EFFECT OF OPIOIDS ON DEVELOPMENT OF PREIMPLANTATION MOUSE EMBRYOS

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Endogenous opioid peptides are distributed not only in the CNS, but also in sex cells: molecules of Met-enkephalin [2] are distributed on the surface of human spermatozoa and the follicular cells surrounding the oocyte containing β-endorphin [4]. β-Endorphin also is present in endometrial cells, from which it is secreted during implantation [7]. It has been shown that hamster embryos at the 2-4-cell stage contain the tetrapeptide kertsin, which posseses opioid-like activity when injected into an animal's brain [1]. It is not yet clear, however, how opioid compounds act on preimplantation embryos.

The aim of this investigation was to study the sensitivity of mouse embryos, developing in vitro, to the mu-opiate receptor agonist D-Ala^2,N-Me-Phe^4-ol-enkephalin (DAGO), the delta-opiate receptor agonist D-Ala^2-D-Leu^5-enkephalin (DADL), and naloxone, an antagonist of mu-, delta-, and kappa-receptors.

EXPERIMENTAL METHOD

Early CBA mouse embryos were used. Single-cell embryos were taken from the oviduct on day 0 of pregnancy, i.e., on the day when a vaginal plug was found. After removal of the cumulative cells with a 0.1% solution of hyaluronidase ("Reanal," Hungary) the embryos were washed out 4 times in Dulbecco's medium and transferred to Whitten's medium in the modification of Hoppe and Pitts [3], saturated with a mixture of 90% N_2 + 5% O_2 + 5% CO_2, and incubated in an ultrothermostat (37°C). The number of late morulas and blastocysts was determined on the 4th and 5th days. The opioids DAGO, DADL, and naloxone were from "Sigma" (USA). Results of one of five experiments in each of which no fewer than 10 embryos developed in the presence of a particular opioid are described.

EXPERIMENTAL RESULTS

The opioid compounds were found to inhibit development of the embryos (Fig. 1). Of all the opioids tested, the mu-, delta-, and kappa-receptor antagonist naloxone had the strongest action: after its addition to embryos at the "zygote" stage in a concentration of 3×10^{-5} M until the "late morula–blastocyst" stage only 6.7% of embryos developed, which is 13.1 times fewer than in the control ($p < 0.001$).

The mu-receptor agonist DAGO had a weaker action, for after its addition (3×10^{-5} M) to zygotes, 36.8% of embryos reached the "late morula–blastocyst" stage ($p < 0.001$), and the rest of the embryos, as after addition of naloxone, remained at the stages of two, four, and, less frequently, eight cells.

When naloxone was added to 4-cell embryos their sensitivity to its action was 7.5 times lower ($p < 0.001$) than that of zygotes: the "late morula–blastocyst" stage was reached by 50% of the embryos.

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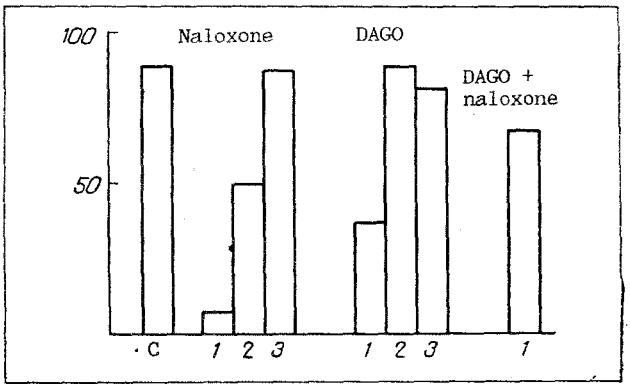

Fig. 1. Effect of opioid compounds on development of preimplantation mouse embryos. C) Control; 1-3: addition of substances in a concentration of 5×10^{-5} M to zygotes (1), to 4-cell embryos (2), and to 8-cell embryos (3). Ordinate, number of late morulas and blastocysts (in %).

Later 8-cell embryos were not sensitive to the action of naloxone and the number of embryos reaching the "late morula–blastocyst" stage was the same as in the control (Fig. 1). The number of late morulas and blastocysts likewise was the same as in the control after addition on DAGO, to both 4- and 8-cell embryos (Fig. 1).

The ability of naloxone and DAGO to inhibit embryonic development was substantially reduced if both opioids were added to the zygotes in equimolar concentrations (3×10^{-5} M) simultaneously: the number of morulas and blastocysts was 66.8%, and not 6.7 and 36.8% as in the presence of naloxone and DAGO respectively. The action of naloxone in the presence of DAGO was 7.95 times weaker ($p < 0.001$) than the action of naloxone alone (Fig. 1).

Opioids can thus inhibit the development of preimplantation mouse embryos. The action of DAGO and naloxone was stage-specific with their maximal effect after addition to embryos at the zygote stage, when the intrinsic genome of the embryo was still inactive [6]. Under these circumstances the opioid peptide DADL, similar to DAGO in its chemical structure, did not inhibit embryonic development, evidence of the selective sensitivity of embryos to these opioids, a fact which points to the specific character of their action.

It is difficult on the basis of the available facts to draw any firm conclusion regarding mediation of the action of opioids by specific opiate receptors of developing mouse embryos. Evidence in support of the possible existence of such receptors is given, on the one hand, by the fact that the effect of the opiate agonist DAGO is weakened in the presence of the opiate antagonist naloxone (Fig. 1), just as takes place during competition between these opioids for interaction with the mu-receptors of the animal brain [5]. On the other hand, however, the antagonist naloxone has an action which is neither opposite (accelerating development) nor neutral (not affecting development), but which is similar to that of the agonist DAGO, i.e., it inhibits embryonic development. If the presence of embryonic opiate receptors is accepted, the simplest explanation of this disagreement may be as follows: the "rules" of function of the opiatergic system of the brain are not observed at the level of opiate receptors of embryonic cells.

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