Molecular Neurobiology

ISSN0893-7648/02/25(2): 133–147/$13.75

Role of the Nitric Oxide/Cyclic GMP/Ca^{2+} Signaling Pathway in the Pyrogenic Effect of Interleukin-1β

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Abstract

Interleukin-1β (IL-1β) has a wide spectrum of inflammatory, metabolic, haemopoietic, and immunological properties. Because it produces fever when injected into animals and humans, it is considered an endogenous pyrogen. There is evidence to suggest that Ca^{2+} plays a critical role in the central mechanisms of thermoregulation, and in the intracellular signaling pathways controlling fever induced by IL-1β and other pyrogens. Data from different labs indicate that Ca^{2+} and Na^{+} determine the temperature set point in the posterior hypothalamus (PH) of various mammals and that changes in Ca^{2+} and PGE_2 concentrations in the cerebrospinal fluid (CSF) of these animals are associated with IL-1β-induced fever. Antipyretic drugs such as acetylsalicylic acid, dexamethasone, and lipocortin 5-(204–212) peptide counteract IL-1β-induced fever and abolish changes in Ca^{2+} and PGE_2 concentrations in CSF. In vitro studies have established that activation of the nitric oxide (NO)/cyclic GMP (cGMP) pathway is part of the signaling cascade transducing Ca^{2+} mobilization in response to IL-1β and that the ryanodine (RY)- and inositol-(1,4,5)-trisphosphate (IP_3)-sensitive pools are the main source of the mobilized Ca^{2+}. It is concluded that the NO/cGMP/Ca^{2+} pathway is part of the signaling cascade subserving some of the multiple functions of IL-1β.

Index Entries: Interleukin-1β; fever; intracellular Ca^{2+}; nitric oxide; cyclic GMP; intracellular Ca^{2+} stores; human astrocytoma cells; rat striatum; neurotoxicity.

Ionic Theory for Temperature Set Point

Regulation of cell-membrane activity in general and neuronal functions in the central nervous system (CNS) depends partially on the metabolism of Ca^{2+}. In the early 1970s, experimental evidence indicated that Ca^{2+} plays an important role in the central mechanisms of thermoregulation. During studies in which the cerebral ventricles of unrestrained cats were perfused, Feldberg, Myers, and co-workers showed that changes in Ca^{2+} concentrations ($[Ca^{2+}]$) in brain tissue affected thermo-regula-

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tion. Omission of Ca^{2+} from medium perfusing from the lateral ventricles to the *cisterna magna* resulted in a rise in body temperature in unanesthetized cats and monkeys, whereas perfusion of the posterior hypothalamus (PH) with high [Ca^{2+}] produced a sharp fall in body temperature accompanied by vasodilation and reduced activity in rabbits and cats. In contrast, intense hyperthermia was observed when endogenous Na^+ was artificially increased in the hypothalamus *(1–3).* These observations led to the hypothesis that the ratio of Na^+ to Ca^{2+} within the PH is the basic factor determining the temperature set point *(4–6),* an inherently established, built-in reference temperature at about 37°C in many mammals, around which regulatory adjustments are made. According to this hypothesis, during fever, pyrogenic signals from the periphery are conveyed to the thermosensitive zone of the hypothalamus, namely the anterior hypothalamic-preoptic area (AH/POA). Generated impulses in this area are relayed to PH to shift upward the Na^+ to Ca^{2+} ratio, which, in turn, activates a coordinated set of physiological responses, such as metabolic heat production, shivering, and vasoconstriction to rise body temperature. Furthermore, shifting of the set point triggers a neuronal mechanism also located in the AH/POA to maintain the new temperature by regulatory processes.

Essentially, when the ionic milieu of the PH was perturbed by perfusion of excess Na^+ or Ca^{2+}, the body temperature of the animal underwent an immediate change that persisted as long as the ion imbalance was maintained. The ratio of Na^+ to Ca^{2+} is functionally and anatomically specific and the functional effect of the ions is universal across the species. In addition, when the temperature set-point was reset to a new high or low level by continual perfusion of either cation, the animal maintained this new set point in response to hot or cold ambient temperature *(7).*

Other experimental tests showed that the efflux of endogenous membrane cations was altered in relation to body temperature, corroborating the ionic theory. Using radiotracers in cats, it was demonstrated that a bacterial pyrogen evoked a reciprocal efflux of $^{22}Na^+$ and $^{45}Ca^{2+}$ as fever developed *(8)* and that an antipyretic reversed the efflux kinetics of the two cations *(9).* The flux of Ca^{2+} was specifically shifted by local warming or heating of the AH/POA, by neurotransmitters that evoke a rise or fall in core temperature and by severe challenges of ambient temperature *(7,10,11).* Even an anesthetic that impairs regulatory and set-point mechanisms, had an equally powerful impact on the kinetics of Ca^{2+} flux within the PH when applied directly to the AH/POA *(7).*

