British Journal of Pharmacology (1996) 118, 1705-1710

Interleukin-1β stimulation of $^{45}Ca^{2+}$ release from rat striatal slices

[1]Mitri Palmi, Maria Frosini & Gianpietro Sgaragli

Istituto di Scienze Farmacologiche, University of Siena, Via E.S. Piccolomini 170, Siena, Italy

1 Previous observations that centrally injected interleukin-1β (IL-1β) into rabbits induces a sustained rise in cerebrospinal fluid (CSF) Ca^{2+} concentration ($[Ca^{2+}]$) as well as fever, prompted us to undertake an *in vitro* study to corroborate the *in vivo* results and gain insight as to the source and mechanism of IL-1β-induced Ca^{2+} mobilization.

2 IL-1β treatment of rat striatal slices preloaded with $^{45}Ca^{2+}$ elicited a rise in spontaneous $^{45}Ca^{2+}$ release which was dose-dependent, delayed in onset and of extended duration. At concentrations of 1, 5 and 10 ng ml^{-1}, the $^{45}Ca^{2+}$ efflux increased by 6.3 ± 1.3 (s.e.mean), 33.4 ± 5.0 and 159 ± 10.5% respectively.

3 At 1 μg ml^{-1}, the specific IL-1 receptor antagonist, IRAP, antagonized the effect induced by, 10 ng ml^{-1} IL-1.

4 Caffeine 10 mM, which failed to release calcium on its own, potentiated IL-1-elicited $^{45}Ca^{2+}$ release.

5 Perfusion with a Ca^{2+}-free medium obtained by use of excess EGTA (3 mM) or in the presence of the Ca^{2+} channel blocker, nifedipine (3×10^{-8} M) abolished the potentiating effect of caffeine without affecting the IL-1-induced $^{45}Ca^{2+}$ release.

6 Preincubation of slices for 4 h with *Bordetella pertussis* toxin (PTX, 1.3 μg ml^{-1}) did not change the pattern of Ca^{2+} efflux in response to IL-1.

7 In conclusion, these data indicate that IL-1 stimulates calcium release from brain tissue by a specific, receptor-mediated mechanism which partly depends on extracellular calcium but does not involve a PTX-sensitive G protein as part of the transducing signal.

Keywords: Interleukin-1β (IL-1β); calcium; release; striatum; caffeine

Introduction

Interleukin-1β (IL-1β) possesses a wide spectrum of inflammatory, metabolic, haemopoietic and immunological properties (for review see Rothwell, 1991). When injected into animals or man, it produces fever and therefore is referred to as an endogenous pyrogen. The pyrogenic activity of IL-1 appears to lie in the cytokine's ability to induce the synthesis/release of prostaglandin E_2 (PGE_2) as demonstrated by the absence of fever when eicosanoid production is inhibited by antipyretic/anti-inflammatory agents (Dinarello & Bernheim, 1981; Davidson *et al.*, 1990; Palmi *et al.*, 1995). The involvement of Ca^{2+} in fever production in mammals was first suggested by Myers & Veale (1970) who found that in the preoptic area of the hypothalamus, the Ca^{2+} concentration ($[Ca^{2+}]$) seems to regulate the thermostatic set point for body temperature in mammals. Furthermore, increases of $^{45}Ca^{2+}$ release from diencephalic tissue into cerebrospinal fluid (CSF) of the third ventricle are reported in cats presenting fever due to bacterial endotoxin (Myers, 1976). The same increase in $^{45}Ca^{2+}$ efflux from the diencephalon and other brain regions has also been observed in various mammals exposed to a cool environment (Gisolfi *et al.*, 1977), a condition that activates thermoregulatory mechanisms identical to those which during fever maintain an elevated body temperature.

Since then experimental evidence from our laboratory corroborates the involvement of Ca^{2+} in thermoregulation. Our previous work established a direct correlation between changes of $[Ca^{2+}]$ in CSF of febrile animals and increased body temperature, independently of the pyrogenic agent involved (Palmi *et al.*, 1992). Under conditions of postsurgical hyperthermia, CSF $[Ca^{2+}]$ increases per unit body temperature were shown to be linear and coincident with values obtained after IL-1β fever induction. As IL-1β is known to be the principal host mediator of febrile response (Rothwell, 1991) it seems reasonable to identify this cytokine as the final common agent of Ca^{2+} change on which different pyrogens converge. Our laboratory postulated the possible sequence of events leading to fever production as follows: pyrogen-IL-1 production-$[Ca^{2+}]$ increase-phospholipase A_2 activation-synthesis of PGE_2 as the final effectors of febrile response (Palmi *et al.*, 1994).

Although our data provide evidence of the involvement of calcium in thermoregulation, they do not address the issue of the sites and mechanism responsible for the increase in CSF $[Ca^{2+}]$ observed in our *in vivo* experiments. To gain insight as to the source and mechanism of IL-1β-induced calcium mobilization, we used slices from rat striatum which is rich in calcium-binding proteins (Baimbridge *et al.*, 1992) as an *in vitro* model specially suitable for observing Ca^{2+} release. The results showed that IL-1 indeed promotes Ca^{2+} release and that the process whereby this occurs is receptor mediated and dose-dependent. The synergistic effect between IL-1 and caffeine suggests that calcium potentiates its own release by a possible calcium-induced-calcium-release (CICR)-process.

Methods

Solutions

PSS (physiological salt solution) had the following composition (mM concentration): NaCl 160, glucose 10, HEPES 5, KCl 4.6, $MgCl_2$ 1, pH = 7.2.

Ca-EGTA PSS (mM concentration): $CaCl_2$ 20.5, EGTA 20, NaCl 135, glucose 10, HEPES 5, KCl 4.6, $MgCl_2$ 1, pH = 7.2. This buffer was calibrated to give a final free $[Ca^{2+}]$ of 0.5 mM (Maxchelator, Dr C. Patton, Stanford University, U.S.A.).

