Volume 323, number 1,2, 163–165 FEBS 12492 May 1993

Direct influence of morphine on the release of arachidonic acid and its metabolites

M.G. Sergeeva, I.V. Terentjeva, A.T. Mevkh and S.D. Varfolomeev

A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow 119899, Russian Federation

Received 17 March 1993

The influence of 10^{-10}–10^{-6} M morphine on the release of [^{3}H]arachidonic acid and its metabolites ([^{3}H]AAM) from prelabeled resident peritoneal murine macrophages was investigated. Morphine enhanced [^{3}H]AAM release from A23187- and LPS-stimulated macrophages, as well as the basal release of [^{3}H]AAM. Dose–response curves showed a maximum at 10^{-8} M morphine. Naloxone had no effect on morphine enhancement of [^{3}H]AAM release. These results are in agreement with the hypothesis that [^{3}H]AAM may be involved in the effects of morphine.

Morphine; Opioid; Arachidonic acid metabolism; Macrophage

1. INTRODUCTION

The mechanism for the inter-relationship between opioids and prostaglandins is one of the most fascinating problems in biochemistry. As early as 1969 Jagues reported that analgesic drugs of both the morphine and aspirin groups inhibit the contraction of the longitudinal muscle of isolated guinea-pig ileum, elicited by arachidonic acid (AA) [1]. Since then the problem has been much studied, and many investigators have amassed a convincing body of evidence that opioid ligands influence the effects of prostaglandins, a cyclooxygenase metabolite of AA [2–5]. Morphine has been shown to inhibit, in many tissues, the effects of prostaglandins [2,3] but stimulate their synthesis [4,5]. The relationship between opioid ligands and prostaglandins, however, has not been studied in cells of the immune system.

The influence of opioid peptides and opioids, like morphine, on the immune and defence functions are under intensive investigation. These substances, introduced in vivo, change various immune functions [6]. On the other hand, AA metabolism has been reported as a factor which modulates cell functions involved in various immunological and inflammatory phenomena [7]. In addition, macrophages isolated from morphine-dependent rats enhanced their release of prostaglandins [8]. The surprising thing is that a direct effect of morphine on the release of AA and its metabolites is as yet unknown.

Over several years we have been investigating exogenous and endogenous regulators of eicosanoid synthesis [9,10]. In addition we have studied opioid receptors on different cell systems [11,12]. The aim of the present work was to study the in vitro effect of morphine on the release of AA and its metabolites from resident peritoneal macrophages.

Correspondence address: M.G. Sergeeva, A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow 119899, Russian Federation.

2. MATERIALS AND METHODS

2.1. *Cell cultures*

Mice were killed by cervical dislocation between 15.00–16.00 h in order to take into account possible circadian changes and their influence on macrophage function. Resident macrophages were obtained by peritoneal lavage of untreated F_1-mice with sterile Dulbecco's modified Eagle's medium (DMEM). Cells were counted, then 2×10^6 cells/ml were suspended in DMEM with 10% heat-inactivated fetal calf serum, L-glutamine (2 mM) and 100 U gentamicin. The viability of these cells, as judged by Trypan blue exclusion, was never below 96%. The suspension (3 ml) was dispensed onto 6-wells culture dishes at 37°C in humidified air containing 5% CO_2. After 2 h preincubation, non-adherent cells were removed by washing with the medium, the adherent cells were supplemented with fresh medium and incubated for 12–16 h at 37°C in humidified air, containing 5% CO_2 before experiments in order to adapt cells to post-isolation conditions.

2.2 *Release of [^{3}H]AA and its metabolites from labelled macrophages*

Macrophages were cultured in 6-well dishes in fresh DMEM supplemented with 1% bovine serum albumin (BSA) and with the addition of 25 nCi of [^{3}H]AA. After 4 h the macrophages were washed three times with DMEM to remove unincorporated radiolabel then incubated for 2 h in DMEM with 1% BSA. The initial time was time of addition of tested ligands and stimulators, A23187 (Sigma) or LPS (Difco). Final concentrations of stimulators were 5 μM and 25 μg/ml, respectively. At that time 200 μl of the medium were removed and the radioactivity determined by scintillation counting.

2.3. *Data processing*

The in vitro effect of morphine was investigated in 8 independent experiments. For each experiment macrophages were pooled from 8 mice. Each point of radioactivity release was duplicated. The results were processed using the Sigmaplot v4.0 program (Jandel Scientific) on an IBM PC.

