Radioimmunoassay of plasma ouabain in healthy and pregnant individuals

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Abstract

Ouabain was recently isolated from human plasma, bovine hypothalamus and bovine adrenal in attempts to identify endogenous substances inhibiting the cell membrane sodium pump. A number of radioimmunoassays have been developed in order to study the clinical significance of ouabain. The results have been controversial with regard to the presence and chemical nature of plasma ouabain-like immunoreactivity. We have now measured ouabain in healthy and pregnant individuals using solid-phase extraction of plasma samples followed by a new radioimmunoassay with the extraordinary sensitivity of at least 2 fmol/tube (5 pmol/l). Plasma extracts, a previously isolated human plasma ouabain-like compound and bovine hypothalamic inhibitory factor displaced the tracer in parallel and eluted identically with ouabain in high-performance liquid chromatography. Plasma ouabain immunoreactivity was found to be much lower than reported previously: 12.6 ± 1.3 pmol/l in healthy men

(mean $\pm$ s.e., $n=20$) and 9.4 ± 0.7 pmol/l in women ($n=14$). In pregnant women ($n=28$) plasma ouabain concentration was 16.3 ± 4.0 pmol/l during the first trimester, 18.8 ± 4.3 pmol/l during the second trimester and 24.3 ± 4.0 pmol/l during the third trimester (all $P<0.01$ compared with non-pregnant women). Plasma ouabain 3–5 days after the delivery was 13.6 ± 1.1 pmol/l ($n=10$, $P<0.05$ – 0.01 compared with second and third trimesters). The pregnancy-related changes in the plasma concentrations of ouabain resembled those of cortisol. Therefore cortisol was measured from the same plasma samples and a significant positive correlation was found ($r=0.512$, $P=0.006$). The similar profiles of plasma ouabain and cortisol during pregnancy and their rapid decreases postpartum are consistent with the adrenal cortical origin of ouabain and also show that the secretions of these hormones are possibly under the control of same factors.

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Introduction

The ubiquitous cell membrane enzyme Na^+/K^+ -ATPase, the sodium pump, regulates the transmembrane sodium and potassium gradients. Inhibitors of the sodium pump such as digitalis glycosides have long been used in the treatment of heart failure and endogenous sodium pump inhibitors have been sought for decades. An endogenous ouabain-related compound has been isolated from human plasma (Hamlyn *et al.* 1991), bovine hypothalamus (Tymiak *et al.* 1993, Zhao *et al.* 1995, Kawamura *et al.* 1999) and bovine adrenals (Schneider *et al.* 1998). The isolated compounds were identical to plant-derived ouabain in several reverse phase HPLCs, mass spectrometry and hydrogen-1 nuclear magnetic resonance and also inhibited the sodium pump and increased the contractile force of atrial muscle. For some time it was believed that the endogenous compound was an isomer of

ouabain (Tymiak *et al.* 1993, Zhao *et al.* 1995), but the isomerism was recently found to be caused by the artificial complexation of ouabain to borate from glassware used in the isolation process (Kawamura *et al.* 1999).

Normal pregnancy involves marked changes in endocrine functions in addition to the blood volume and vascular smooth muscle tone. Considering the observed adrenocortical origin of ouabain (Hamlyn *et al.* 1991, Schneider *et al.* 1998), the association of plasma ouabain with cortisol is of great interest, as the activation of the adrenal cortex during pregnancy is well known. Circulating endogenous cardiac glycoside-like compounds also link the sodium pump to vascular changes. In pregnant women, the lymphocyte sodium pump density and activity (Ang *et al.* 1990) and the erythrocyte sodium pump rate and activity (MacPhail *et al.* 1992, Yoshimura *et al.* 1993) have been found to be greater than in non-pregnant individuals. In contrast, the amount of the 2α subunit of

the sodium pump in the myometrium is smaller in pregnant than in non-pregnant women (Maxwell *et al.* 1998). There are also many studies showing that the circulating concentrations or activities of digitalis- or ouabain-like compounds as measured by receptor assays or by immunoassays are greater in pregnant than in non-pregnant individuals (Graves *et al.* 1984, Valdes *et al.* 1985, Poston *et al.* 1989, Seely *et al.* 1992, Paci *et al.* 1996). However, the circulating concentrations of these compounds have been remarkably high and no detailed analyses concerning the nature of the biological activity or immunoreactivity measured as ouabain have been performed.

