The Possible Role of Digitalislike Factors in Pregnancy-Induced Hypertension

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SUMMARY A group of small, digitalislike compounds has been implicated in some forms of essential hypertension. Because of similarities between these forms of essential hypertension and pregnancy-induced hypertension, the presence of digitalislike factors in pregnancy-related fluids has been investigated. The factors are found in maternal sera with significantly higher levels of digitalislike activity, as monitored by digoxin radioimmunoassay, in the sera of third-trimester women with pregnancy-induced hypertension as compared to normotensive third-trimester controls (315 vs 195 pg digoxin equivalents/ml; \( p < 0.001 \)). Similarly, they are found in amniotic fluid, and significantly higher levels, as measured by radioimmunoassay (760 vs 540 pg digoxin equivalents/ml; \( p < 0.0008 \)) and by inhibition of ouabain-sensitive Na\(^+\),K\(^+\)-adenosine triphosphatase (ATPase) (12.8 vs 2.7% inhibition; \( p < 0.002 \)), are found in those women whose pregnancies are complicated with hypertension. With purification, several digoxinlike immunoactive compounds are detected. Of these, some have a marked ability to inhibit ouabain-sensitive Na\(^+\),K\(^+\)-ATPase. While as yet unidentified, these compounds have properties suggesting that they are not peptides, steroids, or fatty acids and lipids.

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KEY WORDS • preeclampsia • toxemia of pregnancy • hypertension • sodium-potassium-adenosine triphosphatase

PREGNANCY-INDUCED hypertension (PIH) represents a complex hypertensive condition that arises during pregnancy, primarily during the last trimester, and resolves with delivery. Its etiology remains obscure. Frequently, PIH is accompanied by fluid and salt retention, renal abnormalities, and a vascular hyperresponsiveness to angiotensin II (ANG II) and norepinephrine (NE).\(^1\) In many of these hypertensive women, levels of plasma renin activity, ANG II, and aldosterone have been reported to be normal or low,\(^2\) although other studies provide conflicting data.\(^3\) Similarly, other studies found that administration of saralasin or a converting enzyme inhibitor (SQ 20881) was ineffective in treating PIH.\(^1,4\) In other women with PIH, circulating catecholamines were found to be normal.\(^2\) Studies of plasma progesterone and prostaglandin levels, both of which have been postulated to be decreased in PIH, have provided contradictory results.\(^2-5\) Urinary levels of prostaglandin metabolites in general seem to be decreased for women with PIH, but the meaning of such findings in view of the variable plasma levels is unclear.\(^5\) Thus to date, the mechanisms underlying the elevated blood pressures in these women are unresolved.

Similarities Between Pregnancy-Induced and Volume-Expanded Hypertension

Some features of PIH are paralleled in other forms of hypertension, in particular those involving volume-expanded, low renin states. These features include fluid and salt retention and a hyperresponsiveness to ANG II and NE.\(^7,8\) In studying experimental low renin forms of hypertension, evidence has emerged for inhibitors of ouabain-sensitive Na\(^+\),K\(^+\)-adenosine triphosphatase (ATPase) having an important role. These digitalislike factors have been postulated to increase vascular tone directly as vasoconstrictors\(^7\) or indirectly by potentiating the effects of ANG II or NE by increasing sympathetic discharge.\(^8,9\) These proposals await proof. However, research to date has provided evidence for increased circulating levels of digitalislike factors in essential hypertension.\(^9-11\)
Digitalislike Factors in Normotensive and Hypertensive Pregnant Women

The report of digitalislike activity in placental homogenates led us to investigate the possible presence of digitalislike factors in pregnant women. We found that plasma from virtually all women in their third-trimester contained digoxinlike immunoactivity as measured by digoxin radioimmunoassay (RIA). Serum digoxinlike activity increased with gestational age but fell to prepregnancy levels within 24 hours of delivery. In addition, we found digitalislike activity in amniotic fluid, and its RIA-assayed levels increased with gestational age (r = 0.80, p<0.001 for normotensive women). Using inhibition of Na⁺,K⁺-ATPase to assay this activity, we obtained comparable results (r = 0.54, p = 0.008).

