Vol. 152, No. 1, 1988
April 15, 1988

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 392-397

INTERLEUKIN-ONE INDUCED INOSITOL PHOSPHOLIPID BREAKDOWN IN MURINE MACROPHAGES: POSSIBLE MECHANISM OF RECEPTOR ACTIVATION

E.S. Wijelath, A.M. Kardasz, R. Drummond and J. Watson*

Division of Biochemistry, University of Strathclyde, The Todd Centre, Taylor Street, Glasgow G4 0NR

Received March 2, 1988

SUMMARY: Stimulation of mouse peritoneal macrophages by Interleukin-one (IL-1) provoked rapid increases in the levels of inositol mono, bis, tris and tetrakisphosphates (IP_1, IP_2, IP_3 and IP_4 respectively). IP_3 was by far the major metabolite formed and time course studies revealed that IP_2 and IP_3 were formed more rapidly than IP_1 and IP_4 in response to IL-1 stimulation. The IP_2 and IP_3 levels peaked at five seconds while there was a time lag of five seconds in the IP_4 response and the IP_1 levels increased relatively steadily over the time course of the experiments. Levels of IP_2, IP_3 and IP_4 all returned almost to control levels by 60s. The rapid formation of the inositol phosphate metabolites was concomitant with a decrease (84%) in the levels of phosphatidyl-inositol 4,5-bisphosphate (PIP_2) in the macrophages. These results suggest that the mechanism of IL-1 receptor activation is by the rapid hydrolysis of phosphoinositides and generation of the second messenger IP_3.

There is now considerable evidence to suggest that interleukin-one (IL-1) may be a major contributor to the joint destruction associated with rheumatoid arthritis (1,2). Accessory cells of the monocyte-macrophage series are a major source of this cytokine which mediates a diverse range of biological effects including the induction of pyrogenicity (3), fibroblast proliferation (4), lymphocyte activation (5), synthesis of hepatic acute proteins (6) and stimulation of collagenase and prostaglandin release from synovial cells (7). The biochemical mechanism of IL-1 receptor activation however remains obscure.

Many biologically active molecules such as hormones and neurotransmitters operating on cell surface receptors activate a phosphodiesterase enzyme (phospholipase C) which hydrolyses phosphatidylinositol 4,5-bisphosphate (PIP_2) to produce the

*To whom correspondence should be addressed.

0006-291X/88 $1.50

metabolite inositol 1,4,5-trisphosphate (IP_3) as well as diacylglycerol (DAG). Both DAG and IP_3 have been shown to act as second messengers with DAG activating protein kinase C (8) while IP_3 causes the release of Ca^{2+} from the endoplasmic reticulum (9,10).

The purpose of the present study was to investigate whether the biochemical mechanism of IL-1 receptor activation in murine macrophages involved the formation of inositol phosphates as second messengers and the results provide the first evidence that this indeed is the case.

MATERIALS AND METHODS

Materials Dowex (formate) AG-1x8 ion exchange resin was obtained from Biorad Laboratories Ltd., Watford, Herts, U.K. myo [2-^{3}H] Inositol (S.A. 16.6 Ci/mmol) was obtained from Amersham International, UK as were labelled inositol phosphate standards used in the standardisation of the ion exchange columns. Interleukin-1 was obtained from Genzyme, UK. RPMI-1640 culture medium, antibiotics and 35mm culture dishes were all obtained from Flow Laboratories, Rickmansworth, UK. Ecoscint scintillator was obtained from B.S. & S. (Scotland) Ltd., Edinburgh, UK. All other chemicals were of analytical grade.

Isolation and labelling of macrophages. Mouse resident peritoneal macrophages were obtained by peritoneal lavage of CBA mice with sterile phosphate buffered saline (PBS). The cells were washed three times with RPMI-1640 medium before being resuspended in 35mm culture dishes at a density of 3 x 10^6 cells/ml in complete medium consisting of RPMI-1640 containing foetal calf serum (5% v/v), penicillin 100 U/ml), streptomycin (100µg/ml) and Hepes (10 mM).

