Vol. 110, No. 1, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 14, 1983 Pages 61-68

NUCLEAR-HORMONE MEDIATED CHANGES IN CHROMATIN SOLUBILITY

Richard N. Re[1], Ronald A. LaBiche[2], and Sara E. Bryan[2]

[1]Division of Research, Alton Ochsner Medical Foundation
1514 Jefferson Highway, New Orleans, LA 70121

[2]Department of Biological Sciences, University of New Orleans
New Orleans, LA 70148

Received November 23, 1982

SUMMARY: Rat liver nuclei were incubated with either thyroid hormone or angiotensin (AII) at varying concentrations or with buffer (control) prior to digestion with micrococcal nuclease. Concentrations of hormones greater than 10^{-10}M were effective in increasing the solubilization of chromatin with physiological levels (10^{-9}M) of AII showing an approximate 2.4 fold increase over control. Nuclei were also isolated from animals treated in-vivo with either AII or buffer (control) and chromatin solubility was increased in the AII treated nuclei even prior to the addition of exogenous nuclease, presumably from the action of endogenous nucleases. The data suggest that hormone-induced increases in solubility are a reflection of structural changes in chromatin which enhance the accessibility of DNA to endonuclease attack.

INTRODUCTION: The detection of intranuclear hormone receptors has directed attention to the cell nucleus as a potential site of hormone regulation (1-4). Of particular interest has been the isolation and characterization of thyroid hormone (T_3) nuclear receptors which are not uniformly distributed in chromatin fractions of rat liver nuclei but are preferentially excised by micrococcal nuclease (4). In fact, DNA adjacent to T_3 receptors is markedly (approximately 14 fold) more sensitive to endonuclease digestion than DNA in bulk chromatin (4). This finding and other studies utilizing specific nucleic acid probes have promoted the view that T_3 receptors are situated in "active" chromatin (4-6), however, the mechanisms by which thyroid hormone-receptor complexes exert an effect on specific genes are unknown.

The peptide hormone, angiotensin II (AII), is likewise a candidate for binding to intranuclear receptor sites. Although binding to external membrane loci accounts for a large part of AII's action in the body, there exists evidence in the literature suggesting that intracellular

0006-291X/83/010061-08$01.50/0

receptors for this hormone may also be important (7, 8). In the present study we add to the evidence for intranuclear hormone receptors, and in particular for Angiontensin II receptor sites in chromatin, by reporting a direct effect on isolated nuclei: an increased solubilization of chromatin following treatment with hormone (either AII or T_3). This effect for AII was observed in both in-vivo and in-vitro studies; in the latter, changes in solubility were best detected following digestion with micrococcal nuclease.

MATERIALS AND METHODS:

Isolation of Nuclei. Nuclei were isolated from rat liver by a modification of the procedure described by Jackson et al. (9). All operations were carried out at 4°C. In in-vitro experiments livers were isolated fresh from male Sprague-Dawley rats (about 200 grams) and homogenized in 3 volumes of homogenization buffer (0.25 M sucrose, 50 mM Tris, 3 mM KCl, 5 mM $MgCl_2$, 0.2 mM $CaCl_2$, 0.1% Triton X-100, 1 mM PMSF, pH 7.4 w/v with a Willem's Polytron Homogenizer at medium speed for 30 sec to speed the filtering process. The homogenate was first filtered through one layer of pre-washed cheesecloth then finally through three more layers. The homogenate was then centrifuged at 700xg for 5 min to obtain a crude nuclear pellet. The pellet was washed twice by resuspending in the above solution and centrifuging. Further purification was obtained by resuspending in this solution, but containing a 2 M sucrose, and pelleting into a layer of buffered 2.3 M sucrose (without the Triton X-100) by centrifuging at 75,000xg for 1 hr.

The purified nuclear pellet was washed by suspending in one volume of nuclei wash buffer (10 mM Tris, 0.25 M NaCl, 50 mM $NaHSO_3$, 1 mM PMSF, pH 7.5) w/v, polytroning at slow speed for 15 sec, and centrifuging at 700 xg for 5 min. The pellet was then rewashed in the same solution.

Hormone Incubation and Nuclease Digestion. Washed nuclear pellet was suspended in hormone incubation buffer (150 mM NaCl, 10 mM Tris, 5 mM DTT, 1 mM PMSF, pH 7.8) to the desired concentration and hormone was added from a stock solution (0.1 mM AII or T3, 10 mM Tris, 1 mM $MgCl_2$, pH 7.5) to the desired concentration. Hormone incubation was carried out at either 4°C for nine to sixteen hours or room temperature for 15 to 30 min. After incubation with hormone the nuclear suspension was centrifuged at 700xg for 5 min and the supernate discarded.

