ESTROGEN INDUCTION OF SPECIFIC SOLUBLE PROTEINS IN THE HYPOTHALAMUS OF THE IMMATURE RAT[1]

Margery C. Beinfeld and Paul M. Packman[2]

Department of Psychiatry
Washington University School of Medicine
St. Louis, Missouri 63110

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SUMMARY

Estrogen administration to immature female rats results in the increased synthesis of specific soluble hypothalamic proteins which can be detected using double isotope labeling and polyacrylamide electrophoretic fractionation. Increase in hormone-specific protein synthesis is detectable after 15 minutes, is maximal after 1 hour, and declines to control levels 2 hours after hormone administration. Electrophoresis on SDS polyacrylamide gels with other proteins of known molecular weights indicates an approximate subunit molecular weight of 18,000. These data suggest that early estrogen action in the central nervous system may be similar to its action in other target organs.

Estrogen administration to immature (1,2,3) and ovariectomized (3) female rats induces the synthesis of specific cytoplasmic proteins in the uterus. Enhanced synthesis of the uterine "induced protein" (IP) can be seen as early as 15 min after hormone stimulation (3), with a maximum at 1 hour (2), and a decrease to control levels by 2-4 hours (2). Since the synthesis of IP precedes all other protein synthetic changes which occur after hormone administration, it has been suggested that IP plays an early role in the mechanism of action of the hormone in the uterus (1).

The effects of estrogen administration on hypothalamic protein synthesis yield conflicting results. Estrogen administration to ovariectomized animals resulted in increased [^{3}H] leucine incorporation in the hypothalamus (4,5) but in one of these reports (4), the increase was not restricted to the hypo-

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thalamus; increased incorporation also occurred in cerebral cortex. In immature rats, no increase in [^{14}C] leucine incorporation in hypothalamic proteins after estrogen treatment was found (6). The failure to see substantial increases in protein synthesis in the brain following estrogen administration, similar to that observed in the uterus, does not rule out the possibility that the hormone stimulates the synthesis of a specific protein or group of proteins. The possibility that synthesis of specific proteins occurs which is not accompanied by dramatic increases in total protein synthesis can, perhaps, be best investigated by the sensitive method of double isotope incorporation and polyacrylamide gel electrophoresis which has been used to demonstrate IP synthesis in the uterus (1-3).

METHOD

Immature Charles River CD outbred rats 21-28 days of age were used. Estradiol 17-β, 5μg, dissolved in 0.2 ml 10% ethanol was injected intraperitoneally, while control animals received only 10% ethanol.

At the indicated times after injection animals were killed by decapitation, the brains removed, and the hypothalamus and similar size pieces of cerebral cortex were dissected out. The tissues were placed in individual 3 ml tubes containing 1 ml Krebs-Ringer buffer enriched with 11 mM glucose and either 15 μCi/ml [^{14}C] leucine or 150 μCi/ml [^{3}H] leucine. Tissues from hormone treated animals were usually incubated with [^{14}C] leucine while control tissues were incubated with [^{3}H] leucine. In one experiment the hormone treated animals were incubated with [^{3}H] leucine while control tissues were incubated with [^{14}C] leucine. The tubes were placed in a 37°C water bath shaker, gently shaken, and bubbled with 95% O_2-5% CO_2 for 1 hour.

At the end of the incubation period the tubes were put on ice, the buffer was removed and the tissue was rinsed with ice cold buffer without labeled leucine and homogenized in 1 ml of this buffer. The homogenate was spun at 105,000 x g for 1 hour, the supernatant removed, and dialyzed exhaustively against 50 mM Tris pH 7.5, and stored at -20° until electrophoresis was performed.

Cylindrical polyacrylamide gels (4%) containing 1% SDS and 24% urea were prepared by the method of Laemmli (7) with the modification of Willard *et al.* (8). Samples containing 100 μg of soluble protein from both hormone and control animals were TCA-precipitated, washed with absolute ether, dissolved in the sample buffer containing SDS and applied to each gel and run at constant voltage, 8.5 V/gel, at room temperature for 2-3 hours. Some gels were stained with Coomassie Blue. For the determination of radioactivity, the gels were removed from the tubes, frozen and sliced into 1 mm slices with a Canalco Slicer. The slices were placed in scintillation vials, 10 ml Protosol-Omnifluor (New England Nuclear) were added and the vials were tightly capped with teflon lined caps. The vials were then placed in a 50° oven overnight and cooled for at least 1 hour before counting in a Packard Tri-Carb Model 3380 with Automatic External Standard. The efficiency of each sample was calculated from the A.E.S. ratio and the ^{3}H counts were corrected for spillover.

