European Journal of Neurodegenerative Diseases
ISSN 2279-5855 / 2019 vol. 8, ISSUE N. 2, July-December

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

D. Lauritano

Department of Medicine and Surgery, Centre of Neuroscience of Milan, University of Milano-Bicocca, Milan, Italy

**Correspondence to*:
Dorina Lauritano, MD
Department of Medicine and Surgery,
Centre of Neuroscience of Milan,
University of Milano-Bicocca,
Milan, Italy
e-mail: dorina.lauritano@unimib.it

KEYWORDS: *Substance P, PGE2, inflammation, IL-1, mast cells, immunity*

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-1β when it is activated with SP for 24 hours (3). When activated by IgE through their FcεRI receptors, MCs immediately release inflammatory mediators (4) (Fig. 1).

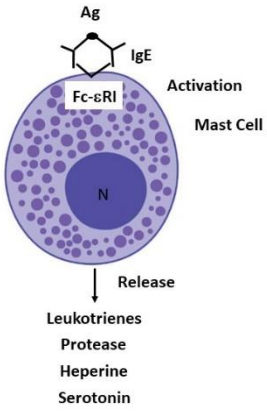

Fig. 1. *This figure depicts the antigen activation mast cell through IgE receptor, releasing leukotrienes, protease, heperine and serotonin.*

Received: 20 May 2019
Accepted: 02 July 2019

ISSN 2279-5855 (2019)

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-1β (10 times more than cells treated with IL-1β 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-1β and causing inflammation (Fig. 2).

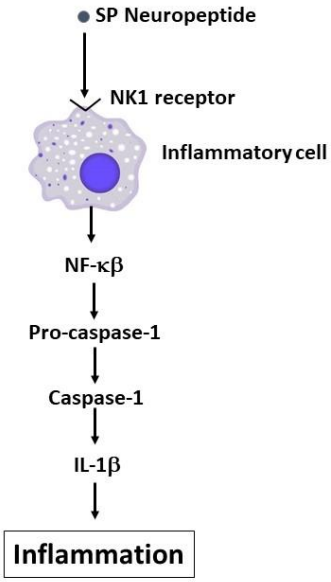

Fig. 2. *Substance P neuropeptide binds its receptor NK1 and activates inflammatory cells to generate IL-1 and therefore inflammation.*

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-1β 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 (± SD) (Fig. 3).

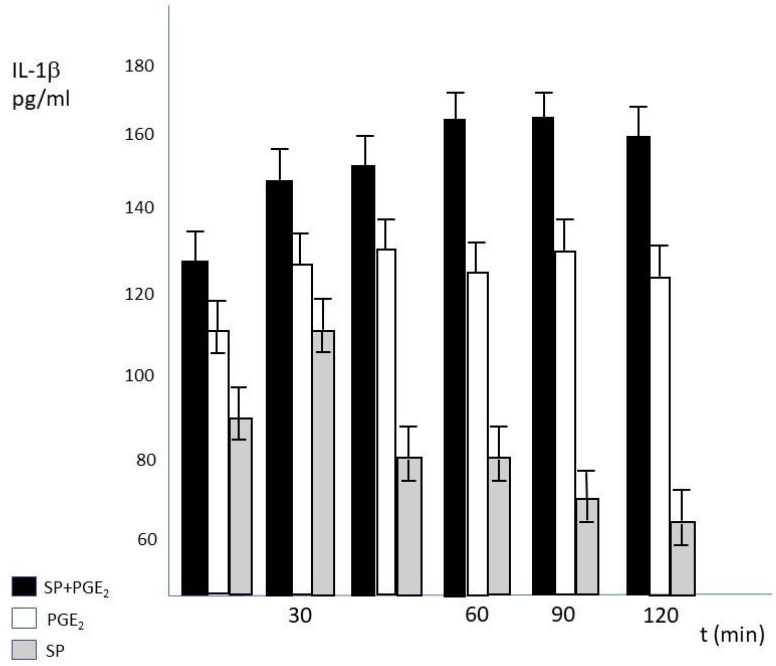

Fig. 3. *In this figure we report the generation of IL-1 after treatment with PGE_2, SP, and SP+PGE_2 in combination. Here it is shown that the combination SP+PGE2 generate larger amounts of IL-1 compared to PGE2 and SP alone.*

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.

