

## SUMMARY

Considering the multiple functions of nitric oxide (NO) as a mediator of interleukin-1 (IL-1)-induced responses, especially those concerning the pathophysiology of arthritic diseases, it is clearly important to understand the mechanisms that regulate its synthesis, both from a pathophysiological and a therapeutic point of view. Therefore, the main objective of this work was to study the role of the transcription factors Nuclear Factor-kappaB (NF- $\kappa$ B) and Activator Protein-1 (AP-1) on the expression of the inducible isoform of the NO synthase (NOS II), induced by IL-1 in articular chondrocytes. In conjunction with this major goal, we also studied the role of reactive oxygen species (ROS), protein tyrosine kinases (PTK) and mitogen-activated protein kinases (MAPK) as mediators of those IL-1-induced responses, in articular chondrocytes. Since, in different cells, NO itself can either induce or repress NF- $\kappa$ B and AP-1 activity, as well as NOS II expression, we also sought to identify positive or negative feedback mechanisms involved in the regulation of NOS II expression, in articular chondrocytes. Thus, we studied the ability of NO to activate those two transcription factors and to modulate NOS II expression induced by IL-1. Finally and to further elucidate their mechanism of action, we investigated the ability of diacerein and rhein, two drugs that exert beneficial effects in the development and course of osteoarthritis, to prevent IL-1-induced NF- $\kappa$ B activation and NOS II expression, in articular chondrocytes.

Using primary confluent cultures of bovine articular chondrocytes, we found that:

- NF- $\kappa$ B mediates IL-1-induced NOS II expression, since all the compounds that prevented IL-1-induced activation of this transcription factor, were equally effective in preventing NOS II mRNA and protein synthesis;
- the superoxide anion and PTK, possibly also including the “Janus kinase 2”, are indispensable for NF- $\kappa$ B activation and for the subsequent expression of NOS II in response to IL-1;

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- the p38<sup>MAPK</sup> is required for NOS II expression, but not for NF-κB activation. Nevertheless, this kinase may be required for NF-κB to acquire transcriptional activity, or otherwise, it may mediate the activation of another transcription factor also essential for IL-1-induced NOS II expression;
- NO inhibits IL-1-induced IκB-α degradation, thereby preventing NF-κB activation and the subsequent expression of NOS II. These results, together with the observation that IL-1 induces the degradation of the constitutive NOS I protein, indicate that NO is involved in a negative feedback mechanism that may prevent accidental, inappropriate or even prolonged NF-κB activation and NF-κB-dependent gene transcription and that may also restrict the activation of this transcription factor in response to a second incoming stimulus;
- diacerhein and rhein inhibit IL-1-induced IκB-α degradation, thus preventing NF-κB activation and the subsequent expression of NF-κB-dependent genes, namely NOS II. These actions of the two drugs can explain, at least in part, their anti-osteoarthritic effects and suggest that diacerhein and rhein may also exert anti-inflammatory and chondroprotective actions, thus being potentially useful in the treatment of inflammatory types of arthritis;
- ROS, particularly H<sub>2</sub>O<sub>2</sub>, and NO mediate IL-1-induced AP-1 activation. Nevertheless, this transcription factor does not modulate NOS II expression, either as an inducer or as a repressor, because inhibition of IL-1-induced AP-1 activation had no effect on NOS II expression and the presence of active AP-1 dimers, in the cell, did not modify the response to IL-1. The temporal gap between H<sub>2</sub>O<sub>2</sub> and NO production, in response to IL-1, suggests that H<sub>2</sub>O<sub>2</sub> is the initial mediator of IL-1-induced AP-1 activation which, later, can be maintained by the NO being produced by the newly synthesized NOS II. Hence, these results suggest that H<sub>2</sub>O<sub>2</sub> can initiate IL-1-induced AP-1-dependent gene transcription and that NO may ensure the prolonged expression of those genes.

In conclusion, the results presented identify some mechanisms relevant to the regulation of NF- $\kappa$ B and AP-1 activity, as well as to the expression of NOS II induced by IL-1, in articular chondrocytes. Considering the role of these two transcription factors in the expression of genes involved in inflammation and cartilage degradation, including NOS II, the inhibition of ROS production may be therapeutically useful by simultaneously exerting anti-inflammatory and chondroprotective actions.

The results obtained also emphasize two opposite roles of NO: on one hand, its ability to activate AP-1 and on the other, its efficacy as a NF- $\kappa$ B inhibitor. These dual functions of NO raise questions as to its true role in the pathophysiology of arthritic diseases. As suggested by Colasanti and Suzuki (2000), the classical concept that low concentrations of NO, produced by the constitutive isoforms, are involved in physiological processes, whereas high concentrations, resulting from the activity of NOS II, participate in pathological responses, is clearly insufficient to explain the molecular mechanisms that regulate both the synthesis and the actions of NO. Despite the huge interest devoted to the research in all areas involving NO, this small mediator still raises numerous questions, whose answers will, certainly, contribute to a better understanding of the pathophysiological processes in which it is involved and, consequently, will allow for the development of more effective therapies that can modulate and take advantage of its multiple capabilities.