Regulation of fetal cardiac and hepatic β-adrenoceptors and adenylyl cyclase signaling: terbutaline effects

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Auman, J. T., F. J. Seidler, and T. A. Slotkin. Regulation of fetal cardiac and hepatic β-adrenoceptors and adenylyl cyclase signaling: terbutaline effects. Am J Physiol Regulatory Integrative Comp Physiol 281: R1079–R1089, 2001.—Terbutaline (Ter), a β2-adrenergic agonist used in preterm labor, stimulates fetal β-adrenoceptors (β-ARs). We administered Ter to pregnant rats on gestational days 17–20 and examined β-ARs and adenylyl cyclase (AC) signaling in heart and liver. Ter produced less downregulation of cardiac β-ARs than in adults, despite a higher proportion of the β2-subtype, and failed to elicit desensitization of the receptor-mediated AC response. AC stimulants acting at different points indicated an offsetting of homologous desensitization at the level of the β-AR by heterologous sensitization at the level of AC: induction of total AC catalytic activity and a shift in the catalytic profile or AC isoform. In fetal liver, Ter produced downregulation of β-ARs, in keeping with the predominance of the β2-subtype; hepatic receptor downregulation was equivalent in fetus and adult. Nevertheless, there was still no desensitization of β-AR-mediated AC responses and again AC was induced. Our results indicate that, unlike in the adult, fetal β-AR signaling is not desensitized by β-agonists and, in fact, displays heterologous sensitization, thus sustaining responses during parturition. At the same time, the inability to desensitize β-AR AC responses may lead to disruption of cardiac, hepatic, or neural cell development as a consequence of tocolytic therapy with β-agonists.

Adenosine 3′,5′-cyclic monophosphate; development; heart; liver; preterm labor; tocolysis

Preterm delivery is a leading cause of neonatal morbidity and mortality, occurring in 8–10% of all births in the United States (4). β2-Adrenoceptor agonists, such as terbutaline (Ter), are widely and successfully used as tocolytics and thus represent a mainstay in the therapy of preterm labor. Ter also crosses the placenta to stimulate fetal β-adrenoceptors (β-ARs) (3, 16, 26), which, to some extent, may provide additional beneficial actions. β-AR stimulation enhances neonatal lung function either by increasing surfactant synthesis (16) or surfactant release (2), resulting in improved lung compliance (17). Furthermore, activation of cardiovascular β-ARs reproduces some of the circulatory changes that ordinarily occur with the profound catecholamine release attending full-term delivery (19). Nevertheless, it is increasingly clear that there are also adverse effects of fetal exposure to β-agonists. Newborns whose mothers received tocolytic therapy exhibit postnatal increases in heart rate, hyperinsulinism, and alterations in glucose metabolism (5). Furthermore, a survey of a large number of infants exposed prenatally to β-agonists indicates an elevated incidence of cardiac anomalies (28), echoing animal studies showing that high doses of Ter can elicit cardiac structural defects (20). Recent studies suggest that tocolytic β-agonists may also result in subsequent cognitive impairment and psychiatric disorders (27), in keeping with earlier work showing Ter-induced changes in brain cell differentiation and synaptic signaling (25, 32, 38).

Both the potential benefits and harms of prenatal exposure to Ter are likely to represent the same cellular target, the β-AR. Excessive β-adrenergic stimulation is known to cause cardiac cell apoptosis (8). Ordinarily, in the adult, tissues are protected from overstimulation by receptor downregulation and desensitization of adrenergic responses (43). However, there is compelling evidence that, at least in the neonate, β-AR systems are resistant to desensitization (13, 42, 48). The current study examines whether these unique features are present in the fetus. We determined whether β-AR downregulation and/or desensitization can be elicited by Ter in the fetal rat and examined the mechanisms underlying the unique regulation of receptors and receptor-mediated signaling mediated through adenylyl cyclase (AC). Heart and liver were studied because they represent major targets for the normal physiological role of catecholamines in the perinatal transition, mediating essential cardiovascular and metabolic adjustments to the neonatal environment (19). In addition, in the adult, the heart and liver differ in the predominant subtype of β-AR, β1 in the heart and β2 in the liver; because Ter is a β2-selective agonist, differential effects on the two tissues might be expected. Accordingly, we also assessed the pattern of receptor subtype expression in the fetus along with the propensity for Ter to cause receptor downregulation in the two tissues. We then contrasted...
β-AR-mediated stimulation of AC with the enzymatic response to stimulants acting at the level of G proteins or directly on AC, as well as with other G protein-linked receptors, to determine whether the resistance to desensitization represents signaling adaptations downstream from the β-AR.

