Regulation of cyclic AMP synthesis and degradation is modified in rat liver at late gestation

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Cyclic AMP (cAMP) is known to play a key role in regulating insulin action, and it is well documented that in several cases of physiological insulin resistance its concentration is increased. Since late pregnancy in the rat is associated with liver insulin resistance, we have studied possible alterations of some cellular mechanisms regulating the cAMP metabolism. (1) Liver cAMP concentration was shown to be increased by some 30% and 50% at 18 and 22 days of pregnancy respectively, compared with virgins. (2) Basal adenylate cyclase activity was higher only in the 18-days-pregnant rat, and the forskolin-stimulated maximal activity was similar in the three groups of animals. (3) α_s protein is decreased in term-pregnant rats; however, coupling between G_s and adenylate cyclase is only impaired in the 18-days-pregnant animals, and stimulation by glucagon is impaired in both groups of pregnant animals. (4) G_i -2 protein was shown to be unable to elicit the tonic inhibition of adenylate cyclase in pregnant rats, although it was only decreased at 22 days of gestation. The increased α_i -2 level detected by immunoblotting at 18 days of gestation did not correlate with its decreased ADP-ribosylation, suggesting that the protein is somehow modified at this stage. (5) Pregnancy is associated with a decrease in membrane phosphodiesterase activity. Our results show that late pregnancy is associated with increases in liver cAMP levels that might be involved in eliciting the characteristic insulin-resistant state, and suggest that mechanisms leading to these increments are changing during this phase of gestation.

INTRODUCTION

During the late phase of gestation in the rat, maternal metabolism of different tissues is modified in order to sustain fetal growth. Thus, despite an increase in plasma insulin concentrations, a state of insulin resistance in liver and peripheral tissues warrants the adequate targeting of substrates to the conceptus [1]. Moreover, this increased transfer of nutrients causes a slight hypoglycaemia in the mother, which is counter-balanced by activation of hepatic glucose production [2]. In fact, it is well documented that rats at term gestation show diminished liver lipogenesis and increased gluconeogenesis, as well as a lesser hepatic glycogen content [3–5].

Cyclic AMP (cAMP) has been identified as an intracellular mediator of hormonal effectors such as glucagon, which induce liver gluconeogenesis and glycogenolysis by stimulation of protein kinase A (cAMP-dependent). Activation of this protein kinase has also been shown to mediate the inhibition of insulin signalling at the insulin-receptor level [6,7]. Concentrations of cAMP within a tissue depend on the rates of formation and degradation, catalysed by hormone-sensitive adenylate cyclase and phosphodiesterase respectively. Adenylate cyclase activity is, in turn, regulated by the stimulatory and inhibitory G-proteins G_s and G_i , which are coupled to different specific cell-surface hormonal receptors, whereas for the phosphodiesterase system a series of regulatory mechanisms has been proposed (see [8] for review). Several insulin-resistant states associated with diabetes mellitus or obesity have been reported to exhibit altered regulation of adenylate cyclase characterized by changes in the expression and functioning of the regulatory G-proteins [9–18].

The aim of the present work was to study the mechanisms controlling the synthesis and degradation of liver cAMP in the late-pregnant rat. Results are discussed with regard to the possible

involvement of alterations in these mechanisms in eliciting the liver insulin resistance characteristic of late pregnancy.

MATERIALS AND METHODS

Materials

[3H]cAMP (32.9 Ci/mmol), [^{32}P]NAD $^+$ (30 Ci/mmol) and the G-proteins-specific antisera RM/1 and AS/7 were obtained from Du Pont–New England Nuclear (Dreieich, Germany). Pertussis and cholera toxins were purchased from List Biological Laboratories (Campbell, CA, U.S.A.). Goat anti-(rabbit IgG)–horse-radish peroxidase conjugate was from Nordic Immunology (Tilburg, The Netherlands). cAMP, cAMP-dependent protein kinase, charcoal, triethanolamine, theophylline, glucagon, NaF, guanosine 5'-[$\beta\gamma$ -imido]triphosphate (p[NH]ppG), forskolin, neutral alumina type WN3, Dowex 1 (200–400 mesh), *Ophiophagus hannah* venom, ATP, GTP, NAD $^+$, dithiothreitol, thymidine, L-arginine and pre-stained molecular-mass standards were all from Sigma. Phosphocreatine and creatine kinase were from Amersham. Reagents for electrophoresis were from Bio-Rad and Serva. All other reagents were of the best grade commercially available.