REFERENCES

1. v. Euler US, Gaddum JH. An unidentified depressor substance in certain tissue extracts. *The Journal of Physiology*. 1931;72(1):74-87. doi:https://doi.org/10.1113/jphysiol.1931.sp002763
2. Chang MM, Leeman SE. Isolation of a Sialogogic Peptide from Bovine Hypothalamic Tissue and Its Characterization as Substance P. *Journal of Biological Chemistry*. 1970;245(18):4784-4790. doi:https://doi.org/10.1016/s0021-9258(18)62860-1

3. Taracanova A, Tsilioni I, Conti P, Norwitz ER, Leeman SE, Theoharides TC. Substance P and IL-33 administered together stimulate a marked secretion of IL-1β from human mast cells, inhibited by methoxyluteolin. *Proceedings of the National Academy of Sciences of the United States of America*. 2018;115(40):E9381-E9390. doi:https://doi.org/10.1073/pnas.1810133115
4. Huber M. Activation/Inhibition of mast cells by supra-optimal antigen concentrations. *Cell Communication and Signaling*. 2013;11(1):7. doi:https://doi.org/10.1186/1478-811x-11-7
5. Denes A, Lopez-Castejon G, Brough D. Caspase-1: is IL-1 just the tip of the ICEberg? *Cell Death & Disease*. 2012;3(7):e338-e338. doi:https://doi.org/10.1038/cddis.2012.86
6. Delgado AV, McManus AT, Chambers JP. Production of Tumor Necrosis Factor-alpha, Interleukin 1-beta, Interleukin 2, and Interleukin 6 by rat leukocyte subpopulations after exposure to Substance P. *Neuropeptides*. 2003;37(6):355-361. doi:https://doi.org/10.1016/j.npep.2003.09.005
7. Ho WZ, Kaufman D, Uvaydova M, Douglas SD. Substance P augments interleukin-10 and tumor necrosis factor-α release by human cord blood monocytes and macrophages. *Journal of Neuroimmunology*. 1996;71(1-2):73-80. doi:https://doi.org/10.1016/s0165-5728(96)00132-4
8. Taracanova A, Alevizos M, Karagkouni A, et al. SP and IL-33 together markedly enhance TNF synthesis and secretion from human mast cells mediated by the interaction of their receptors. *Proceedings of the National Academy of Sciences of the United States of America*. 2017;114(20):E4002-E4009. doi:https://doi.org/10.1073/pnas.1524845114
9. Naukkarinen A, Nickoloff BJ, Farber EM. Quantification of Cutaneous Sensory Nerves and Their Substance P Content in Psoriasis. *Journal of Investigative Dermatology*. 1989;92(1):126-129. doi:https://doi.org/10.1111/1523-1747.ep13071340
10. Theoharides TC, Zhang B, Kempuraj D, et al. IL-33 augments substance P–induced VEGF secretion from human mast cells and is increased in psoriatic skin. *Proceedings of the National Academy of Sciences*. 2010;107(9):4448-4453. doi:https://doi.org/10.1073/pnas.1000803107
11. Leistad L, Feuerherm A, Faxvaag A, Johansen B. Multiple phospholipase A2 enzymes participate in the inflammatory process in osteoarthritic cartilage. *Scandinavian Journal of Rheumatology*. 2011;40(4):308-316. doi:https://doi.org/10.3109/03009742.2010.547872
12. Vane JR. Inhibition of Prostaglandin Synthesis as a Mechanism of Action for Aspirin-like Drugs. *Nature New Biology*. 1971;231(25):232-235. doi:https://doi.org/10.1038/newbio231232a0
13. Ricciotti E, FitzGerald GA. Prostaglandins and Inflammation. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2011;31(5):986-1000.