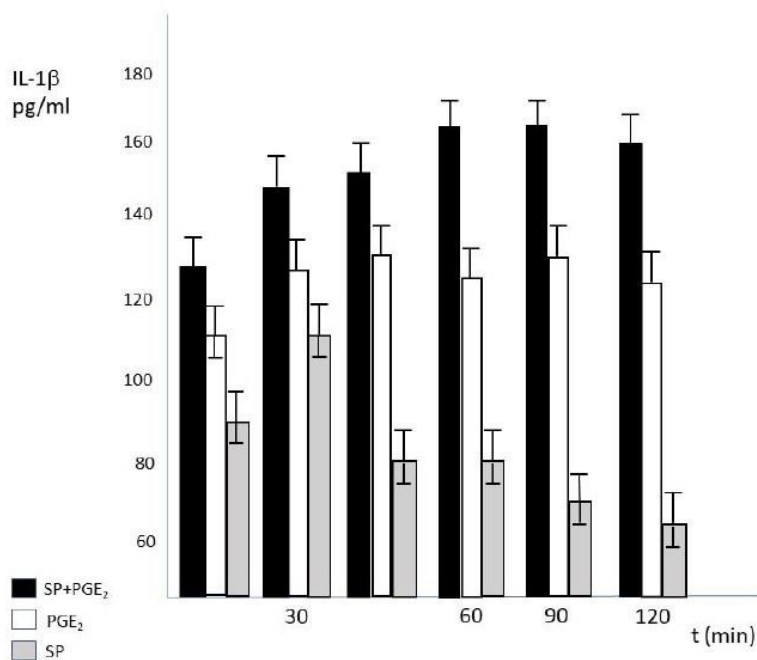
## DISCUSSION

SP mediates the pathogenesis of inflammatory diseases such as psoriasis, where increased levels of this neuropeptide have been found (9). In fact, IL-33 has been reported to enhance the effect of SP inducing vascular endothelial growth factor (VEGF) secreted by MCs, causing increased vascular permeability (10).

Prostaglandins (PGs) are eicosanoids, fatty acids with 20 carbon atoms, and are ubiquitous in the human body. PGs are lipids synthesized from arachidonic acid via constitutive cyclooxygenase-1 (COX-1), or COX-2, which is synthesized after trauma or stimuli. The inflammatory stimulus leads to the activation of phospholipase A2 (PLA2) with release of arachidonic acid and the expression of COX2 (11). PGE2 is a pentanoid PG, the most abundant PG in the human body. It is an important mediator of inflammation, and its pharmacological inhibition represents an important therapeutic strategy. In fact, PGE2, as reported by Sir John Vane in 1971, is inhibited by non-steroidal anti-inflammatory drugs through suppression of the cyclooxygenase enzyme (12). PGE2 is a protector of gastric mucosa and is a mediator of pain, inflammation, fever, and platelet aggregation (13). Gastric erosion and ulcers can occur when PGE2 is inhibited by anti-inflammatory drugs. Therefore, PGE2 plays a protective role in the gastrointestinal tract. In contrast, when PGE2 levels are increased in tissue, it is a strong mediator of inflammation, pain, and fever.

It is known that IL-1 and other inflammatory mediators can regulate the proliferation and differentiation of a number of human cells including immune cells. The modulation of inflammatory mediators can be very useful in therapy for immune and inflammatory diseases. IL-1 is produced by activated monocytes and macrophage cells following inflammatory stimulus, while the levels are low or absent in healthy subjects.

In our study, we used Wistar rats that were put to sleep with CO2, and afterwards, PGE2, SP, and the combination of PGE2 plus SP were injected into the footpads of the rats. After 30, 60, 90, and 120 minutes, inflamed tissue and controls (untreated rat paw) were removed. Pieces of inflamed tissue and controls were minced, placed in medium, stirred for 30 min, and the supernatant was collected for IL-1 testing. The supernatant was filtered and the levels of IL-1 $\beta$  were calculated by ELISA method. The ELISA reader was set at 405 nm absorbance, all the samples were read at room temperature, and the standard curve was constructed. All samples were assayed in triplicate. Results are expressed as pg/ml ( $\pm$  SD) (Fig.3).



**Fig. 3.** In this figure we report the generation of IL-1 after treatment with PGE2, SP, and SP+PGE2 in combination. Here it is shown that the combination of SP+PGE2 generates larger amounts of IL-1 compared to PGE2 and SP alone.

## CONCLUSIONS

In this short article, we report that inflammation occurs when SP and PGE2 are injected alone and in combination into the rat footpad, when compared to the untreated control group. The results have shown that PGE2 is highly inflammatory,

while SP is moderately inflammatory, and when both are combined, levels of IL-1 are higher than PGE2 alone. This effect was shown in all time periods (30 min intervals) of treatment. The inflammatory effect of PGE2 is higher than that of SP because these two compounds act in different ways. The PGE2 plus SP combination is highly inflammatory, higher than PGE2 administered alone, suggesting that SP potentiates the activity of PGE2. In addition, these compounds were found to provoke the generation of IL-1 (Fig.3).

Here, we show that the neurotransmitter SP acts as an inflammatory compound in the first 30 minutes after injection. Afterwards, the inflammatory effect of SP subsequently decreases, while the arachidonic acid product PGE2 remains highly inflammatory. When these two compounds are injected in combination at the same time into the plantar of the rat paw, the inflammatory effect and the level of IL-1 was higher than PGE2 administered alone.

#### *Conflict of interest*

The author declares that they have no conflict of interest.

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