In the decade following formulation of this hypothesis, pharmacological experiments confirmed the ionic theory of the hypothalamic set point but revealed a far more complex relationship between the anterior and posterior hypothalamic areas than had previously been believed. Thus, direct injection of Ca^{2+} antagonist, such as verapamil, differentially altered body temperature in cats depending on the site of injection; hypothermia was elicited when injection was in the AH and hyperthermia when it was in the PH *(12,13).* The authors of this article showed that intracerebroventricular (icv) infusion of verapamil, nifedipine, and cinnarizine evoked hyperthermia in rabbits, whereas Bay K-8644, a dihydropyridine Ca^{2+} channel agonist, elicited a dose-related hypothermic response *(14).*

Role of Interleukin-1 in Fever

Interleukin 1 (IL-1), one of the first identified members of a group of peptides now known as the cytokines, was discovered in the 1940s as a heat-labile protein in acute granulocytic exudate fluid, which had a wide spectrum of biological activities largely related to the immune and inflammatory functions (Fig. 1). When injected into animals or humans, it produced fever and was therefore referred to as an endogenous pyrogen. Fever caused by conditions as diverse as infections or one of the

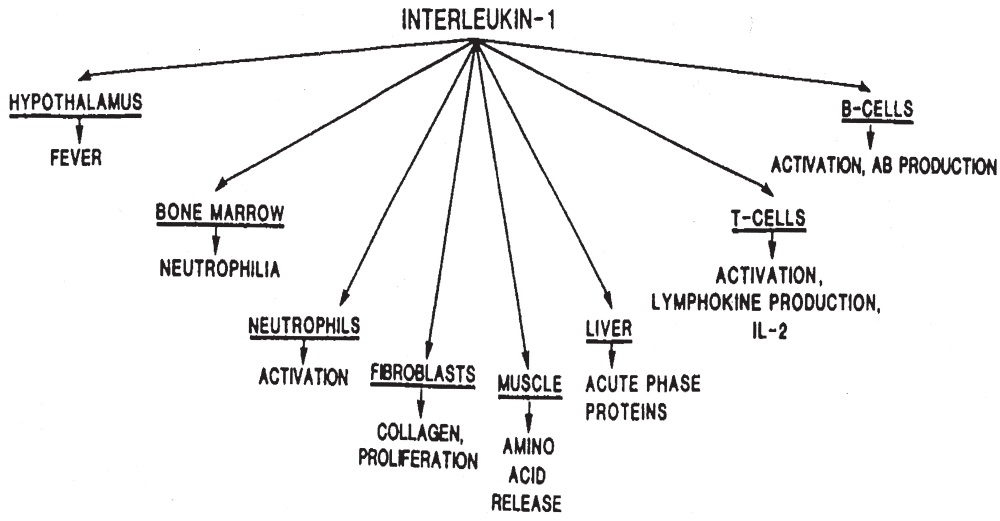

Fig. 1. The multiple biologic activities of interleukin-1. AB, antibody; IL-2, interleukin-2. Reprinted with permission from C. A. Dinarello, *New Engl. J. Med.,* **311,** 1413–1418. Copyright 1984, Massachusetts Medical Society.

sequelae of tissue damage, inflammation, graft rejection, malignancy, and other disease states, has the common feature of enhanced formation of cytokines such as IL-1β, IL-6, interferons-α and -β (IFN-α, IFN-β), and tumor necrosis factor-α (TNF-α).

The pyrogenic effect of these cytokines, and especially IL-1 β, which appears to be one of the most active in this respect, was clearly established to lie in their ability to induce synthesis of prostaglandin E_2 (PGE_2) in circumventricular organs in and near the AH/POA, which in turn triggers the hypothalamus to elevate body temperature, increasing heat generation and decreasing heat loss *(15–18).*

Nonsteroid anti-inflammatory drugs (NSAIDs) suppress this response by inhibiting synthesis of PGE_2 *(19).* Further evidence for this scenario includes the ability of prostaglandins, especially PGE_2, to raise body temperature when infused into the cerebral ventricles or hypothalamus. NSAIDs reduce fever caused by IL-1 and other enhancers of cytokines but do not reduce fever caused by prostaglandins *(20),* suggesting that NSAIDs act by inhibiting PGE_2 synthesis. Although there is now general agreement on the nature of the final mediators of fever, the cellular process transducing pyrogen signaling as well as the role of Ca^{2+} in this context needed to be clarified.