The dispersed cells were incubated for 2h at 37°C in an atmosphere of 5% CO_2 in air. Non-adherent cells were removed and to each dish was then added 1ml of complete medium containing radioactive myo-inositol (20µCi). The cells were incubated for a further 72h. Thereafter the cells were washed with PBS containing lithium chloride (10 mM) and inositol (1 mM) in order to remove radioactive inositol not taken up by the cells. Finally the cells were allowed to equilibrate for 30 min at room temperature in fresh serum free RPMI-1640 medium containing lithium chloride (10mM) prior to the start of the experiment.

Measurement of [^{3}H] inositol phosphates. Cells were stimulated with 2 units of IL-1 at room temperature for fixed periods of time between 0 and 60 seconds. Control cultures contained radioactive inositol but with no addition of IL-1. The incubations were terminated rapidly after the appropriate time by the addition of ice cold trichloroacetic acid (TCA) solution (10%). Removal of the TCA followed by sample preparation and subsequent ion exchange chromatography were carried out according to the procedures of Berridge et al (11) as modified by Downes et al (12). Aliquots (0.5ml) of the eluants from the Dowex (formate) ion exchange Chromatography columns which corresponded to the metabolites IP_1, IP_2, IP_3 and IP_4 (determined using standards) were assessed for radioactivity by dissolving in Ecoscint Scintillator (4ml) and measuring the radioactivity in a Packard tri-carb 1500 scintillation counter.

Measurement of [^{3}H] phosphatidylinositol 4,5-bisphosphate. Inositol phospholipids were extracted according to the method of Mallows et al (13). Separation of the phospholipids was achieved by reversed phase thin layer chromatography on silica gel impregnated with 1%-potassium oxalate (14).

RESULTS

It can be seen from Figure 1 that IL-1 induced rapid formation of the phosphatidyl inositol metabolites IP_1, IP_2, IP_3 and IP_4 in murine macrophages. Within 5s, a rise of almost 4-fold

