Cytochemical bioassay of parathyroid hormone in maternal and cord blood

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SUMMARY Parathyroid hormone and calcium were measured in plasma taken from pregnant women at term and from the umbilical veins of their infants at birth. Three assays were used to measure parathyroid hormone, a cytochemical bioassay of bioactivity and two immunoradiometric assays, one specific for the amino terminus, the other specific for the carboxy terminus of the parathyroid hormone molecule.

Plasma calcium was significantly higher in the infants than in the mothers. Maternal parathyroid hormone bioactivity and the amino terminus were both slightly raised, but the carboxy terminus value was normal; these findings supported the view that late pregnancy is a time of mild physiological hyperparathyroidism. In the infants, the amino terminus was undetectable and the carboxy terminus was either undetectable or towards the lower end of the normal range: bioactivity of parathyroid hormone was considerably raised and was related to the gradient of calcium across the placenta. This suggests that the parathyroid glands are not suppressed during fetal life and that they may play an important part in the maintenance of high fetal plasma calcium concentrations.

It has long been recognised that the total concentration of calcium in maternal plasma falls during pregnancy, particularly during the last trimester.¹ This fall is largely accounted for by a reduction in the plasma albumin concentration,² but there is some disagreement over whether or not the concentration of ionised calcium changes significantly.³⁻⁵ In the fetus, however, the total concentration of calcium rises during fetal life,² and it has been known since 1923 that by term it is significantly greater than that of maternal plasma.⁶ This observation has been confirmed repeatedly,⁷⁻⁸ and it is known to be true also of ultrafilterable⁹ and ionised calcium.¹⁰⁻¹¹ At birth the difference between the total calcium concentration is 0.25 to 0.5 mmol/l even when differences in serum albumin are allowed for. The mechanisms by which this gradient is maintained have not been adequately explained.

Parathyroid hormone has been measured in maternal and cord plasma by immunoassay in several studies. A rise in immunoreactive parathyroid hormone has frequently been described in the maternal circulation during gestation,⁵⁻¹²⁻¹³ although there has been some disagreement on this point.¹¹⁻¹⁴⁻¹⁷ Pregnancy has been described as a

condition of physiological hyperparathyroidism¹² but it has not been established whether this is a primary event associated with the increased calcium retention and bone turnover known to occur from the middle of pregnancy onwards,¹⁸ possibly in anticipation of fetal calcium requirements. It may be a secondary event in response to the demand for calcium by the fetus during the last trimester.

Similar studies in the fetus have produced very conflicting results. In some, the concentrations of parathyroid hormone in cord blood were low or undetectable and maternal concentrations were either not measured,¹⁹⁻²² were normal,¹⁵⁻¹⁶⁻²³ or were raised.⁵⁻¹³ In other studies, the concentrations in both fetal and maternal plasmas were within the normal adult range,¹¹⁻¹⁴⁻¹⁷⁻²⁴ while in others both maternal and cord samples contained high concentrations.²⁵⁻²⁶ In no reports have the concentrations of parathyroid hormone in fetal plasma been higher than those of maternal plasma. It has been suggested that, as a result of the high fetal plasma calcium, the fetal parathyroid glands are suppressed during fetal life²⁴ despite the fact that they are capable, from 12 weeks' gestation, of producing a substance, presumably parathyroid

hormone, which is immunochemically indistinguishable from parathyroid hormone²⁷ and which causes bone resorption *in vitro*.²⁸ Recent evidence in primates²⁹ suggests that the response of fetal glands to induced hypocalcaemia is similar to that of the mother.

We have studied the problem using three different assays for parathyroid hormone, namely a highly sensitive cytochemical bioassay, which measured bioactivity, and two immunoradiometric assays which employed homologous antihuman parathyroid hormone antibodies, one specific for the amino terminus, the other specific for the carboxy terminus of the parathyroid hormone molecule.

Patients and methods

Blood samples were obtained from the umbilical veins of 10 normal term infants immediately after their cords had been clamped and before delivery of the placenta. Simultaneous venous blood samples were obtained from the mothers. All specimens for parathyroid hormone assay were taken into cold heparinised tubes and kept on ice until centrifuged at 4°C. This was done as soon as possible after each specimen had been obtained. Plasma for cytochemical bioassay was snap frozen to -70°C in aliquots of about 150 µl, and those for immunoassay in aliquots of 1 ml. Aliquots of the same samples were also taken for measurement of calcium, albumin, and phosphate.

