CORTICOSTERONE INCREASES THE SYNTHESIS OF A SOLUBLE PROTEIN IN PITUITARY GLANDS FROM ADRENALECTOMIZED MALE RATS WHICH PRECIPITATES WITH ANTI-RAT GROWTH ANTISERUM

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Received 8 July 1980

1. Introduction

The well-documented stimulation of rat growth hormone (rGH) synthesis by corticosterone in rat pituitary GH_3 cells [1] has proven to be a good experimental system for investigating glucocorticoid regulation of protein synthesis. However, as there are substantial differences between GH_3 cells and intact rat pituitary glands, the effect of corticosterone on induction of specific soluble proteins was investigated in intact rat pituitary glands. The methodology of double isotope incorporation and SDS–polyacrylamide gel electrophoresis, successfully used to demonstrate estrogen-induced proteins in the uterus [2] and hypothalamus [3,4], was applied.

We report here that treatment of adrenalectomized male rats with corticosterone increases the synthesis of a specific soluble protein with a relative molecular mass (M_r) 24 000 on SDS–urea polyacrylamide gels, identical in size to the form of rGH made in GH_3 cells [5]. The identity of this induced protein with rGH was tested using a specific anti-rGH antiserum. When pre-labeled pituitary-gland soluble protein was mixed with this antiserum, the 24 000 M_r-induced protein was quantitatively precipitated. The precipitation of the corticosterone-induced protein with anti-rGH antiserum is good evidence that in intact pituitary glands, like GH_3 cells, corticosterone increases rGH synthesis.

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A preliminary report of this work was presented at the ICN–UCLA Winter Conference on Molecular Biology; Keystone, CO, 1979

2. Methods

2.1. *Animals and pituitary incubations*

Charles River Sprague Dawley descended rats were adrenalectomized 5 days prior to sacrifice and maintained on 0.9% NaCl and rat chow. Prior to sacrifice (6 h) the hormone-treated animals were given a single intraperitoneal injection of corticosterone in 0.5 ml 10% ethanol (50 μg/kg) while control animals received 0.5 ml 10% ethanol. After sacrifice, the pituitary glands from similarly treated animals were pooled (3/tube) and incubated at 37°C for 1 h in Krebs-Ringer bicarbonate glucose buffer (KRBG) containing 1 mg/ml glucose and labeled amino acid mixtures. Pituitary glands from hormone treated animals were incubated in ^{14}C-labeled amino acids (10–50 μCi/incubation) while pituitary glands from control animals were incubated in ^{3}H-labeled amino acids (100 μCi/incubation). After incubation, the pituitary glands were washed with ice-cold KRBG, homogenized in 0.1 N HCl and spun at 105 000 × *g* for 1 h. The supernatants from this centrifugation were stored at −70°C until electrophoresis was performed. The protein concentration of the supernatants was determined by the method in [6] with bovine serum albumin as the protein standard.

2.2. *Electrophoresis*

SDS–urea polyacrylamide gel electrophoresis was performed as described with the following modifications:

(a) 6–25% acrylamide gradient slab gels were used;
(b) The polyacrylamide gels were digested with 0.2 ml 30% hydrogen peroxide and counted in 10 ml Scintiverse (Fisher).

Pituitary-gland soluble protein (100 μg) from hormone

treated animals (^{14}C-labeled) and control (^{3}H-labeled) were mixed, trichloroacetic acid-precipitated, washed with absolute ether, dissolved in sample buffer and applied to the gel. After electrophoresis, the gels were stained with Coomassie blue and for determination of radioactivity, individual protein bands were cut out with a razor blade, dissolved, and counted as described.

The hormone-induced protein synthesis (Δ^{14}C) in each gel slice was determined as in [7] using the equation:

$$\Delta^{14}C = {}^{14}C - (R \times {}^{3}H) \quad (1)$$

where ^{14}C is the dpm of ^{14}C, ^{3}H is the dpm of ^{3}H and R is the average (^{14}C/^{3}H) ratio of the gel slices outside the induced protein band.

2.3. *Immunoprecipitation*

Samples of pituitary soluble protein identical to those used for electrophoresis prior to the trichloroacetic acid precipitation were immunoprecipitated with a specific anti-rGH antiserum used in [8] (Minolta 4th serum). Hormone-treated pituitary protein (100 μg) and control protein was mixed with 0.2 ml undiluted antiserum and allowed to precipitate overnight at 4°C. The immunoprecipitate was collected and washed as in [8], dissolved in electrophoresis buffer and 50% of it was applied to gels. An aliquot of the supernatant containing proteins not precipitated by the antiserum was also run on gels.

2.4. *Chemicals*

Corticosterone was from Steraloids (Pawling, NY) and the labeled amino acids were from New England Nuclear (Boston, MA). The monkey anti-rGH antiserum was kindly provided by Dr F. C. Bancroft of Memorial Sloan-Kettering Cancer Center.