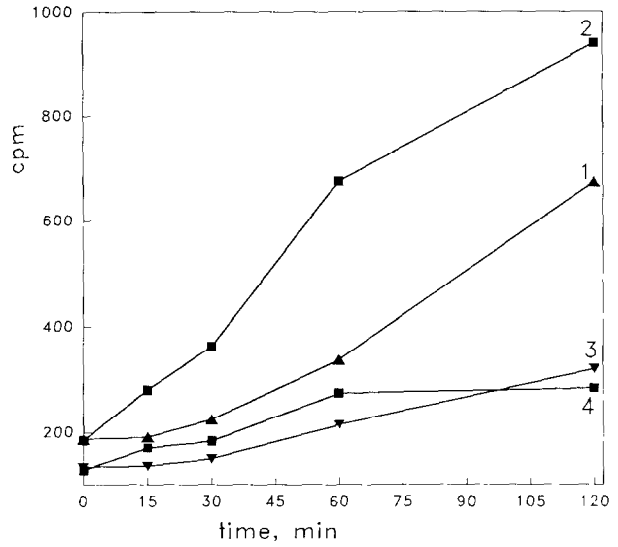

Fig. 1. Kinetics of morphine influence on basal [^{3}H]AAM release from peritoneal murine macrophages. Morphine concentration: 1, 10^{-6} M; 2, 10^{-8} M; 3, 10^{-10} M; 4, without morphine.

3. RESULTS

To investigate the influence of opioid ligands on endogenous AA metabolism in macrophages we measured the radioactivity release from [^{3}H]AA-prelabeled cells. This is the usual method of evaluation of release from cells of AA and its metabolites [13,14]. The detected values constituted the total, [^{3}H]AA plus [^{3}H]AA-metabolite, radioactivity, named hereafter [^{3}H]AAM.

Fig. 1 (curve 1) demonstrates a slight basal release of [^{3}H]AAM from macrophages. Exposure of [^{3}H]AA-labeled cells to morphine resulted in the dose-dependent release of [^{3}H]AAM from membrane phospholipids. Morphine at a concentration of 10^{-6}–10^{-10} M significantly enhanced this release (curves 2–4). The maximal enhancement corresponded to 10^{-8} M morphine.

The dose–response curves for the enhancement of the A23187-induced [^{3}H]AAM release by morphine revealed a bell-shaped dependence as well. Maximal release corresponded to 10^{-8} M morphine but 10^{-6} M morphine had only a slight effect (Fig. 2).

Morphine also enhanced the [^{3}H]AAM release with lipopolysaccharide (LPS) as a stimulator (Fig. 3). The ratio of the effect of morphine plus stimulator to the effect of stimulator itself was remarkably greater for LPS as compared to A23187 (Fig. 3). Naloxone enhanced the [^{3}H]AAM release from the cells and did not abolish the morphine effect (Fig. 3).

4. DISCUSSION

We showed that morphine exerted direct action on peritoneal macrophages. Morphine enhanced the release of the [^{3}H]AAM from non-stimulated and LPS- and A23187-stimulated peritoneal macrophages.

We revealed a bell-shaped dependence of morphine effect on the stimulated AAM release with a maximum at 10^{-8} M morphine. These results may account for the absence of the direct influence of morphine on rat macrophages when 10^{-6} M opioid ligands are used [8]. Morphine was less effective on [^{3}H]AAM release at a concentration of 10^{-6} M than at 10^{-10} or 10^{-8} M. This fact is not surprising as similar results have been found in

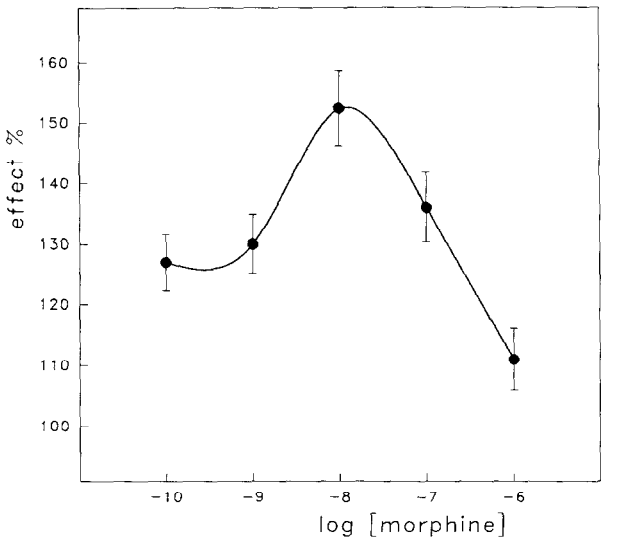

Fig. 2. Dose–response curves for the enhancement of A23187-induced AAM release by morphine. All values have been corrected by subtracting the basal release of [^{3}H]AAM. The values shown are the mean ± S.E.M. [^{3}H]AAM released after 120 min of stimulation and are expressed as a percentage of the [^{3}H]AAM release with A23187 only.