In order to measure accurately cellular Ca^{2+} efflux, Ca-EGTA was used in the perfusion solution to chelate the re-

[1] Author for correspondence.

leased radioactive isotope. This, while maintaining constant the level of free extracellular Ca^{2+}, minimizes the amount of $^{45}Ca^{2+}$ that remains bound in the extracellular space thus reducing the potential for its backflux into the cell (van Breemen & Casteels, 1974).

Tissue preparation

Male albino Wistar rats weighing 200 to 250 g were killed by decapitation and rapidly decerebrated. While the brain was maintained in an oxygenated (95% O_2 + 5% CO_2) PSS, both *corpora striata* were excised, cleaned and cut into slices of about 350 μm by a manual chopper (Stoelting Co. Wood Dale, IL, U.S.A.). Slices were thoroughly washed in a large volume of ice cold PSS at a low (0.2 mM) [Ca^{2+}] and all tissue preparation was carried out in a refrigerated room maintained at a constant temperature of 4°C. The slices were then incubated at 37°C under 95% O_2 + 5% CO_2 bubbling, in 1 ml of an identical low Ca^{2+} PSS solution to which 4 μCi of $^{45}Ca^{2+}$ ($6.02 \cdot 10^{-6}$ M) (specific activity = 532 mCi·mmol^{-1}) had been added.

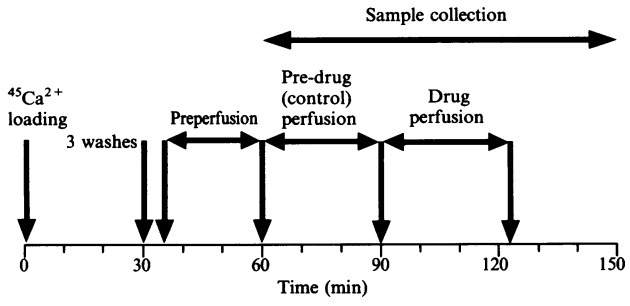
Optimum loading time for the labelled Ca^{2+} was determined by following its uptake over a 60 min period, whereas the Ca^{2+} tissue content was determined by the decreasing radioactivity of the incubation medium over the same time.

At 0, 10, 20, 30, 40, 50 and 60 min following the addition of $^{45}Ca^{2+}$, 1 μl aliquots of the incubation medium were withdrawn and diluted in 4 ml of PSS-EGTA buffer. 0.5 ml of the diluted solution was then added to 3 ml of scintillator to determine radioactivity (c.p.m.). The $^{45}Ca^{2+}$ ratio between tissue and incubation medium (T/M ratio) was calculated at all sampling times with maximum loading occurring at 30 min when the mean T/M ratio is 13.4 ± 2.5 ($n = 10$, range 10–15). After the 30 min point, the T/M ratio either remained constant or slightly decreased; therefore all our Ca^{2+} release experiments began after this loading time.

Slices weighing 16 ± 5 mg were washed three times with 10 to 15 ml of PSS solution and batches of 3 to 5 slices were placed in microperfusion chambers (Vizi *et al.*, 1985) of 300 μl volume and superfused with an oxygenated Ca-EGTA buffered PSS solution at 37°C and at constant rate of 0.5 ml min^{-1} by peristaltic pump.

After $^{45}Ca^{2+}$ release had stabilized in a 25 min period (preperfusion), the perfusion fluid was continuously collected for a period of 90 min in 1.5 ml (3 min) fractions. Aliquots of 0.5 ml from each sample were added to 3 ml of scintillator and the radioactivity measured by liquid scintillation spectrometry. After the full 90 min experimental duration, residual tissue radioactivity was determined: tissue was weighed, homogenized in 1 ml trichloroacetic acid (10%) and kept at room temperature for 30 min. After this acidic precipitation the radioactivity of the supernatant was measured in aliquots of 100 μl supernatant by spectrometry.

Scheme of the experimental protocol is shown below:



Release was expressed as the percentage of residual radioactivity present in the tissue at each sampling interval (fractional release, FR) using the following equation:

$$FR_i = 100 \times X_i \times [(\sum_{j=i+1}^{n} \times X_j + T_{Cont})]^{-1}$$

where X_i is the radioactivity released at the i-fraction, with i = 1, 2, 3.....n and T_{Cont} the residual radioactivity remaining in the tissue at the end of the experimental period.

Chemicals

The following drugs were used: human recombinant IL-1β (hrIL-1β, specific activity 1.0×10^9 u mg^{-1} protein and lipopolysaccharide contamination < 1.2 pg μg^{-1}) was obtained from Prof. L. Parente (Istituto di Ricerca Immunobiologica, Siena, IRIS, Biocine Dipartimento di Farmacologia), IL-1 receptor antagonist protein (IRAP) was a generous gift from Dr L. O'Neill (Biochemistry Department, Trinity College, Dublin, IRL); Pertussis toxin (PTX) was a generous gift from Dr I. Morfili (IRIS, Biocine, Siena, Italy); $^{45}Ca^{2+}$ was obtained from NEN-Du Pont (Cologno Monzese, Italy). All other chemicals were purchased from Sigma Chemical Co. (St Louis, MO, U.S.A.).

Statistical analysis

Values are expressed as means ± s.e.mean. Data were compared statistically by one-way analysis of the variance (ANOVA). Group data of fractional $^{45}Ca^{2+}$ release were compared across all treatment conditions: hrIL-1β alone, hrIL-1β + IRAP, hrIL-1β + caffeine, hrIL-1β + caffeine + EGTA, hrIL-1β + caffeine + nifedipine and hrIL-1β + PTX. In all these conditions pre-drug perfusion (control) values remained unaltered over the 30 min interval preceding the drug perfusion and did not differ significantly one from another. A $P < 0.05$ was considered significant.

Results

Spontaneous and caffeine-induced $^{45}Ca^{2+}$ release

In order to establish whether the basal release of $^{45}Ca^{2+}$ from slices of rat *striatum* was constant over time under conditions of no stimulation, efflux was measured over a 90 min period after preperfusion. As shown in Figure 1a, the decay of $^{45}Ca^{2+}$ radioactivity, expressed as Bq g^{-1} tissue, followed a monoexponential curve (decay rate −0.28 Bq g^{-1} wet tissue min^{-1}) which was converted to a linear plot (b) by expressing it as % of residual radioactivity present in the tissue at each sampling interval (FR) (see Methods). Baseline spontaneous FR was maintained throughout the experimental session providing a benchmark for the effects of different substances on calcium efflux.