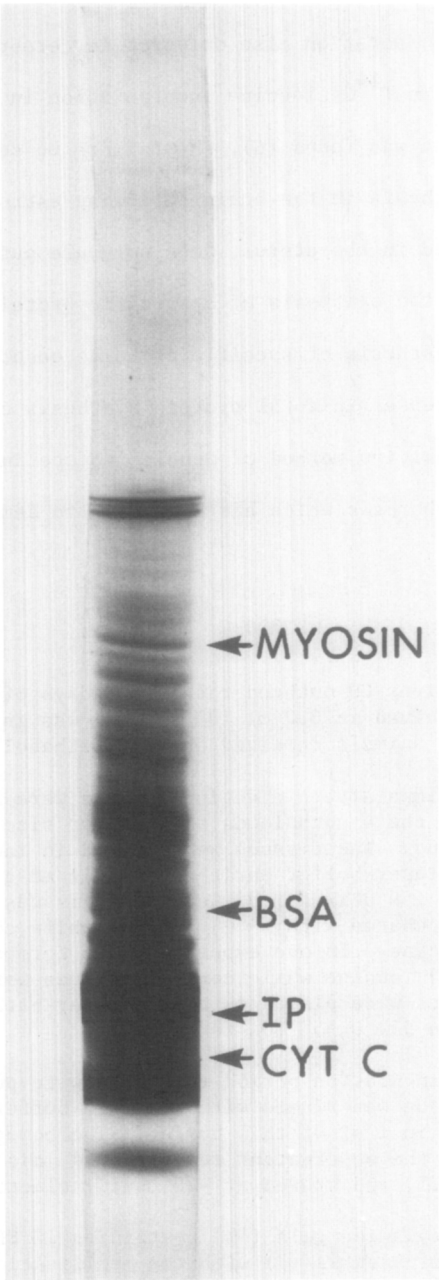

Fig. 1. Polyacrylamide-SDS gel of hypothalamic soluble proteins. Marker protein locations were determined from R_f values of these proteins run on separate gels at the same time. Abbreviations: IP, induced protein(s); BSA, bovine serum albumin; and CYT C, cytochrome C.

The hormone-induced synthesis ($\Delta^{14}C$) in each gel slice was determined by the method of Mayol (9) using the equation:

$$\text{Equation 1:} \quad \Delta^{14}C = {}^{14}C - R \times {}^{3}H$$

where ^{14}C is the disintegrations per minute of ^{14}C, ^{3}H is the disintegrations

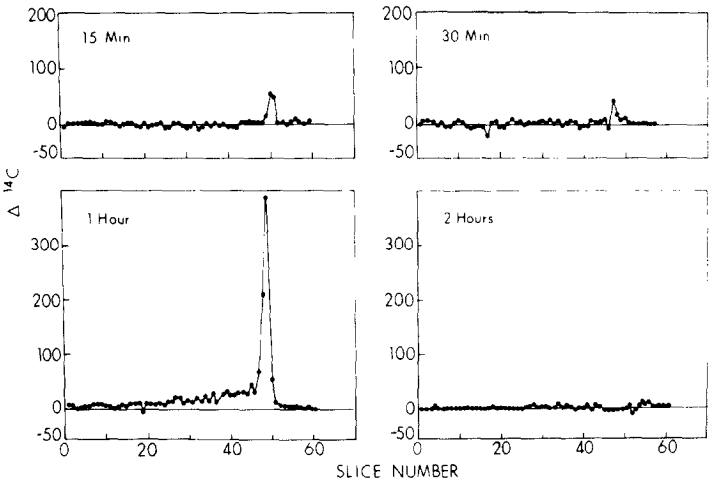

Fig. 2. Time course of hypothalamic IP induction. Immature female rats were sacrificed after estrogen treatment at the times indicated in the panels. Results expressed as $\Delta^{14}C$ according to equation in text.

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per minute of ^{3}H and R is the ratio of $^{14}C/^{3}H$ in all gel slices outside the induced peak.

RESULTS

Fig. 1 shows a typical 4% acrylamide SDS gel of soluble proteins from rat hypothalamus. The arrows indicate the location of molecular weight standards based on calculated R_f values from duplicate gels and the induced protein(s). Since the increased incorporation of labeled leucine occurs in the same region of the gel as several other proteins, the possibility that there is more than one IP with similar molecular weight is not excluded. However, the mixing of other "noninduced" proteins with the induced protein which results from incomplete resolution of the low molecular weight proteins would tend to decrease rather than increase the magnitude of $\Delta^{14}C$ for the induced peak. From the location of the protein band relative to BSA and cytochrome C it is possible to assign an approximate subunit molecular weight of 18,000.