Role of Ca^{2+} Signaling in IL-1-Induced Fever

We investigated the dynamics of Ca^{2+} in CSF during fever of different origins in rabbits with permanent cannulae implanted in the lateral ventricle and *cisterna magna.*

After surgery, these animals presented a wide range of body temperatures (from normothermia to 41°C), different increases in CSF [Ca^{2+}] (Fig. 2A) and a highly significant correlation between these two parameters *(21).* A linear correlation between temperature gain and increase in CSF [Ca^{2+}] was also found when fever was induced in rabbits by icv injection of human recombinant (hr) IL-1β (Fig. 2B) *(22).* The latter experiment showed an 8.5% (0.12 m*M*) increase in [Ca^{2+}] per unit change in temperature, which substantially agrees with data on spontaneous

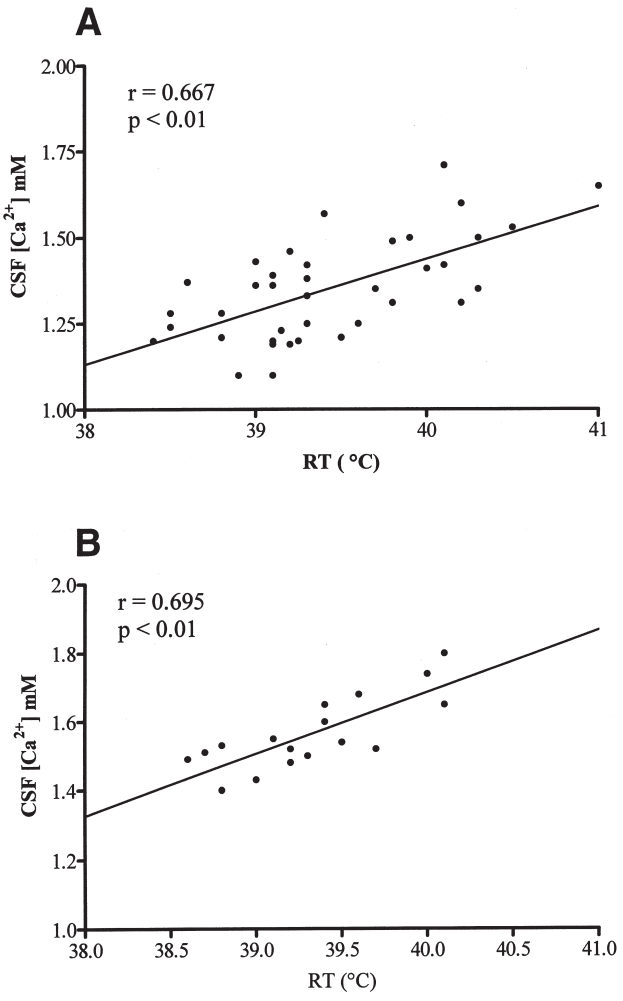

Fig. 2. Correlation between rectal temperature (RT) and cerebrospinal fluid Ca^{2+} concentration (CSF $[Ca^{2+}]$) in rabbits with fever induced by **(A)** surgery (implantation of permanent cannulae in the brain) or **(B)** intracerebroventricular injection of human recombinant IL-1β. r is the correlation coefficient and p the significance of r. Reprinted with permission from refs. *(21)* and *(22).*

postsurgical fever in rabbits, where the average increase in $[Ca^{2+}]$ per unit change in body temperature was 0.15 m*M*, which is a 10% variation in $[Ca^{2+}]$. A close relationship was also found between body temperature, $[Ca^{2+}]$ and PGE_2 production in CSF of rabbits chronically incannulated and perfused with human recombinant (hr) IL-1β from the lateral ventricle to the *cisterna magna.* Perfusion with artificial CSF containing the Ca^{2+} chelator Ethylene glycol-bis (β aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA) blocked the IL-1β-induced elevation in PGE_2 production and concomitantly the rise in body temperature in these animals. A dose-dependent inhibition of the latter response as well as that related to Ca^{2+} and PGE_2 in CSF was also observed when these animals were pretreated with dexamethasone *(22),* which has anti-inflammatory as well as antipyretic properties *(23).*

Icv administration of a nonpyrogenic 9-residue synthetic fragment, corresponding to the highly hydrophilic 163–171 portion of the hr IL-1β-molecule, did not modify CSF $[Ca^{2+}]$, whereas icv injection of PGE_2 promoted an increase in body temperature but failed to modify CSF $[Ca^{2+}]$. This indicated that Ca^{2+} involvement in the signaling pathway leading to fever is upstream of PGE_2 synthesis *(21).* Finally, acetylsalicylic acid (ASA) counteracted fever induced by both endotoxin and hr IL-1β and at the same time abolished increases in CSF $[Ca^{2+}]$

