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Functional Identification of Opioid Receptors In vivo Using <latex>β</latex>-FNA

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Analgesia can be produced by activating both μ and κ opioid receptors (Tyers 1980). Receptor binding experiments indicate that many opioid drugs have comparable affinities for both μ and κ receptors, and in the absence of specific antagonists it is difficult to analyse the contribution of each receptor type to the antinociceptive effect of the drug.

Beta-Funaltrexamine (β-FNA) was synthesized by Portoghese *et al.* (1980) and was reported to be a reversible κ agonist which, on prolonged contact, would alkylate and irreversibly antagonize μ receptors. This occurred both *in vitro* and *in vivo* (Ward *et al.* 1982*a*, *b*). β-FNA may thus be used to antagonize the μ component of an opioid drug, the extent of the antagonism indicating the μ receptor contribution to the effect.

The field-stimulated guinea-pig ileum preparation was used to characterize the effects of β-FNA *in vitro*. This tissue contains only μ and κ opioid receptors (Lord *et al.* 1977). Dose–response curves were constructed to the selective μ agonists [D-Ala, Gly(ol)]enkephalin (DAGO) and morphine, and the selective κ agonist U50488 before and after incubation of the tissue with β-FNA for 30 min at various concentrations. Concentrations of β-FNA up to 10^{-6} M produced dose-dependent parallel shifts of the dose–response curve for DAGO. Treatment with 10^{-6} β-FNA shifted the DAGO dose–response curve by 27-fold and the morphine dose-response curve by 45-fold. This concentration of β-FNA had no effect on the response to U50488, But higher concentrations did shift its dose–response curve to the right, indicating that β-FNA will antagonize κ receptors when used in sufficiently high concentration.

The effects of β-FNA *in vivo* were tested in weanling hooded rats. In rats pre-treated 24 h earlier with β-FNA (20–80 mg kg^{-1}), there was a dose-dependent antagonism of the antinociceptive and other actions of morphine. After 40 mg kg^{-1} β-FNA, the dose ratios in the paw pressure test, hot plate test and tail flick test were 25.4, 11.7 and 16.6 respectively. The hypothermia and inhibition of gut motility induced by morphine were antagonized by a similar ratio, while respiratory depression was shifted by 7.9-fold. The antagonism of morphine by β-FNA was extremely long-lasting: in rats dosed with 80 mg kg^{-1} β-FNA, there was still significant antagonism remaining after 8 days. In contrast, β-FNA did not antagonize the antinociceptive effects of U50488, confirming its lack of activity at the κ receptor.

These results confirm that both μ and κ receptors can independently cause analgesia, and they also demonstrate that the side-effects of morphine such as inhibition of gut motility and respiratory depression are also μ-mediated. Clearly, β-FNA will be a powerful tool for analysing the μ component of analgesic drugs in common clinical use.

References

Lord, J. A. H., Waterfield, A. A., Hughes, J. & Kosterlitz, H. W. 1977 *Nature, Lond.* **267**, 495–499.
Portoghese, P. S., Larson, D. L., Sayre, L. M. & Fries, D. S. 1980 *J. med. Chem.* **23**, 233–234.
Tyers, M. B. 1980 *Br. J. Pharmacol.* **69**, 503–512.
Ward, S. J., Portoghese, P. S. & Takemori, A. E. 1982*a* *Eur. J. Pharmacol.* **85**, 163–170.
Ward, S. J., Portoghese, P. S. & Takemori, A. E. 1982*b* *J. Pharmac. exp. Ther.* **220**, 494–498.