Volume 217, number 1, 6-10 FEB 04750 June 1987

Enhanced prostaglandin E_2 and thromboxane B_2 release from resident peritoneal macrophages isolated from morphine-dependent rats

G.R. Elliott, M.J. van Batenburg and M.R. Dzoljic

Department of Pharmacology, Erasmus University Rotterdam, POB 1738, 3000 DR, Rotterdam, The Netherlands

Received 2 March 1987

Resident peritoneal macrophages from morphine-addicted rats (4 days) released more prostaglandin (PG) E_2 and thromboxane (Tx) B_2, but not 6-keto-PGF_{1a}, than cells from control animals. This effect, which was due to an enhancement of endogenous AA turnover, was not related to any changes in cAMP synthesis or lysososomal enzyme secretion. [D-Ala^2]-Met-enkephalin had no effect on eicosanoid release in vitro. Both morphine and PGE_2 have been shown to depress macrophage functions. We suggest that morphine-stimulated macrophage PGE_2 synthesis, and the consequent inhibition of phagocytosis, could contribute to the decreased resistance to infections associated with opiate addiction.

Morphine; Opiate addiction; [D-Ala^2]-Met-enkephalin; Prostaglandin; Macrophage; (Rat)

1. INTRODUCTION

It is known that morphine addicts have an enhanced risk of infection and reduced capacity to combat disease. This has been mainly ascribed to malnutrition, shared needles, toxic impurities in the drugs and other non-hygienic procedures which could account for the generally poor state of health and low resistance to disease of this group [1]. However, morphine has been shown to reduce the survival time of animals with bacterial and fungal infections indicating that some specific action of morphine is involved [2]. The same authors also found that macrophage phagocytosis, which plays an important role in protecting against extracellular pathogens, is reduced by morphine.

Enkephalins and endorphins, on the other hand, appear to stimulate macrophage functions, for example, chemotaxis, chemiluminescence, antibody-dependent cytotoxicity and superoxide generation [3-6]. Further, spleen macrophages have been reported to synthesise γ-endorphine [7]. These data indicate that opiates and endogenous opioid peptides could modify macrophage functions in vivo. PGE_2, a cyclooxygenase metabolite of AA, is an important regulator of macrophage functions inhibiting, amongst others, chemiluminescence, lysosomal enzyme release and, importantly, phagocytosis [8-10]. It is possible therefore that morphine could suppress the immune response by stimulating macrophage PGE_2 synthesis and opioid peptides could stimulate the immune response by inhibiting it. In order to clarify these possibilities we have investigated the effect of morphine dependence on resident rat peritoneal macrophage cyclooxygenase metabolite release and its modification by [D-Ala^2]-Met-enkephalin.

Correspondence address: G.R. Elliott, Department of Pharmacology, Erasmus University Rotterdam, POB 1738, 3000 DR Rotterdam, The Netherlands

2. MATERIALS AND METHODS

Male Wistar rats (175-200 g) were bought from the Central Laboratory Animal House, Zeist. An-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

tisera against PGE_2 and TxB_2 were obtained from the Institute Pasteur, Paris, and antisera against 6-keto-PGF_{1a} from Seragen (Boston, USA). Standard compounds, eicosanoids, AA and cAMP were ordered from Sigma and standard radiolabelled compounds from New England Nuclear. [D-Ala^2]-Met-enkephalin (enkephalin) was bought from Peninsula Laboratories (Belmont, CA).