Taken together, all these findings point to a role for brain Ca^{2+} in the mechanism of fever induction that is independent of the inducing agent, and indicate that lowering of brain $[Ca^{2+}]$ may represent an additional mechanism of inhibition of prostaglandin synthesis by NSAIDs. The rate-limiting step in prostanoid synthesis is known to be the availability of arachidonate precursor released by cell phospholipids upon activation of phospholipidase A_2 (PLA_2) *(24).* PLA_2 enzyme activity depends on Ca^{2+} *(25),* and its role in inflammation and eicosanoid generation, reviewed by Glaser et al. *(26),* suggests that increased CSF $[Ca^{2+}]$ activates extracellular PLA_2 leading to enhanced PGE_2 synthesis. Experimental evidence exists that IL-1 and glucocorticoid have opposite effects on PLA_2 enzymes, with IL-1 stimulating expression, release, and activity of PLA_2 *(27–29)* and glucocorticoids blocking mRNA synthesis, post-transcriptional expression of

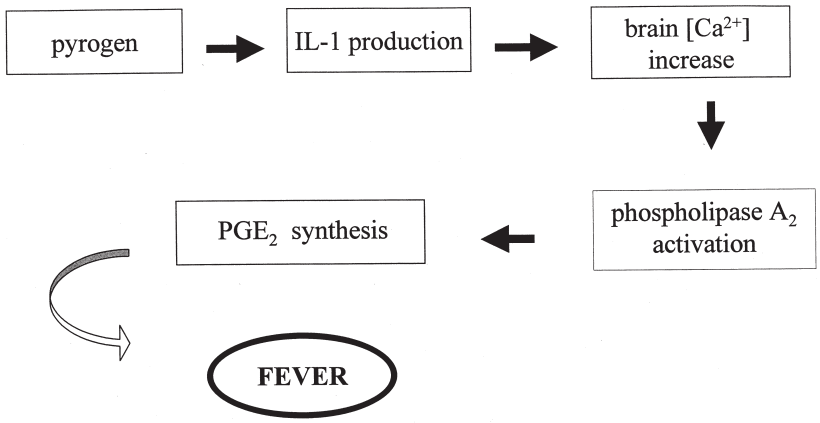

Fig. 3. Schematic representation of fever pathway induced by pyrogens. IL-1, Interleukin-1; PGE_2, Prostaglandin E_2.

PLA_2 *(30,31)*, and enzyme activity by inducing synthesis of lipocortins (annexins), a family of calcium and phospholipid-binding proteins that mediate some of the anti-inflammatory effects of glucocorticoids *(32–36)*.

Reverse modulation of Ca^{2+} by IL-1 and glucocorticoids may be an additional regulatory mechanism of extracellular PLA_2 enzymes and hence a way by which this cation controls fever production. It therefore seems likely that changes in brain Ca^{2+} control the febrile response, via regulation of PLA_2 activity by the following sequence of events (Fig. 3)

Although there is strong evidence in support of this pathway, the intracellular signaling that brings about increased [Ca^{2+}] in CSF after cytokine interaction with the cell membrane is unclear, and is hypothesized to occur via changes in the dynamics of Ca^{2+} fluxes through the cell membrane. By perfusing $^{45}Ca^{2+}$ preloaded striatal slices with IL-1β, we showed that the rate of spontaneous $^{45}Ca^{2+}$ efflux from this tissue was indeed modified. This effect was dose-dependent, delayed in onset, and long-lasting. The concomitant presence of IRAP, a polypeptide that specifically antagonizes IL-1 receptor binding *(38)*, inhibited the Ca^{2+} response to IL-1β, indicating a selective and receptor-mediated mechanism (Fig. 4) *(37)*.

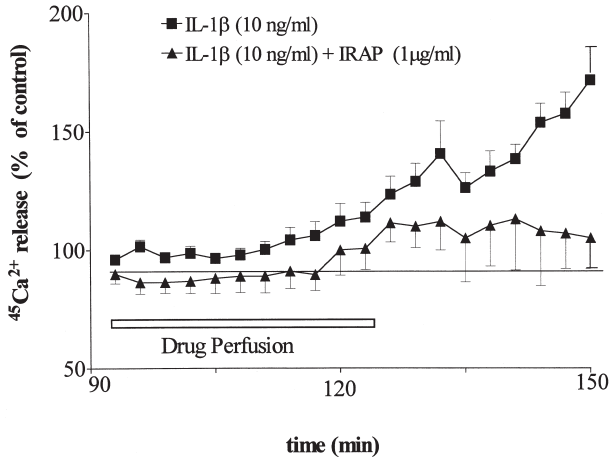

Fig. 4. Effect of human recombinant IL-1β and IL-1β plus IRAP (IL-1β receptor antagonist polypeptide) on Ca^{2+} release from rat striatal slices. Reprinted with permission from ref. *(37)*.

