Opiates induce long-term increases in prodynorphin-derived peptide levels in the guinea-pig myenteric plexus

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Summary. The subcutaneous administration of a single dose of an opiate agonist (levorphanol) or antagonist (naloxone) to guinea pigs results in at least 3-fold elevation of dynorphin and alpha-neoendorphin-immunoreactivity in the longitudinal muscle myenteric plexus preparation. The effects are time- and dose-dependent, significant elevations first being observed 6 h after treatment and lasting for up to 24 h. Pretreatment levels of opioid peptides were observed after 8 days. Combined injection of the narcotic agonist and antagonist, at sufficiently high doses, resulted in an additive effect of the individual drugs. The respective stereoisomers dextorphan and (+)-naloxone did not affect prodynorphin-derived peptide concentrations. An increase of endogenous opioids was also observed after administration of the non-opiate clonidine, a compound which, like opiates, alters the activity of the myenteric plexus. It is suggested that feedback mechanisms in the myenteric plexus are responsible for the elevation of endogenous opioid peptides following exposure to exogenous opiates.

Using a monoclonal antibody (3-E7), which recognizes virtually all endogenous opioid peptides, it was found that levels of higher molecular material were also increased upon opiate challenge. This suggests that a single dose of an exogenous opiate results in an increase in peptide synthesis.

Key words: Guinea-pig myenteric plexus — Prodynorphin — Dynorphin A — Alpha-neoendorphin — Feedback — Opiates

Introduction
Endogenous opioids are believed to have neurotransmitter functions in both the central and peripheral nervous systems (for reviews see Akil et al. 1984; Schulz 1985). Since neuronal activity is controlled by feedback mechanisms, Kosterlitz and Hughes (1975) postulated the occurrence of adaptive processes after the application of exogenous opioids to neuronal systems. This concept has previously been tested by a number of investigators who reported either no change of brain enkephalin levels upon morphine treatment (Wesche et al. 1977; Childers et al. 1977; Fratta et al. 1977; Höltt et al. 1978), a decrease of β-endorphin concentrations following long-term morphine exposure (Przewlocki et al. 1979) or an increase of enkephalin levels during the state of morphine tolerance/dependence (Simantov and Snyder 1976). One investigation of the peripheral nervous system revealed no changes in enkephalin concentrations in the myenteric plexus of the guinea-pig ileum upon morphine exposure; however, moderately increased levels of β-endorphin fragments were observed (Opmeer et al. 1980).

The data reported so far thus suggest that treatment of animals with exogenous opioids may affect the concentrations of some endogenous opioids but not of others. The primary aim of the present investigation was to study more closely the effect of acute actions of an opiate agonist and antagonist upon opioid peptides. We selected the guinea-pig myenteric plexus for these experiments, since it contains functional opioid receptors (Leslie et al. 1980; Paterson et al. 1983; Schulz et al. 1981) which are involved in the control of intestinal propulsion (Kromer et al. 1980, 1981; Clark and Smith 1981). The prodynorphin-derived peptides dynorphin A (DYN) and alpha-neoendorphin (ANE) have been indentified in the myenteric plexus of several species and are believed to be endogenous ligands of these receptors (Goldstein et al. 1979; Tachibana et al. 1982; Watson et al. 1981).

In the present experiments, DYN- and ANE-immunoreactivities (i.r.) were measured following their separation from biological material using the 3-E7 monoclonal antibody (Gramsch et al. 1983) which recognizes virtually all known opioid peptides. In an earlier study we showed the usefulness of this antibody in immunochromatographic studies for the identification of opioid peptides (Dandekar et al. 1985). In the present experiments we demonstrated that a single opiate treatment results in a considerable increase of DYN- and ANE-i.r. in the myenteric plexus-longitudinal muscle preparation of the guinea-pig ileum.

Materials and methods
Male guinea pigs (350—400 g) were housed at 22°C on a 12—12 h light-dark cycle. The animals were treated subcutaneously with the drugs under investigation or with saline. Food was withdrawn 15 h before sacrifice. After decapitation, the jejunum and ileum was dissected rapidly, and the longitudinal muscle with attached myenteric plexus was prepared. These procedures were carried out in the cold (4°C), and the strips were kept in Ringer solution (4°C). After the wet weight was taken, the strips were extracted in 0.1 N HCl (95°C, 0.1 g tissue in 20 ml HCl) for 15 min. Five minutes after starting the extraction the tissue was...
homogenized (Ultra Turrax, rod N 10, 10 s, at maximal speed). The samples were then centrifuged (10,000 x g, 15 min, 4°C) and the pH of the supernatant adjusted to 7.0 (NaOH). The recovery of opioid peptides after this extraction procedure was tested by incubating either 125I-ANE or 125I-DYN with 1 g striated muscle; 75–85% of the radioactive neuropeptides were recovered in the supernatant.

