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# OCCURRENCE OF METHIONINE ENKEPHALIN IN HUMAN PLACENTAL VILLUS

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Acetylcholine (ACh) occurs in high concentrations in human placental villus. It is released into the medium when placental villus tissue is incubated in Krebs-Ringer bicarbonate buffer (pH 7.2 to 7.4) at 37°. Human placenta is not innervated (1). However, the release of ACh from the human placental villus resembles that from the nerve in several respects (2-4).

The discovery of the occurrence of two pentapeptides--methionine enkephalin and leucine enkephalin (5,6)--and β-endorphin in the brain and pituitary gland (7) has opened new avenues of research into the regulation of the functions of neurotransmitters and hormones (8). Generally, it is believed that enkephalins and endorphins may serve as neuromodulators and may regulate the neurotransmitter or hormone release by positive or negative feedback systems. More specifically, enkephalins may regulate neuronal release of acetylcholine and norepinephrine by negative feedback systems (9,10). Similarly, enkephalins may regulate ACh release in placenta. However, there is no evidence for the occurrence or role of enkephalins in human placenta. Therefore, human placental villus was extracted and investigated for enkephalins and endorphins. Our investigations indicate that human placental villus contains methionine enkephalin and β-endorphin.

## MATERIALS AND METHODS

Methionine enkephalin and leucine enkephalin were used as standards in the bioassay. They were purchased from the Pierce Chemical Co., Rockford, IL. Rabbit anti-met-enkephalin, anti-leu-enkephalin, anti-β-endorphin, [$^{125}$I]-methionine enkephalin, [$^{125}$I]-leucine enkephalin and [$^{125}$I]-β-endorphin were supplied by the Immuno Nuclear Corp., Stillwater, MN.

Extraction of enkephalin- or endorphin-like peptides from human placenta. Full-term human placentae were collected from Vanderbilt University Hospital and kept at 4°. The two umbilical arteries were cannulated and perfused with 2 liters of cold isotonic saline containing heparin (0.5 units/ml) until the effluent from the umbilical vein was free of blood. The villus tissue was dissected from the chorionic and basal plates, umbilical cord, and amnionic sac as described previously (3). The villi (100 g) were diced into small pieces (0.5 to 1.0 cm longest axis) and homogenized in cold acetone (500 ml) containing hydrochloric acid (0.02%). The homogenate was filtered under vacuum, and the residue was rehomogenized in a cold acetone-water (80/20) medium (500 ml) and filtered.

The combined filtrates (1000 ml), containing enkephalin-like peptides, were evaporated at 25° to about 100 ml to remove the acetone, and the aqueous extract was centrifuged at 10,000 g for 30 min.

The pellet was discarded and the supernatant fraction was evaporated to dryness under vacuum at 34°. The residue was extracted with methanol (20 ml), and the methanol extract was filtered free of salts and protein. The methanol filtrate was evaporated. The waxy residue was resuspended in 2 vol. of distilled deionized water in a centrifuge tube, heated in a boiling water bath for 15 min, and centrifuged for 15 min at 4°, 100,000 g. The super-

natant fraction was lyophilized, and the resulting powder was analyzed by bioassay and radioimmunoassay or stored at -20°.

Radioimmunoassay for enkephalins and β-endorphins. The radioimmunoassay for methionine enkephalin employed the simultaneous addition of placental extract (200 µl), rabbit anti-met-enkephalin antibody (100 µl) and [$^{125}$I]-methionine enkephalin (100 µl; 50 pg/ml, 0.3 µCi/ml). The mixture was vortexed and incubated overnight at 4°. Phase separation was accomplished by the addition of an equal volume of saturated ammonium sulfate (500 µl) in the presence of carrier gamma globulin (100 µl; 0.02%). The mixture was vortexed vigorously, allowed to stand for 15 min, and centrifuged at 760 g for 10 min. Both the supernatant fraction and the precipitate were counted for $^{125}$I. Values for the methionine standard curve (0.4 to 12.5 ng/ml; % vs ng/ml) were determined at the same time. All solutions for this assay were made in boric acid (0.001 M)-NaOH buffer (pH 8.4) with boiled bovine serum albumin (0.01%) and merthiolate (0.0001%).

There were significant differences between the assays for methionine enkephalin, leucine enkephalin and β-endorphin which will be described elsewhere.

Characterization of the biological activity of enkephalin-like peptides from placental villus. Extracts were characterized for enkephalin-like activity on three preparations: (a) guinea pig longitudinal ileal muscle (11,12); (b) mouse vas deferens (13); and (c) rat vas deferens. The conditions of the assay for the rat vas deferens were similar to those used for the mouse vas deferens (13). The first two are classical preparations and respond to both enkephalins and morphine, which decrease the output of the chemical transmitter. The rat vas deferens was found to be sensitive to both enkephalins, which were partially antagonized by naloxone (60 nM).

