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RAT VAS DEFERENS: A SPECIFIC BIOASSAY FOR ENDOGENOUS OPIOID PEPTIDES

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The electrically-evoked contractions of the rat vas deferens were selectively inhibited by β-endorphin, the preparation being much less sensitive to enkephalins and narcotic analgesic drugs. However, introduction of D-Ala in position 2 of [Leu]-enkephalin enhanced the activity of the opioid peptide to the order of that of β-endorphin. It is concluded that the rat vas deferens preparation constitutes a specific bioassay for endogenous opioid peptides and related compounds.

Introduction Since the mouse vas deferens was found to contain a morphine-sensitive neuroeffector junction (Henderson, Hughes & Kosterlitz, 1972), it was chosen as a suitable preparation for the measurement of the activity of opioid compounds, including the newly discovered endogenous opioid peptides: enkephalins (Hughes, Smith, Kosterlitz, Fothergill, Morgan & Morris, 1975) and endorphins (Coy, Goldstein & Li, 1976; Guillemin, Ling & Burgus, 1976). On the other hand, the vas deferens of other species, namely the rat, cat, rabbit and guinea-pig were demonstrated to be almost insensitive to the presence of morphine (Hughes, Kosterlitz & Leslie, 1975). After the recent discovery of multiple opiate receptors for endogenous opioid peptides (Lord, Waterfield, Hughes & Kosterlitz, 1977), it became interesting to investigate the action of β-endorphin and related peptide compounds on these latter preparations. In this paper we show that the rat vas deferens preparation constitutes a specific sensitive bioassay for endogenous opioid peptides and related compounds.

Methods Rat, cat, rabbit and guinea-pig vasa deferentia were prepared as described by Hughes *et al.* (1975). They were mounted in 10 ml organ baths containing Krebs solution (bubbled with 95% O_2 and 5% CO_2) at 37 ± 0.5°C and contracted by electrical stimulation at supramaximal voltage (0.1–0.2 Hz, 1 ms). A 1 g tension was applied on the tissues and was adjusted during a 30 min equilibration period. Contractions were recorded isometrically with Grass force-displacement transducers (FT 0.03C) on a Grass model 7D polygraph.

Synthetic β-endorphin (sheep) was prepared in our laboratory (Lemaire, Berube, Derome, Lemaire, Magnan, Regoli & St-Pierre, 1978) as described by Li, Lemaire, Yamashiro & Doneen (1976) using preformed symmetrical anhydrides of Boc amino-acids for the solid-phase peptide synthesis. The purified peptide was found homogeneous and identical with the natural hormone (from Dr Michel Chrétien, Montréal) by amino acid analysis, thin layer chromatography (1-butanol-pyridine-acetic acid-water, 6:6:1.2:4.8, R_F: 0.45), partition chromatography (1-butanol-pyridine-4.6% ammonium acetate in 0.1% aqueous acetic acid, 5:3:11, R_F: 0.2), paper and gel electrophoreses. [D-Ala^2]-β-endorphin was also synthesized by the same methods and its purity was confirmed by the same analytical criteria. The enkephalins were kindly supplied by Dr S. Wilkinson (Wellcome Research Laboratories). Naloxone (Narcan), nalorphine (Nalline), pethidine (maperidine, Demerol), levorphanol (L-Dromoran) and pentazocine (Talwin) were obtained from Endo Laboratories, Frosst, Winthrop Labs, Hoffman-LaRoche and Winthrop Labs respectively. Ketazocine and cyclazocine were generous gifts of Dr F.C. Nachod of the Sterling-Winthrop Research Institute, New York. Male wistar rats (200 to 250 g) were obtained from Canadian Breeding Farm & Laboratories Ltd.

Relative potencies of the opioid compounds were estimated by measurement of their ID_{50} values, i.e. the concentration of the opiate agonist that reduces the electrically-evoked contractions of the rat vas deferens by 50 percent. These values were obtained from log-probit plots of five concentrations of the opiate, each representing the means calculated from eight different tissues. The ability of opiate antagonists to reverse the action of opiate agonists was estimated by calculation of their *Ke* value, i.e. the concentration of antagonist that requires a doubling of the agonist dose to give the same pharmacological response (Lord *et al.*, 1977).

Results The ability of β-endorphin to inhibit the electrically-evoked contractions of the vas deferens was estimated with preparations from the cat, rat, rabbit and guinea-pig. Among these preparations, only the rat and rabbit vasa deferentia were sensitive to the action of β-endorphin with respective ID_{50} values of 130 and 110 nM. This activity of β-endorphin was completely reversed by naloxone (50 nM). On the rat vas deferens preparation, naloxone was found