Animals

Albino Wistar rats fed on standard laboratory chow and water *ad libitum* were used for the experiments. The presence of spermatozoa in the vagina was assumed to indicate conception, and gestational age was confirmed by the fetal weight. Age-matched virgin rats were used as controls [4].

Assay of cAMP

Freeze-clamped liver was pulverized under liquid N $_2$, weighed, and homogenized in 10 vol. of cold 10% (w/v) trichloroacetic

Abbreviations used: cAMP, cyclic AMP; cGMP, cyclic GMP; G_s , GTP-binding protein coupled to the stimulation of adenylate cyclase; G_i , GTP-binding protein coupled to the inhibition of adenylate cyclase; p[NH]ppG, guanosine 5'-[$\beta\gamma$ -imido]triphosphate.

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acid. After 10 min at 0 °C, precipitated protein was removed by centrifugation at 3000 *g* for 20 min. The acid was extracted from the supernatant by washing with 6 × 2 vol. of water-saturated ether. Then cAMP was measured as described in [19].

Plasma-membrane preparation

Isolation of plasma membranes was performed as described in [20], by using the proteinase inhibitors phenylmethanesulphonyl fluoride (1 mM), iodoacetamide (5 mM), bacitracin (0.1 mg/ml), trypsin inhibitor (0.1 mg/ml) and benzamidine (0.2 mg/ml) throughout all centrifugations. Membranes were finally resuspended in 50 mM-Tris (pH 7.4)/2 mM-EDTA/1 mM-phenylmethanesulphonyl fluoride at a final concentration of 10–20 mg of protein/ml.

Assay of adenylate cyclase

Adenylate cyclase activity was assayed as described by Houslay *et al.* [21]. Protein concentration in the assay was 1 mg/ml (100 µg/assay).

Assay of cAMP phosphodiesterase

cAMP phosphodiesterase activity was measured as described in [22]. Incubations were carried out over periods of 15 min in a final volume of 0.1 ml with 50–60 µg of membrane protein at 30 °C. For these assays, membranes were prepared in the absence of EDTA and NaCl throughout the process, in order to avoid losses of the low-*K_m* peripheral enzyme.

ADP-ribosylation

For ADP-ribosylation of membrane proteins, bacterial toxins were preactivated just before use by incubation with 20 mM-dithiothreitol for 10 min at 37 °C.

Membranes (100 µg of protein) were incubated in a final volume of 50 µl containing 100 mM-potassium phosphate buffer (pH 7.5), 2.5 mM-MgCl₂, 1 mM-ATP, 10 mM-thymidine, 10 mM-L-arginine, 100 µg of activated cholera toxin/ml or 45 µg of activated pertussis toxin/ml, 10 µCi of [³²P]NAD⁺ (30 Ci/mmol), 1 µM-NAD⁺, 0.1 mM-GTP and 10 mM-NADP⁺ to inhibit endogenous NAD⁺ glycohydrolase activity [23]. The reaction was allowed to proceed for 90 min at 30 °C and stopped by addition of 1 ml of ice-cold 100 mM-KH₂PO₄, pH 7.5.

Membranes were pelleted at 12000 *g* for 10 min at 4 °C and, after removing the supernatant, were dissolved in Laemmli sample buffer [24] at 100 °C for 5 min. Proteins were separated by SDS/PAGE and radioactive bands were identified by autoradiography of the stained and dried gel, and quantified by integrating densitometric scans.