The hormone treated pellet was suspended to the desired concentration in digestion buffer (0.25 M sucrose, 15 mM Tris, 15 mM NaCl, 60 mM KCl, 10 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM PMSF, pH 8.0 adjusted with solid cacodylic acid) by polytroning at low speed for 15 sec. The nuclear suspension was treated with micrococcal nuclease (Sigma Chemical Co., St. Louis, MO, 0.059 units of activity per unit of absorbance at 260 nm) as indicated in results but usually for either 30 min at 30° C or for approximately 16 hours at 4°C and was centrifuged again at 700xg for 5 min prior to removal of supernatant. Aliquots of the supernatant were diluted with 8 M urea, 1% SDS, 0.05 M Tris pH 8.0 and assayed for DNA by optical density at 260 nanometers. The pellet was also solubilized in this 8 M urea solution and assayed for DNA.

In-Vivo Experiments. For in-vivo experiments rats were injected in tail vein 30 min before sacrifice with either 0.2 ml of 0.1 mM AII in a 1 mM $MgCl_2$, 10 mM Tris, pH 7.5 buffer or with buffer (1 mM $MgCl_2$) containing no hormone

(control). The nuclei were washed after isolation as before, but were then suspended directly in digestion buffer and treated as described above.

Chromatin Solubility Determination. The pellet (P) from the above isolation was dissolved, in urea and the 260 nm absorbance measured along with corresponding supernatant(S) using 8 M urea solution as a blank. Chromatin solubility was estimated from the ratio: supernatant A_{260}/(supernatant A_{260} + pellet A_{260}) x 100 or $S/_{(S+P)}$ x 100.

Unless specified, EDTA was omitted throughout the nuclei isolation and digestion procedures; digestion was terminated by transferring to an ice bath.

RESULTS:

In-vitro Studies

Nuclei were incubated with hormone at varying concentrations (hormone treated) or in buffer (150 mM NaCl, 10 mM Tris, 5 mM DDT, 1 mM PMSF, pH 7.8) without hormone (control)prior to digestion with micrococcal nuclease. The extent of digestion (% solubility) was linear over 24 hrs following a 19 hr incubation at 4°C for control (data not shown). After 4 hrs of nuclease digestion, there was a progressive increase in solubility with hormone-treated nuclei at T_3 concentrations greater than 10^{-10}M. There was essentially no change in solubility when T_3 incubation concentration were less than 10^{-10}M, indicating a dose-related effect on solubility (Figure 1).

When nuclei were treated with angiotensin II, increased solubilization of chromatin was again detected (Table 1) and the effect could be demonstrated under various hormone concentration and incubation conditions. The competitive angiotensin II inhibitor, saralasin, and teprotide, a nonapeptide converting

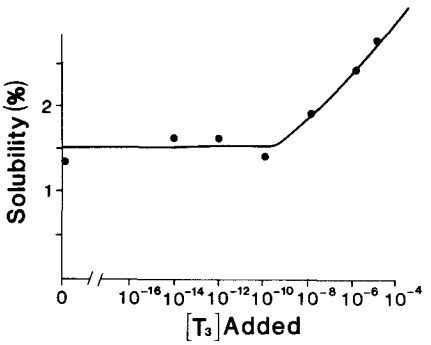

Figure 1. Dose response curve of DNA liberated by micrococcal nuclease digestion after treatment of nuclei (48 A_{260}/ml) with (molar concentrations) T_3.

Table 1
In Vitro Effects of Hormone on Solubility

Hormone or Buffer Incubation Conditions	% Solubility[d] (n)	Hormone or Inhibitor / Buffer Control
WITHOUT EDTA		
10^{-6}M AII[a]	22.9 ± 2.0 (4)	1.3 (p <0.02)
Buffer Control[a]	17.3 ± 3.3 (3)	
10^{-9}M AII[b]	6.3 ± 0.7 (8)	2.4 (p <0.001)
Buffer Control[b]	2.6 ± 0.6 (6)	
10^{-9}M Saralasin[b]	3.2 ± 0.9 (4)	1.2 (NS)
10^{-9}M Terpotide[b]	3.5 ± 1.3 (4)	1.3 (NS)
WITH EDTA		
10^{-9}M AII[b]	5.6 ± 0.8 (8)	1.1 (NS)
Buffer Control[b]	5.1 ± 0.7 (5)	
WITHOUT MICROCOCCAL NUCLEASE		
10^{-9} AII[c]	0.9 ± 0.4 (10)	1.1 (NS)
Buffer Control[c]	0.8 ± 0.2 (4)	

[a]Nuclei (39 A_{260}/ml) were incubated in 10^{-6}M AII or buffer (control) for 21 hrs at 4°C prior to digestion at 20°C for 6 hrs.

