Inhibition of [^{3}H]-naloxone binding by opiate agonists

H.W. KOSTERLITZ & FRANCES M. LESLIE

Unit for Research on Addictive Drugs, University of Aberdeen

It has been shown that the relative receptor affinities of most opiates, as measured by their inhibition of [^{3}H]-naloxone binding to rat brain homogenates, correlate well with their pharmacological actions in the guinea-pig ileum, which is a good predictor of their analgesic action in man (Kosterlitz & Waterfield, 1975; Creese & Snyder, 1975). Of particular interest is the fact that Na^+ decreases the binding of agonists whilst not affecting, or slightly enhancing, that of antagonists (Pert & Synder, 1974). The purpose of this communication is to draw attention to the great variability of the sodium shift.

A modification of the method of Pert & Snyder was used. Guinea-pig brain homogenates, prepared by means of an Ultraturrax, were spun at 600 *g* to remove cell debris. The supernatant was centrifuged at 49,000 *g* and the resulting pellets resuspended in either 50 mM Tris buffer (pH 7.4 at 37°C) or in Krebs-Tris. Aliquots of homogenate (2 ml) were incubated with 1 nM [^{3}H]-naloxone (4 Ci/mmol, NIDA) plus the inhibiting drug at 37°C for 20 min, the samples then being cooled on ice for 30 min before filtration through Whatman GF/B filters. Stereospecific naloxone binding was determined by subtracting the binding in the presence of 50 nM MR 2266 {(–)-α-5,9-diethyl-2-(3-furylmethyl)-2′-hydroxy-6,7-benzomorphan} from that in its absence. For each drug, the concentration required to produce a 50% inhibition of binding was calculated from log-probit regression lines.

The degree of sodium shift was not constant for all agonists since a 7 to 150 fold decrease in potency was observed. Pert & Snyder (1974) found that agonists with an antagonist component have a small sodium shift but the great variation found in this study cannot be explained on this basis, as only pure agonists were used. It is of interest that the three drugs with the smallest sodium shifts belong to a group of benzomorphans which are unusual in that they do not cause morphine-like dependence in monkeys and require more naloxone than does normorphine to antagonize their actions in the guinea-pig ileum and mouse vas deferens (Hutchinson, Kosterlitz, Leslie, Waterfield & Terenius, 1975). They are (–)-α-(1R, 5R, 9R)-5,9-dimethyl-2-(L-tetrahydrofurfuryl)-2′-hydroxy-6,7-benzomorphan (MR 2034, C.H. Boehringer Sohn), (±)-α-5,9-dimethyl-2-(3-methyl-furfuryl)-2′-hydroxy-6,7-benzomorphan (MR 1353) and (±)-9-methyl-5-ethyl-8-oxo-2-cyclopropyl-methyl-2′-hydroxy-6,7-benzomorphan (ethylketocyclazocine, Win 35,197-2, Sterling-Winthrop).

For most of the agonists studied there is good correlation between the relative potencies of inhibition of binding and those found in the pharmacological models, the guinea-pig ileum and mouse vas deferens. However, some compounds, notably Win 35,197-2, were less potent in the binding assay than in the pharmacological models. The reason for this discrepancy is, as yet, unclear. It would, however, appear to be advisable to employ several parallel *in vitro* assays, using pharmacological responses and inhibition of binding, for the prediction of potencies *in vivo*.

Supported by a grant from the National Institute on Drug Abuse (DA-00662). F.M.L. is a MRC Scholar.

References

CREESE, I. & SNYDER, S.H. (1975). Receptor binding and pharmacological activity of opiates in the guinea-pig intestine. *J. Pharmacol. exp. Ther.*, **194**, 205–219.

HUTCHINSON, M., KOSTERLITZ, H.W., LESLIE, F.M., WATERFIELD, A.A. & TERENIUS, L. (1975). Assessment in the guinea-pig ileum and mouse vas deferens of benzomorphans which have strong antiociceptive activity but do not substitute for morphine in the dependent monkey. *Br. J. Pharmac.*, **55**, 541–546.

KOSTERLITZ, H.W. & WATERFIELD, A.A. (1975). *In vitro* models in the study of structure-activity relationships of narcotic analgesics. *Ann. Rev. Pharmac.*, **15**, 29–47.

PERT, C.B. & SNYDER, S.H. (1974). Opiate receptor binding of agonists and antagonists affected differentially by sodium. *Mol. Pharmacol.*, **10**, 868–879.

Dopamine utilization in the posterior pituitary gland of the rat

URMA GODDEN, MARGARETHE HOLZBAUER & D.F. SHARMAN

Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge CB2 4AT

Dopamine is present in the posterior lobe of the pituitary gland where it is thought to be contained in nerve fibres (Björklund, 1968; Saavedra, Palkovits, Kizer, Brownstein & Zivin, 1975). As a first step in the study of the function of dopamine (DA) in this tissue, its utilization was investigated. Albino rats (Wistar, Porton strain) were injected with α-methyl-*p*-tyrosine (AMPT), an inhibitor of tyrosine hydroxylase and were killed 30, 60 and 120 min later. Control rats were injected with a 0.9% NaCl solution and killed after the same time intervals. The pituitary gland was dissected out, the anterior lobe separated from the