Immunoblotting of membranes

Samples (30–100 µg) of membrane proteins were run on SDS/PAGE (10% acrylamide). Prestained molecular-mass standards were run in parallel. The proteins were electrophoretically transferred on to nitrocellulose sheets (0.2 µm; Schleicher & Schuell) in methanol buffer containing 48 mM-Tris, 39 mM-glycine, 0.01% SDS and 10% methanol at 1 A for 2 h at 4 °C. After blocking with 5% (w/v) dried skimmed milk in Tris-buffered saline (TBS; 50 mM-Tris/HCl/150 mM-NaCl, pH 7.4) for 1 h at room temperature, the sheets were incubated with the first antibody (diluted 1/1000 in TBS) overnight at 4 °C. The nitrocellulose membranes were then washed three times with TBS containing 0.5% Tween-20 and 5% dried skimmed milk before incubation for 2 h at room temperature with the second antibody (goat anti-rabbit IgG coupled to horseradish peroxidase, diluted 1/15000 in rinse buffer). The sheets were again washed as detailed above. The bound antibody was detected by using the ECL Western-blotting detection system (Amersham)

and quantified by densitometric scanning of blots which employed non-saturating amounts of membrane protein.

Statistical analysis of the data

Statistical significance of differences between values was calculated by Student's paired *t* test.

RESULTS

cAMP content of rat liver

Since increases in cAMP content have been observed in some insulin-resistant states [8,25], we have studied the possible association of pregnancy in the rat with altered levels of liver cAMP. Results shown in Table 1 indicate that in pregnant rats there is in fact an increase in liver cAMP content (30% and 50% for 18 and 22 days pregnant respectively) compared with that observed in virgins.

Phosphodiesterase activity of liver membranes

Degradation of cAMP is achieved by the phosphodiesterase system, which is composed of multiple isoenzymes differing in kinetic properties, substrate specificity and localization in the cell [8]. Theoretical studies have suggested that low-*K_m* phosphodiesterase bound to the plasma membrane plays a pivotal role in regulating overall and local cAMP concentration changes, even though it represents only some 10% of the total cellular activity [26]. Moreover, Heyworth *et al.* [27] have reported the involvement of this enzyme in decreasing hepatocyte cAMP concentration in response to insulin. Table 2 shows the phosphodiesterase activity associated with liver plasma membranes from virgin and pregnant rats, measured at 1 µM-cAMP. A significant decrease in this activity is observed at 18 and 22 days of pregnancy relative to that found in virgins, a fact that might contribute to the elevation of liver cAMP concentration during late gestation.

Since the membrane fraction used in these assays was taken from the upper part of the Percoll gradient, it should be essentially free from the more dense microsomal fraction [28]. Consequently, it is unlikely that the 'dense vesicle' phosphodiesterase isotype contributes to the activity found in our membranes. In fact, this isotype is known to be inhibited by cyclic GMP (cGMP), whereas the presence of 2 µM-cGMP in the assay induces the stimulation of the membrane activity (results not shown).

Adenylate cyclase activity of liver membranes

Synthesis of cAMP is catalysed by the plasma-membrane enzyme adenylate cyclase, whose activity is regulated by the G-proteins G_s and G_i, which mediate the coupling of the enzyme to occupied receptors for stimulatory and inhibitory agonists respectively. Data shown in Table 3 indicate that the basal activity

Table 1. Liver cAMP content of virgin and pregnant rats

Liver cAMP content was determined as indicated in the Materials and methods section. Values, expressed as pmol of cAMP/g dry wt., are means ± S.E.M. of five separate determinations using different animals: * *P* < 0.01, ** *P* < 0.001, significantly different with respect to virgin rats. No significant differences were found between the dry weights of the liver of the three groups of animals.

Animals	cAMP content
Virgin	1600 ± 40
18 days pregnant	2166 ± 41*
22 days pregnant	2368 ± 37**

Table 2. Phosphodiesterase activity of liver membranes from virgin and pregnant rats

Liver membranes were obtained as indicated in the Materials and methods section, with EDTA and NaCl omitted throughout the process in order to avoid losses of the low- K_m peripheral enzyme. Phosphodiesterase activity was assayed at physiological cAMP concentrations (1 μ M) in the K_m range of the low- K_m enzyme [36]. Values are means $\pm$ S.E.M. of three separate determinations using different animals: * $P < 0.1$, ** $P < 0.025$, significantly different with respect to the virgin rats.