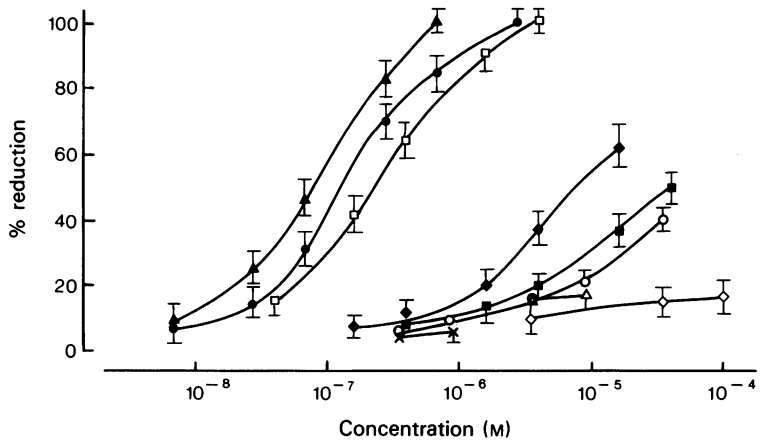

Figure 1 Dose-response curves of the different agents tested on the rat vas deferens. Points indicate the means and vertical lines represent the s.e. means of at least 8 experiments. Ordinate scale: % reduction of the electrically-evoked contractions of the vas deferens; abscissa scale: concentration of the drugs, (M). (▲) [D-Ala^2]-β-endorphin; (●) β-endorphin; (□) [D-Ala^2, Leu^5]-enkephalin; (◆) [Met^5]-enkephalin; (■) [Leu^5]-enkephalin; (○) pethidine; (△) levorphanol; (×) pentazocine; (◇) morphine.

to be 58 fold more potent than nalorphine in reversing the effect of β-endorphin, with a *K*e value of 27 nM compared to 1,600 nM for nalorphine. Neither of the opiate antagonists displayed any agonist activity.

In order to characterize further the opiate activity of β-endorphin, the rat vas deferens was chosen for further studies. Figure 1 shows the inhibition of electrically-evoked contractions of the rat vas deferens by increasing concentrations of β-endorphins, enkephalins, and various narcotic analgesic drugs. Substitution of Gly by D-Ala in position 2 of β-endorphin induced a 1.6 fold increase in its biological activity, with a final ID_{50} value of 82 nM compared with 130 nM for β-endorphin. [Met]- and [Leu]-enkephalins were approximately 600 and 3,000 fold less potent than β-endorphin respectively ([Met]-enkephalin, ID_{50}:8,000 nM; [Leu]-enkephalin, ID_{50}:40,000 nM). When Gly^2 of [Leu]-enkephalin was replaced by D-Ala, the resulting compound was 250 fold more potent than the parent peptide ([D-Ala^2, Leu^5]-enkephalin, ID_{50}:160 nM).

In general, the non-peptidic narcotic analgesics were much less potent than endogenous opioid peptides in the rat vas deferens bioassay. Figure 1 shows that morphine, at 10^{-4}M, produced only a 10% to 20% inhibition of the electrically-evoked contractions of the rat vas deferens. This action of morphine was also reversed by naloxone (50 nM). Pethidine at concentrations ranging from 10^{-6} to 5×10^{-5}M reduced the contractions of the rat vas deferens up to 40%; but this effect was not specific since it was not reversed by naloxone. Pentazocine and levorphanol at concentrations ranging from 10^{-7} to 10^{-6}M had some inhibitory effect (6 and 16%, respectively). However, when the concentrations of these drugs were further increased, a contractor rather than a relaxant effect was observed on the muscle preparation. Naloxone (50 nM) antagonized the small inhibitory effect of levorphanol but not that of pentazocine; the contractor effect of both drugs was not modified by naloxone. Ketazocine and cyclazocine, two benzomorphan derivatives, did not display any kind of activity.

Discussion Previous studies have demonstrated that among various preparations of vas deferens from different species, only the mouse vas deferens was sensitive to morphine (Lord *et al.*, 1977). In contrast, we have shown that the rat and rabbit vas deferens preparations are highly sensitive to the presence of β-endorphin. The specificity of the effects of β-endorphin on these tissues was confirmed by their reversal with naloxone, a pure antagonist of narcotic analgesic drugs. On the other hand, the enkephalins were much less potent than β-endorphin in inhibiting the electrically-evoked contraction of the rat vas deferens, whereas [D-Ala^2, Leu^5]-enkephalin was almost as active as β-endorphin. These data further indicate the importance of the resistance of the Tyr-Gly bond to enzymatic degradation for the biological activity of enkephalin (Pert, Pert, Chang & Ford, 1976). The same change in the structure of β-endorphin, introducing D-Ala in position 2, induced a much smaller increase in its biological activity, suggesting that the Tyr-Gly bond in β-endorphin may be already fairly

resistant to the action of aminopeptidases (Doneen, Chung, Ymashiro, Law, Loh & Li, 1977).

Earlier reports (Lord, Waterfield, Hughes & Kosterlitz, 1976; Lord *et al.*, 1977) have demonstrated the existence of multiple receptors for endogenous opioid peptides and it was then suggested that they should be classified according to their specific sensitivity to known opiate agonists or antagonists such as μ for morphine, κ for ketazocine or σ for N-allylcyclazocine or nalorphine, as antagonist (Martin, Eades, Thompson, Huppler & Gilbert, 1976). Our studies suggest that the rat vas deferens must contain very few μ, κ or σ receptors since it is poorly or not sensitive to morphine, ketazocine, nalorphine and other non-peptide narcotic analgesic drugs. It is concluded that the rat vas deferens provides a specific sensitive bioassay for naturally occurring opioid peptides and analogues.

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