BIOGENIC MONOAMINES IN MOUSE OOCYTES AND PREIMPLANTATION EMBRYOS

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UDC 612.646.019.08

KEY WORDS: biogenic monoamines; early mouse embryos; histochemical detection.

Biogenic monoamines and acetylcholine, which are neurotransmitters in adult organisms, are known to appear and to begin to function in the earliest stages of embryogenesis, long before formation of the nervous system [1, 3]. The character of the functional activity of biogenic monoamines changes in the course of individual development. These substances are involved in the regulation of cleavage divisions and early interactions between blastomers [2], but in the later stages of preneural embryogenesis they require morphogenic movements of the cells and embryonic motor activity [1, 3, 8].

The basic data on "preneural" functions of mediators have been obtained on early embryos of invertebrates and amphibians. Meanwhile, there have been virtually no investigations into the functional role of biogenic monoamines in early mammalian embryogenesis.

In this paper we describe the histochemical detection of biogenic amines in mouse oocytes and preimplantation embryos. For this purpose, a histochemical method [12] was used, having previously been successfully used on embryonic material [5, 6].

EXPERIMENTAL METHOD

(CBA × C57BL) hybrid mouse embryos were used.

To synchronize ovulation and to increase the number of ovulating oocytes, the females were treated with hormones. Initially 10 units of pregnant mare's serum was injected initially, followed after 48 h by 10 units of chorionic gonadotrophin (CGT) intraperitoneally.

Newly fertilized eggs were obtained 16-20 h after injection of CGT. Unicellular zygotes were flushed out on the 1st day of pregnancy, which was identified by the appearance of a vaginal plug in the females. Embryos of two and four cells were obtained on the 2nd day of pregnancy, 8-cell embryos during the first half of the 3rd day of pregnancy were obtained by flushing out the oviducts with culture medium. Morulas and blastocysts were flushed out of the oviducts and uterine cornua on the 4th and 5th days of pregnancy respectively.

Biogenic monoamines were demonstrated by the method in [12] using glyoxalic acid. Control embryos were treated with buffer not containing glyoxalic acid. The preparations were examined in the "Lyuman R-2" luminescence microscope with primary FS-1 and SZS-24-1 filters, giving green fluorescence of flurophores of catecholamines and yellow fluorescence of indoles.

To verify the effect of monoamine oxidase (MAO) inhibitors and inhibitors of serotonin synthesis on the intensity of the histochemical reaction these substances were injected intraperitoneally into pregnant animals. The MAO inhibitors (iproniazid, pargyline, transamine, in concentrations of 2 mg/mouse, the inhibitor of serotonin synthesis parachlorophenylalanine, in 2 doses: 0.6 mg/mouse initially, and 1.2 mg/mouse 20-24 h later; after a further 3 h the animals were killed. Control animals were given an injection of physiological saline. The experimental results were recorded by microfilming.

N. K. Kol'tsov Institute of Developmental Biology, Academy of Sciences of the USSR, Moscow. Institute of Experimental Biology, Academy of Sciences of the Kazakh SSR, Alma-Ata. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 6, pp. 577-578, June, 1990. Original article submitted September 22, 1989.

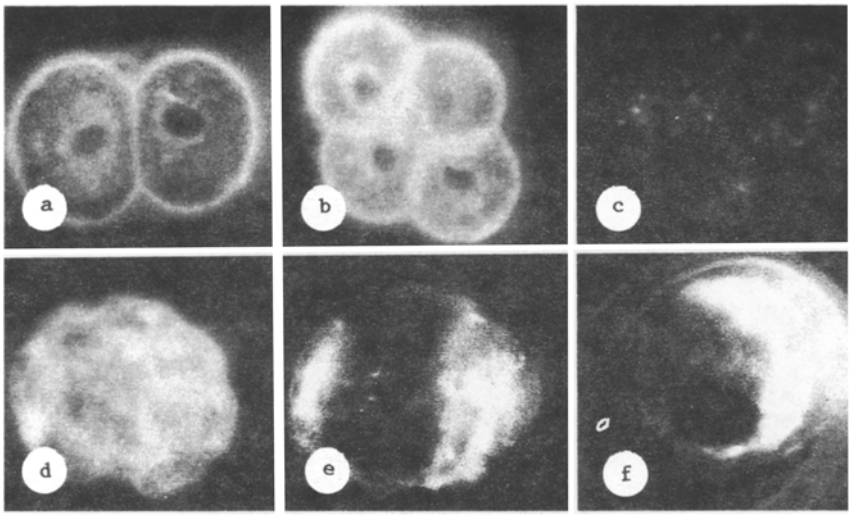

Fig. 1. Specific fluorescence of early mouse embryos: a) embryo at 2 blastomere stage, b) 4 blastomeres, c) control embryo at 4 blastomere stage; d) morula stage, e) early blastocyst, f) late blastocyst. Ocular 7, objective 40.