[b]Nuclei (less than 15 A_{260}/ml) were incubated in 10^{-9}M hormone for 15 min at 30°C prior to digestion at 30°C for 30 min. In all cases the ratio of micrococcal nuclease to nuclear DNA was constant (0.059 units/A_{260}).

[c]Nuclei (less than 15 A_{260}/ml) were incubated with hormone for 15 min at 30°C prior to incubation at 30°C for 30 min, but without enzyme and without EDTA.

[d]Mean values ± sample standard deviation. (n) = Number of assays; NS = No significant difference between control and hormone-treated.

enzyme inhibitor structurally unrelated to angiotensin, are shown to be ineffective (under the conditions tested), in increasing the solubilization of chromatin whereas, physiological levels of AII (10^{-9}M) showed an approximate 2.4-fold increase over control. This increased solubility by AII treatment, however, was not detected when micrococcal nuclease reactions were stopped by the addition of EDTA. Apparently, the chelation of divalent metals by EDTA increases the solubilization of chromatin for both control and hormone-treated nuclei to the extent that the effect of hormone pretreatment is masked.

In vivo Studies

Nuclei isolated from animals previously injected with hormone or buffer (see Methods) were suspended in digestion buffer, and digested with micrococcal nuclease for indicated times (Figure 2A) and DNA concentrations (Figure 2B). The extend of digestion for control was essentially a constant at DNA concen-

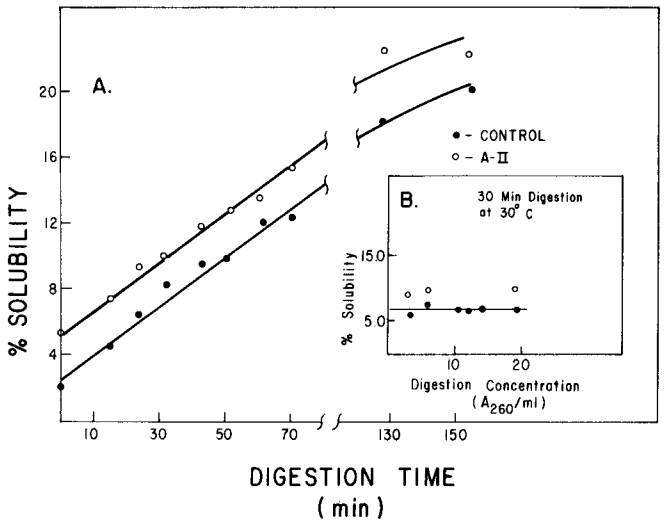

Figure 2. In-Vivo effects of A II on solubility. A. Nuclei (6.74 A_{260}/ml) were digested at 30° with micrococcal nuclease for indicated times. B. Nuclei (concentrations as indicated) were digested for 30 min at 30°C.

trations less than 1 mg/ml (or 20 A_{260}/ml) as shown in Figure 2B. Nuclei obtained from AII-treated animals reflected increased solubility over control after 30 min digestion at 30°C; this effect of AII can best be visualized in the time-course curve plotted in Figure 2A, where the difference in solubility appears to remain constant throughout the course of the digestion. Differences between AII and control could be detected at a zero time point (no digestion) where solubility was found to be 5.01% for AII and 2.94% for control. The extent of digestion appeared to be linear up to 72 min; a linear regression analysis of these data (Figure 2A) showed the following:

	Intercept	Slope	Correlation Coefficient
Control	2.54	0.1444	0.9779
AII	5.43	0.1327	0.9878

Thus, after the initial digestion, the solubilization of chromatin in each case appeared to proceed in parallel as evidenced by the two almost identical slopes. The calculated intercepts reflected approximately the same difference between AII-treated and control, corresponding with the measured zero time point values.

DISCUSSION: Results of the present study provide further evidence that hormone-nuclear interactions increase the solubilization of chromatin and raise the possibility that these hormone dependent effects are brought about, at least in part, by changes in chromatin structure. Other investigators have reported

similar effects with the peptide hormone, TSH (6, 10). It is noteworthy that alterations due to TSH were clearly observed only when nuclease digestion was limited and the effects were most pronounced during the first minute of digestion (10). This is in agreement with results reported in the current study with AII, where changes in solubility were most apparent under conditions favoring limited digestion: at DNA concentrations less than 1 mg/ml and at less than 6% solubilization. After initial digestions, release of solubilized chromatin into the supernatants from in-vivo AII-treated and control nuclei appeared to proceed in parallel, as was the case with TSH-treated tissue slices (6, 10). Recently it has been demonstrated that early solubilized chromatin fragments following micrococcal nuclease treatment are enriched in actively transcribed sequences (5). Thus, our studies demonstrating a hormone effect on solubility early digestion points, suggest a hormonal effect on transcriptions.