These data received further support from studies showing that IL-1β promoted an increase in [Ca^{2+}] in pre-B-like cells and human fibroblasts and that, in the latter, the effect was antagonized by antibody specific for anti-type 1 IL-1 receptors *(39,40)*. Moreover, the dose at which IRAP antagonized the activity of cytokine in various cell types *(41–43)* and ani-

mal models *(44–46)* was consistent with the range of concentrations required to antagonize the Ca^{2+} response in our experiments. The same IRAP-to-IL-1 dose ratio was found to almost completely counteract the fever induced in rabbits by iv injection of IL-1 *(47).*

Although the possibility remains that in vivo, IL-1 enhances the access of blood Ca^{2+} to the brain by modifying pemeability of the blood-brain barrier (BBB), our in vitro model, showed that IL-1 induced a rise in $^{45}Ca^{2+}$ efflux from rat striatal slices, indicating that one source of Ca^{2+} that contributes to its increase in CSF is certainly brain tissue.

The presence of caffeine in the perfusing medium potentiated IL-1-induced release of Ca^{2+}, whereas excess EGTA and the Ca^{2+} channel blocker, nifedipine, abolished the potentiating effect of caffeine. Caffeine had no effect when tissues were perfused with Ca^{2+}-free medium, indicating that the availability of extracellular Ca^{2+} is of primary importance for the booster effect of caffeine. Caffeine is known to activate the Ca^{2+}-induced Ca^{2+} release (CICR) process, a Ca^{2+}-regenerative releasing mechanism by which Ca^{2+} itself amplifies its own release *(48,49).* Since rapid removal of external Ca^{2+} prevented the caffeine-induced spikes right up to the onset of the regenerative process in sympathetic ganglion neurons, external Ca^{2+} was concluded to play a role in activating the CICR process *(48–53).* Accordingly, the difference in amplitude of Ca^{2+} mobilization between IL-1 plus caffeine and IL-1 alone observed in our experiments is due to the presence of extracellular Ca^{2+} and its boosting effect on the CICR process.

Modulation by Nitric Oxide of IL-1-Induced Ca^{2+} Signaling

The Ca^{2+} response to IL-1 with its lag time and long-lasting profile indicated the presence of an intermediate messenger mediating this effect. Fertilization studies have shown that a "sperm factor," later identified as cyclic ADP ribose (cADPR), induced a release of Ca^{2+} from caffeine/ryanodine-sensitive stores when added to sea urchin and golden hamster eggs *(52,54,55).* On one hand, the formation of this NAD^+ derivative was enhanced by cyclic GMP (cGMP) *(48),* on the other, nitric oxide (NO) upregulated synthesis of cGMP. These findings and the notion that IL-1 induces expression of the inducible isoform (iNOS) of NO synthase, suggest a link between cADPR and NO on the one hand and IL-1 and Ca^{2+} release on the other. IL-1 may therefore upregulate cGMP synthesis via intermediate synthesis of NO and hence enhance levels of cADPR mobilizing Ca^{2+} from caffeine/ryanodine-sensitive intracellular stores (Fig. 5).

Further support to the hypothesis that NO is the intermediate messenger mediating the IL-1-evoked Ca^{2+} response is the typical kinetic pattern of IL-1-induced NO production in neurons *(56)* and other cell types *(57,58),* which mirror the profile of Ca^{2+} release during IL-1 stimulation in our experiments. NO is also involved in functions and molecular mechanisms controlling Ca^{2+} homeostasis in many different cell systems *(59),* and increased synthesis/release of nitrite and nitrate, the breakdown products of NO, have been found in patients with fever *(60)* or septic shock *(61).* Another relevant observation was that dexamethasone inhibited induction of NOS *(62)* and, as we previously reported, antagonized the fever and the increase in CSF [Ca^{2+}] induced by IL-1β.

A series of experiments carried out by us with striatal slices and astroglial U-373 MG cells demonstrated that NO did in fact mediate the Ca^{2+} response elicited by IL-1β *(63).* Thus in rat striatum, where a population of NOS-containing neurons has been demonstrated *(64,65),* an increase in substrate (L-arginine) availability for NOS potentiated the effect of IL-1β on Ca^{2+} release, whereas the competitive NOS inhibitor L-NAME dose-dependently antagonized this effect. Two different NO donors, diethylamine/NO and spermine/NO