Animals	Phosphodiesterase activity (pmol/min per mg of protein)
Virgin	8.9 $\pm$ 1.4
18 days pregnant	5.1 $\pm$ 1.1*
22 days pregnant	3.6 $\pm$ 0.9**

Table 3. Adenylate cyclase activity in liver plasma membranes from virgin and pregnant rats

Data show adenylate cyclase activity in liver membranes in pmol of cAMP produced/min per mg of membrane protein. Mean values $\pm$ S.E.M. are given for five separate experiments using different animals. Values in parentheses indicate the fold stimulation elicited by each ligand with respect to the basal activity, for each group of rats: * $P < 0.05$, ** $P < 0.025$, *** $P < 0.0125$, significantly different with respect to the virgin rats.

Modulating ligand	Adenylate cyclase		
	Rats...	Virgin	18 days pregnant 22 days pregnant
None (basal)		1.8 $\pm$ 0.4	4.5 $\pm$ 0.8** 1.6 $\pm$ 0.7
Forskolin (0.1 mM)		41.2 $\pm$ 11.1	57.8 $\pm$ 8.7 45.8 $\pm$ 12.7
NaF (15 mM)		13.9 $\pm$ 1.1 (7.5)	22.1 $\pm$ 3.5 (4.9)* 10.1 $\pm$ 2.0 (6.1)
p[NH]ppG (0.1 mM)		7.3 $\pm$ 0.3 (4.0)	9.5 $\pm$ 1.0 (2.1)* 5.2 $\pm$ 1.5 (3.1)
Glucagon (1 μ M)		13.9 $\pm$ 1.6 (7.5)	13.7 $\pm$ 2.3 (3.0) 6.7 $\pm$ 2.1 (4.1)***

of adenylate cyclase is increased in liver in 18-days-pregnant rats, whereas at term gestation it reaches similar values to those observed in virgins. When stimulated by the diterpene forskolin, which acts directly on the catalytic subunit of adenylate cyclase, no significant differences between the three groups of rats were observed, suggesting that the level of expression of the catalytic unit is not affected by pregnancy.

Adenylate cyclase was studied after constitutive stimulation of G_s by NaF or p[NH]ppG. As shown in Table 3, both effectors led to stimulation of adenylate cyclase, the effect being greater with NaF. The activity was higher in liver membranes from 18-days-pregnant rats in both cases, but no differences were observed between 22-days-pregnant and virgin rats. However, since the basal adenylate cyclase activity is increased in the 18-days-pregnant rats, a direct comparison of specific activities does not allow us to assess the effectiveness of the G_s -adenylate cyclase coupling. Thus, when the ratio of ligand-stimulated to basal activities is calculated, we observe that the degree of stimulation is significantly decreased in 18-days-pregnant rats (Table 3, values in parentheses).

The ability of hormones to stimulate adenylate cyclase was assessed by incubation of liver membranes with glucagon. In this case, both groups of pregnant rats show a decrease in the ability

Fig. 1. Dose-effect of p[NH]ppG on forskolin-stimulated adenylate cyclase activity in liver membranes

Liver membranes from virgin (○), 18-days-pregnant (●) and 22-days-pregnant (▲) rats were stimulated with 0.1 mM-forskolin and different concentrations of p[NH]ppG, and adenylate cyclase activity was monitored as reported in [21]. Values are expressed as percentages of that found in the presence of forskolin and in the absence of p[NH]ppG for each group of animals, and are means $\pm$ S.E.M. of four experiments performed with different membrane preparations.

of glucagon to stimulate adenylate cyclase compared with virgin rats (Table 3).

