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MULTISTAGE REGULATORY SYSTEM TRANSMITTING THE ACTION OF ESTRADIOL IN THE RAT UTERUS

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Estradiol induces histidine decarboxylase in the tissues of the uterus, and histamine activates adenylate cyclase. Cyclic AMP, like histamine, stimulates the action of estradiol by stimulating RNA synthesis and inducing enzymes of glycolysis and hydration of the uterus. Autoradiography showed that [^{3}H]estradiol is accepted by the nuclei of some myometrial cells, and [^{3}H]histamine by the cytoplasm of these cells; [^{3}H]cyclic AMP is selectively bound by the endothelial cells of the uterine capillaries. Estradiol mediators (histamine and cyclic AMP) evidently spread the effect of the hormone to heterofunctional cells of the uterus which together constitute a unique type of multicellular functional assemblage.

KEY WORDS: Estradiol; histamine; cyclic AMP; induction.

Investigations have shown that histamine and cyclic AMP simulate the principal physiological effects of estradiol: hyperemia, hydration of the uterus, etc. [6, 12, 14]. It was shown recently in the writers' laboratory that histamine and cyclic AMP are mediators in the action of pentagastrin. Together with certain enzymes, they constitute a unique type of amplification cascade, spreading the action of the hormone to various adjacent cells forming a multicellular functional assemblage [10]. It might be supposed that this principle of regulation is used in several systems of multicellular organisms and that, in particular, a similar amplification cascade of enzymes and mediators, including histamine and cyclic AMP, transmits the action of estradiol. The investigation described below was carried out in order to test these hypotheses.

EXPERIMENTAL METHOD

Sexually mature female Wistar albino rats weighing 140-160 g were used and ovariectomized bilaterally three weeks before the experiment. Each variant of the experiment was carried out on three or four animals. Estradiol-17β (10 μg/100 g), histamine·2HCl (250 μg/100 g), and cyclic AMP (10 mg/100 g) with theophylline (10 mg/100 g) were injected intraperitoneally. Actinomycin D was injected 15 min before the test substances. [^{14}C]Uridine (specific activity 13.9 μCi/mmole) was injected intraperitoneally in a dose of 20 μCi/100 g body weight 1 h before sacrifice. RNA synthesis was determined from incorporation of [^{14}C]uridine into the acid-insoluble fraction of uterine tissue [5]. Hexokinase and pyruvate-kinase activity was determined by Singhal's method [12]. Histamine-decarboxylase activity, detectable in segments of uterus, was judged from the quantity of [^{14}C]histamine formed from [^{14}C]histadine [11]. Adenylate cyclase activity in uterine tissue homogenates was determined by the method of Krishna et al. [7] and the DNA concentration in the tissues by the method of Dishe and Rosenfeld [3]. The distribution of ^{3}H-labeled estradiol, histamine, and cyclic AMP in the uterine tissues was investigated by autoradiography. Segments of uterus were incubated for 30 min in the presence of 10 μCi/ml [^{3}H]estradiol (specific activity 9.3 Ci/mmole; USSR), [^{3}H]histamine (specific activity 9.4 Ci/mmole; Radiochemical Centre, Amersham, England), or [^{3}H]cyclic AMP (specific activity 20.7 Ci/mmole; Radiochemi-

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TABLE 1. Effect of Histamine, Cyclic AMP, and Actinomycin D (25 μg/100 g) on RNA Synthesis, Hexokinase and Pyruvate Kinase Activity, and Weight of Uterus 6 h after Injection

Variant of experiment	RNA synthesis, cpm/mg DNA of homogenate	Hexokinase activity, nmoles of NADPH/min/mg DNA of homogenate	Pyruvate kinase activity, nmoles NADH/min/mg DNA of homogenate	Weight of uterus, mg
Control	1504±146 (6)	38±2,3 (9)	330±44 (9)	76±2,6 (32)
Histamine	3443±462 (5)	60±2,6 (7)	610±39 (7)	91±4,8 (17)
Histamine + actinomycin	963±443 (3)	35±1,9 (6)	340±59 (6)	82±1,6 (16)
Cyclic AMP	2626±284 (3)	46±1,1 (7)	520±10 (7)	91±2,8 (13)
Cyclic AMP + actinomycin D	1370±383 (3)	36±0,9 (6)	310±16 (6)	81±2,5 (6)
Actinomycin D	786±124 (3)	31±2,5 (6)	260±42 (6)	82±2,4 (9)