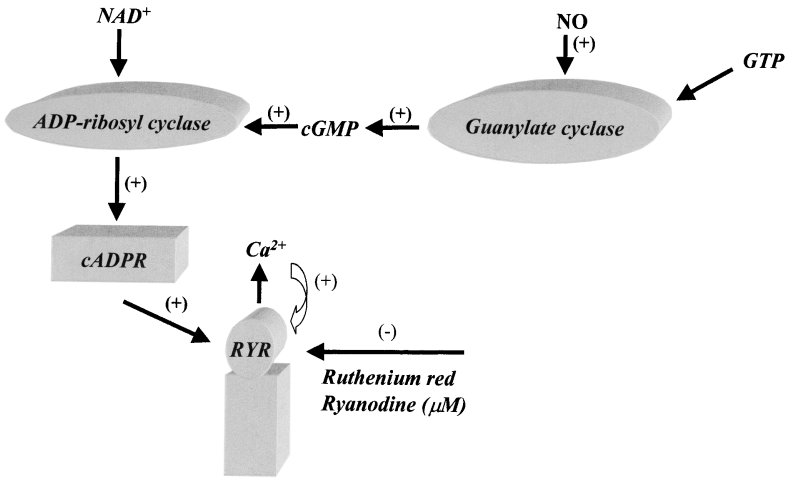

Fig. 5. Schematic representation of signaling pathway activating the ryanodine receptor (RYR). NO, nitric oxide; cADPR, cyclic ADP ribose; cGMP, cyclic GMP. (+) activation, (–) inhibition.

complexes *(66,67)*, mimicked the effect of IL-1β on Ca^{2+} efflux. The kinetic profiles of NO release from these two NO donors showed faster release of NO by diethylamine than by spermine, which resulted in differences in their relative effects on tissue Ca^{2+} response. Thus spermine/NO induced rapid release of Ca^{2+}, whereas an equivalent concentration of diethylamine/NO gave rise to a delayed effect similar to that induced by IL-1β. Data from spermine/NO experiments revealed a dual role of NO in the Ca^{2+} response, which depended on the amount released. Low concentrations of NO therefore stimulated and excessive NO levels inhibited Ca^{2+} release (Fig. 6). Although the mechanism underlying this effect is not known, this finding is of great importance for the different and often opposite effects on a number of cell systems and physiopathological processes attributed to NO. Thus NO appears to alternatively promote or inhibit inflammation, angiogenesis, and cancer *(68,69)*. In blood vessels and neurons, low NO concentrations transduce signals *(70)* but high concentrations damage cells *(71–73)*. Furthermore, in different cell types, both NO and cGMP were shown to be involved either in the

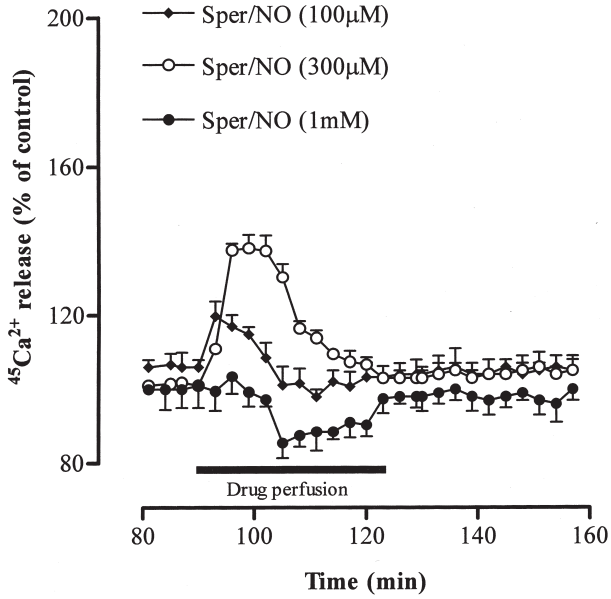

Fig. 6. Effect of different concentrations of Spermine/NO complex on the rate of Ca2+, release from rat striatal slices. Sper, spermine. Reprinted with permission from ref. *(63)*.

suppression *(74)* or in induction of apoptosis *(75,76)*. Similar to NO, changes in intracellular Ca^{2+} may alternatly block *(77)* or activate the cell cycle *(78)*.

Even though these controversial reports may, at least in part, be attributed to the concentration-dependent effect discussed earlier, further experiments are necessary to clarify the mechanism of NO-mediated modulation of cell Ca^{2+} signaling and to establish whether this may be part of a physiological process controlling the cell cycle and apoptosis.

Role of Cyclic GMP in IL-1-Induced Ca^{2+} Signaling

Many of the actions of NO in different tissues are elicited via activation of soluble guanylate cyclase. Although in the majority of these actions the precise signaling pathways involved are still primarily unknown, NO-mediated generation of cGMP and activation of a G kinase are generally accepted as part of the overall mechanism *(55)*.