The cytochemical bioassay was performed as previously described³⁰ on samples from eight of the mother and baby pairs. Parathyroid hormone bioactivity was measured by its ability to stimulate glucose 6-phosphate dehydrogenase (G6PD) in distal convoluted tubules of guinea pig kidneys. Samples were assayed against a standard preparation of bovine hormone (77/533 NIBSC) and all results were expressed as pg/ml of this standard. The limit of sensitivity of this assay is 0.1 pg/ml when the initial dilution is 1:100 and the normal range for young adults is 1.1 to 5.9 pg/ml. Immunoradiometric assays for N-PTH and C-PTH were performed as previously described^{31, 32} using homologous antibodies selected for high affinity to the respective terminals of the parathyroid hormone molecule. The limit of detection of the amino terminus assay is 40 pg/ml of 1-34 human parathyroid hormone standard and the upper limit of normal for adults is 120 pg/ml. Two thirds of normal subjects have undetectable amino terminus concentrations in plasma. The limit of detection of the carboxy terminus assay is 100 pg/ml of 53-84 human parathyroid hormone standard and the upper limit of normal is 800 pg/ml; the carboxy terminus can be

detected in all normal subjects. Calcium, phosphate, and albumin were measured by automated techniques on a Vickers M300 autoanalyser and all calcium values were corrected for plasma albumin.

To assess the nature of parathyroid hormone bioactivity in fetal plasma, a time course of the activity of G6PD in guinea pig kidney renal tubules in response to exposure to fetal plasma was compared with that of the response to maternal plasma. In another experiment antibody was added to the fetal plasma before assay to see if parathyroid hormone bioactivity was eliminated. The antibody used for this was the same as that used for the amino terminus immunoradiometric assay, and was added to the plasma in a dilution of 1:100. A similar concentration of extraction medium was added to the plasma being assayed.

Results

The concentrations of calcium, corrected for albumin; the amino terminus; the carboxy terminus; and parathyroid hormone bioactivity found in maternal and cord plasma are shown in Figs. 1 and 2. The calcium concentrations were mean (SEM), 2.46 (0.02) mmol/l in maternal plasma and 2.70 (0.04) mmol/l in cord plasma. This difference was highly significant ($P < 0.001$). The amino terminus was raised in four of the maternal samples (mean (SEM), 173 (27) pg/ml) but was undetectable in all the cord plasma samples. Even if it is assumed that undetectable values were at the limit of detection of the assay, the difference between these two groups was significant ($P < 0.001$). The carboxy terminus of parathyroid hormone was within the normal range in all the maternal samples, and within the normal range in five, but undetectable in two, of the fetal samples. Bioactivity of parathyroid hormone was significantly raised (mean (SEM), 7.1 (1.4) pg/ml; $P < 0.02$) in maternal plasma, although four of the values were within the normal range. In cord plasma, however, in contrast to the findings with immunoassay, concentrations were considerably raised (mean (SEM) 41 (6.5) pg/ml), and the difference between maternal and cord samples was highly significant ($P < 0.001$).

The time courses of response of G6PD to maternal and cord plasma are shown in Fig. 3. The response was parallel in the two samples, with an initial peak response at six minutes. Addition of antibody against parathyroid hormone to cord plasma before assay (Fig. 4) eliminated the bioactivity.

There was no relation between plasma calcium and bioactivity of parathyroid hormone in either group of subjects. There was also a significant

Fig. 1 Plasma calcium, amino terminal parathyroid hormone (N-PTH), and carboxy terminal parathyroid hormone (C-PTH) in maternal (open circles) and cord (closed circles) venous plasma.

The normal ranges for adults are shown by the rectangles and the limits of detection of the immunoassays by horizontal interrupted lines. For purposes of data analysis, undetectable values were assumed to be at the limit of detection of the assays.

Fig. 2 Parathyroid hormone bioactivity (Bio-PTH) in maternal (open circles) and cord (closed circles) venous plasma.

The upper limit of normal for young adults is represented by the horizontal dotted line.

Fig. 3 Time course of the response (measured as mean integrated extinction) of glucose 6-phosphate dehydrogenase activity to parathyroid hormone in maternal (open circles) and cord (closed circles) venous plasma of one mother-baby pair.

The pattern of response in both is identical, declining from an initial high value at six minutes followed by a second peak at 12 minutes.

Fig. 4 Effect of addition of amino terminal specific anti-human parathyroid hormone antibody (AB) to cord plasma.