In order to study the secretion profile of ouabain and to understand the changes in blood pressure that occur during pregnancy, we decided to measure plasma concentrations of ouabain and cortisol in pregnant women. For this purpose we set up a new sensitive radioimmunoassay for ouabain and calibrated it with the previously isolated ouabain preparations of human plasma (Hamlyn *et al.* 1991) and bovine hypothalamus (Tymiak *et al.* 1993). We observed that plasma ouabain concentrations in healthy individuals were much lower than reported previously and that plasma ouabain and cortisol concentration profiles change in parallel during pregnancy, possibly reflecting a common regulation of these hormones.

Materials and Methods

Study participants

Informed consent was obtained from all the participants in the study. Blood samples were obtained from 14 non-pregnant female (mean age 32.7 years) and 20 male laboratory workers (43.2 years), and from 28 pregnant women (28.9 years) from the first ($n=10$), second ($n=8$) and third ($n=10$) trimesters (gestational weeks 1–13, 14–25 and 26–40 respectively). A blood sample was also taken from 10 women (25.5 years) at 3–5 days after delivery. All the blood samples were taken between 0900 h and 1200 h, into polypropylene or polystyrene test tubes containing EDTA as an anticoagulant. Plasma was stored at -20°C . Umbilical cord plasma and amniotic fluid samples were collected from six mothers (30.2 years) after the delivery or amniotomy. Blood pressure was recorded by a sphygmomanometer with the person sitting after a 10-min rest. The pregnant women had normal pregnancies and delivered healthy babies.

Chemicals

Ouabain (G-strophantin) octahydrate, ouabagenin, digoxin, digitoxin and related steroids (aldosterone, cortisol, hydrocortisone, progesterone, β -estradiol, testosterone), bovine thyroglobulin and sodium cyanoborohydride were

obtained from Sigma Chemical Company (St Louis, MO, USA). Freund's incomplete and complete adjuvants were purchased from Difco Laboratories (Detroit, MI, USA), L-tyrosine and polyethylene glycol 6000 from Fluka AG (Buchs, Switzerland), radioiodinated (^{125}I) ouabain derivative (70–80 MBq/nmol) from Biotop Ltd (Oulu, Finland), Sephadex G-50F from Pharmacia (Uppsala, Sweden). All other chemicals were obtained from E Merck AG (Darmstadt, Germany).

Reference compounds

Lyophilized preparations of the ouabain-like compound from human plasma (Hamlyn *et al.* 1991), approximately 1 pmol, and from bovine hypothalamus (Tymiak *et al.* 1993), 171 pmol, were generously supplied by Dr J M Hamlyn and Dr G T Haupt Jr respectively.

Radioimmunoassays

Ouabain was coupled to bovine thyroglobulin using the periodination and sodium cyanoborohydride method according to Masugi *et al.* (1986). The ouabain-thyroglobulin conjugate was purified by Sephadex G-50 gel filtration. The conjugate was emulsified in Freund's complete adjuvant and injected subcutaneously at multiple sites on the backs of five rabbits (1 mg immunogen/rabbit). Boosters (0.5 mg/rabbit in Freund's incomplete adjuvant) were given at monthly intervals. All five rabbits produced antisera against ouabain after the primary immunization. The antiserum '199' at a final titer of 1:750 000, the ouabain tracer and ouabain standards or samples in duplicate were dissolved in the radioimmunoassay buffer (PBS with 0.1% gelatin and NaN_3 , pH 7.4) and each pipetted in 100 μl aliquots into polystyrene test tubes. After an overnight incubation at 6°C the bound and free fractions were separated by double antibody precipitation in the presence of 8% polyethylene glycol, and the precipitates were counted for radioactivity. The sensitivity of the ouabain radioimmunoassay calculated at a level of 5% displacement of the tracer with antiserum 199 was 2 fmol/tube or 5 pmol/l (Fig. 1). The ouabain concentrations in normal human plasma extracts varied between 3 and 10 fmol/tube.

The within and between assay coefficients of variation were 3.8% and 13.4% at 15 pmol/l and 3.7% and 9.4% at 42 pmol/l respectively ($n=10$). All the samples from pregnant and postpartum women were assayed in the same assay. Of the compounds tested, ouabagenin showed the greatest cross-reactivity (52%); digoxin and digitoxin cross-reacted slightly (1.7%), and other less relative compounds (butaline 1%, dihydro-ouabain 0.7% and digoxigenin 0.5%). Naturally occurring steroids cross-reacted very little: cortisol 0.0005%, aldosterone 0.0008%, progesterone 0.00017%, estrone 0.00027%, β -estradiol 0.0006%, dehydroepiandrosterone 0.0003%, hydroxyandrostenone 0.0001% and testosterone 0.0005% (Table 1).