The time course of stimulation of the induced protein is shown in Fig. 2. Increased synthesis can be detected after 15 minutes. About the same level of

TABLE I

^{3}H AND ^{14}C INCORPORATION INTO SOLUBLE HYPOTHALAMIC PROTEIN.*

Slice No.	Time of In Vivo Estrogen Treatment: 15 min		30 min		60 min		120 min	
	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C
1	24	2	22	0	3	7	44	0
3	32	0	10	10	0	0	38	0
5	11	0	27	2	12	7	39	0
7	23	7	22	2	23	9	45	2
9	19	2	30	0	12	11	64	4
11	23	8	21	6	12	7	52	2
13	40	0	48	2	20	7	57	2
15	51	0	40	4	25	9	57	2
17	47	4	179	4	10	10	43	2
19	43	2	39	7	134	4	59	0
21	56	2	43	2	18	12	90	0
23	43	0	42	12	20	14	50	2
25	67	4	45	8	10	12	69	2
27	45	8	52	8	22	23	76	4
29	64	0	61	10	32	19	76	2
31	68	10	84	12	40	19	92	10
33	91	14	63	11	38	21	125	0
35	90	12	82	8	51	21	83	0
37	84	10	66	18	38	19	89	0
39	75	8	229	20	74	37	118	0
41	172	16	166	24	93	23	94	8
43	200	25	119	20	193	44	116	8
45	174	24	199	31	111	51	175	2
46	164	22	455	51	110	44	165	4
47	246	29	2560	375	190	84	158	4
48	236	26	1139	171	806	276	150	2
49	1108	136	60	12	2020	551	172	4
50	6240	725	15	12	694	111	199	7
51	1553	220	21	4	99	21	301	10
52	137	14	27	4	13	4	1598	36
53	98	14	16	2	10	7	2940	86
55	83	12	3	2	8	2	10	10
57	23	8	8	2	0	2	10	7
59	58	12	0	0	0	2	11	0

*Results expressed as dpm ^{3}H or ^{14}C/gel slice. Every other slice is shown except at the peak areas. The 1 to 2mm difference in location of the peak at the various time periods is due to slight variation of individual gel lengths.

synthesis is detectable after 30 min. and a much larger peak at 1 hour. The induced peak is gone by 2 hours. This time course is similar to the induction of uterine IP (1-3). The dpm/gel slice for each isotope and each time period are summarized in Table I.

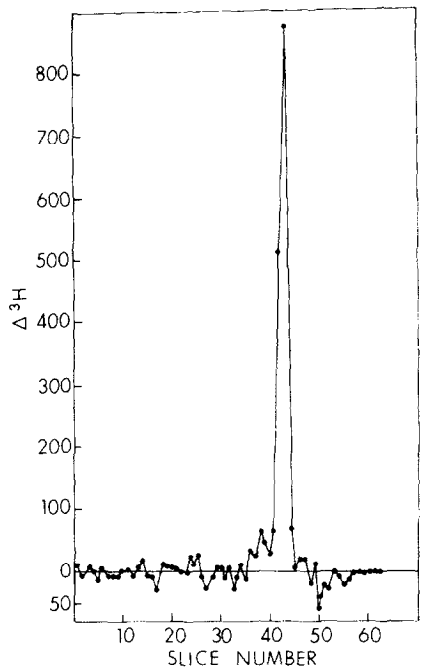

Fig. 3. Reverse isotope experiment. Hypothalami from estrogen treated rats were incubated with [^{3}H] leucine, controls with [^{14}C] leucine. Animals were sacrificed at the time of peak induction, 1 hour.

Fig. 3 shows the results of the experiment in which the hypothalami from hormone treated animals were incubated with [^{3}H] leucine instead of [^{14}C] leucine, while the control animals were incubated with [^{14}C] leucine (reverse isotope experiment). An induced peak under these conditions would have an elevated Δ^{3}H rather than Δ^{14}C. The induced peak occurs at the same region of the gel (relative to the tracking dye) and the magnitude of Δ^{3}H is larger. The increased height of the peak is due to the higher specific activity of the [^{3}H] leucine added. The persistence of the induced peak with reversal of the isotopes indicates that the induced peak is not due to differential incorporation of the [^{14}C] leucine *vs* the [^{3}H] leucine into protein in the hypothalamic samples No induced protein peaks were observed in the samples from cerebral cortex.

DISCUSSION

These data indicate that estrogen treatment stimulates the synthesis of

specific soluble protein(s) in the hypothalamus of immature rats. This is the first direct demonstration of an effect of estrogen on the synthesis of particular proteins in the brain and suggests that early estrogen action in the central nervous system may be similar to its action in other target organs. This finding is in general agreement with previously observed increases in amino acid incorporation with estrogen treatment (4,5). The time course of the response is somewhat similar to the induction of uterine IP (1-3). The smaller apparent molecular weight of the induced protein in brain (about 18,000 vs 44,000) suggests that it may not be the same protein. On 4% acrylamide gels the protein is not resolved into a single band, and hence it is possible that there may actually be more than one induced protein species.

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