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THE 'ESTROGEN-INDUCED PROTEIN': QUANTITATION BY AUTORADIOGRAPHY OF POLYACRYLAMIDE GELS

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SUMMARY

The increased rate of synthesis of the "estrogen-induced protein" (IP) first detected by Notides and Gorski is one of the earliest macromolecular responses to estrogen by the rat uterus. The IP detected by autoradiography of sodium dodecyl sulfate polyacrylamide gel electropherograms was shown to correspond to a stainable protein band in electropherograms of cytosol from uteri of both untreated and estrogen-treated rats. The uteri of 9–10-day-old rats showed a maximum rate of IP synthesis 1 h after estrogen-treatment, followed by a decrease to constitutive levels by 24 h. The rate of synthesis of IP was doubled by estrogen administration to 10–30-day-old rats. The improved sensitivity of the autoradiographic method permitted the detection of some stimulation of IP synthesis in uteri of 5-day-old rats, in contrast to our previous findings in Wistar rats using a double isotope labelling technique.

The increased rate of synthesis of "estrogen-induced protein" (IP) first described by Notides and Gorski [1] has proven to be a reproducible marker of the early response to estrogen by the rat uterus [2,3]. However, the use of the double isotope ratio method [4–6] makes quantitative interpretation of results difficult [7] and has the serious disadvantage that it can measure only the increase in IP after estrogen treatment and is therefore unable to determine whether IP is present in untreated animals. We have recently adopted the method of autoradiography of [^{35}S]-methionine labeled proteins separated by SDS-polyacrylamide electrophoresis [8] for quantitation of IP and for the demonstration of IP synthesis in untreated immature rats.

In this communication, we show that autoradiographically detected IP corresponds to a stainable protein band, present a summary of our initial experiments on the time course and age dependence of stimulation of IP synthesis and report the detection of a protein in rat hypothalamus which shows the same characteristics as IP in two dissimilar gel electrophoretic systems.

For stimulation of IP synthesis, 5–30-day-old female rats were injected intraperitoneally with a 10 μg/ml solution of estradiol-17β in 1% ethanol (1 μg estradiol/7 g body weight) or with 1% ethanol. After 1 h or longer, uteri were excised, rinsed in phosphate-buffered saline (PBS) [9] and incubated at 37°C for 2 h in a 95% O_2–5% CO_2 atmosphere in 1 ml PBS containing 50 μCi [^{35}S]-methionine. Cytosol was prepared by homogenization of uteri in an all-glass Potter–Elvejehm homogeniser (0.005–0.007 in. clearance) in 5 vol. of 0.05% disodium EDTA·2H_2O, and centrifugation for 50 min at 50,000 rev./min in the Spinco No. 50 rotor at 2°C. Samples of cytosol were made up to a concentration of 10% glycerol, 5% 2-mercaptoethanol, 3% sodium dodecyl sulfate (SDS), 0.0625 M Tris–HCl (pH 6.8), 0.001% bromphenol blue and heated for 5 min at 100°C. Samples containing less than 10 μg protein were subjected to electrophoresis at 3 V/cm at 20°C for 14–16 h on 0.75 mm thick polyacrylamide gel slabs [10], containing a 10–20% polyacrylamide gradient and 0.1% SDS [11]. Gels were stained with Coomassie Brilliant Blue, impregnated with a scintillator [12], dried and exposed to Kodak RP 54 X-ray film for 1–3 days at −70°C. The exposure was adjusted to ensure linearity of response of the film to increasing amounts of ^{35}S. Developed films were scanned at 560 nm using a Gilford gel scanner and the amount of IP synthesis relative to total protein synthesis was determined by planimetry of the tracings using a Keuffel and Esser planimeter.

The most striking difference between the autoradiographic pattern of uterine cytosol from estrogen treated and untreated animals is an increase in the intensity of a band appearing between tubulin and actin (Fig. 1), with an apparent molecular weight of 46,000 corresponding to previous estimates for the molecular weight of IP [3]. Our identification of this band with IP rests, in addition, on experiments [8] in which uterine cytosol protein eluted from the IP region after electrophoresis on cellogel (a separation based on net charge) shows an identical band on SDS polyacrylamide gel electrophoresis.

