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STIMULATION OF UTERINE ORNITHINE DECARBOXYLASE IN ORGAN CULTURE BY DECREASING OSMOLALITY: POSSIBLE RELATION TO IN VIVO MECHANISMS

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Summary

Ornithine decarboxylase (ODC) activity increases during many growth responses. Some cells and tissues in culture also exhibit elevated enzyme activity with decreasing osmolality of the culture medium. We have found that this also occurs with uterine tissue from ovariectomized rats. Organ culture incubation under hypotonic conditions caused maximal stimulation of uterine ODC activity at 4 hr. This stimulation was observed when either NaCl or sucrose was used to adjust the osmolality. Incubation under isotonic conditions also increased ODC activity relative to hypertonic conditions. This increase was similar in magnitude to that seen with unincubated uterine tissue from animals receiving systemic estradiol or intrauterine cholera toxin. Both estradiol and cholera toxin increase vascular permeability, and the resultant edema changes the extracellular microenvironment of the uterine cells. We suggest that this change somehow is mimicked by organ culture under hypotonic or isotonic conditions and is responsible for the stimulation of uterine ODC activity.

Ornithine decarboxylase (ODC) is the first and rate-limiting enzyme in the synthesis of polyamines, which have been implicated in many growth processes (1-3). ODC activity is elevated dramatically by many growth promoting stimuli (4), including estrogen acting in the uterus (5). In HeLa cells and thyroid tissue in culture, ODC activity also increases in response to a decrease in the osmolality of the culture medium (6,7). We report here the same effect with uterine tissue from ovariectomized rats. In addition, we have compared the stimulation of uterine ODC activity in organ culture with that seen when animals are treated with estradiol systemically or cholera toxin intraluminally.

Materials and Methods

Chemicals and Media. Bovine serum albumin, calf thymus DNA, estradiol-17B, and cholera toxin were purchased from Sigma Chemical Co. Amersham was the source of the L[1-^{14}C] ornithine-HCl (57 mCi/mmole), and the premixed liquid scintillation cocktail (3a70B) was obtained from Research Products International. A specially formulated culture medium was purchased from GIBCO. This medium contained all the components of Eagle's Minimum Essential Medium (with Earle's salts) at twice the normal concentration, but lacked glutamine, leucine, and NaCl. For usage, this medium was diluted 1:1 with a mixture of

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water, 4 mM leucine (final concentration 0.4 mM), and 1 M NaCl or 2 M sucrose to obtain the approximate desired osmolality. The actual osmolality was determined with an Osmette osmometer (Precision Systems).

Animals and Organ Culture. Ovariectomized adult rats (Sprague-Dawley) were purchased from Hormone Assay Laboratories, Chicago, IL. The animals were not used in experiments until at least 3 weeks after castration. Estradiol was administered by subcutaneous (s.c.) injection of 10 ug in 0.5 ml of 99% saline, 1% ethanol. Control animals received only the vehicle. The procedure for the transcervical, intrauterine (i.u.) injection of cholera toxin was that described by Barker (8). Cholera toxin was administered in 30 ul at a concentration of 0.4 ug/ul in a buffer containing 10 mM Tris, 40 mM NaCl, 0.6 mM NaN_3, 0.2 mM EDTA, pH 7.5. Animals receiving cholera toxin had only one horn injected, with the contralateral horn serving as an uninjected control.

Animals were killed by cervical dislocation. In the cases of estradiol-treated and cholera toxin-treated animals, sacrifice was at 4 hr after injection. The uteri were quickly removed, placed on ice, and trimmed free of fat. Uteri from animals treated with estradiol or cholera toxin were not incubated in organ culture. Uteri used for organ culture were obtained from animals that received no previous treatment (i.e. no estradiol, cholera toxin, or vehicle). Prior to organ culture incubation, individual uterine horns were cut in half and placed in cold medium. Organ culture was carried out under an atmosphere of 95% O_2-5% CO_2 in tightly capped glass scintillation vials (20 ml), with each vial containing 6 half horns (from 5 or 6 different animals) in 2.0 ml medium. The vials were incubated at 37°C with gentle shaking.