Pellets, control or morphine-impregnated (5×75 mg pellets/rat, 35 mg morphine/pellet), were implanted subcutaneously in the backs of rats under light ether anaesthesia [11]. After 4 days the resident peritoneal macrophages were isolated from Geys balanced salt solution (GBSS) washes of rat peritonea, by density gradient centrifugation over Ficoll, and suspended at 1×10^6 viable cells/ml GBSS. The macrophage preparation was greater than 85% pure and greater than 95% viable, as assessed by trypan blue exclusion. Aliquots (1 ml) were added to Plastibrand polypropylene reaction vials and incubated for 1 h at 37°C in a water bath. The cells were then centrifuged down and the medium stored at −70°C for later analysis of PGE_2, TxB_2, 6-keto-PGF_{1a}, lactate dehydrogenase (LDH) and β-glucuronidase (GUR). LDH was assayed by the method of Wroblewsky and LaDue [12], and GUR activity was quantified using the conversion of phenolphthalein glucuronic acid to phenolphthalein, which was then measured spectrophotometrically (555 nm).

The cell pellet was heated for 5 min at 95°C in 150 μl Tris-EDTA buffer and the medium cAMP assayed using a protein binding method [13].

When used, AA (stored as an ethanolic solution under nitrogen) was diluted with GBSS to the required concentration (final ethanol concentration 0.001%) and naloxone hydrochloride and enkephalin were dissolved in GBSS. 10 μl volumes were then added to 1 ml of the cell suspension at the beginning of the incubation. In some experiments, to investigate the effect of enkephalin, macrophages were isolated and used 24 h after injecting 5 ml of a 1% starch suspension intraperitoneally.

3. RESULTS

The effect of morphine ex vivo was investigated in 3 separate experiments. For each experiment macrophages were pooled from 5 control and 5 morphine rats. The in vitro experiments were performed twice. Results given are typical of the other experiments performed. Student's *t*-test was used to assess significance.

Resident macrophages isolated from peritonea of morphine-treated rats released more PGE_2 and TxB_2, but not 6-keto-PGF_{1a}, than cells isolated from control animals. No differences in eicosanoid formation were observed when the macrophages were incubated with exogenous AA (table 1). As changes in PGE_2 and TxB_2 were parallel we decided to assay only TxB_2 in the following experiments. No changes in basal or AA-stimulated TxB_2 synthesis were found when morphine or control macrophages were incubated in vitro with either naloxone or enkephalin (table 2). Enkephalin (10^{-8}–10^{-4} M) had no consistent effect on the basal synthesis of TxB_2 and cAMP and secretion of GUR by resident peritoneal macrophages. Similarly, AA- and carrageenin-stimulated TxB_2 formation was also unaffected (not shown).

Enkephalin (10^{-8}–10^{-4} M) was also without ef-

Table 1

Effect of morphine (ex vivo) on basal and arachidonic acid-stimulated release of PGE_2, TxB_2 and 6-keto-PGF_{1a} from resident rat peritoneal macrophages

Treatment	PGE_2	TxB_2	6-Keto-PGF_{1a}
Control	0.88 ± 0.06	4.80 ± 1.14	23.60 ± 3.60
Morphine	2.13 ± 0.35[a]	9.80 ± 0.35[a]	25.30 ± 0.90
Control + AA	27.30 ± 2.30	31.00 ± 1.73	137.5 ± 10.6
Morphine + AA	26.30 ± 1.50	43.00 ± 11.0	146.7 ± 20.0

[a]Significantly different ($P < 0.01$) from control values

Results (ng/ml) are means ± SD of triplicate incubations. AA concentration was 1 μg/ml

Table 2

Effect of naloxone and enkephalin (in vitro) on basal and arachidonic acid-stimulated TxB_2 synthesis using resident rat peritoneal macrophages from morphine-treated and control rats

Treatment	TxB_2(ng/ml)	
	Control	Morphine
Control	5.92 ± 1.13	8.00 ± 0.53[a]
+ naloxone	7.00 ± 0.50	9.75 ± 1.30[a]
+ enkephalin	6.92 ± 1.18	9.42 ± 2.24
Naloxone + enkephalin	6.00 ± 0.87	8.77 ± 1.25[a]
Control + AA	19.83 ± 1.26	18.90 ± 2.95
Naloxone + AA	20.33 ± 1.60	19.67 ± 2.90
Enkephalin + AA	17.83 ± 2.84	19.33 ± 1.89
Naloxone + enkephalin + AA	20.50 ± 1.32	21.80 ± 3.00