TABLE 2. Effect of Estradiol-17β and Actinomycin D (150 μg/100 g) on Histidine-Decarboxylase Activity in Tissues of Uterus 3 h after Injection

Variant of experiment	Number of experiments	Weight of uterus, mg	Histidine-decarboxylase activity, cpm/100 mg [^{14}C]benzenesulfohistamine/mg DNA
Control	6	75±2,8	367±71
Estradiol	5	136±5,9	806±67
Estradiol + actinomycin D	4	86±5,2	412±51
Actinomycin D	4	80±3,0	418±119

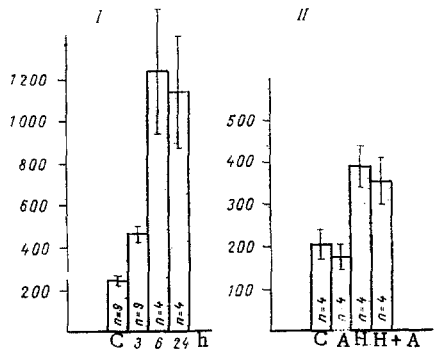

Fig. 1. Effect of histamine and actinomycin D on adenylate cyclase activity in uterus of ovariectomized rats. I: C) control; 3, 6, 24) hours after injection of histamine. II: C) control; A) actinomycin D; H) histamine (3 h after injection); H+A) histamine + actinomycin D. Ordinate, adenylate cyclase activity (in pmoles [^{14}C]cyclic AMP/mg DNA).

cal Centre, Amersham, England). The control for the experiments with [^{3}H]cyclic AMP consisted of segments of uterus which were incubated in medium with theophylline (1 mM) and with 10 μCi [^{3}H]5'-AMP (specific activity 22 Ci/mmole; Radiochemical Centre, Amersham, England). Pieces of uterus incubated with [^{3}H]-estradiol were fixed in Bouin's fluid and, after incubation with [^{3}H]histamine, [^{2}H]cyclic AMP, or [^{3}H]5'-AMP, they were fixed in Carnoy's fluid. Dewaxed sections were stained by the usual methods.

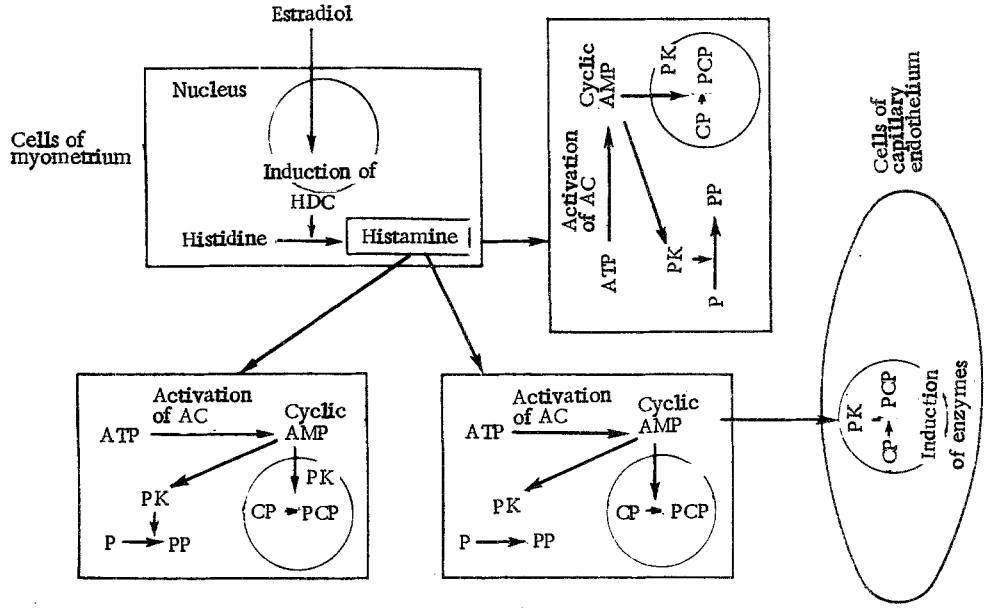

Fig. 2. Scheme of potentiation and spread of action of estradiol in uterine cells. HDC) histidine decarboxylase; AC) adenylate cyclase; PK) protein kinase; P) enzyme and other functional proteins; PP) phosphorylated proteins; CP) chromatin proteins; PCP) phosphorylated chromatin proteins.