To test if the rate of Ca^{2+} efflux is a reflection of an intracellular release, we assayed the effect of caffeine, a known mobilizer of intracellular Ca^{2+} on spontaneous $^{45}Ca^{2+}$ release. Figure 2 shows that the addition of 40 mM caffeine at 69 min of efflux for a period of 9 min caused a transient increase in the rate of $^{45}Ca^{2+}$ release as shown by peak Bq g^{-1} wet tissue (a) and FR (b) values.

Effect on $^{45}Ca^{2+}$ release of hrIL-1β alone and in combination with IRAP

Figure 3 shows that the addition of hrIL-1β (10 ng ml^{-1}) to the perfusion liquid for 33 min, induced a slow and significant ($P < 0.01$) increase in the rate of $^{45}Ca^{2+}$ efflux. This effect started 30 min after cytokine addition and grew in strength after wash-out. At the end of the experiment, the percentage increase of Ca^{2+} release was 71.6 ± 13.9 over the basal value (6.57 ± 0.33). IRAP (1 μg ml^{-1}) did not modify spontaneous $^{45}Ca^{2+}$ efflux (data not shown), however when perfused in combination with hrIL-1β, IRAP drastically antagonized the hrIL-1β effect ($P < 0.01$). During the initial 30 min of perfusion with IRAP, the booster effect of hrIL-1β on calcium release

was reduced by 12% (6.27±0.20) and returned to levels of spontaneous release after washing.

In order to confirm further the specificity of IL-1 on $^{45}Ca^{2+}$ release, a dose-response relationship was investigated with three different dose levels of hrIL-1β (Figure 4). After 30 min stimulation with 1, 5 and 10 ng ml^{-1} hrIL-1β, Ca^{2+} efflux increased by 6.3±1.3, 33.4%±5% and 159±10.5% respectively over resting efflux values (6.31±0.55, 6.87±0.45 and 6.53±0.23 respectively). At low hrIL-1β concentrations the onset of response was delayed as compared to the highest dose condition. Statistical analysis revealed that the Ca^{2+} responses to the various IL-1 concentrations were significantly different.

Effect of hrIL-1β on $^{45}Ca^{2+}$ release in caffeine-primed tissue

Contrary to the 40 mM (see Figure 2) concentration, caffeine at only 10 mM, failed to boost calcium release (data not shown). However, when added prior to hrIL-1β, 10 mM caffeine significantly ($P<0.01$) potentiated and modified the kinetic of calcium efflux from brain tissue (Figure 5) in a synergistic manner. With caffeine, after a lag phase of 9–12 min, calcium efflux was characterized by periodic stepwise surges at intervals of 15–20 min preceded by a lag phase of 9–12 min duration. With each surge, calcium release increased by 10.2±3.4, 25.7±6.2 and 52.5±8.1% over spontaneous efflux values (5.94±0.17) to a total of 90.0±17.4% over baseline values at the end of the experimental session.

To substantiate further the actions of IL-1β and IL-1β+caffeine on $^{45}Ca^{2+}$ efflux, we determined the total radioactivity (T) released during the 90 min of the 'sample collection' period (see Methods) and the residual radioactivity (R)

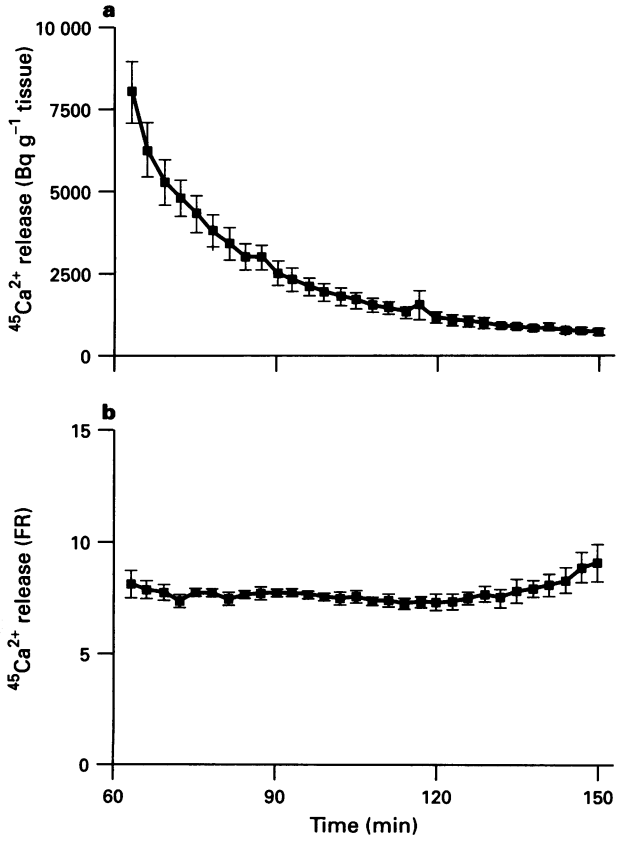


Figure 1 Time course of spontaneous $^{45}Ca^{2+}$ efflux from rat striatal brain slices. The release values were expressed as Bq g^{-1} tissue (a) or as percentage of residual radioactivity present in the tissue at each sampling interval (fractional release, FR) (b). Values represent the mean±s.e.mean of triplicate determinations from 3 separate experiments.