[a]Significantly different ($P<0.01$) from control values
Results are means ± SD of triplicate incubations. Naloxone concentration was 10^{-6} M and enkephalin concentration, 10^{-8} M. AA concentration was 1 μg/ml

Table 3

Effect of enkephalin on TxB_2 and cAMP synthesis and the release of β-glucuronidase from 24 h starch-elicited rat peritoneal macrophages

Enkephalin(M)	TxB_2(ng/ml)	GUR (mU)	cAMP (pmol)
Control	0.28 ± 0.03	0.060 ± 0.004	4.74 ± 0.1
10^{-4}	0.26 ± 0.04	0.064 ± 0.003	7.50 ± 2.0[a]
10^{-6}	0.27 ± 0.05	0.062 ± 0.002	5.45 ± 0.5[a]
10^{-8}	0.25 ± 0.04	0.063 ± 0.002	4.30 ± 1.0

[a]Significantly different from control value ($P<0.01$)
Results are means ± SD of triplicate incubations

fect on the basal synthesis of TxB_2 and the release of GUR from 24 h starch-elicited peritoneal macrophages. However, there was a dose-response stimulation of cAMP synthesis (table 3).

4. DISCUSSION

The basal release of eicosanoids is linear over a 1 h incubation period (unpublished) and chronic morphine dependence enhanced this synthesis. AA-stimulated release of PGE_2 and TxB_2 from resident peritoneal macrophages was not affected however (AA-stimulated eicosanoid release is not saturated at 1 μg/ml AA; unpublished). That is, morphine had no effect on cyclooxygenase activity directly but enhanced AA availability. Unlike many mediators, PGs are not thought to be stored intracellularly. Thus synthesis is synonymous with release.

A small inflammatory reaction was initiated by implantation of the pellets. We are unable to say, therefore, whether the morphine effect was direct, i.e. an interaction between morphine and macrophages, or indirect, i.e. morphine modulated the inflammation or acted via central opioid receptors. The finding that morphine treatment had no effect on either cAMP levels or GUR secretion indicates that morphine did not stimulate macrophage functions in general. Morphine has been reported to alter the body's defence system by decreasing the

number of circulating polymorphonuclear leukocytes and inhibiting macrophage phagocytosis [2]. The mechanism by which morphine modifies this macrophage function is not known but our results indicate a possible mechanism. PGE_2 has been shown in a number of models to be anti-inflammatory, for example it inhibits carrageenin-induced granuloma formation [15]. It also specifically inhibits oxygen radical formation and macrophage phagocytosis [8,10]. PGE_2 synthesises from exogenously added AA also inhibited carrageenin-induced granuloma formation [16]. The latter finding is particularly interesting as it demonstrated that the anti-inflammatory action of PGE_2 predominates although other cyclooxygenase (e.g. TxB_2) and lipoxygenase (e.g. LTs) metabolites are formed. PGE_2 is, thus, a potent regulator of macrophage functions and it is feasible that morphine could depress various aspects of the body's defence system by stimulating PGE_2 synthesis. Collier et al. [17] have reported that morphine (3.5×10^{-4} M) and naloxone (2.8×10^{-4} M) stimulate PGE_2 synthesis, from exogenous AA, by bull seminal vesical homogenate. The stimulatory effect of morphine was not naloxone-sensitive. Similarly, naloxone did not inhibit the increase in eicosanoid release observed by us using macrophages from dependent rats. Naloxone (10^{-6} M), however, did not stimulate eicosanoid release in our experiments. We are now investigating the possibility that naloxone can stimulate macrophage AA turnover at higher concentrations. Enkephalin had no effect on resident cell functions tested. There was however a stimulation of starch-elicited macrophage cAMP synthesis at extremely high concentrations. A similar, although temporary, increase in macrophage cAMP levels, induced by 10^{-6} M met-enkephalin has previously been demonstrated [5]. Our results indicate that any effects of endogenous opioid peptides on macrophage function are mediated by a PG-independent mechanism. It is surprising that we found no increase in AA turnover as enkephalins have been reported to stimulate Na^+ and Ca^{2+} influx [5], which might be expected to stimulate phospholipase activity and hence AA release, and endothelial cell prostacyclin formation [18].