We then studied digitalislike factors in women manifesting PIH. Serum levels of digitalislike activity as assessed by digoxin RIA were significantly higher in women with PIH than in sera of matched normotensive pregnant women. Mean values (± SE) for these two groups were 315 ± 29 versus 195 ± 27 pg RIA digoxin equivalents/ml, respectively (p<0.001). Similar findings were reported by others. We also found significantly higher factor levels in amniotic fluids from women with PIH than in those from matched normotensive pregnant women. The RIA levels of 760 ± 52 versus 540 ± 30 pg digoxin equivalents/ml (p<0.0008) and Na⁺,K⁺-ATPase inhibitions of 12.8 versus 2.7% (p<0.002) were obtained for the two populations, respectively. When gestational age was taken into consideration, better separation of these values for the two populations was achieved (p<9 × 10⁻⁶). In addition, we found a significant correlation between digitalislike factor levels as measured by either assay and diastolic blood pressure (RIA: r = 0.55, p<0.001; ATPase: r = 0.44, p<0.002). The RIA results are shown in Figure 1.

Characterization of Digitalislike Factors from Pregnancy-Related Fluids

Purification of digitalislike factors, including fractionation by high-performance liquid chromatography (HPLC), from either pools of serum or amniotic fluid from pregnant women demonstrated several fractions of eluate having digoxinlike immunoactivity. This is typified by the bottom panel of Figure 2. Of the immunoactive fractions, some had significant Na⁺,K⁺-ATPase inhibitory activity, as shown in the upper panel.

Using further purified preparations of the two digitalislike factors having the most inhibitory activity, we have begun studying their physicochemical properties. A summary follows.

Figure 1. Relationship between digitalislike factor levels in amniotic fluid and diastolic blood pressure. Amniotic fluids were drawn by amniocentesis from normotensive (n = 25) and preeclamptic (n = 24), undigitalized women in the third trimester of pregnancy and assayed in duplicate by digoxin RIA per manufacturer's protocol. Line of least-squares linear regression is plotted.

Figure 2. Digitalislike activity in fractions of HPLC eluate. Fractionation of partially purified digitalislike factors from amniotic fluid was carried out on a C₁₈-reversed phase column (30 cm, μ-Bondapak, Waters Associates, Milford, MA, USA) eluted with an acetonitrile/water gradient (room temperature, 1.0 ml/min). The eluate was monitored at 225 nm, 0.02 absorbance unit full scale. No ultraviolet absorbance was detected except for the void volume (fractions 2–3). Fractions were collected (1.0 ml), taken to dryness to remove the organic solvent, and reconstituted in H₂O. Ten percent of the fraction was assayed by digoxin RIA, and an equivalent amount of each RIA-active fraction was reassayed using a ouabain-sensitive Na⁺,K⁺-ATPase assay, as described elsewhere.
Gel filtration and ultrafiltration both indicate the small size of these factors (<5000 molecular weight). Partitioning experiments employing sizing membranes and dyes of known molecular weight predicted molecular weights of about 200. These compounds are highly polar and are only partially removed by organic solvent extraction (~30% extracted by 1:1 methylene chloride or ethyl acetate, <5% by 1:1 chloroform or hexane). The factors demonstrated no detectable loss of RIA activity (<10%) after heating at 100°C for 2 hours or exposure to 6 N HCl at 100°C for 2 hours. Extending the acid hydrolysis period to 24 hours resulted in about 60 to 70% loss of immunoactivity. The compounds we studied appear to be neutral, as evidenced by no appreciable binding (<5%) to strong cation or anion exchange resins over the pH range of 4 to 9. In addition, no fluorescence or reduction in activity was detectable after reaction with fluorescamine, suggesting further the absence of primary amines. Incubation of these compounds with either trypsin or pronase caused no loss of activity. While digitalislike factors in serum are predominantly bound to proteins, they are not bound in amniotic fluid, even though albumin is present in significant concentrations. Similarly, addition of 1% by volume bovine serum albumin to aqueous solutions of digitalislike factors caused no change in their detected levels.

In summary, the results argue against these compounds being peptides. The apparent absence of amines would exclude digitalislike factors from being catecholamines. Similarly, their solubility patterns are not consistent with recognized steroids, prostanoids, or other nonpolar compounds. Because lipids and fatty acids have recently been shown to have digitalislike activity, the following observations seem pertinent. Chloroform-methanol extractions removed only 30 to 40% of digitalislike activity. These techniques are commonly used to remove lipids quantitatively. The dose-activity response of these digitalislike factors, as measured by digoxin RIA or by inhibition of ATPase, paralleled the dose-response curve for ouabain (data not shown), although our curve was incomplete due to limited material. The increase in this inhibition from 0 to 50% of ATPase activity required a 2-log increase in concentration. This differs from results obtained with fatty acids or lipids. These findings, coupled with those previously mentioned, would argue strongly against these digitalislike factors being fatty acids or lipids. Thus they may represent previously unrecognized vasoactive compounds, but identification is needed to resolve the question finally.

References

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