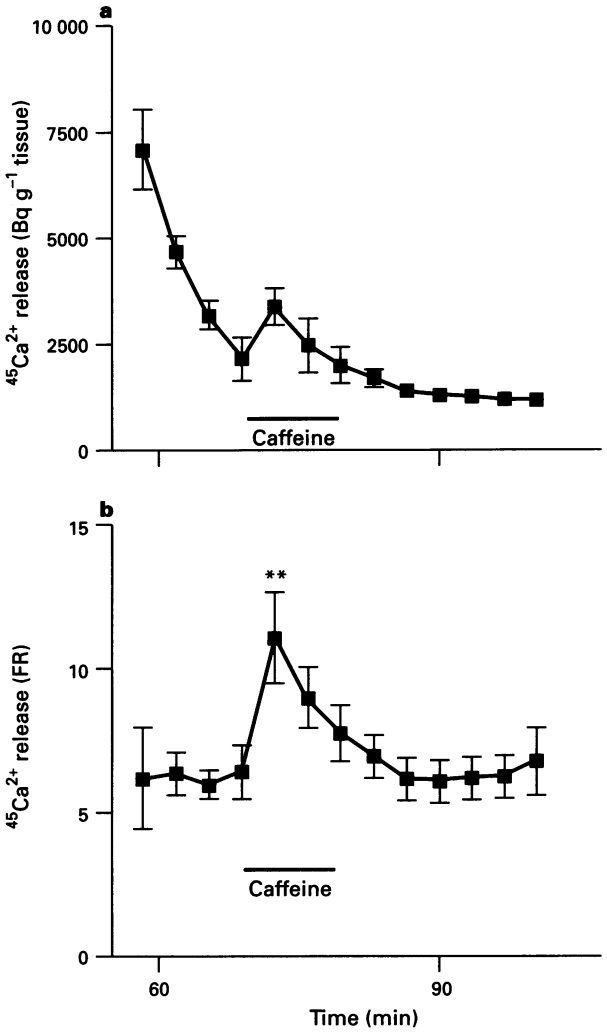


Figure 2 Effect of caffeine stimulation on $^{45}Ca^{2+}$ efflux from rat striatal brain slices. The release values were expressed as Bq g^{-1} tissue (a) or as fractional release (FR, b). Tissue stimulation was obtained by perfusing slices with 40 mM caffeine in the 69–78 min interval within the pre-drug perfusion period (see Methods). Values are the means±s.e.mean of triplicate determinations from 3 separate experiments. $^{**}P<0.01$.

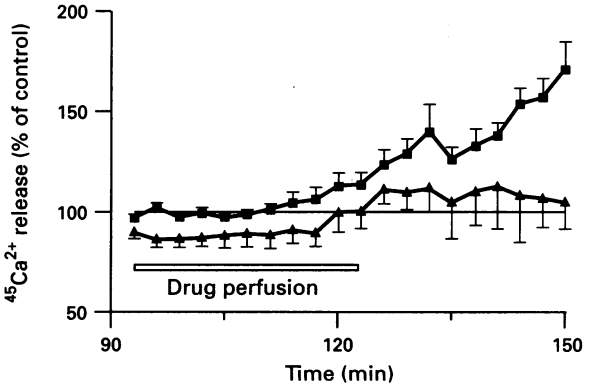


Figure 3 Effect of perfusion of hrIL-1β (10 ng ml^{-1}) alone (■) and in combination with IRAP (1 μg ml^{-1}) (▲) on $^{45}Ca^{2+}$ release (FR) from rat striatal brain slices. Values (mean of triplicate determinations from 5 separate experiments) with s.e.mean show percentage deviations in $^{45}Ca^{2+}$ efflux over controls. Controls (100%) represent the mean release value from 10 fractions collected over the 60–90 min (pre-drug perfusion) period (see Methods). Group data of hrIL-1β and hrIL-1β+IRAP were compared by ANOVA: hrIL-1β alone vs hrIL-1β+IRAP, FR = $P<0.01$.

remaining in the tissue after this time. As shown in Table 1, treatment with 10 ng ml^{-1} IL-1β induced an increase in the total released radioactivity and a corresponding decline in its tissue content as compared to controls. This effect was more pronounced in tissue pretreated with caffeine (10 mM). With IL-1β and IL-1β+caffeine the R/T ratio decreased significantly ($P<0.01$) by 43.8 and 63.3% respectively.

Effect of EGTA and nifedipine on hrIL-1β-induced $^{45}Ca^{2+}$ release in caffeine-primed tissue

In order to study the interdependence of intracellular and extracellular calcium, a nominally Ca^{2+}-free PSS (2×10^{-8} M, Maxchelator, Dr C. Patton, Stanford University, U.S.A.) was obtained by adding 3 mM EGTA to PSS containing 1 mM Ca^{2+}. Perfusion with this solution countered the potentiating effect of caffeine on Ca^{2+} release leaving unaltered the effect of IL-1β alone. Figure 5 shows the time course of caffeine+hrIL-1-induced calcium release, superimposed on the unprimed effects of hrIL-1β alone (dotted line transposed from Figure 3). Figure 5 also shows convergent results obtained when tissue was incubated in the presence of nifedipine (3×10^{-8} M). This calcium channel blocker, like the calcium chelator, EGTA, suppressed the synergistic effects of caffeine on hrIL-1β-induced $^{45}Ca^{2+}$ release as can be seen from the suppression of Ca^{2+} surges evoked by caffeine + hrIL-1β. Responses to Ca^{2+}-free PSS and nifedipine perfusion were statistically similar.

These data indicate that extracellular Ca^{2+} exerts a positive feedback control on hrIL-1β-induced $^{45}Ca^{2+}$ release and that drugs affecting the availability of extracellular Ca^{2+} crucially affect calcium release from the cell.

Effect of pertussis toxin on hrIL-1-induced $^{45}Ca^{2+}$ release

A possible involvement of a G-protein in the signal transduction mechanism of IL-1-induced $^{45}Ca^{2+}$ release was investigated. Tissues were preincubated for 4 h in the presence of 1.3 μg ml^{-1} of PTX, then washed extensively and submitted to the loading procedure as previously described. At the end of this time, the radioactive content in PTX-treated tissues did not differ significantly from those of controls (tissues preincubated for 4 h without PTX), suggesting that PTX treatment did not modify the $^{45}Ca^{2+}$ loading capacity. As shown in Figure 6, pretreatment of tissue with PTX failed to evoke a change in the rate of Ca^{2+} release compared to controls, thus suggesting that IL-1 evoked Ca^{2+} release is not mediated by a PTX-sensitive G-protein.