Ornithine Decarboxylase Assay. ODC activity was assayed by the method of Russell and Snyder (9) with minor modification. The uterine tissue was minced finely and homogenized with a Polytron Homogenizer (Brinkman Instruments). Homogenization was carried out in 4.5 ml cold 50 mM TES buffer, 5 mM dithiothreitol, 1 mM glutamate, 0.5 mM pyridoxal-5-phosphate, 0.1 mM EDTA, pH 7.5. The homogenate was centrifuged at 35,000 x g for 10 min at 4°C.

Each supernatant liquid was assayed in quadruplicate for ODC activity. The 0.5 ml reaction mixture contained 0.4 ml of the supernatant liquid plus 0.1 uCi ^{14}C-ornithine at 0.2 mM, and it was incubated for 60 min at 37°C in a 16x100 mm culture tube. Each tube was capped with a rubber stopper, from which was suspended a polypropylene well (Kontes Glass Co.) containing 0.2 ml ethanolamine to trap $^{14}CO_2$. Assay "blanks" were incubated at 4°C. The reactions were terminated by addition of 0.5 ml 4 M citric acid. The tubes were allowed to set at 22°C overnight. The next day the entire well was placed in a scintillation vial, and 0.4 ml methoxyethanol plus 4.0 ml scintillation cocktail were added. Radioactivity was measured in a Packard 3375 liquid scintillation spectrometer. The liberation of $^{14}CO_2$ from ^{14}C-ornithine was linear with respect to time and protein concentration. Protein and DNA were determined by the methods of Bradford (10) and Burton (11), respectively.

Results

Fig. 1 shows that uterine ODC activity was maximally stimulated at 4 hr of organ culture incubation in a hypotonic medium. The activity at 4 hr was elevated 64-fold relative to the 0 hr (unincubated) control. By 8 hr of incubation, the ODC activity had dropped significantly, but it was still 16-fold greater than the control. Addition of the antibiotics streptomycin and tetracycline (each at 100 ug/ml) did not antagonize the increase observed at 4 hr (data not shown), indicating that it was not due to bacterial contamination. Cycloheximide (4 ug/ml), on the other hand, caused 98% inhibition of the stimulation of ODC activity at 4 hr (data not shown), indicating that protein synthesis was required for the effect.

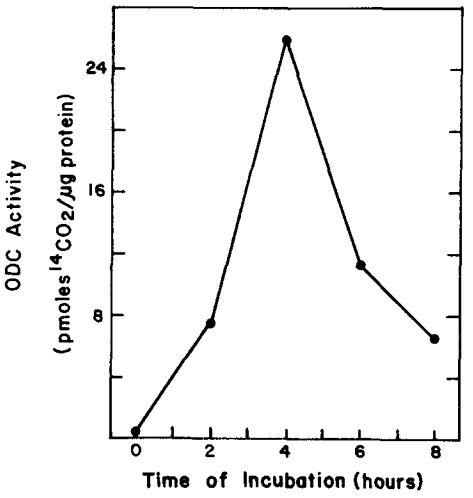

FIG. 1

Time course for the stimulation of uterine ornithine decarboxylase (ODC) activity by organ culture incubation in a hypotonic medium. The osmolality of the medium was adjusted with NaCl to 202 mOsm/kg.

The same dramatic increase of ODC activity under hypotonic conditions was seen whether NaCl or sucrose was used to adjust the osmolality of the incubation medium. This is shown in Fig. 2. When NaCl was used, the maximum hypotonic condition (189 mOsm/kg) stimulated ODC activity 95-fold relative to the maximum hypertonic condition (440 mOsm/kg). With sucrose, ODC activity increased 52-fold going from maximum hypertonicity (442 mOsm/kg) to maximum hypotonicity (222 mOsm/kg). These results suggest that the stimulation of ODC activity was due to a change in osmolality and not to a change in ionic conditions.