EXPERIMENTAL RESULTS

Estradiol-17β is known to induce RNA synthesis and hexokinase and pyruvate kinase activity in the rat uterus and to cause an increase in weight of the uterus [12]. As Table 1 shows, histamine and cyclic AMP have a similar action, simulating the effects of estradiol completely. It seemed likely that estradiol induces histidine decarboxylase in the tissues of the uterus, and the histamine thus formed acts as mediator for estradiol. It was in fact found that estradiol increases histadine decarboxylase activity and that this effect is inhibited by actinomycin D (Table 2). In various tissues histamine exerts its action through activation of adenylate cyclase, and the cyclic AMP thus formed acts as mediator for histamine [2, 8-10]. The present experiments showed that histamine activates adenylate cyclase in the tissues of the uterus. This effect of histamine is not inhibited by actinomycin D and probably takes place through activation of adenylate cyclase [1], and not through induction of that enzyme (Fig. 1). As Table 1 shows, cyclic AMP simulates the action of estradiol, by inducing RNA synthesis, activation of the key enzymes of glycolysis, and hydration of the uterus; this action of cyclic AMP is inhibited by actinomycin D. It seems likely that the cyclic AMP formed under the influence of histamine, probably through activation of protein kinase and phosphorylation of chromatin proteins, leads to derepression of the genes responsible for some of the effects of estradiol in the uterus. These results enable the events initiated by estradiol in the tissues of the uterus to be represented by the scheme in Fig. 2, in which estradiol induces histadine decarboxylase, the histamine thus formed activates adenylate cyclase, the cyclic AMP arising as a result of this activates protein kinase, which phosphorylates histones, leading to induction of enzymes responsible for the physiological effects of estradiol. However, histamine is known to be an intercellular, and not an intracellular, mediator in the tissues of animals. The action of estradiol may perhaps begin with the target cells, where it induces histidine decarboxylase; the histamine later spreads the action of estradiol to other cells of the uterus, in which activation of adenylate cyclase takes place; the cyclic AMP activates certain physiological reactions in the cells in which it arises, and it may perhaps act simultaneously at a distance also [4].

To test these hypotheses the distribution of ^{3}H-labeled estradiol, histamine, and cyclic AMP was studied in the uterine tissues of ovariectomized rats by means of autoradiography. The results showed that [^{3}H]-estradiol accumulates selectively over the nuclei of some smooth-muscle cells of the myometrium and above the nuclei of some stromal cells of the endometrium (Fig. 3a). Information on the selective uptake of estradiol by cell nuclei in the uterus is also to be found in the literature [13]. After incubation with [^{3}H]histamine grains of silver were distributed uniformly above the cytoplasm, the nuclei, and also, evidently, the membranes of the myometrial cells (Fig. 3b). During incubation with [^{3}H]cyclic AMP tracks were clearly found above the endothelial cells of the capillaries and small vessels in all layers of the uterus and above the round cells of the endothelium (tentatively lymphocytes and granulocytes, Fig. 3c). Unlike during incubation with [^{3}H]cyclic