Discussion

Our previous work on the sequencing of the fever process showed that the administration of IL-1β and other pyrogens consistently prompted an increase in [Ca^{2+}] in CSF, leading us to inquire as to the source and kinetics of the calcium mobilization and release. While the possibility remains that *in vivo* IL-1 enhances the access to brain of blood calcium, thereby contributing to the rise in CSF [Ca^{2+}], our *in vitro* model, by showing that IL-1 induces a rise of $^{45}Ca^{2+}$ efflux from rat striatal slices indicates that one source of calcium which contributes to CSF [Ca^{2+}] increase is certainly brain tissue. Furthermore the finding that caffeine, a known mobilizer of Ca^{2+} from intracellular stores (Fleischer & Inui, 1989; Tsien & Tsien, 1990) transiently enhances the rate of $^{45}Ca^{2+}$ efflux, suggests that in our *in vitro* model, Ca^{2+} efflux is directly related to the increase in free cytoplasmic [Ca^{2+}]. That this is indeed the case is supported by data shown in Table 1 showing that $^{45}Ca^{2+}$ content remaining in the tissue at the end of IL-1β stimulation was significantly lower than that of control with a proportional decrease almost equalling the concomitant increase of total $^{45}Ca^{2+}$ efflux. Even though the rate of Ca^{2+} efflux is indicative of an intracellular Ca^{2+} release, as observed with other *in vitro* preparations (Blaustein & Hodgkin, 1969; Deth & Van Breemen, 1977; Deth & Casteels, 1977) our study does not fully identify the site of Ca^{2+} mobilization induced by IL-1β as Ca^{2+} may have originated from intracellular (1,4,5, inositoltrisphosphate- or ryanodine-sensitive) stores, or from various sequestration zones within the cell. In addition, the

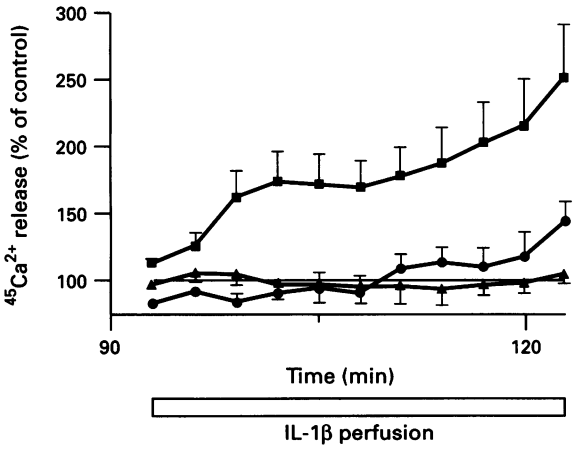


Figure 4 Effect of different doses of hrIL-1β on $^{45}Ca^{2+}$ release (FR) in rat striatal tissue primed with 10 mM caffeine. Tissue priming was obtained by perfusing tissue with caffeine (10 mM) in the 66–75 min interval within the pre-drug perfusion period (see Methods): (■) 10 ng ml^{-1} hrIL-1β; (●) 5 ng ml^{-1} hrIL-1β and (▲) 1 ng ml^{-1} hrIL-1. Values (mean of triplicate determinations from 5 separate experiments) with s.e.mean show percentage deviations in $^{45}Ca^{2+}$ efflux over controls. Controls (100%) represent the average release value from 10 fractions collected over the 60–90 min (pre-drug perfusion) period (see Methods). Groups data of $^{45}Ca^{2+}$ release, comprised in the 90–123 min (drug perfusion) interval, were compared statistically by ANOVA across the following treatment conditions: hrIL-1β (1 ng ml^{-1}) vs hrIL-1β (5 ng ml^{-1}), FR = $P<0.05$. hrIL-1β (5 μg ml^{-1}) vs hrIL-1β (10 ng ml^{-1}), FR = $P<0.01$.

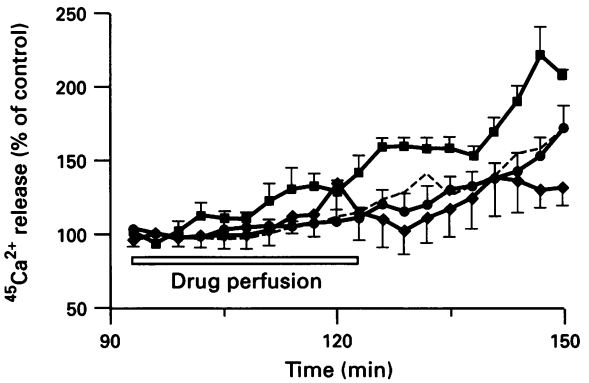


Figure 5 Effect of hrIL-1β (10 ng ml^{-1}) perfusion on $^{45}Ca^{2+}$ release (FR) in caffeine-primed rat striatal tissue (■) and effect of EGTA (3 mM) (●) and nifedipine (3×10^{-8} M) (◆) on this hrIL-1β-induced $^{45}Ca^{2+}$ release. Tissue priming was obtained by perfusing tissue with caffeine (10 mM) in the 66–75 min interval within the pre-drug perfusion period (see Methods). The broken line reproduced from Figure 3 represents the effect of hrIL-1β on $^{45}Ca^{2+}$ release in non-primed tissue. Values (mean of triplicate determinations from 3 to 5 separate experiments) with s.e.mean show percentage deviations in $^{45}Ca^{2+}$ efflux over controls. Controls (100%) represent the average release value from 10 fractions collected over the 60–90 min (pre-drug perfusion) period (see Methods). Group data of FR were compared statistically by ANOVA across the following treatment conditions: hrIL-1β vs hrIL-1β+caffeine FR = $P<0.01$; hrIL-1β vs hrIL-1β+caffeine+EGTA or nifedipine, FR = NS; hrIL-1β+caffeine+EGTA vs hrIL1β+caffeine+nifedipine, FR = NS; hrIL-1β+caffeine vs hrIL-1β+caffeine+EGTA or nifedipine, FR = $P<0.01$.