Hormone mediated changes in solubility can easily be masked. In our experiments, the addition of a chelation agent seemed to abolish the effect which could be demonstrated over a rather wide range of conditions without EDTA. It should be pointed out that TSH pretreatment of tissue slices was effective in increasing solubility even when EDTA (7.5 mM) was added to terminate nuclease digestion (10).

We have previously characterized a chelation sensitive nucleoprotein particle (B_2) which is not readily detected in nuclease solubilized chromatin when chelation agents are used (11, 12). Chromatographic and compositional properties of this B_2 particle are compatible with an internucleosomal species of linker DNA; the particle's sensitivity to chelation is one of its most unique properties (11, 12). Preliminary and unpublished results in our laboratory have indicated that AII binds with a fraction of chromatin that is either associated with or perhaps adjacent to this chelation sensitive B_2 particle which we believe to be linker DNA.

Likewise, T_3 receptors are found in a fraction of chromatin having some of the characteristics of active chromatin. Since micrococcal nuclease is effective in excising over 90% of T_3 receptors (4), it is reasonable to

postulate that T_3 receptors are associated with expressed regions of chromatin. Thus, if these hormone receptor sites are located in the vicinity of a chelation sensitive region of chromatin, they could be easily altered and as a result, the subtle effect of hormone chromatin binding would be either masked or abolished.

Hormone binding to active regions of native chromatin appears to bring about a conformational change which enhances the accessibility of DNA to RNA polymerase thereby increasing RNA initiation sites. Such a structural alteration can be inferred from an analysis of the solubilization of chromatin following digestion with micrococcal nuclease. The observation that treatment of rats with albeit large concentrations of angiotensin II increases chromatin solubility gives additional support to our in-vitro observations. The finding that chromatin solubility is enhanced even prior to the addition of exogenous nuclease, implies that endogenous nuclease serves to detect hormone-induced structural changes in chromatin.

The present study suggests that the peptide horone, angiotensin II, either following internalization or generation within the cytoplasmic compartment, can act directly on the nucleus. As such,it lends support to the concept that peptide hormones or fragments generated from them may produce physiologic effects following internalization.

ACKNOWLEDGMENTS: We thank Mrs. Jean Brown for her excellent technical assistance. Supported by National Institute of Health (NIH) research grants ES00802-10, H.L. 26101, a Grant in Aid from the American Heart Association and the American Heart Association of LA, Inc.

REFERENCES:

1. MacLeod, K.M., and Baxter, J.D. (1976) J. of Biol. Chem., 251, 7380-7387.
2. Purrello, F., Vigneri, R., Clawson, G.A., and Goldfine, I.D. (1982) Science, 216, 1005-1007.
3. Samuels, H.H., Stanley, F., Casanova, J., and Shao, T.C. (1980) J. of Biol. Chem. 255, 2499-2508.
4. Jump, D.B., Seelig, S., Schartz, H.L., and Oppenheimer, J.H. (1981) Biochemistry 20, 6781-6789.
5. Kitzis, A., Leibovitch, S., Leibovitch, M., Tichonicky, L., Harel, J., and Kruh, J. (1982) Bioch. and Biophy. Acta (1982) 697, 60-70.

6. Abe, Y., Cooper, E., and Spaulding, S.W. (1982) Endocrinology 110, 2118-2123.

7. Re, R.N. (1982) Clinical Science. In Press.

8. Re, R.N., MacPhee, A.A., and Fallon, J.F. (1981) Clinical Science 61, 245S-247S.

9. Jackson, J.B., Pollock, J.M. Jr., and Rill, R.L. (1979) Biochemistry 18, 3739-3748.

10. Abe, Y., Cooper, E., and Spaulding, S.W. (1982) BBRC, 105, 1102-1108.

11. Beary, D.A., Vizard, D.L., Labiche, R.A., Hardy, K.J. and Bryan, S.E. (1982) BBRC 104, 491-499.

12. Bryan, S.E., Vizard, D.L., Beary, D.A., LaBiche, R.A., and Hardy, K.J. (1981) Nucleic Acid Res. 9, 5811-5821.