3. Results

In fig.1, the results of SDS–urea polyacrylamide gel electrophoresis of pituitary gland soluble protein from adrenalectomized male rats sacrificed 6 h after corticosterone treatment or 10% ethanol treatment is shown. Corticosterone treatment causes an increase in the synthesis of the protein in gel slice 37 which is indicated by a high Δ^{14}C value (A), increased incorporation of amino [^{14}C] acids relative to [^{3}H] amino acids (B), and an increase in the ratio of ^{3}H/^{14}C

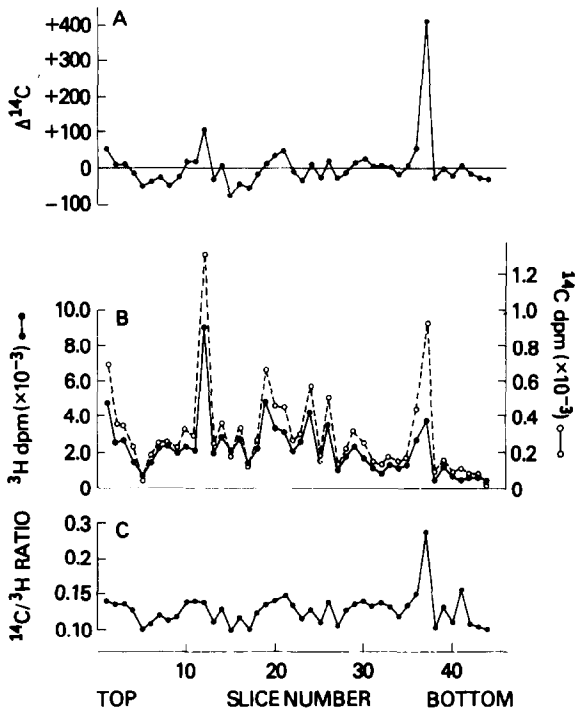

Fig.1. Results of electrophoresis of pituitary gland soluble protein from 120-day-old adrenalectomized male rats sacrificed 6 h after corticosterone or 10% ethanol treatment. The electrophoresis was performed on 6–25% acrylamide gradient slab gels containing SDS and urea. In this figure the 3 ways of expressing double label data are compared: (A) the parameter Δ^{14}C; (B) dpm of ^{3}H and ^{14}C; (C) ^{14}C/^{3}H ratio.

(C). In fig.1, the ^{14}C/^{3}H dpm ratio and dpm of ^{14}C and ^{3}H are plotted along with Δ^{14}C to demonstrate that the 3 methods of expressing double label induced protein results are equivalent.

The protein band in fig.1 (slice 37) judged by the density of the protein staining, is one of the major protein bands in rat pituitary soluble protein and has M_r 24 000 on SDS–urea gels. The experiment shown in fig.1 is from animals sacrificed 6 h after corticosterone treatment. The induced protein can also easily be observed 1 and 3 h after hormone treatment (not shown).

Since corticosterone stimulates rGH synthesis in GH_3 cells [1] the possibility that the induced protein is an rGH-like material was evaluated using a highly specific anti-rGH antiserum as in [8]. In [8] this antiserum selectively and quantitatively precipitated rGH and this method was utilized to measure rGH biosynthesis in GH_3 cells [6].

When this anti-rGH antiserum was mixed with

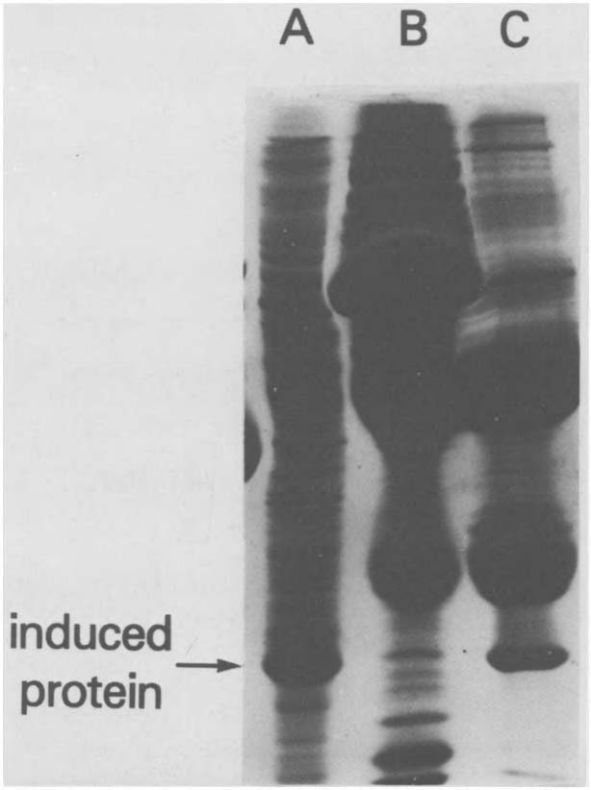

Fig.2. Comparison of electrophoresis of pituitary soluble protein (A) with protein precipitated with anti-rGH antiserum (C) and supernatant not precipitated with anti-rGH antiserum (B), run on 6–25% gradient acrylamide slab gel. The induced protein band is indicated by an arrow in the left-hand margin.