Table 1 Effect of perfusion with IL-1β (10 ng ml^{-1}) alone and in combination with caffeine (10 mM) on total $^{45}Ca^{2+}$ release (T) and on residual $^{45}Ca^{2+}$ tissue content (R) in rat striatal slices

	n	*Residual (R)* $^{45}Ca^{2+}$ *tissue content* (Bq g^{-1} wet tissue)	*Total (T)* $^{45}Ca^{2+}$ *release* (Bq)	R/T
Control	9	17855±1452.6	70964.3±2067.4	0.251±0.024
IL-1β	15	11569±1024.3	79537.0±2214.6	0.141**±0.011
Caffeine + IL-1β	15	9178±896.4	89392.4±3025.6	0.102**±0.009

The values represent the total $^{45}Ca^{2+}$ radioactivity (T) released during 90 min period (sample collection time, see Methods) and the residual (R) radioactivity remaining in the tissue at the end of the experiment. Group data from treatments (IL-1β alone and in combination with caffeine) were compared statistically with the control groups by ANOVA test, n = number of experiments, $^{**}P<0.01$.

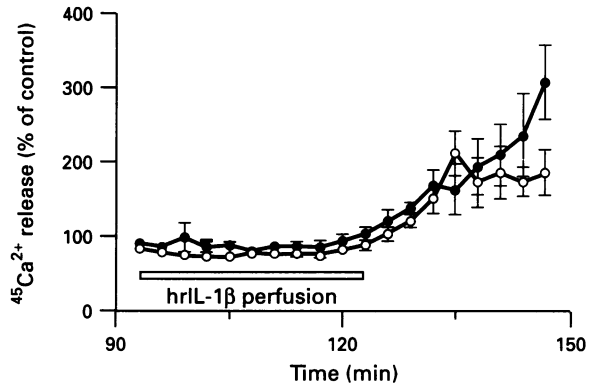


Figure 6 Effect of pertussis toxin (PTX) pretreatment (○) on hrIL-1β-induced $^{45}Ca^{2+}$ release (FR) from rat striatal slices. Tissues were preincubated for 4 h with PTX (1.3 μg ml^{-1}) before submission to the $^{45}Ca^{2+}$ loading procedure. Values (mean of triplicate determinations from 5 separate experiments) with s.e.mean show percentage deviations in $^{45}Ca^{2+}$ efflux over controls (●). Controls (100%) represent the average release value from 10 fractions collected over the 30 min (pre-drug perfusion) period (see Methods). Group data of hrIL-1β vs hrIL-1β+PTX compared by ANOVA showed no statistically significant difference.

possibility that IL-1β stimulates an ATP-dependent Ca^{2+}-pump or various Ca^{2+} exchangers in the plasma membrane that act to expel Ca^{2+} cannot be ruled out.

The calcium response to IL-1β is related to the dose of the cytokine and mediated by receptors as shown by its inhibition by IRAP, a specific IL-1β receptor antagonist polypeptide (Carter, 1990). This receives further support from studies showing that IL-1β promoted a dose-dependent increase of intracellular Ca^{2+} in human fibroblasts and that this effect was antagonized by anti-type 1 IL-1 receptor antibody (Arora *et al.*, 1995). Furthermore, in a pre-B-like cell line IL-1β was shown to induce changes in total intracellular Ca^{2+} (Stanton *et al.*, 1986). The dose at which IRAP antagonizes IL-1-induced Ca^{2+} efflux i.e. 100 fold that of IL-1, is consistent with the range of concentration required to antagonize the cytokine's activity both *in vitro* and *in vivo* in various animal models (Arend *et al.*, 1990; Ohlsson *et al.*, 1990; Von Uexküll *et al.*, 1992). Interestingly, the same IRAP to IL-1 ratio also counteracts 95% of the fever in rabbits caused by i.v. injection of IL-1 (Coceani *et al.*, 1992). Finally, IRAP at a concentration two order of magnitudes higher than that of the IL-1, blocks IL-1 binding to type I and type II receptors in different cultured cells (Rambaldi *et al.*, 1990; Hannum *et al.*, 1990; Dripps *et al.*, 1991). The specificity of the IL-1 action on Ca^{2+} release is further outlined by the fact that levels of other cations (Na^+, K^+ and Mg^{2+}) in rabbit CSF are unaffected by central IL-1 injection (unpublished observations).

The kinetics of IL-1-induced Ca^{2+} release may be better appreciated when caffeine is added as a primer. Caffeine, in fact potentiates the release of Ca^{2+}; however, this booster effect is strictly dependent on the availability of the extracellular Ca^{2+} ($[Ca^{2+}]_o$) as shown by the experiments where EGTA and nifedipine were used. Thus incubation in a Ca^{2+}-free medium obtained with excess of EGTA or Ca^{2+} entry blockade by the voltage-operated Ca^{2+} channel antagonist nifedipine, abolishes the caffeine effect on IL-1-induced Ca^{2+} release. It is known that caffeine activates the Ca^{2+}-induced Ca^{2+} release (CICR) process, a Ca^{2+} regenerative releasing mechanism by which Ca^{2+} generates intracellular $[Ca^{2+}]$ oscillations and amplifies its own release (Nathanson *et al.*, 1992; Berridge, 1993). The observation that in sympathetic ganglion neurones, the rapid removal of external calcium prevented the caffeine-induced spikes right up to the onset of the regenerative process led to the conclusion that external Ca^{2+} played a crucial role in activating the CICR process (Miyaraki, 1988; Swann, 1992; Friel & Tsein, 1992a,b; Berridge, 1993). Thus it might be hypothesized that in our experiments, the difference in amplitude of Ca^{2+} mobilization between IL-1-induced Ca^{2+} responses in Ca^{2+}-containing and Ca^{2+}-free media during caffeine stimulation might be a consequence of the presence of $[Ca^{2+}]_o$ and its boosting effect on the CICR process. The presence of Ca^{2+} and activation of CICR process might also be claimed to explain the oscillatory-like kinetic pattern of Ca^{2+} mobilization shown in Figure 5 by a series of periodic and regenerative surges of Ca^{2+} waves. As shown in the same figure, incubation in the $[Ca^{2+}]_o$-free medium indeed reduces the Ca^{2+} response to IL-1β+ caffeine; however, this condition fails to counter Ca^{2+} release induced by the cytokine alone. This rules out the hypothesis that IL-1 may promote the increase of Ca^{2+} efflux via an enhanced $[Ca^{2+}]_o$ influx and a consequent exchange of it for the $^{45}Ca^{2+}$ located within the cell.

