A Study of Nonpathogenic *Francisella, Brucella, and Yersinia* Strains as Producers of Recombinant β-Endorphin

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*Yersinia pseudotuberculosis, Brucella abortus, and Francisella tularensis* strains producing a recombinant β-endorphin have been obtained. The highest production of this peptide, which displays physiological activity, was recorded for cells of the *Y. pseudotuberculosis* strain 2243 (pSK95E).

**Key Words:** β-endorphin; producer strains; synthesis; biological activity

In our previous study [2], in which the regulatory peptide β-endorphin additively synthesized by a microbial donor was evaluated for its influence on the physiological state of recipient organisms, we established that the psychoemotional status and nociceptive responses of CBA mice depend on the reproductive dynamics in these hosts of the *Francisella tularensis* strain generating a recombinant β-endorphin. The prolonged effect induced by this strain exceeded by 25-50% the mean index of pain sensitivity inhibition [15]. Native β-endorphin (8-10 µg/kg) elicited in mice only a short-term effect that varied from 0 to 200-300% [2].

In an effort to further boost the additive action of recombinant β-endorphin, we have now explored the possibility of producing a number of strains capable of more intensive and more prolonged synthesis of this peptide in vitro and in the vaccinated animal host.

With this aim in view, we selected known vaccine strains of *Brucella abortus* and *Francisella tularensis* as well as avirulent *Yersinia pseudotuberculosis* strains. These microorganisms are all capable of prolonged survival in the laboratory animals used in our studies [1,3,7].

**MATERIALS AND METHODS**

In the study, we used *Escherichia coli* strains C600 and HB101 [6], *F. tularensis* strain 15 [2], *B. abortus* strain 19-BA [3], and *Y. pseudotuberculosis* strains 149 and 2243 [9]. *E. coli*, *Y. pseudotuberculosis*, and *B. abortus* cells were cultured in L-broth or L-agar [6]. *F. tularensis* cells were grown on erythrite agar with black albumin [5]. The β-endorphin gene within the pSK95E plasmid was kindly provided by O. I. Serpinskii and V. V. Kravchenko (from the Research Institute of Microbiology, Koltsovo, Novosibirsk Region, Russia). This plasmid was used to transform *E. coli* and *Y. pseudotuberculosis* cells as described previously [6]. *Y. pseudotuberculosis* cells, unlike those of *E. coli*, were cultured at 28°C. The β-endorphin gene was transferred to *B. abortus* cells by the conjugation technique [11]. For the transfers, the Sa plasmid was used as the vector [14]. All manipulations with DNA were carried out in accordance with the guidelines set out by Mazin *et al.* [6]. In addition, *F. tularensis* 15 cells inheriting the β-endorphin gene in the chromosome [2] were used for analysis.
Fig. 1. Schematics of the genetic constructs providing for β-endorphin gene expression in E. coli (pSK95E), Y. pseudotuberculosis (pSK95E), F. tularensis (pSKFT5E), and B. abortus (pSaE) cells. The broad open arrows denote the lac UV5 promoter; the broken arrows with dots, the β-endorphin gene; the continuous arrows, the tet gene of the pBR322 plasmid; the continuous line, the sequence of the pBR322 plasmid; the wavy arrow, a fragment of the F. tularensis chromosomal DNA; and the broken line, the SA plasmid sequence. Restriction endonucleases are designated by symbols as follows: E: EcoRI, B: BamHI, S: SalI.

β-Endorphins synthesized by producer cells were assayed radioimmunologically using a standard reagent kit (INC, USA). Assamples, cell lysates of each of the producers were used in a dose of 2×10⁹ CFU (colony-forming units). The lysates were prepared by treating cells with 10 mM KNa-phosphate buffer containing 0.9% NaCl and 2 mg/ml lysozyme. To lower proteolytic activity of the lysates against the recombinant β-endorphin, lysis was carried out at 4°C for 30 min. Additional disintegration of the cells was accomplished through freezing-thawing in cycles (-70°C - room temperature). The thawed-out samples were centrifuged and the supernatants, diluted if necessary 10-fold in 1% BSA (bovine serum albumin)-borate buffer, were used for further analysis. Samples with a known β-endorphin concentration were employed as standards.

Opioid activity of the recombinant β-endorphins relative to the native peptide was determined as described elsewhere [10] using isolated smooth-muscle preparations of guinea pig ileum. Test samples were prepared from lysates used in the radioimmunoassay by double extraction with acetone followed by evaporation in a rotor evaporator and resuspension in Krebs-Henseleit's solution [12]. The prepared samples were added directly to a bath for isolated organs. Lysates of cells that did not inherit the β-endorphin gene served as negative controls. As positive controls, β-endorphin (Serva) in a concentration of 0.3 µg/ml and morphine (Serva) in a concentration of 0.6 µg/ml were used. Ileum contractions were recorded using an isometric transducer (HSE). Specificity of the test preparations' activity was assessed by blockage of their effects on ileum contractility by naloxone (Endo Laboratories), a specific antagonist of opioid peptides (2 mg/ml).

The stability of β-endorphin gene inheritance by Y. pseudotuberculosis 2243 (pSK95E) cells was determined by evaluating the β-endorphin-producing capacity of a culture isolated from organs of rats infected with that strain. For this, 20 white rats were each injected with 1 ml of physiological saline containing a culture of the test strain, after which two rats were decapitated each day for a total of 10 days and cultures from their liver, lung, and spleen were seeded on L-agar. If cultures were consistently recovered, they were assayed for β-endorphin as indicated above, and the percentage of those cells in a cell population that had retained tetracycline resistance was also determined.

RESULTS

The schemes of genetic constructs used in this study for obtaining a β-endorphin-producing strain are shown in Fig. 1. The pSK95E plasmid used for transforming E. coli and Y. pseudotuberculosis cells contained the β-endorphin gene together with the tet gene of the pBR322 plasmid in one reading frame. Processing of the peptide was accomplished using the translation terminator TAG which separates these genes. The expression of both genes was controlled by the lac UV5 promoter inserted at EcoRI sites directly before the initiating ATG codon of the β-endorphin gene. To transform F. tularensis cells, the pSKFT5E plasmid was used, in which a fragment of the F. tularensis chromosomal DNA served as the promoter [8]. Transformants of E. coli, Y. pseudotuberculosis, and F. tularensis cells were selected on the basis of tetracycline resistance of their clones, as the location of the β-endorphin and tet genes in the same operon with a single promoter and single terminator indicated that these genes should be expressed simultaneously in the microorganisms under study.

The β-endorphin gene was introduced into B. abortus cells by conjugation within the pSaE plasmid in which the pSK95E plasmid had been incorporated into the single restriction site SalI of