Figure 1 Displacement curves of the ouabain-like compound from human plasma ($\blacktriangle$, A; Hamlyn *et al.* 1991), the bovine hypothalamic inhibitory factor ($\blacksquare$, B; Tymiak *et al.* 1993) and commercial plant-derived ouabain ($\bullet$, C) in the ouabain radioimmunoassay. Each point represents the mean of two measurements. B/B_0 , Percentage binding ratio over binding of the zero standard sample.

Plasma cortisol was measured using a Cortisol (^{125}I) kit from Orion Diagnostica, Espoo, Finland. According to the manufacturer, the reference limits for samples taken between 0800 h and 1000 h are 189 and 682 nmol/l and

the maximum intra-assay and interassay coefficients of variation are 4.1 and 9.0%, respectively. Cortisol was measured from the 27 plasma samples available to us at the time of the assay.

Solid-phase separation of plasma and amniotic fluid samples

Ouabain was extracted from 1–2 ml plasma or amniotic samples with Sep-Pak-Vac 500 mg C18 cartridges (Waters, Milford, MA, USA) using an automated Gilson 5100 Aspec system and polypropylene test tubes. Briefly, the cartridge, preconditioned with 2 ml 2-propanol and 4 ml 0.1% aqueous trifluoroacetic acid (TFA), was loaded with the plasma sample to which 0.2 ml 1 M HCl/1.6% glycine/ml was added. The cartridge was washed with 0.1% TFA. Ouabain was eluted with 2 ml 40% acetonitrile in 0.1% TFA and evaporated to dryness. The dried residue was reconstituted with 250 μl radioimmunoassay buffer (see above). The recoveries of ouabain added to plasma were $103 \pm 2.5\%$ at 43 pmol/l and $94 \pm 1.3\%$ at 130 pmol/l (mean $\pm$ s.e., $n=6$).

HPLC analysis of the reference materials and plasma extracts

Aliquots of human plasma ouabain-like compound (Hamlyn *et al.* 1991) and bovine hypothalamic inhibitory factor (Tymiak *et al.* 1993) and Sep-Pak extracts of plasma or amniotic fluid (see above) were dissolved in 0.1% TFA, centrifuged, filtered and subjected to reverse-phase HPLC using a Vydac C8 218TP column (0.46×15 cm, Hesperia, CA, USA). A 30-min linear gradient from 5 to 30% acetonitrile in 0.1% TFA was run at a flow rate of

Table 1 Comparison of immunoassays developed for ouabain with respect to specificity (cross-reactivity was determined at 50% displacement of tracer on a weight basis), sensitivity (read at 95% binding level of tracer, or as mean for the zero calibrator -2SD) and plasma ouabain (concentrations in healthy adults)

Authors	Assay method	Specificity (cross-reactivity %)					Sensitivity (pmol/l)	Plasma ouabain (pmol/l)
		Ouabagenin	Dihydro-ouabain	Digoxin	Digitoxin	Steroids*		
Harris <i>et al.</i> (1991)	ELISA	40	0.16	5.2	28	≤ 0.01	100	100–180
Doris <i>et al.</i> (1994)	^3H -RIA	56.2	—	4.5	—	≤ 0.025	80	<80
Gomez-Sanchez <i>et al.</i> (1994)	ELISA	2.2	0.02	<0.001	0.12	≤ 0.001	30	30–50
Lewis <i>et al.</i> (1994)	ELISA	77.2	—	1.25	—	≤ 0.07	60	<60
Naruse <i>et al.</i> (1994)	^3H -RIA	—	—	9.2	—	≤ 0.01	20	20–60
Worgall <i>et al.</i> (1996)	^3H -RIA	88	—	0.06	<0.1	<0.1	20	30–100
Balzan <i>et al.</i> (1997)	^3H -RIA	—	—	3.8	36	≤ 0.18	10	15–30
Ferrandi <i>et al.</i> (1997)	^3H -RIA	21	—	0.4	9	≤ 0.9	20	50–750
Harwood <i>et al.</i> (1997)	EIA	55	0.3	5.3	78	≤ 0.02	40	60–170
Komiyama <i>et al.</i> (1997)	ELISA	72	0.82	6.4	84.6	<0.01	10	20–140
Bernini <i>et al.</i> (1998)	ELISA**	—	—	3	5	—	10	10–175
Butt <i>et al.</i> (1998)	^3H -RIA	4.0	2.0	0.4	0.67	≤ 0.016	60	400–800
Present study	^{125}I -RIA	52.2	0.7	1.7	0.6	<0.001	5	10–20

*Aldosterone, cortisol, progesterone, β -estradiol, testosterone and other common steroids.