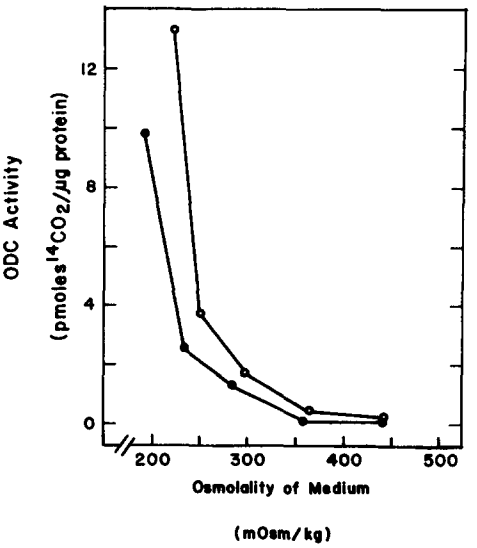

FIG. 2

Effect of the osmolality of the organ culture medium on uterine ornithine decarboxylase (ODC) activity. The osmolality was adjusted with NaCl (•) or sucrose (o). The incubation time was 4 hr.

Fig. 2 also shows that incubation of uterine tissue in a medium approximately isotonic with plasma caused a rather substantial elevation of ODC activity relative to incubation in a hypertonic medium. With NaCl, for

example, this represented a 12-fold increase (286 vs 440 mOsm/kg). In addition, the ODC activity of unincubated tissue was essentially the same as that of tissue incubated for 4 hr under hypertonic conditions (>400 mOsm/kg). Thus, organ culture incubation under isotonic conditions stimulated uterine ODC activity.

This latter stimulation was comparable to that seen after uterine exposure in vivo to systemic estradiol or intraluminal cholera toxin. This is demonstrated in Fig. 3. As shown in the upper panel, organ culture incubation under isotonic conditions (302 mOsm/kg) increased ODC activity 10-fold relative to hypertonic conditions (501 mOsm/kg). Estradiol administered by s.c. injection and cholera toxin given by i.u. injection stimulated ODC activity 16-fold and 9-fold, respectively, relative to vehicle (s.c.) and uninjected (i.u.) controls. In addition, the specific activity of ODC was similar for isotonic organ culture and estradiol treatment, while that for cholera toxin treatment was about 50% less. The specific activities for all three controls were very similar.

All three treatments in Fig. 3, however, affected the protein concentration of the supernatant liquid that was assayed for ODC activity. Organ culture reduced the protein concentration, apparently by extraction during incubation of extracellular protein from the uterine stroma. Conversely, estradiol and cholera toxin increased the protein concentration, apparently because they increase uterine vascular permeability and thus cause accumulation of plasma protein in the tissue (12-14). These changes in protein concentration would affect the value obtained for the specific activity of ODC.

When uterine ODC activity was expressed per unit mass DNA, however, as shown in the lower panel of Fig. 3, all three treatments still caused dramatic increases. Relative to the respective controls, ODC activity was elevated 10-fold by organ culture at 302 mOsm/kg, 16-fold by estradiol, and 12-fold by cholera toxin. Absolute levels of ODC activity were similar for estradiol and cholera toxin treatment, while that for isotonic organ culture (302 mOsm/kg) was about 50% less. Again, the control activities were all very similar.

Discussion

Maximal elevation of ODC activity almost always occurs at 4-6 hr after exposure to the stimulus, whatever that may be (4). This is also true for the stimulation of uterine ODC activity by estradiol in vivo (15,16) and by organ culture under hypotonic conditions (Fig. 1). The latter effect appears to be due to the change in osmolality and not ionic conditions (Fig. 2), which confirms results reported for thyroid tissue (7), but not for HeLa cells (6).

Systemic estradiol and i.u. cholera toxin cause similar increases not only in uterine ODC activity at 4 hr (Fig. 3), but also in the uterine protein/DNA ratio and radiolabeled precursor incorporation into protein and DNA at 24 hr (17). This estrogen-like growth response induced by cholera toxin is not accompanied by nuclear accumulation of estrogen receptor (submitted for publication). About 50% of the cholera toxin-induced increase in uterine ODC activity is due to the buffer vehicle. This increase, in turn, is probably due to uterine distension, since the i.u. injection of water or air causes the same degree of stimulation (submitted for publication).

ODC is not the only uterine enzyme whose activity is elevated in response to the types of treatments described here. Systemic estradiol and the i.u. injection of saline cause similar increases in RNA polymerase I activity of uterine nuclei (18,19), and organ culture incubation of uteri under isotonic conditions stimulates total nuclear RNA polymerase activity (20).

