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SIGMA RECEPTORS OF LOACH EMBRYOS CONTROL ORNITHINE DECARBOXYLASE ACTIVITY

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UDC 615.357:577.175.829].065:618.33].076.9

KEY WORDS: embryonic development; sigma receptors; ornithine decarboxylase

The sigma receptor agonist SKF 10.047 (N-allylnormethazocine), unlike certain other neurotransmitters and ligands of opioid, mu, delta, and kappa receptors, has a specific teratogenic action on embryos of the loach (*Misgurnus fossilis*) [1]. The primary mechanism of the morphogenic action of this compound is to potentiate aggregation of the embryonic shield cells during gastrulation, which in turn is brought about by clustering of the cell surface receptors. The action of SKF 10.047 has been shown to be mediated by specific binding centers, which are similar in their biochemical properties to the sigma receptors of the mammalian brain [9, 10] and of the adult female loach brain [2]. An important role in the maintenance of growth and differentiation of embryonic cells is played by endogenous polyamines, namely putrescine, spermine, and spermidine [3, 5], and this paper gives data indicating that the action of SKF 10.047 on embryonic cells may be linked with modulation of activity of the key enzyme involved in the biosynthesis of these compounds, i.e., ornithine decarboxylase (ODC).

EXPERIMENTAL METHOD

Ripe oocytes were obtained, fertilized, and incubated and the stages of their development were identified in accordance with the scheme described in [7]. The serial number of the stage of development corresponded to the number of hours of development at 21°C. Reagents (±)-SKF 10.047, (+)-SKF 10.047, and (−)-SKF 10.047 were generously provided by A. Hertz (Max Planck Institute of Psychiatry, West Germany). The L-1-^{14}C-ornithine was obtained from "Amersham International" (England) and the remaining reagents from firms in the USA. ODC activity was determined by the method suggested previously [8].

EXPERIMENTAL RESULTS

In the presence of (±)-SKF 10.047 (10^{-5} M), added immediately after fertilization, ODC activity increased more rapidly than in the control, and at stage 4 (eight blastomeres) it reached its highest value, more than $1^1/_2$ times greater than in the control (Fig. 1). Later, however, ODC activity declined, and by stage 8 (middle blastula) it had returned to the control level. Between stages 8 and 10 (late blastula to beginning of gastrulation) activation of ODC by the action of (±)-SKF 10.047 did not take place. However, ODC activity after stage 10 increased again in the presence of this substance and reached a maximum which was about 1.3 times higher than the ODC level in the control at stage 12 (formation of the embryonic shield). Activity of the enzyme then decreased and at stage 14 (separation of the notochord from the mesoderm) it returned to the control level. If (±)-SKF 10.047 was added at stage 7 to embryos developing under normal conditions, activation of ODC, just as with addition at stage 10 or with continuous incubation with this substance, did not begin until stage 10. Thus, the

All-Union Research Center for Maternal and Child Health, Ministry of Health of the USSR. All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow.
(Presented by Academician of the Academy of Medical Sciences of the USSR, D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 7, pp. 97-98, July, 1989. Original article submitted October 21, 1988.

 0007-4888/89/0007-1020$12.50

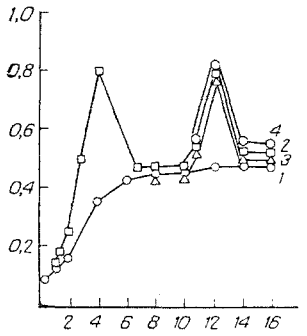

Fig. 1

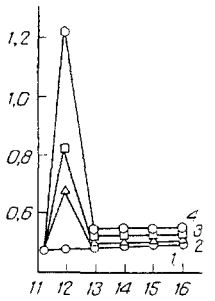

Fig. 2

Fig. 1. Changes in ODC activity in loach embryos on addition of (±)-SKF 10.047. Abscissa, stages of embryonic development; ordinate, ODC activity (in nmoles CO_2/mg protein). 1) Control; 2) embryos developed constantly in the presence of SKF 10.047 when added after fertilization; 3) SKF 10.047 was added at stage 7; 4) SKF 10.047 was added at stage 10. Results of one of three experiments are shown.

Fig. 2. Stereospecificity of ODC-stimulating action of SKF 10.047 on the loach embryo. 1) Control; 2) (−)-SKF 10.047; 3) (±)-SKF 10.047; 4) (+)-SKF 10.047. Substances added at stage 11 in a concentration of 10^{-5} M. Results of one of three experiments are given. Remainder of legend as to Fig. 1.

ODC-stimulating action of (±)-SKF 10.047 depends on the stage of development. Rapid activation of ODC after the addition of (±)-SKF 10.047 at stage 10 was due in all probability, not to intensification of the de novo synthesis of this enzyme, but to activation of existing molecules of it, just as is observed during interaction of lectins with receptors of T lymphocytes [7]. With a decrease in the concentration of (±)-SKF 10.047 to 10^{-6}-10^{-7} M, activation of ODC did not take place. With an increase in concentration of the substance to 10^{-3} M ODC was not activated, but inhibition at stage 12 also was only half of that observed in the control, probably as a result of nonspecific suppression of the general metabolism of the embryos (data not given).