**Du Pont–New England Nuclear ELISA kit.

RIA, radioimmunoassay; ELISA, enzyme-linked immunosorbent assay; EIA, enzyme immunoassay.

1 ml/min. Fractions of 1 ml were collected into polypropylene test tubes, dried in Speed Vac, reconstituted with 250–500 µl radioimmunoassay buffer as described before and subjected to the ouabain radioimmunoassay. In the reverse-phase HPLC, 90% of the ouabain-like compound (Hamlyn *et al.* 1991) and 78% of the bovine hypothalamic inhibitor (Tymiak *et al.* 1993) could be recovered.

Statistical analyses

The data are expressed as mean $\pm$ s.e. For comparison of the ouabain or cortisol concentrations and blood pressure recordings between various experimental groups, one-way ANOVA followed by Newman–Keul's multiple comparison test was used. Spearman coefficients of rank correlation and linear correlation were calculated with immunoreactive plasma ouabain concentration as independent and systolic and diastolic blood pressure recordings or plasma cortisol as dependent variables.

Results

Validation of the radioimmunoassay for use with human plasma samples

The displacement curves of the bovine hypothalamic inhibitory factor and human plasma ouabain-like substance were parallel with that of plant-derived ouabain (Fig. 1). The apparent cross-reactivity of the hypothalamic inhibitory factor obtained from Dr Haupert (Tymiak *et al.* 1993) was 70% and that of plasma ouabain-like compound obtained from Dr Hamlyn (Hamlyn *et al.* 1991) was 52%. Endogenous immunoreactivity present in human male plasma Sep-Pak extracts displaced the tracer in parallel with the ouabain standard (Fig. 2). The Sep-Pak extracts of the reference preparations from bovine and human plasma origin eluted identically with plant ouabain in the reverse phase HPLC system (Fig. 3). The recovery of the added or endogenous materials in the HPLC run was 78–103%. When the ouabain-like compound isolated from human plasma was subjected to HPLC, a very small immunoreactive peak was found to elute after ouabain (Fig. 3B). Immunoreactive ouabain in Sep-Pak extracts of human pregnant plasma, umbilical cord plasma and amniotic fluid eluted in the reverse-phase HPLC system identically with plant ouabain (Fig. 4). These findings show that our radioimmunoassay is suitable for the measurement of endogenous ouabain.

Plasma immunoreactive ouabain in reference and pregnant individuals

The mean plasma ouabain concentrations measured by our radioimmunoassay were 12.6 ± 1.3 pmol/l in healthy men and 9.4 ± 0.7 pmol/l in women. The mean plasma

Figure 2 Displacement curves of ouabain (●) and a human plasma extract (■) in the ouabain radioimmunoassay. Each point represents the mean of two measurements. B/B₀, Percentage binding ratio over binding of the zero standard sample.

ouabain concentrations in healthy pregnant women during the first, second and third trimesters were 16.3 ± 4.0 , 18.8 ± 4.3 and 24.3 ± 4.0 pmol/l respectively; all these values are significantly greater than those in non-pregnant women ($P < 0.01$; Fig. 5). Plasma ouabain concentration during the second and third trimesters was also significantly greater than that during the first trimester ($P < 0.05$ – 0.01). After the delivery, plasma ouabain concentrations were 13.6 ± 1.1 pmol/l, significantly ($P < 0.05$) lower than those during the second and third trimesters. Amniotic fluid and umbilical cord plasma ouabain levels were 25.5 ± 4.1 and 22.0 ± 2.0 pmol/l respectively.

The mean systolic and diastolic blood pressures in healthy women were 127 ± 4 and 82 ± 1 mmHg respectively. In pregnant women the systolic blood pressure during the third trimester was significantly ($P < 0.05$) greater than that during the first or second trimesters (124 ± 3 mmHg compared with 110 ± 3 and 118 ± 5 mmHg respectively). The mean systolic and diastolic blood pressures in postpartum women were 125 ± 3 mmHg and 69 ± 5 mmHg. There was a significant rank correlation between plasma ouabain concentration and the systolic blood pressure in pregnant women ($r = 0.434$, $P = 0.0269$, $n = 26$; Fig. 6). The correlation between plasma ouabain and diastolic blood pressure in the same individuals was not significant ($r = 0.08$, $P > 0.05$).