The delay in onset of Ca^{2+} release induced by IL-1 and the prolongation of the action after its withdrawal as described in Figure 3, suggest the presence of an intermediate messenger. Observations from fertilization studies have shown that a sperm factor is the agent potentiating the process of CICR (Swann, 1992). Cyclic ADP ribose (cADPR) (Galione *et al.*, 1991; 1993) has been suggested to be this factor. While the addition of cADPR to sea urchin egg homogenate induces a release of Ca^{2+} from caffeine-ryanodine sensitive stores, the formation of this NAD^+ derivative seems to be enhanced by cyclic GMP (Berridge, 1993). These data suggest a possible link between cADPR and the local hormone nitric oxide (NO) on the one hand and IL-1 and Ca^{2+} release on the other. We may, in fact, speculate that in our experimental model, IL-1, via its intermediate production of NO might synthesize cyclic GMP and this in turn induce Ca^{2+} release through cADPR production. The characteristic kinetic pattern of IL-1 induced NO production in neurones (Bredt *et al.*, 1991) and other cell types (Hibbs *et al.*, 1990; Inoue *et al.*, 1993), with its delayed onset and progressively prolonged activity, is reminiscent of the kinetic of Ca^{2+} release during IL-1 stimulation shown in our experiments.

The series of experiments presented in this paper demonstrate that IL-1 increases spontaneous Ca^{2+} release from brain tissue and that the Ca^{2+} response to IL-1 is receptor-mediated,

delayed in onset and persistent. It was also shown that PTX failed to antagonize the IL-1 effect, thereby excluding the involvement of a PTX-sensitive G-protein as part of the transducing mechanism of intracellular mediators. Though the manner whereby IL-1 increases spontaneous Ca^{2+} release is not fully clarified, the present studies contribute to the understanding of the way in which Ca^{2+} potentiates and regenerates its own release in response to IL-1 stimulation. This may have possible implications for the management and containment of neurotoxic damage due to cellular calcium overload.

This work was supported by a contribution from MURST (40+60%), CNR (Roma, Italy) and by grants of the European Community (BIOMED I, CT-94-1402). Dr R. Raikes is acknowledged for her help in improving the manuscript.

References

AREND, W.P., WELGUS, H.G., THOMPSON, R.C. & EISENBERG, S.P. (1990). Biological properties of recombinant human monocyte-derived interleukin-1 receptor antagonist. *J. Clin. Invest.*, **85,** 1694–1697.

ARORA, P.D., MA, J., MIN, W., CRUZ, T. & MCCULLOCH, C.A. (1995). Interleukin-1-induced calcium flux in human fibroblasts is mediated through focal adhesions. *J. Biol. Chem.*, **270,** 6042–6049.

BAIMBRIDGE, K.G., CELIO, M.R. & ROGERS, J.H. (1992). Calcium-binding proteins in the nervous system. *Trends Neurosci.*, **15,** 303–308.

BERRIDGE M.J. (1993). Inositol trisphosphate and calcium signalling. *Nature*, **361,** 315–324.

BLAUSTEIN, M.P. & HODGKIN, A.L. (1969). The effect of cyanide on the efflux of calcium from squid axons. *J. Physiol.*, **200,** 497–506.

BREDT, D.S., GLATT, C.E., HUWANG, P.M., FATUHI, M., DAWSON, T.M. & SNYDER, S.H. (1991). Nitric oxide synthase protein and mRNA are discretely localized in neuronal population of the mammalian CNS together with NADPH diaphorase. *Neuron*, **7,** 615–624.

CARTER, D.B. (1990). Purification, cloning, expression and biological characterization of an interleukin-1 receptor antagonist protein. *Nature*, **344,** 633–640.

COCEANI, F., LEE, J., REDFORD, J. & ISIS BISHAI. (1992). Interleukin-1 receptor antagonist: effectiveness against interleukin-1 fever. *Can. J. Physiol. Pharmacol.*, **70,** 1590–1596.

DAVIDSON, J., MILTON, A.S. & ROTONDO, D. (1990). A study of the pyrogenic action of interleukin-1 and interleukin-1β: interaction with a steroidal and non steroidal antipyretic/antiinflamatory agents. *Br. J. Pharmacol.*, **100,** 542–546.

DETH, R. & CASTEELS, R. (1977). A study of releasable Ca fractions in smooth muscle cells of the rabbit aorta. *J. Gen. Physiol.*, **69,** 401–412.

DETH, R. & VAN BREEMEN (1977). Agonist induced Ca^{2+} release. *J. Memb. Biol.*, **30,** 363–380.

DINARELLO, C.A. & BERNHEIM, H.A. (1981). Ability of human leukocite pyrogen to stimulate brain prostaglandin synthesis in vitro. *J. Neurochem.*, **37,** 702–707.

DRIPPS, D.A., VERDEBER, E., RAY, K.N.G., THOMPSON, R.C. & EISEMBERG, S.P. (1991). Interleukin-1 receptor antagonist binds to the type II interleukin-1 receptor on B cells and neutrophils. *J. Biol. Chem.*, **266,** 20311–20315.

FLEISCHER, S. & INUI, M. (1989). Biochemistry and biophysics of excitation-contraction coupling. *Annu. Rev. Biophys. Chem.*, **18,** 333–364.

FRIEL, D.D. & TSEIN, R.W. (1992a). A caffeine and ryanodine sensitive Ca^{2+} stored in bullfrog sympathetic neurones modulates effects of Ca^{2+} entry on $[Ca^{2+}]$. *J. Physiol.*, **450,** 217–246.

FRIEL, D.D. & TSEIN, R.W. (1992b). Phase dependent contributions from Ca^{2+} entry and Ca^{2+} release to caffeine-induced $[Ca^{2+}]_i$ oscillations in bullfrog sympathetic neurons. *Neuron*, **8,** 1109–1125.