The function of the G_i protein was studied by analysis of the inhibitory effect elicited by low concentrations of p[NH]ppG on forskolin-stimulated adenylate cyclase activity, as reported by others [29]. As shown in Fig. 1, this agent is able to inhibit the forskolin-stimulated adenylate cyclase in membranes from virgin rats, at low concentrations, suggesting the presence of a functional G_i protein. On the contrary, this inhibitory effect is hardly observed in membranes from 18-days-pregnant rats, and is totally absent from membranes from term-pregnant rats, suggesting an association between pregnancy and the loss of G_i function.

Determination of G_s and G_i abundance

Changes in adenylate cyclase responsiveness to stimulatory or inhibitory stimuli have been postulated to occur owing to variations in the expression of the corresponding regulatory G-proteins [9,10,30]. We have assessed the abundance of the α subunits of G_s and G_i (α_s and α_i) in liver membranes by immunodetection, and by specific ADP-ribosylation with cholera and pertussis toxins respectively. Even though these two methods are only semi-quantitative, when used under appropriate conditions they allow comparison of the relative abundance of G-proteins in different membrane preparations. Antibody AS/7 can be used to recognize specifically the α -subunits of transducin (α_t), α_i -1 and α_i -2. Since no detectable α_t and α_i -1 have been found in liver [11,29], this antibody is a valid tool with which to evaluate the relative abundance of α_i -2, the G_i isoform suggested to be involved in adenylate cyclase inhibition [29]. On the other hand, antibody RM/1 recognizes the 42 and 45 kDa forms of α_s ,

Fig. 2. Immunoblot of liver plasma membranes from virgin and pregnant rats

Liver membranes were immunoblotted as described in the Materials and methods section. In (a) RM/1 antibodies were used to detect levels of the 42 and 45 kDa subtypes of the α_s protein; in (b) AS/7 antibodies were used to detect the immunoreactive α_i -2 protein. In each case equal amounts of liver membrane protein from (1) virgin, (2) 18-days-pregnant and (3) 22-days-pregnant rats were used. Bands of molecular mass > 60 kDa whose nature is unknown were always observed to cross-react with the AS/7 antibody. The Figure shows a representative experiment repeated three times.

Fig. 3. ADP-ribosylation of proteins from liver membranes isolated from virgin and pregnant rats

Equal amounts of membrane protein from (1) virgin, (2) 18-days-pregnant and (3) 22-days-pregnant rats were ADP-ribosylated as described in the Materials and methods section, by using cholera and pertussis toxins to detect α_s and α_i -2 respectively. After SDS/PAGE, ADP-ribosylated proteins were detected by autoradiography. Control experiments in the absence of toxins were conducted in parallel to observe unspecific ADP-ribosylation (results not shown). Bands not marked with kDa values in the Figure were observed to undergo ADP-ribosylation in the absence of the corresponding toxins. The Figure shows a representative experiment repeated three times.

and allows its quantitative determination. Fig. 2 shows immunoblots of liver membrane proteins resolved by SDS/PAGE, performed with the above-mentioned antibodies. Levels of both α_s forms in liver membranes from 18-days-pregnant rats are some $111 \pm 2.5\%$ of that observed in virgins (means $\pm$ S.E.M., $n = 3$ different membrane preparations). On the contrary, in membranes from 22-days-pregnant rats a marked decrease in α_s forms is observed ($52 \pm 18\%$; $n = 3$).

Regarding the α_i -2 subunit, pregnancy seems to influence its presence differently, depending on the day of gestation. Thus, whereas 18-days-pregnant rats show a considerable increase in α_i -2 compared with virgins ($259 \pm 91\%$; $n = 3$), the level detected in term-pregnant rats is markedly decreased ($54 \pm 25\%$; $n = 3$).