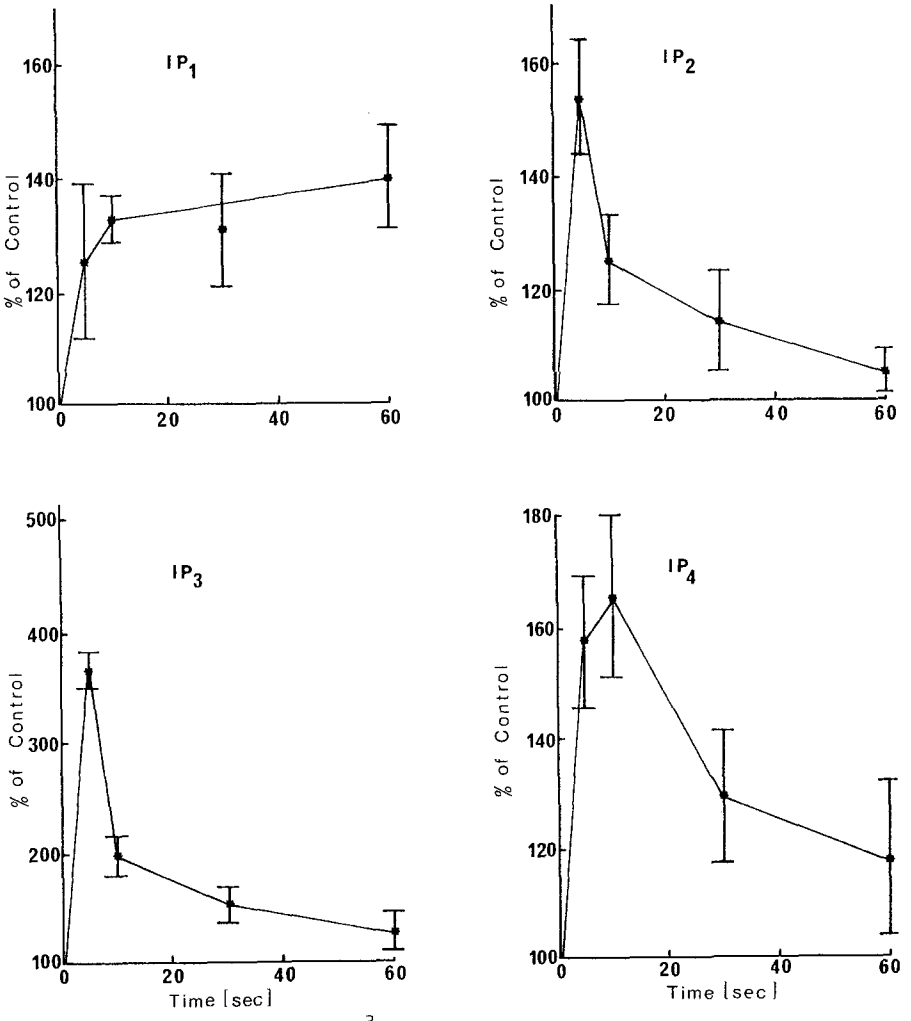

Fig. 1. Time course of [^{3}H] inositol phosphate formation in murine macrophages following stimulation with interleukin-1. Mean control levels (100%) of [^{3}H] IP_1, IP_2, IP_3 and IP_4 were 2602 ± 37, 1393 ± 62, 704 ± 123 and 445 ± 42 dpm respectively. Each point represents the mean ± SEM of four independent determinations carried out in duplicate.

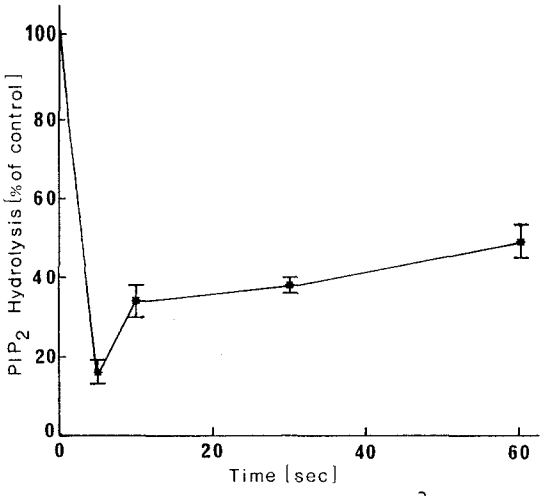

Fig. 2. Time course of the hydrolysis of [^{3}H] PIP_2 in murine macrophages following stimulation with interleukin-1. Each point represents the mean ± SEM of four experiments carried out in duplicate.

was observed in the major second messenger metabolite IP_3 while a 1.5-fold increase in IP_2 was evident. A 1.7-fold increase in IP_4 synthesis was also observed, although in the case of this metabolite it did not reach its maximum until 10s after the addition of IL-1. All three metabolites IP_2, IP_3 and IP_4 returned almost to control levels by 60s whereas the IP_1 continued to rise throughout the experiment without showing any maximum value. PIP_2, the immediate precursor of all the above sequence of metabolites demonstrated a dramatic 84% decrease from control levels after 5s (Figure 2) and thereafter a slight elevation occurred during the remainder of the experiment.

DISCUSSION

The production of the cytokine IL-1 by cells of the monocyte-macrophage series initiates a series of inflammatory processes and plays a number of important roles such as T lymphocyte activation (15). The compound exists in two main forms, IL-1 and IL-1 (16) and recent evidence suggests that both forms share the same receptor (16,17). Although the biological responses of IL-1 are well documented, information concerning IL-1 receptor activation and the biochemical transmembrane signalling events are poorly understood.

A great variety of external signals use the inositol lipid transduction mechanism to convey information into the cell (18) and the results of the present study demonstrate that this is

probably the case in the IL-1 stimulation of murine macrophages. The major second messenger of the above mechanism is IP_3 and since this is maximally stimulated (4-fold) by IL-1 in macrophages within five seconds, it would suggest that the mechanism of action of IL-1 (at least in part) involves mobilisation of calcium ions, now regarded universally as the principal effect of IP_3. It is interesting to note that stimulation of the other inositol polyphosphates IP_2 and IP_4 peaked at five and ten seconds respectively indicating that these may also play a role as second messengers in macrophages. The mechanism by which these compounds perform their second messenger function remains obscure although Irvine and Moor (20) have suggested that in sea urchin eggs, IP_4 may control cellular Ca^{2+} homoeostasis at the plasma membrane. Such a mechanism would be in keeping with the delayed response in IP_4 secretion observed in the present study.