We showed that the membrane-permeant analog of cGMP, dibutyryl-cGMP, induced a concentration-dependent increase in Ca^{2+} release from striatal slices with a time-course similar to that observed with IL-1β. Moreover, both IL-1β and the NO donor diethylamine/NO produced two- to threefold increases in cGMP, which preceeded the onset of Ca^{2+} release. Two selective inhibitors of guanyl cyclase, LY 83,583 and ODQ *(79–83)*, antagonized IL-1β-induced Ca^{2+} release but were only effective when the Ca^{2+} response was promoted by low, not high, IL-1β concentrations *(63)*. Although these data indicated that the IL-1β signaling cascade involved the synthesis of cGMP upstream of the Ca^{2+} response, the partial inhibition by LY 83,583 and ODQ at increasing cytokine concentrations suggested that the cGMP-dependent mechanism does not totally account for the effect of NO on Ca^{2+} release in the striatum and that besides the cGMP-dependent pathway, a cGMP-independent one may also operate. This meant direct interactions of NO with cellular and extracellular proteins or nitrosylation of receptors, or else production of NO-derived products, such as peroxynitrite *(84–86)* If higher IL-1β concentrations induced maximal Ca^{2+} release by either pathway, then inhibiting the cGMP-dependent pathway with guanyl cyclase inhibitors would not give, as observed by us, a modified response.

Role of Fast-Exchanging Ca^{2+} Pools in IL-Induced Ca^{2+} Signaling

The source of the Ca^{2+} released was the next aspect to investigate. Cells have two principal intracellular calcium channels responsible for mobilizing stored Ca^{2+}: inositol-(1,4,5)-trisphosphate (IP_3)- and ryanodine (RY)-sensitive receptors. In many cells, including neurons, these pools occupy specialized compartments of the endoplasmic reticulum and NO plays a key role in modulating intracellular Ca^{2+} release from IP_3- and RY-sensitive stores. Depending on the store type and cell system, NO may induce different and even opposite effects. NO inhibits Ca^{2+} release from IP_3-sensitive stores of smooth-muscle cells *(87)*, platelets *(88)*, and neurosecretory PC12 cells *(89)* while it enhances Ca^{2+} efflux in hepatocytes *(90)*. On the other hand, NO consistently activates release of Ca^{2+} from the RY-pool of urchin oocytes and PC12 cells *(59)*. To determine whether these stores were involved in Ca^{2+} response to IL-1β, we investigated the effect of inhibitors of IP_3- and RY-sensitive receptors, namely heparin and ruthenium red (RR), respectively. Combined administration of RR and heparin completely abolished the rise in Ca^{2+} release induced by IL-1β, whereas administration of RR alone only partially inhibited the Ca^{2+} response. Curiously, heparin alone did not affect the Ca^{2+} response to IL-1β.

Although these findings indicate that both these deposits contribute to the IL-1β Ca^{2+} response, they do not clearly address the issue of whether they are functionally separate or interacting subcellular compartments. One possibility is that Ca^{2+} released from RY stores is the priming event that triggers Ca^{2+} release from the other pool, via the known process of calcium-induced calcium release. Alternatively, the two pools may be regarded as independent Ca^{2+} stores with different intracellular locations *(91)* as shown in sea urchin eggs where the RY receptors are mostly concentrated in endoplasmic reticulum areas of the subplasmalemma cytoplasm and IP_3 receptors in the deep cytoplasm *(92)*. The much slower time-course of Ca^{2+} release in the presence of RR would be consistent with lower accessibility of the heparin-sensitive receptors.

Results obtained with human astrocytoma U-373 MG cells are in line with those obtained in the tissue slices and demonstrate that IL-1β increases intracellular [Ca^{2+}] and that L-NAME counteracts this effect. In contrast to the sustained effect observed in tissue, the Ca^{2+} response in astroglial cells was transient dropping to basal value after prolonged IL-1β stimulation (Fig. 7). In terms of the concentration-dependent effects of NO discussed earlier, the longer Il-1β stimulation in cells than in tissue could have resulted in sufficiently high steady-state NO levels to inhibit the Ca^{2+} response. Alternatively, a process resulting in inhibition of NO production in glial cells may be activated. A neurotrophic factor that markedly reduces NO release in glial cells and protects against ischemia-induced infarction in rat cerebral cortex has indeed been reported *(93)*.

Taken together, all these data demostrate that control of Ca^{2+} homeostasis by the NO signaling system, besides vascular cell physiology, solid tumor progression, and angiogenesis *(68,69)* plays a crucial role also in the pyrogenic response induced by IL-1β. The established sequence of events, previously shown, which from exogenous pyrogen leads to fever, now has to take more recent findings into account and as such can be redefined as shown in Fig. 8.

