Opioid Effects in Developing Rat Vas Deferens

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The inhibitory effects of various opiates on developing rat vas deferens were studied by determining the degree of depression of mechanical responses elicited by electrical field stimulation. All agonists showed decreased effects with maturation, but the decrease occurred at different times. With normorphine the loss of agonist activity was greatest at days 12–16, while with β-Met¹,Pro⁵-enkephalinamide it was greatest at days 16–20. β-Endorphin also was less effective in adult than 30-day preparations, but methionine enkephalin was ineffective at all ages. Morphine and normorphine were weak antagonists of opiate agonists in the adult preparations. These results indicate that the nature and pharmacologic sensitivity of opiate actions change with development.

KEY WORDS: opioid mechanisms; opioid peptides; rat vas deferens; postnatal development.

INTRODUCTION

The vas deferens of adult rats is practically insensitive to the opioid agonist actions of morphine and normorphine and very moderately susceptible to the inhibitory action of Met-enkephalin (ME) but is a sensitive, specific assay system for porcine β-endorphin (β-EP) (Lemaire et al., 1978; Schulz et al., 1979; Wüster et al., 1979). Naloxone is an effective antagonist of β-endorphin in this preparation. Furthermore, ethylketazocine, which is a pure agonist at the so-called k receptors (Lord et al., 1977) in other in vitro assays, is a pure opioid antagonist in isolated rat vas deferens (Gillan et al., 1981).

The present study was undertaken to characterize the postnatal development of opioid mechanisms in rat vas deferens using four different types of opioid agonists, β-endorphin, normorphine, D-Met²,Pro⁵-enkephalinamide (D-Met,Pro-EA), and methionine-enkephalin, and the opiate antagonist naltrexone (Ntx). The agonist activities of these compounds and their sensitivity to antagonism by naltrexone were determined on the 12th, 16th, and 30th postnatal days and in adulthood.

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METHODS

Male CFY rats were used. The weight range of pups and young animals, depending on the age, was from 18 to 100 g. The adult animals weighed 160–190 g. Each compound was tested in vas deferens taken from animals chosen from at least two different litters.

The vas deferens were dissected rapidly; the cleaning procedure (removal of connective tissue by "stripping" and expulsion of seminal content from "adult" preparations) was carried out at room temperature (24–26°C) in Mg²⁺-free Krebs' solution containing 0.25 mM (-)-tyrosine (Schulz et al., 1979), aerated with 5% CO₂, 95% oxygen.

The experiments were carried out in Krebs' solution of the same composition as above at 31°C, in an organ bath of 6.4-ml capacity.

In preparations taken from young animals (up to 30 days old) the initial tension applied to the organs was as high as 0.1 g. The organs were equilibrated for 25–30 min under resting conditions, followed by electrical stimulation until the contractions became stabilized, which usually took a further 20–30 min. Field electrical stimulation (Paton and Vizi, 1969) was used; paired shocks with 100 msec between the individual pulses were delivered every 20 sec through platinum wire electrodes. The square-wave pulses were 1 msec in impulse duration and 8 V/cm. In some cases at the end of an experiment, after washing the preparation repeatedly for at least 15 min, a single high-frequency, long-train stimulus (50 Hz, 200 shocks) was applied (the parameters of individual pulses were the same as above).

In adult preparations the initial tension was 1.0 g. The equilibration period was 120 min, under stimulation (Schulz et al., 1979). Field stimulation was applied according to two different schedules. The first comprised continuous stimulation at 0.1 Hz, where the voltage drop of individual square-wave pulses of 1-msec duration was 3 V/cm. The second was the same as that used in the case of young animals (see above) except that the paired shocks were delivered every 10 sec.

The electrically induced contractions of the longitudinal musculature were detected under near-isometric conditions.

MATERIALS

Porcine β-endorphin was kindly supplied by L. Gráf (Institute for Drug Research, Hungary). Methionine-enkephalin and d-Met²,Pro⁵-enkephalinamide were synthesised by S. Bajusz (Institute for Drug Research, Hungary) as described previously (Bajusz et al., 1977). Naltrexone hydrochloride was donated by J. Fishman and E. F. Hahn (Rockefeller Institute, New York). Normorphine (base) was supplied by the Alkaloida Works (Hungary).

The agonist activities of opioid compounds are characterized by their dose–response curves and, where possible, by the 50% inhibitory concentrations (IC₅₀) expressed as nanomoles per liter (nM). To characterize the antagonist–agonist interaction, either the Kₑ or the pA₂ values were determined for the antagonist (Arunlakshana and Schild, 1959; Paton, 1961; Kosterlitz and Watt, 1968) and are expressed also as nanomoles per liter. The equilibration time with the antagonist was