Without antibody the response (measured as mean integrated extinction) is parallel to that of the standard curve and gives a value of 36 pg/ml. After the addition of antibody the activity falls to below 0.1 pg/ml.

correlation between the gradient of calcium across the placenta and the concentration of bioactive parathyroid hormone in cord blood ($r=0.83$ $P<0.01$) (Fig. 5).

Discussion

The cytochemical bioassay has been shown to be a highly sensitive method of measuring parathyroid hormone,³³⁻³⁵ and we have applied it to the measurement of concentrations in fetal and maternal plasma. Activity in the two groups showed parallelism with the standard curve and the similar time courses of response to maternal and fetal plasma indicated that the same substance was being measured in each. The disappearance of activity from fetal plasma when amino terminal specific anti-human parathyroid hormone antibody was added to it confirmed that this substance was parathyroid hormone.

Fig. 5 Correlation between parathyroid hormone (Bio-PTH) bioactivity of cord venous plasma and the transplacental gradient of plasma calcium.

There is a significant positive relation.

Intact human parathyroid hormone contains 84 amino acids.³⁶ Only the first 34, however, are required for full biological activity.³⁷ The concentration of the amino terminus in plasma is normally less than that of the carboxy terminus. This is partly a reflection of the different half lives of fragments from opposite ends of the molecule in plasma.³⁸ Our results showed that in maternal plasma both the amino terminus and bioactivity were slightly raised, and support the view that late pregnancy is a time of mild physiological hyperparathyroidism. The carboxy terminus however, was not raised. This difference between the amino and carboxy termini of parathyroid hormone helps to explain some of the conflicting results found previously when parathyroid hormone has been measured by immunoassay, and may be related in the pregnant compared with the non-pregnant female to reduced generation or more rapid clearance of carboxy terminus fragments.

Immunoreactive parathyroid hormone, particularly the amino terminus, was lower in fetal than in maternal plasma, an observation which is consistent with the hypothesis that the parathyroid glands are suppressed during fetal life. The greatly increased concentrations of bioactive parathyroid hormone in fetal plasma, however, were surprising and suggested that, despite the low or undetectable concentrations of immunoreactive parathyroid hormone in the fetus, the parathyroid glands are not in fact suppressed. Furthermore, an inverse relation between bioactive parathyroid hormone and plasma calcium, which might be expected to exist if high fetal plasma calcium concentrations do suppress fetal parathyroid gland activity, was not shown. Similarly there was no relation between these two variables in the mothers.

The positive relation between bioactive parathyroid hormone in the fetus and the difference between fetal and maternal calcium was of considerable interest. Since plasma calcium concentrations are higher in the human fetus than in the mother, an active calcium pump clearly exists, and from animal studies, mainly in sheep,^{39 40} it is thought that the fetus is largely responsible for this pump. The results of the cytochemical bioassay support this view and suggest that, rather than being suppressed, the fetal parathyroid glands have considerable activity which contributes to the maintenance of the transplacental calcium gradient, possibly by acting, directly or indirectly, on the placenta.

Allowing for the different molecular weights of the standard reference preparations used in the two assay systems, the ratio of the mean values of the amino terminus to bioactivity was no more than 2.4:1 in fetal plasma and 61:1 in maternal plasma. It

therefore seems that, while in normal adults and pregnant women only a very small proportion of circulating amino terminus is biologically active, in cord plasma this proportion is greater than 40%. The reasons for this difference are not clear, but are presumably due to the presence, in adult and maternal plasma, of high concentrations of biologically inactive parathyroid hormone which is also recognised by the immunoassay. The relatively low concentrations of biologically inactive amino terminus of parathyroid hormone in fetal plasma may be due either to a very rapid rate of removal of these fragments from fetal plasma (for example by the placenta), or to differences between mother and fetus in the rate of metabolism of intact parathyroid hormone.

Whatever the explanation for the differences observed in these studies, it is clear that the measurement of parathyroid hormone by cytochemical bioassay has resulted in data which conflict strongly with those of immunoassay, both in the same infants in whom it was measured and when related to the results of previously reported studies. In particular, this is the first report of parathyroid hormone concentrations which were significantly higher in cord blood than in corresponding maternal blood. If parathyroid hormone acted in some way on the placenta, the high concentrations of bioactive parathyroid hormone in cord blood might help to explain how the well recognised gradient of calcium across the placenta is maintained.

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