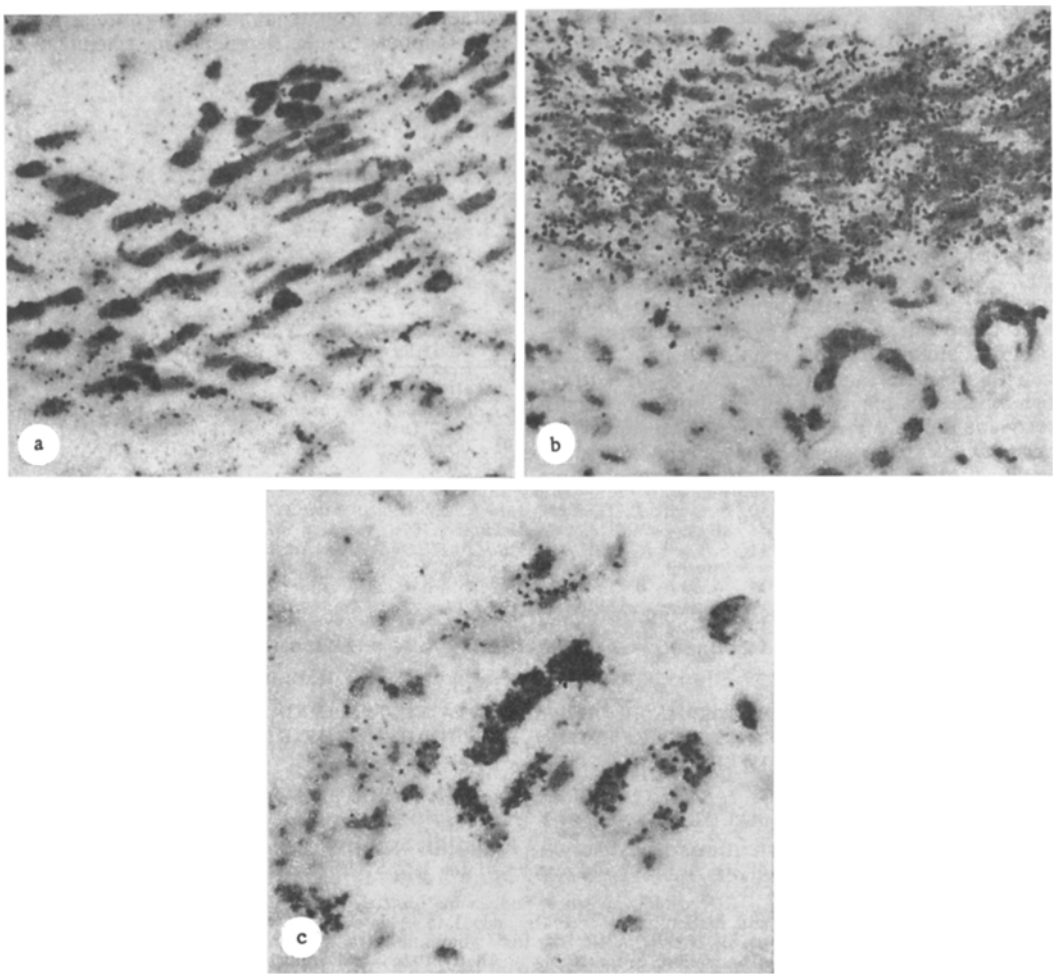

Fig. 3. Grains of silver above nuclei of muscle cells of myometrium after incubation of uterine tissues with [^{3}H]estradiol (a), above myometrium after incubation of uterine tissue with [^{3}H]histamine (b), and above endothelial cells of capillaries and small vessels of endometrium after incubation of uterine tissues with [^{3}H]cyclic AMP (c). a) 300×, b) 192×, c) 450×.

AMP, on incubation of the pieces of uterus with [^{3}H]5'-AMP grains of silver were found uniformly above all cells of the uterine wall except muscle fibers. These findings indicate that [^{3}H]cyclic AMP and not its hydrolysis product, [^{3}H]5'-AMP, binds with the cells of the uterus. It can be concluded from these autoradiographic studies that estradiol and its hypothetical mediators – histamine and cyclic AMP – interact specifically with different cells of the uterine tissue and with different intracellular structures in them.

It seems likely that estradiol, after its acceptance by the nuclei of target cells, induces the synthesis of histidine decarboxylase in them, thus promoting synthesis of histimine which is liberated into the intercellular space. This mediator, binding with receptors of the myometrial cells, activates adenylate cyclase in them, leading to synthesis of cyclic AMP, which is bound by the endothelial cells of the capillaries. These cells evidently contain proteins which recognize cyclic AMP. It is perhaps in the endothelium of the blood vessels that cyclic AMP activates transcription of genes for synthesis of the key enzymes of glycolysis and ion transport. By means of the multistage system of mediators described above the action of estradiol can not only be amplified many times over, but it can also be spread to various adjacent cells, which together form a multicellular functional assemblage, in the cells of which the distribution and integration of functions take place.

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