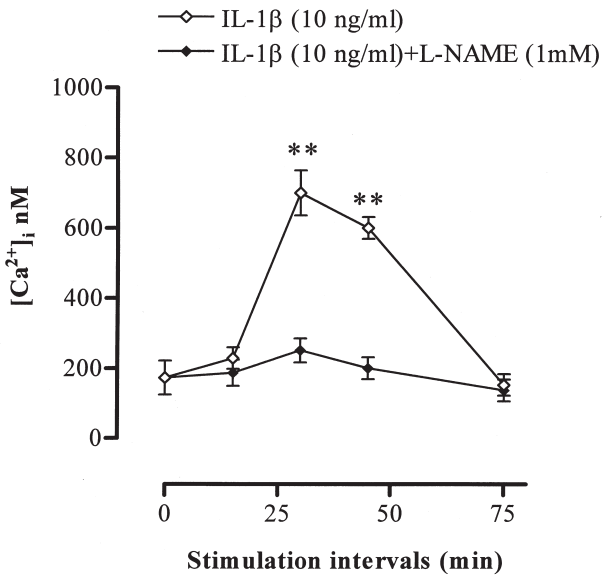

Fig. 7. Effect of IL-1β and IL-1β plus L-NAME on intracellular [Ca^{2+}] in human astrocytoma U-373 MG cells. Min, minutes; nM, nMolar. Reprinted with permission from ref. *(63)*.

Conclusions

After the discovery of the pyrogenic role of IL-1, it was found that, in addition to its well-known immune and inflammatory functions, this cytokine also had potent effects on neuronal, metabolic, behavioral, and neuroendocrine functions, all of which were related by direct actions on the CNS (Table 1). Moreover, there is increasing evidence that IL-1 contributes to many neurological responses, such as those in multiple sclerosis, AIDS, dementia complex, stroke, and Alzheimer's disease *(113,114)*. It is therefore possible that the NO/cGMP-dependent modulation of intracel-

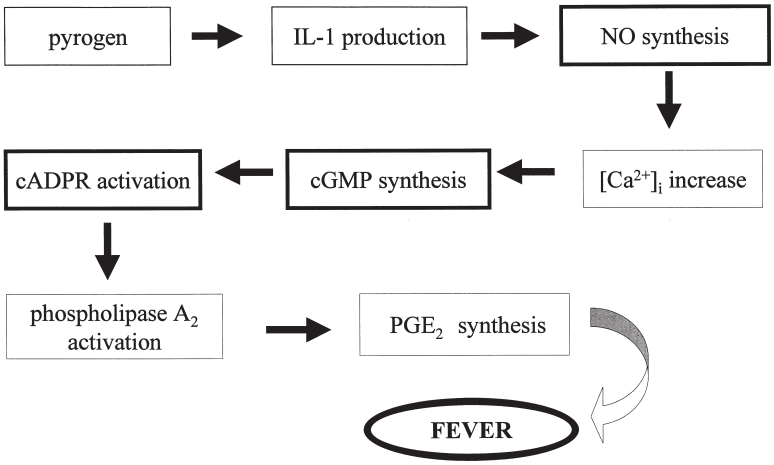

Fig. 8. Detailed representation from Fig. 3 of fever pathway induced by pyrogen.

Table 1
Central Action of IL-1

Action	References
Local role in brain	
Altered EEG and neuronal activity	*(94)*
Inhibition of long-term potentiation	*(95)*
Cortical inhibitory postsynaptic function	*(96)*
Neurotransmitter release/turnover	*(94)*
Induction of NGF	*(97)*
Self-induction (of IL-1β)	*(97)*
Astrogliosis	*(98)*
Neovascularization	*(98)*
Metabolic role	
Sympathetic activation of brown fat	*(99)*
Hypophagia	*(100)*
Altered gastric function	*(101)*
Endocrine role	
Hypothalamic-pituitary hormone release (CRF, GnRH, TSH, ACTH)	*(102)*
Pituitary-adrenal activation	*(102)*
Insulin release	*(103)*
Behavioral role	
Sleep	*(104)*
Sickness behaviour (e.g., reduced exploration)	*(105)*
Functional role in neurodegeneration	
Brain injury	*(106)*
Stroke	*(107)*
Excitotoxic brain damage	*(108)*
Multiple sclerosis and experimental allergic encephalomyelitis	*(109)*
Down's syndrome	*(110)*
Alzheimer's disease	*(111)*
Parkinson's disease	*(112)*

lular [Ca^{2+}] may be part of a more generalized signaling cascade subserving some of the multiple functions of IL-1.

Acknowledgments

This study was supported by contributions from the Ministero dell'Università e della ricerca Scientifica (Cofin, 2000), Fondazione Monte dei Paschi di Siena and a grant from Ministero degli Affari Esteri (Rome, Italy under Law 212 [26102011992]).

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