The separation of opioid peptides, including DYN and ANE, was achieved by immunoaffinity chromatography, employing the 3-E7 monoclonal antibody, as previously described (Dandekar et al. 1985). Briefly, the neutralized supernatant (20 ml) was run in the cold over an immunoaffinity column (3-E7 antibody linked to Sepharose). Thereafter, the affinity gel was washed with 0.1 M ammonium acetate (pH 7.0, 5 ml), and the opioid peptides eluted using 2 M acetic acid (2 ml). The eluted material was lyophilized, taken up in 1 M acetic acid and submitted to gel chromatography (Sephadex G 50, superfine, column 10 x 900 mm; 1 M acetic acid, containing 0.1% bovine serum albumin; flow rate 10 ml/h). One milliliter fractions were collected and lyophlized (Speed Vac Concentrator, Savant Instruments). β-Endorphin eluted in fractions 33–37, DYN in 39–44 and ANE in 50–54. Specific RIA's were employed to detect the individual peptides. Details of the RIA-technique and characteristics of the antisera have been published (Dandekar et al. 1985). The detection limit for DYN and ANE-i.r. was 5 and 8 fmol per tube, respectively. High pressure liquid chromatography (HPLC) was employed in a few cases in order to verify DYN- and ANE-i.r. was 5 and 8 fmol per tube, respectively. High pressure liquid chromatography (HPLC) was employed in a few cases in order to verify DYN- and ANE-i.r. was 5 and 8 fmol per tube, respectively. High pressure liquid chromatography (HPLC) was employed in a few cases in order to verify DYN- and ANE-i.r. was 5 and 8 fmol per tube, respectively. High pressure liquid chromatography (HPLC) was employed in a few cases in order to verify DYN- and ANE-i.r. was 5 and 8 fmol per tube, respectively. High pressure liquid chromatography (HPLC) was employed in a few cases in order to verify DYN- and ANE-i.r. was 5 and 8 fmol per tube, respectively. High pressure liquid chromatography (HPLC) was employed in a few cases in order to verify DYN- and ANE-i.r. was 5 and 8 fmol per tube, respectively. High pressure liquid chromatography (HPLC) was employed in a few cases in order to verify DYN- and ANE-i.r. was 5 and 8 fmol per tube, respectively. High pressure liquid chromatography (HPLC) was employed in a few cases in order to verify DYN-

The following drugs were used: Dynorphin A, alpha-neoendorphin, met-enkephalin, β-endorphin (all from Bachem, Bubendorf, Switzerland), bovine serum albumin (Sigma, St. Louis, MO, USA), morphine-HCl (Merck, Darmstadt, FRG), levorphanol and dextrorphan (Roche, Grenzach-Wyhl, FRG), ethylketazocine methanesulphonate (Sterling-Winthrop, Rensselaer, NY, USA), fentanyl-dicyclodihydrogentartrate (Janssen, Düsseldorf, FRG), (−)-naloxone-HCl (Endo, Garden City, NY, USA), clonidine-HCl (Boehringer, Mannheim, FRG), (−)-naloxone-HCl (a gift of Dr. A. Jacobson).

Results

3-E7 “opioid-screening”

The 3-E7 monoclonal antibody recognizes all endogenous opioid peptides. Therefore, it was here utilized to screen for these neuropeptides in preparations of the longitudinal muscle/myenteric plexus. Figure 1 demonstrates the results of screening in extracts of strips from naive (upper panel) and morphine treated (lower panel) guinea pigs. When 125I-β-endorphin was used as the tracer, a considerable increase in the i.r. profile was observed 6 h after morphine treatment (20 mg/kg). Since pilot studies also suggested an elevation of i.r. DYN and ANE, the relevant fractions were subjected to the appropriate RIA’s so as to determine the amount of these peptides. There was indeed a significant increase in the levels of i.r. DYN and ANE in morphine exposed preparations.

Effect of narcotic agonists

Figure 2 displays the temporal profile of the effect of a single dose of the µ-ligand levorphanol (1 mg/kg) on DYN- and ANE-i.r. Neither peptide-concentration was affected by levorphanol 1 h after injection. In contrast, 6 h following levorphanol challenge, i.r. of both DYN and ANE were significantly increased (3-fold for DYN, 6-fold for ANE), and similar increases were seen 24 h later. Dextrorphan (inactive isomer of levorphanol) treatment did not alter peptide levels.

Figure 3 shows that the effect of levorphanol upon DYN- and ANE-i.r. in strips prepared 6 h after treatment is dose-dependent. Controls were treated with dextrorphan. While no effect was observed at 0.01 mg/kg levorphanol on