pre-labeled pituitary gland soluble protein containing the induced protein, an immunoprecipitate was formed. This immunoprecipitate was run on a polyacrylamide slab gel in comparison with untreated soluble protein and the supernatant from the immunoprecipitate (protein not precipitated by the antiserum). The removal of the induced protein from the soluble protein fraction by this antiserum and its quantitative recovery in the immunoprecipitate can be seen in the protein staining patterns in fig.2 and in the pattern of radioactivity in fig.3.

In fig.2, the induced protein, indicated by an arrow in the left margin, is absent from (B) (supernatant from the immunoprecipitation) but is present in (C) (pellet from immunoprecipitation). The induced protein in (C) is less intensely stained than the induced protein in (A) as only 50% of the immunoprecipitate was applied to (C).

In fig.3, the $\Delta^{14}C$ values of the gel displayed in fig.2 are shown to demonstrate the quantitative precipitation of the induced protein counts. The induced protein is not present in the supernatant (C) and is entirely recovered in the immunoprecipitate (B). As in fig.2, 50% of the immunoprecipitate is run on the polyacrylamide gel (B) so that it contains 50% of the amount of induced protein as (A). The immunoprecipitation is selective since all the radioactivity in the induced protein band is recovered in the precipitate, while only 15% of the total counts added to the antiserum are recovered in the immunoprecipitate.

4. Discussion

Treatment of adrenalectomized male rats with low doses of corticosterone (50 μg/kg) causes an increase in the synthesis of a specific soluble protein in their pituitary glands of M_r 24 000. The induction of the soluble protein is similar to that of other steroid-induced proteins, in that it can be detected 1 h after hormone treatment [3–5] and it can be induced by a comparatively small dose of hormone.

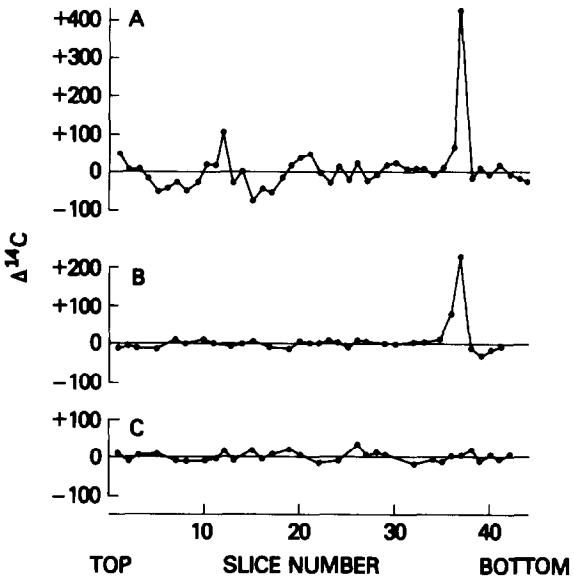

Fig.3. The protein bands in fig.2 were cut with reference to the photograph and $\Delta\ ^{14}C$ value were aligned in fig.3 relative to the bands in (A). In (A) the $\Delta\ ^{14}C$ values of pituitary soluble protein from animals sacrificed 6 h after hormone or 10% ethanol treatment are shown. In (B) the $\Delta\ ^{14}C$ values of 50% of the protein in the pellet precipitated by anti-rGH antiserum is shown and in (C), the $\Delta\ ^{14}C$ values of an aliquot of proteins not precipitated by anti-rGH antiserum is shown.

The observation that corticosterone induces the synthesis of a protein which precipitates with anti-rGH antiserum is good evidence that intact pituitary glands from adrenalectomized rats, like GH_3 cells, synthesize rGH under corticosterone stimulation.

The magnitude of the increase in rGH synthesis by corticosterone in intact rat pituitaries is smaller than that seen in GH_3 cells (a 2–3-fold increase as opposed to 5–15-fold increase in GH_3 cells [9]). This could be caused by the presence of some inhibitory control mechanism or substance in intact animals which is missing in GH_3 cells. Also corticosterone could be more rapidly metabolized and eliminated from circulation by intact rats than by GH_3 cells in culture.

Acknowledgements

We thank Leonard Tolmach of the Anatomy and Neurobiology Department of Washington University, St Louis, MO, for the use of his scintillation counter and computer. This work was supported by grants MH 30140, MH 19624 and a National Research Service Award to M. B. (NIMH).

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