GALIONE, A., LEE, H.C. & BUSA, W.B. (1991). Ca^{2+}-induced Ca^{2+} release in sea urchin egg homogenates: modulation by cyclic ADP-ribose. *Science*, **253,** 1143–1146.

GALIONE, A., WHITE, A., WILLMOTT, N., TURNER, M., POTTER, B.V.L. & WATSON, S.P. (1993). cGMP mobilizes intracellular Ca^{2+} in sea urchin eggs by stimulating cyclic ADP-ribose synthesis. *Nature*, **365,** 456–459.

GISOLFI, G., MIRA, F. & MYERS, R.D. (1977). Diencephalic efflux of calcium ions in the monkey during exercise, thermal stress and feeding. *J. Physiol.*, **273,** 617–630.

HANNUM, C.H., WILCOX, C.J., AREUD, W.P., JOSLIN, F.G., DRIPPS, D.J., HEIMDAL, P.L., ARMES, L.G., SOMMER, A., EISENBERG, S.P. & THOMPSON, R.C. (1990). Interleukin-1 receptor antagonist activity of human interleukin-1 inhibitor. *Nature*, **343,** 336–340.

HIBBS JR, J.B., TAINTOR, R.R., VAURIN, Z., GRANGER, J-C., DRAPIER, K.J., AMBER, K.J. & LANCASTER JR, J.R. (1990). Synthesis of nitric oxide from terminal guanidino nitrogen atom of L-arginine: a molecular mechanism regulating cellular proliferation that targets intracellular iron. In *Nitric Oxide from L-Arginine: a Bioregulatory System*, ed. Moncada, S. & Higgs, E.A. pp. 189–223. New York: Elsevier, Science Publishing Co., Inc.

INOUE, T., FUKUO, K., MORIMOTO, S., KOH, E. & OGIHARA, T. (1993). Nitric oxide mediates interleukin-1-induced prostaglandin E_2 production by vascular smooth muscle cells. *Biochem. Biophys. Res. Commun.*, **194,** 420–424.

MIYARAKI, S.I. (1988). Inositol 1, 4, 5-trisphosphate-induced-calcium release and guanine nucleotide-binding protein-mediated periodic calcium rises in golden hamster eggs. *J. Cell. Biol.*, **106,** 345–353.

MYERS, R.D. (1976). Diencephalic efflux of ^{22}Na and ^{45}Ca ions in the febrile cat: effect of an antipyretic. *Brain Res.*, **103,** 412–417.

MYERS, R.D. & VEALE, W.L. (1970). Body temperature: possible ionic mechanism in the hypothalamus controlling the set-point. *Science*, **170,** 95–97.

NATHANSON, M.H., PADFIELD, P.J., O'SULLIVAN, A.J., BURSTAHLER, A.D. & JAMIESON, J.D. (1992). Mechanism of Ca^{2+} wave propagation in pancreatic acinar cells. *J. Biol. Chem.*, **267,** 18118–18121.

OHLSSON, K., BJORK, P., BERGENFELDT, M., HAYEMAN, R. & THOMPSON, R.C. (1990). Interleukin-1 receptor antagonist reduces mortality from endotoxin shock. *Nature*, **348,** 550–552.

PALMI, M., FROSINI, M., BECHERUCCI, C., SGARAGLI, G.P. & PARENTE, L. (1994). Increase of extracellular brain calcium involved in interleukin-1β induced pyresis in the rabbit: antagonism by dexamethasone. *Br. J. Pharmacol.*, **112,** 449–452.

PALMI, M., FROSINI, M. & SGARAGLI, G.P. (1992). Calcium changes in rabbit CSF during endotoxin, IL-1β and PGE_2 fever. *Pharmacol. Biochem. Behav.*, **43,** 1253–1262.

PALMI, M., FROSINI, M., SGARAGLI, G.P., BECHERUCCI, C., PERRETTI, M. & PARENTE, L. (1995). Inhibition of Interleukin-1β-induced pyresis in the rabbit by peptide 204-212 of lipocortin 5. *Eur. J. Pharmacol.*, **281,** 97–99.

RAMBALDI, A., TORCIA, M., BETTONI, S., BORBUNI, T., VANNIER, E., DINARELLO, C.A. & COZZOLINO, F. (1990). Modulation of cell prolification and cytokine production in acute myeloblastic leukemia by interleukin-1 receptor antagonist and lack of its expression by leukemic cells. *Blood*, **76,** 114–121.

ROTHWELL, N.J. (1991). Functions and mechanisms of interleukin-1 in the brain. *Trends Pharmacol. Sci.*, **12,** 430–435.

STANTON, T.H., MAYNARD, M. & BOMSRTYK, K. (1986). Effect of interleukin-1 on intracellular concentration of sodium, calcium and potassium in 702/3 cells. *J. Biol. Chem.*, **261,** 5699–5701.

SWANN, K. (1992). Different triggers for calcium oscillations in mouse eggs involve a ryanodine-sensitive calcium store. *Biochem. J.*, **287,** 79–84.

TSIEN, R.W. & TSIEN, R.Y. (1990). Calcium stores and oscillations. *Annu. Rev. Cell. Biol.*, **6,** 715–760.

VAN BREEMEN, C. & CASTEELS, R. (1974). The use of Ca-EGTA in the measurement of ^{45}Ca efflux from smooth muscle. *Pflügers Arch. Eur. J. Physiol.*, **348,** 239–245.

VIZI, E.S., HARSING, L.G., ZIMANYI, I. & GAAL, G. (1985). Release and turnover of noradrenaline in isolated median eminence: lack of feedback modulation. *Neuroscience*, **16,** 907–915.

VON UEXKÜLL, C., NOURSHARGH, S. & WILLIAMS, T.J. (1992). Comparative responses of human and rabbit interleukin-1 in vivo: effect of a recombinant interleukin-1 receptor antagonist's. *Immunology*, **77,** 483–487.

(Received March 5, 1996
Revised April 10, 1996
Accepted April 23, 1996)