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Murine macrophage cell lines contain μ_3-opiate receptors

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Abstract

Opiate alkaloid-selective, opioid peptide-insensitive μ_3 receptors are present in three murine macrophage cell lines (J774.2; RAW 264.7; BAC1.2F5). The receptor binds morphine, its active metabolite morphine 6-glucuronide and certain other alkaloids, but not morphine 3-glucuronide or any of the opioid peptides tested. The cell lines thus provide valuable model systems for investigation of μ_3-opiate receptors, previously demonstrated to mediate inhibitory effects of morphine on activation of human peripheral blood macrophages (monocytes).

Keywords: Opiate receptor; Macrophage; Morphine 6-glucuronide

Opiate alkaloid-selective, opioid peptide-insensitive receptor binding sites, designated μ_3, have been detected in human peripheral blood macrophages (monocytes) (Stefano et al., 1993) and granulocytes (Makman et al., 1995). These receptors have been postulated to mediate inhibition by morphine and other opiate alkaloids of monocyte/macrophage or granulocyte activation induced by interleukin-1α, tumor necrosis factor-α, interleukin-8 or *N*-Formyl-Met-Leu-Phe (chemotactic peptide). The μ_3 receptor has been proposed to function in vivo in response to endogenously formed as well as to exogenously administered opiate alkaloids (Stefano et al., 1993). Investigation of the properties and function of this newly discovered and unusual opiate receptor would be greatly facilitated by the availability of an appropriate immunocyte cell culture system containing this receptor. We report here that three murine macrophage cell lines contain opiate alkaloid binding sites similar or identical to the immunocyte μ_3 receptor sites previously described. Also this receptor is shown for the first time to bind morphine 6-glucuronide, an important active metabolite of morphine, but not morphine 3-glucuronide, a metabolite inactive with respect to analgesic effect and binding to brain opioid receptors (Mulder, 1992).

J774.2 cells (Muschell et al., 1977; Nagata et al., 1984) and RAW 264.7 cells (Burkhart et al., 1994) were grown on plastic dishes in Dulbecco's modified Eagle's medium (high glucose) plus 5% NCTC 109 medium, glutamine, non-essential amino acids with 10% heat-inactivated fetal calf serum (Life Technologies) in an atmosphere of 5% CO_2 and 95% air. BAC1.2F5 cells were grown in the presence of colony stimulating factor-1 (required for growth of these cells) (Boocock et al., 1989). Cell membranes were prepared and receptor binding assays carried out at 25°C for 40 min in 50 mM Tris buffer (pH 7.5) containing 0.1% bovine serum albumin (Cruciani et al., 1994; Makman et al., 1995). [^{3}H]Morphine (83 Ci/mmol) [^{3}H][D-Ala^2,D-Leu^5]enkephalin (DADLE) (40 Ci/mmol) and [^{3}H][D-Ala^2,H-Me-Phe^4,Gly^5-ol]enkephalin (DAGO) (54.4 Ci/mmol) were from New England Nuclear; [^{3}H]diprenorphine (56 Ci/mmol) was from Amersham. Specific binding represented total binding minus that with 10 μM unlabeled ligand.

In each of the cell lines studied, J774.2, RAW 264.7 and BAC1.2F5, saturable [^{3}H]morphine and [^{3}H]diprenorphine binding sites were detected at high concentration, and competition studies (> 70% specific binding with 3 nM radioligand) indicated drug affinities similar to those of monocyte and granulocyte μ_3 recep-

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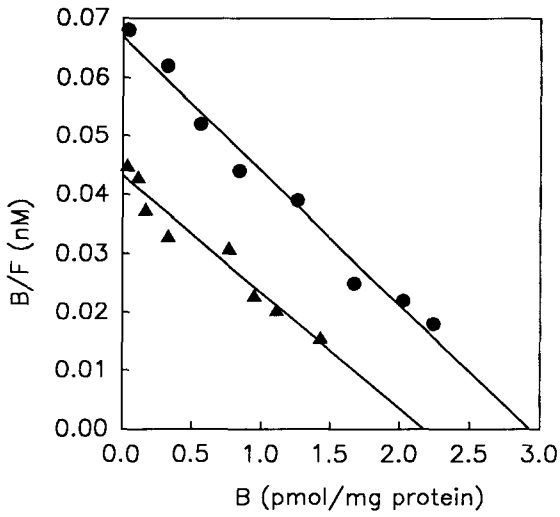

Fig. 1. Saturation studies of [^{3}H]morphine binding sites in macrophage membranes. Representative Scatchard plots are shown for J774.2 cells (circles) and for RAW 264.7 cells (triangles). Mean values for B_{max} and K_d are given in the text. Data were analyzed with the computer program LIGAND (Cruciani et al., 1994).

tors. Thus for [^{3}H]morphine saturation binding, for J774.2 cells $K_d = 36.9 \pm 5.6$ nM and $B_{max} = 2250 \pm 410$ fmol/mg protein ($n = 7$); for RAW 264.7 cells $K_d = 34 \pm 7$ nM and $B_{max} = 2140 \pm 240$ fmol/mg protein ($n = 3$) (representative Scatchard plots shown in Fig. 1).