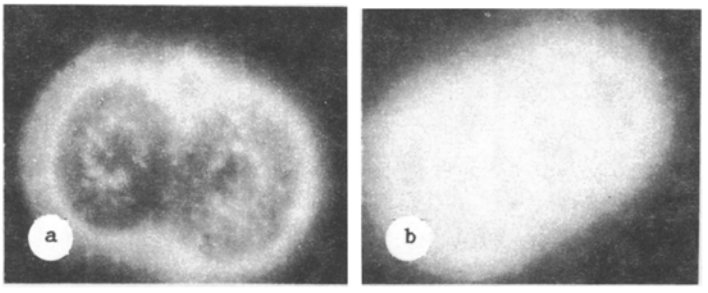

Fig. 2. Effect of MAO inhibitor, pargyline, on intensity of specific fluorescence of 2-cell mouse embryo. a) Normal, b) action of pargyline injected intraperitoneally.

EXPERIMENTAL RESULTS

Living embryos in a drop of culture medium had virtually no background fluorescence. On treatment with glyoxalic acid, very weak fluorescence was observed over the whole surface of the cell in the unfertilized eggs, with more intense fluorescence in the follicular cells. After fertilization its intensity increased somewhat. In 2- and 4-cell embryos yellow fluorescence was observed, evidence of the presence of indole derivatives, and was localized on the surface of the blastomeres. Punctate fluorescence also was observed in the cytoplasm around the cell nuclei. This fluorescence, by contrast with the specific kind, also was observed in the control (Fig. 1a-c). The cell nuclei did not fluoresce. Starting with the 8-cell stage of development the character and location of the fluorescence changed. It became green in color, which is characteristic of catecholamines, and was concentrated not on the cell surface, but in the cytoplasm. At the morula stage its intensity increased (Fig. 1d) to reach a maximum by the blastocyst stage. In early blastocysts flushed out of the uterus in the morning of the 4th day of pregnancy, fluorescence was located in the cells of the inner cell mass, and also to some extent in cells of the trophoblastic ectoderm (Fig. 1e). In late blastocysts flushed out of the uterine cornua on the 5th day of pregnancy only cells of the inner cell mass, from which the embryo subsequently develops, were fluorescent (Fig. 1f).

MAO inhibitors, injected intraperitoneally into the pregnant animals 2-3 h before flushing out of the embryos affected the intensity of monoamine-induced fluorescence of the early mouse embryos. Of the three inhibitors tested, the clearest effect was given by pargyline and transamine. They greatly increased the intensity of fluorescence both on the cell surface (stage of 2-4

blastomeres) and in the cytoplasm (stage of 8 blastomeres; Fig. 2a, b). Incidentally, in the concentrations used, MAO inhibitors injected in vivo had no effect on the development of early embryos. Moreover, as was shown previously in model experiments, these substances, on interaction with glyoxalic acid, did not form fluorescent fluorophores.

Injection of *p*-chlorophenylalanine, an inhibitor of serotonin synthesis, into the pregnant animals caused disturbances of development in many of the embryos. Abnormal fragmented embryos, failure of cleavage of the zygote, and embryos with disaggregated blastomeres were observed. The intensity of fluorescence was reduced in the embryos studied. Because there were too few experiments these results must be regarded as provisional.

It can be concluded from the results as a whole that even in the earliest stages of development of mouse embryos biogenic monoamines are present. During the first cleavage divisions yellow fluorescence of the cell surface predominates, disappearing under the influence of ultraviolet radiation. This fluorescence is evidence of the presence of indole derivatives. Later, starting with the 8-blastomere stage, green fluorescence of the cytoplasm appears and becomes stronger in the course of development. This is evidence of the appearance of catecholamines.

Changes in expression of biogenic monoamines in the course of early embryogenesis of the mouse are indirect evidence of the functional activity of these substances. Judging by the results of experiments with MAO inhibitors and *p*-chlorophenylalanine, these changes are linked with monoamine metabolism in cells of early embryos.

Data in the literature on biogenic monoamines in early mammalian embryos are very scanty. The presence of serotonin and noradrenalin in early rat embryos has been demonstrated by means of the histochemical formaldehyde method [6]. According to the authors cited, the intensity of histochemical expression of monoamines increases after fertilization, and it also increases after incubation of early embryos in solutions of these substances. Evidence of the presence of enzymes of catecholamine synthesis and breakdown in early mouse embryos was obtained in [9]. Functional activity of these substances has been studied [4, 10, 11] in early mammalian embryogenesis.

Our results, together with these data in the literature, can be regarded as proof of the presence and metabolism of biogenic monoamines in mammalian eggs and early embryos. Our information on changes in the expression of these substances in the course of development and during the action of inhibitors also is evidence of their functional activity. Elucidation of the actual character of this activity will be the aim of future investigation.

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