The mean morning plasma cortisol concentrations were 183 ± 17 nmol/l during the first trimester, 214 ± 44 nmol/l during the second trimester, 278 ± 25 nmol/l during the third trimester and 146 ± 12 nmol/l

Figure 3 Reverse-phase HPLC of the bovine hypothalamic inhibitory factor (A) (Tymiak *et al.* 1993) and the ouabain-like compound from human plasma (B) (Hamlyn *et al.* 1991). The elution of plant-derived commercial ouabain is marked by an arrow. For HPLC conditions see methods. Dotted line represents the detection limit in the ouabain radioimmunoassay.

at 3–5 days after the delivery. The cortisol concentrations during the third trimester were significantly greater ($P < 0.05$) than those during the first trimester or postpartum. There was a significant linear correlation between plasma ouabain and plasma cortisol concentrations in pregnant and postpartum women ($r = 0.512$, $P = 0.006$, $n = 27$; Fig. 7). It should be stressed that our ouabain antiserum does not have any detectable cross-reaction with cortisol or the naturally occurring steroid hormones.

Discussion

In order to validate our ouabain radioimmunoassay for the analysis of human plasma samples, we compared the displacement curves of the human plasma endogenous ouabain-like compound (Hamlyn *et al.* 1991) and the bovine hypothalamic inhibitor (Tymiak *et al.* 1993) with commercial plant-derived ouabain. Both endogenous sub-

Figure 4 Reverse-phase HPLC of Sep-Pak extracts of plasma from a pregnant woman in the third trimester (A), an amniotic fluid sample (B) and cord plasma (C). The elution of plant-derived ouabain is marked by an arrow in (C).

stances diluted in parallel with ouabain and their potencies compared with commercial plant ouabain were 52% and 70% respectively. Considering the inaccuracies inherent in weighing small amounts of natural materials (1 pmol and 171 pmol), both endogenous substances probably are equipotent with ouabain in our radioimmunoassay. In addition, we wanted to see if the endogenous materials were homogenous. Reverse-phase HPLCs of both endogenous substances were performed and demonstrated almost

Figure 5 Plasma ouabain concentrations in non-pregnant women (CON, ○, $n=14$), and in pregnant women during gestational weeks 8–40 (●, $n=28$) and 3–5 days after the delivery (PP, △, $n=10$). There was a significant linear correlation between plasma ouabain and the duration of gestation (solid line). Prediction lines with 95% probability are shown (dotted lines).

complete recovery; in addition, the great majority (78–90%) of the immunoreactivity eluted identically with ouabain. However, a small broad immunoreactive peak eluted after ouabain in the human plasma preparation. This finding and the slightly lower than expected immunoreactivity of the human plasma and bovine hypothalamic preparations that we observed in this study may have been presumed to reflect the complexation of ouabain with borate from glassware that was recently shown to be possible (Kawamura *et al.* 1999); however, we did not use glassware in this study. Our radioimmunoassay is thus valid for the measurements of endogenous ouabain in biological fluids.

In the present study, the mean plasma ouabain concentrations in non-pregnant women were found to be 9.4 pmol/l and those in men were 12.4 pmol/l. Previously, an enzyme-linked immunosorbent-assay (ELISA) method gave plasma ouabain concentrations of 138 pmol/l (Hamlyn *et al.* 1991). In other studies even greater concentrations have been reported, but the identity of the immunoreactivity was not studied (Masugi *et al.* 1986,

Figure 6 Systolic blood pressure as a function of plasma ouabain concentration in pregnant women. There was a significant positive rank correlation between the parameters (solid line: $r=0.434$, $P=0.0269$, $n=26$).

Harris *et al.* 1991, Gottlieb *et al.* 1992, Rossi *et al.* 1995). In a more recent radioimmunoassay study, plasma ouabain concentrations in healthy volunteers (sex not stated) were found to be 25 pmol/l and the immunoreactivity eluted in HPLC in the same way as ouabain (Balzan *et al.* 1997). In contrast, plasma ouabain concentrations less than 5 pmol/l have also been reported (Gomez-Sanchez *et al.* 1994, Doris *et al.* 1994, Lewis *et al.* 1994). A summary of the results of previous ouabain immunoassays was presented in Table 1. It is our opinion that the use of tritiated or enzyme-labeled tracers and the resulting low sensitivity of the previously used ouabain antisera are major reasons why greatly variable ouabain concentrations in human plasma have been reported. In addition, no attempts to compare the chemical and immunological properties of the endogenous ouabain-like substances with commercial ouabain have been made in any of the previous studies.