In summary our results indicate that [D-Ala^2]-Met-enkephalin, in vitro, had no effect on macrophage cyclooxygenase metabolite synthesis while morphine, ex vivo, induced, directly or indirectly, enhancement of PGE_2 and TxB_2 formation. Stimulation of PGE_2 synthesis by morphine and the consequent inhibition of macrophage phagocytic functions, together with the other immunosuppressive actions of this analgesic, might contribute to the decreased resistance of opiate addicts exposed to infections.

ACKNOWLEDGEMENT

We wish to thank Mrs L. van de Poel for implanting the pellets.

REFERENCES

[1] Jaffe, J.H. (1985) in: Pharmacological Basis of Therapeutics (Gillman, A.G. et al. eds) 7th ed., pp. 532–581, MacMillan, New York.
[2] Tubaro, E., Boreli, G., Croce, C., Cavallo, G. and Santiangeli, C. (1983) J. Infect. Dis. 148, 656–666.
[3] van Epps, D.E. and Saland, L. (1984) J. Immunol. 132, 3046–3053.
[4] Foris, G., Medgyesi, G.A., Gyimesi, E. and Hauck, M. (1984) Mol. Immunol. 21, 747–750.
[5] Foris, G., Medgyesi, G.A. and Hauck, M. (1986) Mol. Cell Biochem. 69, 127–137.
[6] Sharp, B.M., Keane, W.F., Suh, H.J., Gekker, G., Tsukayama, D. and Peterson, P.K. (1985) Endocrinology 117, 793–795.
[7] Lolait, S.J., Clements, J.A., Markwick, A.J., Cheng, C., McNally, M., Smith, A.I. and Funder, J.W. (1986) J. Clin. Invest. 77, 1776–1779.
[8] Schenkelaars, E.J. and Bonta, I.L. (1986) Int. J. Immunopharmacol. 8, 305–313.
[9] Weidemann, H.J., Peskar, B.A., Wrogemann, K., Rietschel, E.T., Staudinger, H. and Fischer, H. (1978) FEBS Lett. 89, 136–140.
[10] Oropeza-Rendon, R.L., Speth, V., Hiller, G., Weber, K. and Fischer, H. (1979) Exp. Cell Res. 119, 365–371.
[11] Blasig, J., Herz, A., Reinhold, K. and Zieglgansberger, S. (1973) Psychopharmacology 33, 19–38.
[12] Wroblewsky, F. and LaDue, J.S. (1955) Proc. Soc. Exp. Biol. Med. 20, 210–213.
[13] Gilman, A.G. (1970) Proc. Natl. Acad. Sci. USA 67, 305–312.
[14] Shavit, Y., DePaulis, A., Martin, F.C., Terman, G.W., Pechnick, R.N., Zane, R.P., Gale, R.P. and Liebeskind, J.C. (1986) Proc. Natl. Acad. Sci. USA 83, 7114–7117.

[15] Bonta, I.L., Adolfs, M.J.P. and Parnham, M.J. (1981) Prostaglandins 22, 95–103.
[16] Elliott, G.R., Adolfs, M.J.P. and Bonta, I.L. (1985) Eur. J. Pharmacol. 110, 367–371.
[17] Collier, H.O.J., Francis, D.L., McDonald-Gibson, W.J., Roy, A.C. and Saeed, S.A. (1975) Life Sci. 17, 85–90.
[18] Boogaerts, M.A., Vermylen, J., Deckmyn, H., Roelant, C., Verwilghen, R.L., Jacob, H.S. and Moldow, C.F. (1983) Thromb. Haemostas. 50, 572–575.