Fig. 3 shows an autoradiogram of the ADP-ribosylated liver membrane proteins resolved by SDS/PAGE. Cholera toxin elicits the specific ADP-ribosylation of two bands whose molecular masses coincide with those of the two α_s forms. In agreement with results obtained by immunoblotting, the level of α_s appears to be slightly increased in membranes from 18-days-pregnant rats compared with virgins ($144 \pm 38\%$; $n = 3$), but is

severely decreased at term gestation ($22 \pm 6\%$; $n = 3$). On the other hand, pertussis toxin catalyses the specific ADP-ribosylation of a protein whose relative electrophoretic mobility coincides with that of α_i -2. Surprisingly, relative amounts of this protein in both groups of pregnant rats, detected by ADP-ribosylation, are considerably decreased ($55 \pm 19\%$ and $16 \pm 4\%$ for 18- and 22-days-pregnant rats, respectively, of values detected in virgins; $n = 3$), compared with that observed by immunoblotting. This suggests that, during pregnancy, some modification of the α_i -2 protein occurs that impedes its ADP-ribosylation.

DISCUSSION

Although molecular mechanisms responsible for the insulin resistance observed in different physiological situations are not wholly understood, there are some common features to most of the models so far studied. Thus it appears that the insulin-receptor kinase activity is generally decreased, and, on the other hand, there exist tissue-dependent alterations of the cAMP metabolism [9–18] leading to changes in its cellular concentration. Late pregnancy in the rat is associated with a marked hepatic insulin resistance, and we have previously reported [31] that liver insulin-receptor tyrosine kinase activity is significantly inhibited in term-pregnant rats.

Here we show that in 18- and 22-days-pregnant rats there is an increase in the liver content of cAMP compared with virgin rats. A similar increment in cellular cAMP was previously reported to occur in alloxan- and streptozotocin-diabetic insulin-resistant rats [8,25]. Stadtman & Rosen [7] have shown that, in IM-9 cells, agents that increase intracellular cAMP or that directly activate the adenylate cyclase cause serine and threonine phosphorylation of the insulin receptor and inhibition of its intrinsic tyrosine kinase activity. Häring *et al.* [6] have also demonstrated that treatment of adipocytes with isoprenaline (an agonist of the adenylate cyclase system) leads to a decreased insulin-receptor kinase activity. Consequently it seems reasonable to postulate that, at the end of gestation in the rat, the observed increment in liver cAMP concentration could cause the inhibition of insulin-receptor kinase and the subsequent impairment of insulin signal transduction.

Increased basal cAMP levels could reflect an increased basal adenylate cyclase activity. Our results show that this is indeed the case in 18-days-pregnant rats, but not in term-pregnant rats (Table 2). These differences cannot be accounted for by different levels of expression of the adenylate cyclase itself, since after stimulation of the enzyme with forskolin no significant variations in enzyme activity were observed between the three groups.

Changes in adenylate cyclase activity could be a consequence of modifications in the abundance of, or the coupling efficiency to, G_s and G_i regulatory proteins. The presence of α_s in liver membranes, determined by two different methods, is slightly greater in 18-days-pregnant rats than in virgins, whereas a dramatic decrease in this subunit occurs at the end of gestation, a feature not observed in liver in other insulin-resistant states [12,15,32]. Coupling between G_s and adenylate cyclase seems to be functional in both groups of pregnant rats, although the degree of stimulation is significantly decreased in membranes from 18-days-pregnant rats, and similar to that in virgins in term-pregnant rats. There is no correlation between the G_s -adenylate cyclase coupling efficiency and the relative abundance of the α_s subunit. This fact raises the question whether changes in α_s abundance like those observed in this work are of functional importance, an issue previously discussed by Chang & Bourne [33].

The ability of glucagon to stimulate adenylate cyclase is clearly

impaired in liver membranes from term-pregnant rats, and the same is observed in 18-days-pregnant rats when the stimulated/basal activity ratio is considered. From our results it is not possible to conclude whether this impairment is due to specific alterations of glucagon receptors, or is a consequence of a dysfunction in receptor- G_s coupling. Our data differ from those reported in the other cases of insulin resistance, where an increased [11,15] or unchanged [12] response of liver adenylate cyclase to glucagon was observed. Summarizing, these observations suggest that changes in the stimulatory pathway of adenylate cyclase do not play a key role in elevating cAMP concentrations in liver during late pregnancy.