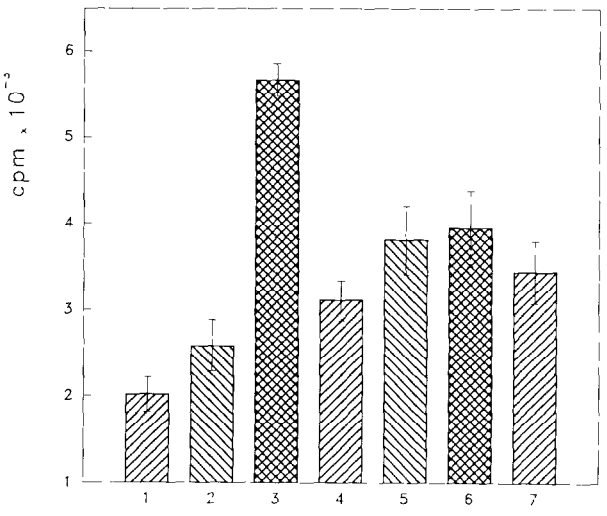

Fig 3. Effect of 10^{-8} M morphine and 10^{-8} M naloxone on A23187- and LPS-induced [^{3}H]AAM release. All values have been corrected by substracting the basal release of [^{3}H]AAM. Bars represent mean ± S.E.M. [^{3}H]AAM released after 120 min of stimulation and correspond to (1) basal, (2) LPS only, (3) LPS and morphine, (4) A23187 only, (5) A23187 and morphine, (6) A23187, morphine and naloxone, (7) A23187 and naloxone.

the opioid peptide modulation of other immune parameters [16,17].

Naloxone, which is regarded as a classical antagonist of opioid receptors, under our experimental conditions acted as an agonist of morphine, i.e. enhanced [^{3}H]AAM release from cells and did not abolish the morphine effect. Specific opiate receptors have been demonstrated on mononuclear cells [18], as well as non-classical binding sites for opioid ligands [19]. Our results confirm the suggestion [20,21] that naloxone can influence some functions of the immune system unrelated to its opioid receptor antagonism.

Stimulation of AA metabolism, along with other immunosuppressive effects of morphine, can aid in suppressing the resistance of drug-dependent animals and people to infections, and also promote regulation of other functions of the immune system. The elucidation of the mechanism of morphine effect on different enzymatic systems of AA metabolism is the subject of our further investigations.

Acknowledgements: This work was suppressed in part by Grant 173-101-89 from the state programme 'Development of Fundamental Research' (Ministry of Science and Education of Russia).

REFERENCES

[1] Jaques, R. (1969) Experientia 25, 1059–1060.
[2] Faletti, A., Chaud, M.A., Gimeno, M.A. and Gimeno, A.L. (1988) Prost. Leuk. Essential Fatty Acids 34, 147–151.
[3] Widy-Tyszkiewicz, E., Luczar, A., Gumulka, W. and Szreniawski, Z. (1978) Arch. Int. Pharmacodyn. Ther. 232, 53–57.
[4] Bekemeier, H., Giessler, A.J. and Vogel, F. (1977) Pharmacol. Res. Commun. 9, 587–598.
[5] Collier, H.O.J., Francis, D.L., McDonald-Gibson, W.J., Roy, A.C. and Saeed, S.A. (1975) Life Sci. 17, 85–90.
[6] Yahua, M.D. and Watson, R.R. (1987) Life Sci. 41, 2503–2510.
[7] Davies, P., Bailey, P.J., Goldberg, M.M. and Ford-Hutchinson, A.W. (1984) Annu. Rev. Immunol. 2, 335–357.
[8] Elliott, G.R., van Batenburg, M.J. and Dzoljic, M.R. (1987) FEBS Lett. 217, 6–10.
[9] Beisembaeva, R.U., Mursagalieva, A.T., Dzhumalieva, L.M., Shaikenov, T.E. and Mevkh, A.T. (1990) FEBS Lett. 269, 125–127.
[10] Varfolomeev, S.D. and Mevkh, A.T. (1985) Prostaglandins as Molecular Bioregulators: Biokinetics, Biochemistry and Medicine, Moscow University PH, Moscow.
[11] Zaitsev, S.V., Sergeeva, M.G., Chichenkov, O.N., Petrov, V E. and Varfolomeev, S.D. (1986) Biokhimiia (Russ.) 51, 1334–1340.
[12] Ilyina, A.D., Zaitsev, S.V. and Varfolomeev, S.D. (1991) Biosystems 25, 193–203.
[13] Bonney, R.J., Wightman, P.D., Davies, P., Sharon, J.S., Kuenl Jr., F.A. and Humes, J.L. (1978) Biochem. J. 176, 433–442.
[14] Fernandez, B. and Balsinde, J. (1990) FEBS Lett. 268, 107–109.
[15] Weidemann, H.J., Peskar, B.A., Wrogemann, K., Rietschel, E.T., Staudinger, H. and Fischer, H. (1978) FEBS Lett. 89, 136–140.
[16] Brown, S.L. and Van Epps, D E. (1985) J. Immunol. 134, 3384–3390.
[17] Prieto, J., Subira, M.L., Castilla, A., Arroyo, J.L. and Serrano, M. (1989) Scand. J. Immunol. 29, 391–398.
[18] Lopker, A., Abood, L.G., Hoss, W. and Lionetti, F.J. (1980) Biochem Pharmacol. 29, 1361–1365.
[19] McCain, H.W., Lamster, I.B., Bozzone, J M. and Gebic, J.T. (1982) Life Sci. 31, 1619–1624.
[20] Kay, N.E., Morley, J.E. and Allen, J.I. (1990) Immunology 70, 485–491.
[21] Marcoli, M., Ricevuti, G. and Mazzone, A. (1988) Int. J. Immunopharmacol. 10, 425–433.