With [^{3}H]morphine (3 nM) and J774.2 membranes, K_i values (nM) were 19 ± 2.3 for morphine, 75 ± 11 nM for morphine 6-glucuronide, 25 ± 6 for etorphine, 56 ± 7 for diprenorphine and 120 ± 18 for naloxone ($n = 3$). None of the opioid peptides or a number of other drugs tested, even at 3–10 μM, caused significant displacement of radioligand. Thus for each cell line, K_i values were greater than 3000 nM for DADLE, DAGO, [Met^5]enkephlin, [Leu^5]enkephalin, dynorphin-(1–13), dynorphin-(1–17), ß-endorphin, [D-Ala^2]metenkephalinamide, [D-Ala^2]deltorphin I, 3,4-dichloro-*N*-methyl-*N*[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide (U50,488H), cyclazocine, ketocyclazocine and morphine 3-glucuronide.

Similar selectivity was obtained in competition experiments with [^{3}H]diprenorphine, and no specific binding was obtained with [^{3}H]DADLE or [^{3}]DAGO for any of the cell lines (not shown). Thus, classical opioid peptide-sensitive μ, δ and κ receptors were not detected with any of the radioligands used.

In conclusion, macrophage cell lines contain opiate alkaloid-selective binding sites closely resembling or the same as the μ_3 receptor sites of human peripheral blood monocytes. Furthermore, the macrophage cell lines lack classical opioid-peptide sensitive receptor subtypes. Selectivity of binding for morphine 6-glucuronide versus the 3-glucuronide further supports receptor specificity, and also indicates efficacy of a metabolite present in the circulation at a much greater concentration than morphine itself shortly following morphine administration (Mulder, 1992). It is of particular interest that the macrophage cell lines respond to growth factors and cytokines known or likely to interact with the peripheral blood monocyte μ_3 receptor system; also, mutant cell lines with altered responsiveness are available. Thus, these cell lines provide model systems for functional studies, as well as relatively homogeneous and consistent preparations for biochemical, pharmacological and molecular studies of the μ_3 receptor.

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References

Boocock, C.A., G.E. Jones, E.R. Stanley and J.W. Pollard, 1989, Colony-stimulating factor-1 induces rapid behavioral responses in the mouse macrophage cell line, BAC1.2F5, J. Cell Sci. 93, 447.

Burkhart, C.A., J.W. Berman, C.S. Swindell and S.B. Horwitz, 1994, Relationship between the structure of taxol and other taxanes on induction of tumor necrosis factor-α gene expression and cytotoxicity, Cancer Res. 54, 5779.

Cruciani, R.A., B. Dvorkin, H.P. Klinger and M.H. Makman, 1994, Presence in neuroblastoma cells of a μ_3 receptor with selectivity for opiate alkaloids but without affinity for opioid peptides, Brain Res. (in press).

Makman, M.H., T.V. Bilfinger and G.B. Stefano, 1995, Human granulocytes contain an opiate alkaloid-selective receptor mediating inhibition of cytokine-induced activation and chemotaxis, J. Immunol. (in press).

Mulder, G.J., 1992, Pharmacological effects of drug conjugates: is morphine 6-glucuronide an exception?, Trends Pharmacol. Sci. 13, 302.

Muschell, R.J., N. Rosen and B.R. Bloom, 1977, Isolation of variants in phagocytosis of a macrophage-like continuous cell line, J. Exp. Med., 145, 175.

Nagata, Y., O.M. Rosen, M.H. Makman and B.R. Bloom, 1984, Biochemical analysis of a mutant macrophage cell line resistant to the growth-inhibitory activity of interferon, J. Cell Biol. 98, 1342.

Stefano, G.B., A. Digenis, S. Spector, M.K. Leung, T. Bilfinger, M.H. Makman, B. Scharrer and N.N. Abumrad, 1993, Opiate-like substances in an invertebrate, an opiate receptor on invertebrate and human immunocytes, and a role in immunosuppression, Proc. Natl. Acad. Sci. USA 90, 11099.