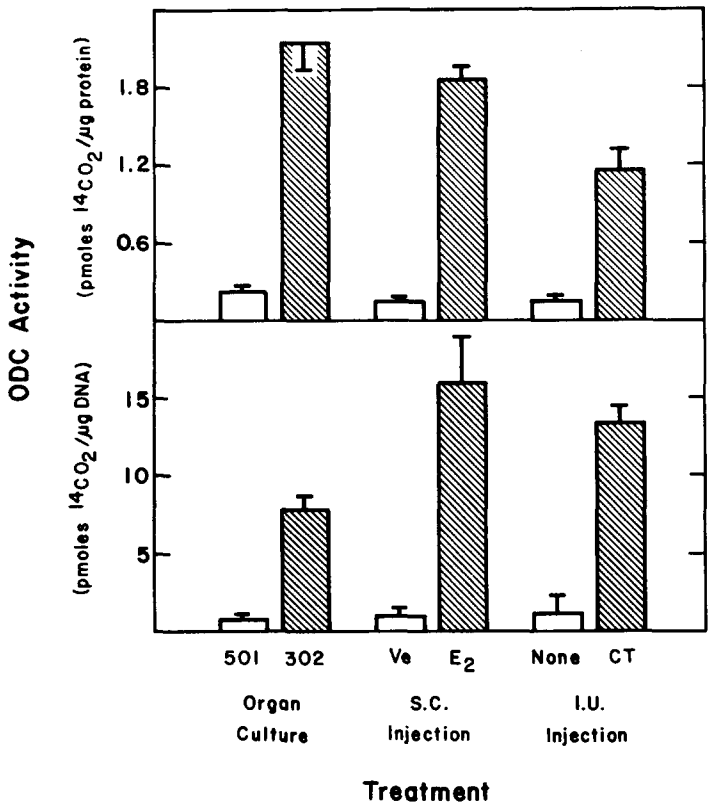

FIG. 3

Comparative effects of organ culture, systemic estradiol, and intraluminal cholera toxin on uterine ornithine decarboxylase (ODC) activity. Organ culture was carried out in an incubation medium having an osmolality of either 302 or 501 mOsm/kg (adjusted with NaCl). Estradiol (E_2) and vehicle (Ve) were injected subcutaneously (s.c.). Cholera toxin (CT) was administered by intrauterine (i.u.) injection, with each animal receiving only one such injection, and the contralateral horn thus serving as the control ("none"). The bars represent the mean ± SEM for 3 separate groups. Each group consisted of: for organ culture, half uterine horns pooled from 6 animals; for s.c. injection, whole uteri pooled from 2 animals; for i.u. injection, whole uterine horns pooled from 3 animals. All treatment times were 4 hr. ODC activity is expressed per ug protein (upper panel) and per ug DNA (lower panel).

A common effect of these treatments (organ culture, estradiol, cholera toxin) is alteration of the extracellular microenvironment of the uterine cells. With estradiol and cholera toxin, this is due to increased vascular permeability and resultant edema (12-14,21). The effect of this edema on the interstitial fluid of the uterus may be mimicked somehow by organ culture incubation under hypotonic or isotonic conditions. By 4 hr after treatment, estradiol causes a dramatic increase in the water content of the uterus (22). Although this does not necessarily mean that the osmolality of the interstitial fluid actually decreases, it is not inconsistent with that

possibility. The extracellular space of the uterine endometrium is very large (23), and in the absence of estrogen, hydration of the large amount of extracellular protein of the stroma could make the interstitial fluid effectively hypertonic. It may be relevant that liver and kidney cells have been reported to be isotonic with, not 0.15 M saline, but rather 0.2-0.3 M saline (24). Exactly how a change in the extracellular microenvironment might stimulate uterine ODC activity remains to be determined, but the stimulatory effect on adenyl cyclase of estradiol in the uterus (25,26) and cholera toxin in other systems (27) suggests that cAMP may be involved, and the inhibitory effect of cycloheximide in organ culture indicates that new enzyme synthesis may be required.

Acknowledgments

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