The correspondence between the IP band seen in autoradiograms with a stained protein band, suggests

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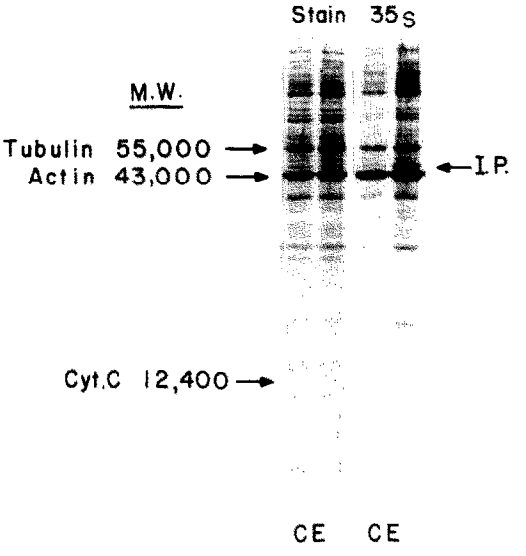

Fig. 1. SDS-polyacrylamide gel electrophoresis of cytosol from uteri of 12-day-old rats. Uteri from rats killed 1 h after injection of 1% ethanol (C) or 5 μg estradiol-17β (E) were incubated in 1 ml PBS (3 uteri/ml) containing 50 μCi [^{35}S]-methionine. After 2 h incubation at 37°, uterine cytosol was prepared as described in the text and subjected to electrophoresis on SDS 10–20% polyacrylamide gradient gels, which were stained with Coomassie Brilliant Blue. Autoradiograms of the dried gels were evaluated by planimetry of densitometric tracings as described in the text.

that IP may represent a significant percentage of cytoplasmic protein. The rate of synthesis of IP doubled 1 h following estrogen administration. However, this additional synthesis of IP did not significantly increase the total amount of this protein at 1 h following treatment (Fig. 1) or at 4, 12 or 24 h (data not shown).

Further characteristics of IP are the rapidity of its induction [1] and the early age at which its induction may be observed [6]. The rate of IP synthesis in uteri of 9- and 10-day-old animals was approximately double that in untreated rats at 1 h (Fig. 2), remained significantly elevated at 4 h and returned to the constitutive level of approximately 3% by 24 h. Using the response at 1 h after estradiol injection as the measure of the inducibility of IP, the autoradiographic method detected some stimulation of IP synthesis as early as 5 days postnatally, with the maximal response of a doubling of synthetic rate reached by approximately 10 days.

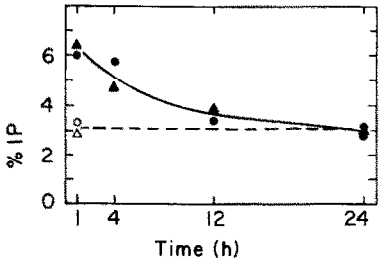

Fig. 2. Time course of IP synthesis. Nine-day-old (triangles) or 10-day-old (circles) rats, were injected with 1% ethanol (open symbols) or 2.4 μg estradiol in 1% ethanol (filled symbols). Uteri, excised at the indicated time, were incubated in 1 ml of PBS (5 uteri/ml) containing 50 μCi [^{35}S]-methionine. Incubation, preparation of cytosol, electrophoresis and evaluation were as described in the text.

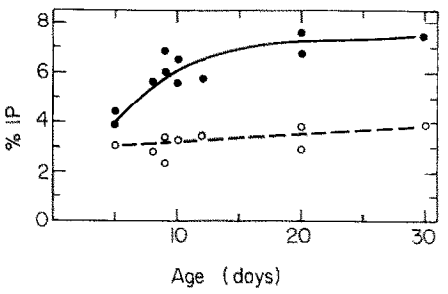

Fig. 3. Age dependence of IP synthesis. Groups of immature rats (ranging from 10/group for 5-day-old rats to 2/group for 30-day-old rats), were injected with 1% ethanol (open circles) or estradiol-17β (1 μg/7 g body weight; (filled circles) and killed after 1 h. Excised uteri were incubated in 1 ml PBS containing 50 μCi [^{35}S]-methionine. Incubation, preparation of cytosol, electrophoresis and evaluation were as described in the text.

The comparison of results obtained using the autoradiographic or the double isotope ratio method and the current status of ideas concerning a function for IP will be discussed elsewhere [8]. The finding of a significant constitutive level of IP in immature uterus (Figs. 2 and 3) suggests that IP may have a function which is not limited to the estrogen stimulated state. Nor may it be limited to the uterus and vagina [13]. In fact, we have recently observed in rat hypothalamus cytosol the presence of a protein with properties similar to IP. This protein showed identical electrophoretic mobility both in preparative cellogel and SDS polyacrylamide gels. We are currently surveying both estrogen-responsive and unresponsive organs in the rat and other species for the presence of IP.

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