There are no previous studies reporting plasma immunoreactive ouabain concentrations in pregnant women. Using a radioreceptor assay, plasma ouabain concentrations in non-pregnant individuals were found to be 204 pmol/l; those in pregnant women were up to 2000 pmol/l (Paci

Figure 7 Plasma cortisol as a function of plasma ouabain in pregnant (●) and postpartum (○) women. There was a significant positive linear correlation between the parameters (solid line: $r=0.512$, $P=0.006$, $n=27$). Prediction lines with 95% probability are shown (dotted lines).

et al. 1996). In contrast, plasma digitalis-like immunoreactivities have been measured in several studies. The concentrations in pregnant women have varied from 10 to 930 pmol/l, but in none of these studies were analyses of the chemical identity of the measured immunoreactivity performed (Graves *et al.* 1984, Valdes & Graves 1985, Poston *et al.* 1989, Seely *et al.* 1992, Paci *et al.* 1996). We have recently measured immunoreactive digitalis-like substances in human pregnant plasma and placenta after solid-phase extraction and HPLC and observed that the amount of immunoreactive digitalis-like substance is much smaller than that of immunoreactive ouabain-like substance (S S Árnason, unpublished observation).

Systolic but not diastolic blood pressure in our pregnant women was significantly greater in the third trimester than in the first or second trimesters. We wanted therefore to know whether plasma ouabain concentrations correlated to blood pressure during pregnancy, and we observed a significant rank correlation between plasma ouabain concentration and systolic blood pressure but not between diastolic blood pressure. We also performed a linear

correlation analysis between plasma ouabain and systolic blood pressure, but this did not reach statistical significance, indicating that the correlation is complex. It has been proposed that plasma ouabain could interfere with the sodium pump of vascular smooth muscle, resulting in increased vascular tone and increased blood pressure (Hamlyn & Manunta 1992, Blaustein 1996). Our findings are consistent with this concept during pregnancy but not after the delivery, when plasma ouabain decreased but systolic blood pressure remained at increased values. The significant correlation between plasma ouabain and systolic blood pressure during pregnancy may still impart some role for ouabain in regulating blood pressure, but other factors have also to be taken into account.

In view of the observed adrenal origin of ouabain (Hamlyn *et al.* 1991, Schneider *et al.* 1998) and the finding that the stimulation of adrenal cells by adrenocorticotrophic hormone (ACTH) *in vitro* increases release of ouabain to the medium (Laredo *et al.* 1994), it was interesting to note that there was a gradual increase in maternal plasma ouabain during pregnancy and a rapid decrease within 3–5 days after the delivery in the present study. These changes resemble those in maternal plasma total cortisol concentration (Brien & Dalrymple 1976, Cousins *et al.* 1983, Allolio *et al.* 1990). In our study, the mean plasma total cortisol concentration was 48% less in the postpartum women than in the third trimester women; in a similar study, a 25% decrease was observed (Allolio *et al.* 1990). The difference is evidently due to the fact that our postpartum women were different from the third trimester ones.

We detected in this study high concentrations of ouabain in the amniotic fluid and cord plasma, indicating an association of ouabain with the fetoplacental unit. The significant positive linear correlation between the concentrations of ouabain and total cortisol in samples taken during pregnancy and postpartum further strengthens the proposal that ouabain and cortisol are under similar regulation in these conditions. The fluctuations in maternal cortisol have been accounted for by stimulation of adrenal cortex by ACTH and corticotropin-releasing hormone (Sasaki *et al.* 1989), an increase in cortisol-binding globulin, or an antiglucocorticoid action of progesterone (Brien & Dalrymple 1976, Cousins *et al.* 1983, Allolio *et al.* 1990). The potential roles of these factors in determining plasma ouabain concentrations are not known.

In conclusion, we have developed a sensitive radioimmunoassay for ouabain that detects equally well plant-derived ouabain and the endogenous ouabain-like substance previously isolated from human plasma and bovine hypothalamus. Using the assay, we demonstrated that plasma ouabain concentrations in healthy individuals are much lower than reported previously and that plasma ouabain in pregnant women increases towards the delivery, abruptly decreases after it and correlates with plasma total cortisol, suggesting that the secretions of ouabain and

cortisol during pregnancy may be regulated by the same factors.

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