The decrease in PIP_2 in the macrophages at five seconds, concomitant with IP_3 and IP_2 stimulation, suggests that IL-1 receptor activation causes the rapid hydrolysis of PIP_2 by activation of phospholipase-C and the fact that PIP_2 levels begin to rise again after five seconds would indicate that very rapid depletion of PIP_2 occurs before it can be replenished by synthesis via PI and PIP. Additional evidence for this latter suggestion arises from the delayed formation of IP, which shows that, at least at very early times, IL-1 does not induce phospholipase-C mediated hyrolysis of PI. Similar pathways of inositol phospholipid metabolism following receptor activation have been observed in other tissues (11,19). Further studies are in progress to determine whether a similar mechanism of IL-1 receptor activation occurs in other cell types.

ACKNOWLEDGEMENTS

This work was supported by grants from the Medical Research Council, the Scottish Hospital Endowments Research Trust and the Carnegie Trust.

REFERENCES

1. Wood, D.D., Ihrie, E.J., Dinarello, C.A. and Cohen, P.L. (1983) Arthritis & Rheumatism, 26, 975-983
2. Nouri, A.M.E., Panayii, G.S. and Goodman, S.M. (1984) Clin. Exp. Immunol., 55, 2965-302
3. Dinarello, C.A. (1982) Am. J. Med., 72, 799-819.
4. Schmidt, J.A., Mizel, S.B., Cohen, D. and Green, I. (1982) J. Immunol. 128, 2177-2182.
5. Oppenheim, J.J., Krovacs, E.J., Matsushima, K. and Durum, S.K. (1986) Immunol. Today, 7, 45-56.
6. Pepys, M.B. and Batty, M.L. (1983) Adv. Immunol., 34, 141-212

7. Mizel, S.B., Dayer, J.M., Krane, S.M. and Mergenhagen, S.E. (1981) Proc. Natl. Acad. Sci. USA, 78, 2474-2477
8. Nishizuka, Y. (1984) Nature, 308, 693-698.
9. Streb, H., Irvine, R.F., Berridge, M.J. and Schultz, I. (1983) Nature, 306, 67-69.
10. Burgess, G.M., Godfrey, P.P., McKinney, J.S., Berridge, M.J., Irvine, R.F. and Putney, J.W. (1984) Nature, 309, 63-66.
11. Berridge, M.J., Dawson, R.M.C., Downes, C.P., Heslop, J.P. and Irvine, R.F. (1983) Biochem. J., 212, 473-482.
12. Downes, C.P., Hawkins, P.T. and Irvine, R.F. (1986) Biochem. J., 238, 501-506.
13. Mallows, R.S.E. and Bolton, T.B. (1987) Biochem. J., 244, 763-768.
14. Gonzalez-Sastre, F. and Folch-Pi, J. (1968) J. Lipid Res., 9, 532-533.
15. Durum, S.K., Schmidt, J.A. and Oppenheim, J.J. (1985) Ann. Rev. Immunol., 3, 263-287.
16. Bird, T.A. and Saklatvala, J. (1986) Nature, 324, 263-265.
17. Dower, S.K., Kronheim, S.R., Hopp, T.P., Cantrell, M., Deeley, M., Gillis, S., Henney, C.S. and Urdal, D.L. (1986) Nature, 324, 266-267.
18. Berridge, M.J. (1986) In: Phosphoinositides and Receptor Mechanisms pp25-46. Eds. J.W. Putney Jr., Alan R. Liss Inc., New York.
19. Downes, C.P. and Wusterman, M.W. (1983) Biochem. J., 216, 633-640.
20. Irvine, R.F. and Moor, R.M. (1986) Biochem. J., 240,917-920.