Levels of α_1 -2 in liver membranes determined by immunoblotting suggest that a considerably greater expression of this protein occurs in 18-days-pregnant rats, whereas its presence is decreased at term gestation. Previous studies on other insulin-resistant states associated with altered cAMP metabolism reported unchanged [10,15,34] or diminished [9,12,32] expression of α_1 -2 in different tissues. Gawler *et al.* [11] have postulated that the expression of G_i in liver is regulated, among other factors, by circulating insulin concentrations. In the rat, late pregnancy is associated with hyperinsulinaemia [2,3], which might explain the elevated expression of G_i at day 18 of pregnancy. Plasma insulin levels return to near the values observed in virgins at the end of gestation, and consequently a decrease in G_i is observed. The increase in G_i at 18 days of pregnancy militates against the notion that the increment of adenylate cyclase basal activity is due to the absence of this regulatory protein. Interestingly, when the amount of G_i protein is determined by ADP-ribosylation, decreased levels of this protein are detected in both groups of pregnant rats. These results suggest that the α_1 -2 subunit is somehow modified during pregnancy, making it unable to undergo ADP-ribosylation, and it could be speculated that its function might be impeded by this modification. The former speculation is strongly supported by the data shown in Fig. 1, which demonstrate the insensitivity of forskolin-stimulated adenylate cyclase to treatment with low concentrations of p[NH]ppG, a feature previously observed in liver from type I diabetic rats [11], in liver and adipose tissue from genetically diabetic *db/db* mice [10,12], and in liver and adipose tissue from genetically obese Zucker rats [18,34]. From these data it appears that late pregnancy shares with other insulin-resistant states a common mechanism resulting in the impairment of the ability of G_i to elicit the tonic inhibition of adenylate cyclase. Whether this modification is caused by serine phosphorylation as proposed in [35] remains to be established. Our results do not rule out the possibility that G_i agonists could overcome the impairment of G_i , as discussed in [29].

Finally, our data shown in Table 2 demonstrate that late pregnancy is associated with a decrease in membrane phosphodiesterase activity, assayed at physiological cAMP concentrations (1 μ M). Under these conditions, values are likely to reflect mainly the activity of the low- K_m membrane peripheral phosphodiesterase, with some contribution of the cGMP-stimulated isotype [36]. Since this activity has been suggested to play a key role in regulating cAMP levels in the cell, and near the plasma membrane [26], its inhibition will probably be an important factor in elevating the cAMP levels in liver from pregnant rats, especially at term gestation when no obvious stimulation of cAMP synthesis can be observed. Preliminary results suggest that the diminished membrane phosphodiesterase activity found in late-pregnant rats is due to a decreased V_{max} without alterations in substrate affinity, which could reflect a decrease in the amount of a particular enzyme isotype. Quantification of the peripheral enzyme by immunoblotting will help considerably to clarify this point.

In conclusion, our results show that hepatic cAMP metabolism undergoes changes during late pregnancy in the rat that lead to increased cellular cAMP levels. Concerning the mechanism leading to rises in cAMP, there exist differences between 18- and 22-days-pregnant rats that are probably consequences of the profound hormonal changes that take place in the mother during this final phase of pregnancy. Thus at 18 days of pregnancy cAMP synthesis is stimulated owing to a slight increase in G_s levels and to the dysfunction of tonic inhibition elicited by G_i , its degradation being partially decreased by inhibition of phosphodiesterase activity. At term gestation, however, both G_s and G_i proteins are decreased, and the rise in cAMP level is likely to be due to its decreased degradation rate. The impairment of stimulatory impulse signalling observed in both groups of pregnant rats does not contribute to elevation of cAMP, and probably represents a feedback-regulatory mechanism to avoid a still greater increase in cellular cAMP. From our data it can be concluded that at late gestation some of the mechanisms involved in alterations of the cAMP metabolism in other insulin-resistant states are effective; however, there are also significant differences that might be responsible for the transitory character of these changes in the pregnant rat.

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