To solve the problem of specificity of the ODC-stimulating action of SKF 10.047, we used the (+)- and (−)-stereoisomers of this compound. It was found that (+)-SKF 10.047 activated ODC 1.5 times more strongly than (±)-SKF 10.047 and almost twice as strongly as the (−)-isomer (Fig. 2). These observations are evidence that the ODC-stimulating action correlates with the teratogenic action of SKF 10.047. First, with this compound in a concentration of 10^{-6}-10^{-7} M activation of ODC did not take place, correlating with the absence of teratogenic activity of SKF 10.047, when added to embryos in those concentrations [1]. Second, it was not (−)-SKF 10.047 which had a teratogenic action on the embryos, but (±)-SKF 10.047 and (+)-SKF 10.047: the (+)-isomer was more active under these circumstances than the racemate (data not given), and they bind more specifically with embryonic sigma receptors [2].

What is the mechanism of the ODC-stimulating teratogenic action of SKF 10.047? As was shown previously [1], under the influence of SKF 10.047 cells of the embryonic shield began to condense, and the same process also took place in the control during gastrulation, but less intensively. In other words, SKF 10.047 apparently accelerates the normal course of aggregation of the cells during the formation of the embryonic shield. Assuming that aggregation of cells of the embryonic shield depends directly on the concentration of endogenous polyamines, the mechanism of the ODC-dependent teratogenic action of SKF 10.047 may be as follows. In the presence of SKF 10.047, added to the embryos, synthesis of the key enzyme in polyamine synthesis, namely ODC, is intensified. As a result of this the concentration of the polyamines is increased, and this leads to intensification of the process of aggregation of the embryonic shield cells controlled by them, as the writers established previously [1]. It can also be tentatively suggested that it is not the polyamines themselves, but their derivatives that are the controllers of aggregation of embryonic cells; it has been found [4] that the neurotransmitter GABA can be formed from putrescine (a precursor of spermine and spermidine).

Thus, SKF 10.047, a sigma receptor agonist, can activate the ODC of loach embryos. The ODC-activating action of SKF 10.047 is stereospecific and it depends on the stage of embryonic development. A further study of the character of aggregation of embryonic cells under the influence of polyamines (and/or their derivatives) and discovery of the mechanism of their action on embryonic cells would seem to be important.

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DETERMINATION OF AMYLOID P-COMPONENT IN BLOOD PLASMA BY ELISA

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UDC 616.153.96-078.73

KEY WORDS: amyloid P component; enzyme immunoassay; serum amyloid P component

The amyloid P component (AP), found in amyloid deposits, is structurally and antigenically similar to serum amyloid P-component (SAP), which is a normal blood plasma glycoprotein [15]. It is claimed that SAP is the precursor of AP in systemic amyloidosis [9]. There is evidence that AP is not only formed in the tissues during the development of systemic amyloidosis, but it is also a normal component of the basement membranes of the renal tubules, skin, lungs, and intestine [2].

The disappearance of AP from the basement membrane of the renal glomeruli is associated with the development of an inherited nephropathy (Alport's disease) [10]. SAP is a glycoprotein with mol. wt. of 250 kD, consisting of 10 identical subunits which, by noncovalent binding, form two pentameric structures. C-reactive protein and certain other mammalian protein molecules, linked together on this basis to form a class of proteins known as pentraxins [11], have a similar structure. A characteristic feature of AP is its conservatism in evolution, for identical proteins are found in all vertebrates, amphibians, and fishes investigated, and this has attracted attention to the study of its biological function [13]. It has been shown that SAP is produced by hepatocytes, it takes part in immunoregulation, by inhibiting the proliferative response of lymphoid cells to mitogenic stimulation [8], and it can undergo calcium-dependent binding with various ligands [1]. Normally the SAP concentration remains quite stable at 50-70 μg/ml [3]. Serum levels of SAP are extremely low in neonates and a little higher in children [12]. The SAP level is lower in women than in men [12]. Several methods have been developed to determine the SAP concentration, including rocket immunoelectrophoresis, nephelometry, radioimmunoassay, and enzyme immunoassay [3, 4, 7, 14]. However, there is little information on the SAP concentration in diseases in man. Some preliminary results indicate an increase in the SAP concentration in Waldenstrom's macroglobulinemia, rheumatoid arthritis, and malignant neoplasma [6, 7]. This confirms the need for further improvement in methods used to determine SAP in order to estimate its concentration in various human diseases.

Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, A. I. Vorob'ev.)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 7, pp. 99-101, July, 1989. Original article submitted July 4, 1988.

0007-4888/89/0007-1022$12.50