## RESULTS AND DISCUSSION

Using highly sensitive and specific radioimmunoassays, the occurrence of methionine enkephalin and β-endorphin in human placental villus was demonstrated (Table 1). The content was variable from placenta to placenta. Part of the enkephalins and β-endorphins might have been released during labor and washing. Therefore, the figures in Table 1 should be considered as minimal values. The presence of leucine enkephalin in human placental villus could not be demonstrated in our experiments. The occurrence of immunoreactive corticotropin, lipotropin and β-endorphin in whole placental extracts was demonstrated by Odagiri et al. (14). But, they did not demonstrate the presence of enkephalins.

When the floating villi, chorionic plate and basal plate of the same human placenta were extracted and analyzed, the methionine enkephalin concentration was lower in the chorionic and basal plates. This suggests that the distribution of methionine enkephalin is similar to that of ACh (15).

The occurrence of opioid peptides was further demonstrated by the biological activity of the placental peptides on the intramurally stimulated rat vas deferens, mouse vas deferens and longitudinal ileal muscle of the guinea pig. A biphasic response, an initial phase of facilitation followed by a second phase of the inhibition of transmission, was obtained when the placental extract was added to the intramurally stimulated longitudinal ileal muscle of the guinea pig. The initial phase of facilitation was not prevented by subjecting the placental extracts to hydrolysis by cholinesterases prior to the test. Therefore, the initial facilitation was not due to acetylcholine-like esters but due to unidentified peptides or

other endogenous substances. The second phase of the inhibition of transmission was antagonized by naloxone (60 nM). Therefore, this phase of the inhibition of transmission was due to the occurrence of enkephalin- and endorphin-like peptides in the placental extracts. Placental extract inhibited the transmission in the mouse and rat vas deferens which was antagonized by naloxone (60 nM).

Table 1. Methionine enkephalin and β-endorphin in human placental villus

| Placenta number | Methionine enkephalin (ng/mg protein) | β-Endorphin (ng/mg protein) |
|---|---|---|
| 1 | 0.27 | 0.17 |
| 2 | 0.46 | 1.70 |
| 3 | 0.49 | 1.22 |
| 4 | 0.22 | 0.44 |
| 5 | 0.54 | 1.07 |
| 6 | 1.05 | 0.40 |
| 7 | 1.14 | 0.34 |
| 8 | 0.60 | 0.49 |
| mean ± S.E. | 0.60 ± 0.12 | 0.73 ±0.19 |

According to our investigations, the rat vas deferens is the most convenient tissue for the assay of placental extracts. Leucine enkephalin, methionine enkephalin and placental extracts inhibited transmission in rat vas deferens (Fig. 1). Naloxone blocked their effects by about 44-51 percent. The rat vas deferens contains presynaptic muscarinic receptors. Acetylcholine activated these presynaptic receptors and facilitated transmission. Full-term human placenta contains 5-7 times higher levels of acetylcholine than brain. Extracts of placenta which were contaminated with acetylcholine could also be analyzed by atropinized rat vas deferens.

All of the above studies indicate that human placental villus contains biologically active opioid peptides. These peptides were identified as methionine enkephalin and β-endorphin. Methionine enkephalin and β-endorphin may exert negative feedback control on the placental release of acetylcholine. Or, the local release of methionine enkephalin and β-endorphin by placenta may regulate sensory transmission (or pain impulses) to the central nervous system from the distended uterus and vaginal tract during childbirth.

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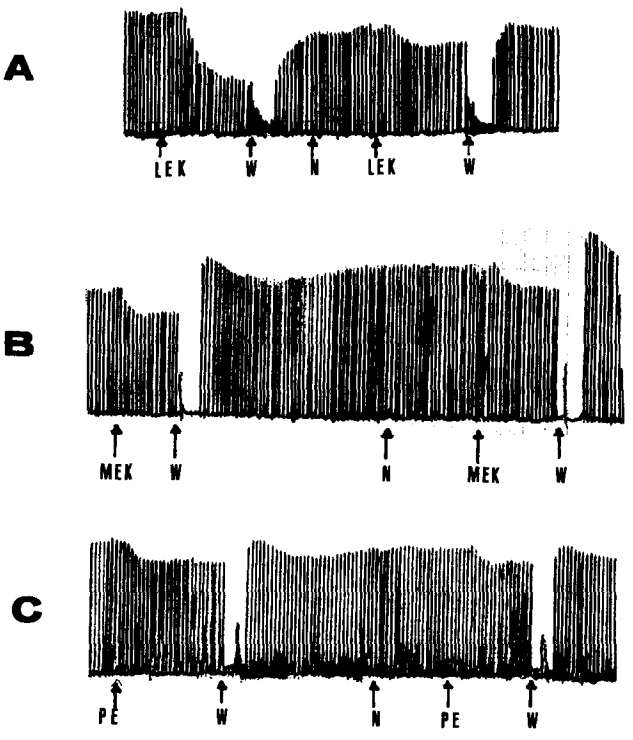


Fig. 1. Effects of leucine enkephalin (LEK, 10 μg/ml), methionine enkephalin (MEK, 10 μg/ml) and human placental extract (0.2 ml) on the rat vas deferens. All three inhibited transmission. The effects of all three were blocked partially (44-51 percent) by naloxone (60 nM). W, wash.

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