Fas-mediated cell death promoted by opioids

Opioids have been used as potent analgesics for centuries, but their abuse has deleterious physiological effects beyond addiction[1] — for instance, they can somehow alter the function of the immune system[2]. Here we show that morphine induces the expression of the protein Fas (also known as CD95 or APO-1), a receptor on the cell surface that triggers the cell's suicide by apoptosis when it binds to its ligand, FasL. This induction of Fas expression by opioids appears to prime lymphocytes for elimination by apoptosis.

Several tissues and organs possess opioid receptors, but most of the information we have about their function comes from studies of neuronal cells. Lymphocytes have several different types of opioid receptor, making the immune system susceptible to perturbation by opioids[2].

Fas is a transmembrane protein that belongs to the family of receptors for tumour-necrosis factor and nerve-growth factor, and which helps to regulate the immune response[3,4]; mice and humans with mutations in Fas or FasL both develop lymphocyte-accumulation diseases[4]. As the Fas–FasL system is crucial for cellular homeostasis of the immune system, we reasoned that it might participate in the opioid-mediated alteration of the immune response.

The tightly regulated expression of Fas and FasL determines whether a lymphocyte survives or dies[5,6]. We found that in a T-cell hybridoma (A1.1), in mouse splenocytes, and in freshly isolated human peripheral blood lymphocytes, morphine dramatically increases the expression of Fas (Fig. 1a) by activating the opioid receptors on these cells. This morphine-induced expression of Fas is blocked by naloxone, an antagonist of the opioid receptor.

Although morphine did not significantly affect the viability and proliferation of these cells, it primed them to undergo Fas-mediated apoptosis. Morphine-treated human peripheral blood lymphocytes underwent apoptosis when they were stimulated by L cells expressing Fas ligand (as indicated by the appearance of the hypodiploid peak in Fig. 1b). This morphine-primed, Fas-mediated apoptosis must be specific, because morphine-treated cells did not undergo apoptosis when co-cultured with L cells transfected with FasL in the antisense orientation. Results were similar when morphine-treated cells were incubated with Fas-specific antibodies. In addition, morphine did not promote apoptosis in splenocytes from Fas-deficient mice, confirming that Fas is necessary for this process (data not shown).

It has been claimed that morphine can reduce the number of lymphocytes *in vivo*[7], although in our *in vitro* experiments it was not sufficient to induce apoptosis. We confirmed this with splenocytes in mice *in vivo*, finding that morphine sulphate reduced the number of cells by 30% in 24 hours. We administered morphine together with either soluble Fas fusion protein (Fas-Ig) or normal mouse serum to test whether morphine caused loss of cells *in vivo* as a result of Fas–FasL interaction on these cells[8]: Fas-Ig prevented the morphine-induced loss of splenocytes, whereas normal serum did not (Fig. 1c). Morphine also induced Fas messenger RNA expression after 12 hours in the spleen, heart and lungs (Fig. 1d). As the morphine-induced loss of splenocytes *in vivo* is blocked by naloxone, this effect is presumably exerted through their opioid receptors, contributing to the immunosuppressive effects of morphine.

One way in which opioids perturb the immune system is by suppressing cytokine production, thereby inhibiting activation-induced proliferation. Our results indicate that they can also do this by directly inducing Fas expression and increasing apoptosis. Because Fas-mediated apoptosis requires FasL, which is expressed on a limited set of cells, only a small portion of cells undergo opioid-induced apoptosis under physiological conditions. But in pathological settings where the amount of FasL may increase, opioid-induced apoptosis could also increase.

Our discovery that opioids induce Fas expression not only bears upon the consequences of taking morphine, but should also help our understanding of the interaction between the nervous and immune systems.

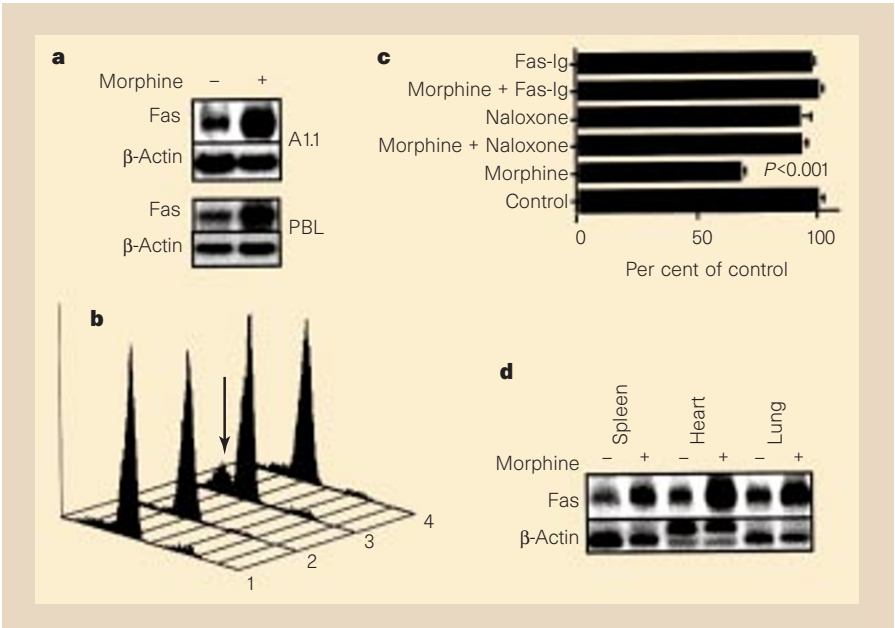

Figure 1 Morphine induces Fas expression and promotes FasL-mediated apoptosis. **a,** Morphine induces Fas expression. Northern-blot analysis of Fas expression in A1.1 T-cell hybridoma and human peripheral blood lymphocytes (PBL) treated with 3 μM morphine sulphate for 2 hours. **b,** Morphine primes human PBL to FasL-mediated apoptosis. PBL were treated with (peaks 1 and 3) or without (peaks 2 and 4) morphine for 2 hours and then co-cultured with mouse fibroblasts (L929) transfected with constitutively expressed FasL complementary DNA in the sense (peaks 3 and 4) or antisense (peaks 1 and 2) orientation. DNA content was analysed by flow cytometry after 12 hours. Arrow indicates the hypodiploid peak of apoptotic cells which was not evident in controls. **c,** The effects of *in vivo* administration of morphine sulphate on the cellularity of mouse spleens. BALB/c mice six weeks old were treated with morphine sulphate (50 mg per kg), Fas-Ig (150 μg per mouse) or naloxone (100 mg per kg). Total splenocytes were counted at 24 hours. **d,** Morphine injection induces Fas expression in different tissues. Fas expression was analysed by northern blotting 12 hours after morphine treatment.

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1. Roy, S. & Loh, H. H. *Neurochem. Res.* **21,** 1375–1386 (1996).
2. Nestler, E. J. & Aghajanian, G. K. *Science* **278,** 58–63 (1997).
3. Mariani, S. M., Matiba, B. & Krammer, P. H. *Behring Inst. Mitt.* **97,** 12–23 (1996).
4. Nagata, S. & Golstein, P. *Science* **267,** 1449–1456 (1995).
5. Shi, Y. F., Sahai, B. M. & Green, D. R. *Nature* **322,** 625–626 (1989).
6. Brunner, T. *et al. Nature* **373,** 441–444 (1995).
7. Stefano, G. B. *et al. Crit. Rev. Immunol.* **16,** 109–144 (1996).
8. Suda, T., *et al. J. Immunol.* **154,** 3806–3813 (1995).