METHODS

Animal treatments. Studies were carried out in accordance with the declaration of Helsinki and with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health. Adult male and timed pregnant female Sprague-Dawley rats (Zivic-Miller Laboratories, Allison Park, PA) were shipped by climate-controlled truck (transit time 12 h) and housed with free access to food and water. Dams were given daily subcutaneous injections of 10 mg/kg of Ter hemisulfate or an equivalent volume (1 ml/kg) of isotonic saline vehicle on gestational days (GD) 17–20. This Ter regimen has been shown to elicit robust β-AR stimulation in the fetus, including cardiac activation and enhancement of lung surfactant synthesis (15, 16, 26). Twenty-four hours after the third or fourth injection, dams were decapitated, fetuses were removed, and hearts and livers were dissected, frozen in liquid nitrogen, and stored at −45°C until assayed. The fetuses from each dam were considered to be a single determination so that the number of determinations is the number of dams; two fetal hearts were combined for each determination.

Studies in adult male rats were conducted similarly, except that an additional treatment group received 1.25 mg/kg of l-isoproterenol (Iso) HCl, and the isotonic saline vehicle for all treatment groups contained 0.1% ascorbic acid to prevent decomposition of Iso. Because of the size of the adult liver, only the median lobe was taken for analysis. The Iso regimen has been shown to downregulate cardiac β-ARs and to elicit desensitization of the receptor-mediated response of AC (13).

Membrane preparation. Tissues were thawed and homogenized (Polytron, Brinkmann Instruments, Westbury, NY) in 39 volumes of ice-cold buffer containing (in mM) 145 NaCl, 2 MgCl₂, and 20 Tris (pH 7.5) strained through several layers of cheesecloth to remove connective tissue and sedimented at 40,000 g for 15 min. The pellets were washed twice by resuspension (Polytron) in homogenization buffer followed by resedimentation and were then dispersed with a homogenizer (smooth glass fitted with a Teflon pestle) to achieve a final protein concentration of 0.5–1 mg/ml in a buffer consisting of (in mM) 250 sucrose, 1 EGTA, and 10 Tris (pH 7.4).

AC activity. Aliquots of membrane preparation containing 25–50 μg protein were incubated for 30 min at 30°C, with final concentrations of (in mM) 100 Tris-HCl (pH 7.4), 10 theophylline, 1 ATP, 2 MgCl₂, and 1 mg/ml bovine serum albumin, and a creatine phosphokinase-ATP-regenerating system consisting of 10 mM sodium phosphocreatine and 8 IU/ml phosphocreatine kinase, with or without 10 μM GTP, in a total volume of 250 μl. The enzymatic reaction was stopped by placing the samples in a 90–100°C water bath for 5 min, followed by sedimentation at 3,000 g for 15 min, and the supernatant solution was assayed for cAMP using radioimmunoassay kits. Preliminary experiments showed that the enzymatic reaction was linear well beyond the assay period and was linear with membrane protein concentration; concentrations of cofactors were optimal, and, in particular, the addition of higher concentrations of GTP produced no further augmentation of activity.

We assessed the contributions of G protein-linked processes to AC in several ways. First, we contrasted basal AC activity in the presence or absence of GTP. Second, we determined the response of AC to 100 μM forskolin or 10 mM MnCl₂ in the presence of GTP. Forskolin requires association of G proteins with AC for maximal effect (29), whereas manganese activates AC by replacing magnesium at the active site (22) and shows decremental effects when G proteins are associated with the enzyme (46); the preference for one stimulant over the other also reflects shifts in the subtype of AC being expressed (46). Third, β-adrenergic stimulation of activity via Gₛ was determined with 100 μM L-iso in the presence of GTP. Fourth, to determine the net G protein-linked response of AC activity with maximal activation of all G proteins, samples were prepared containing 10 mM NaF in addition to GTP (47). Finally, to determine whether effects on β-AR signaling represented heterologous changes influencing multiple receptor inputs, we assessed the response to clonidine, an α₂-AR agonist, using a concentration (500 μM) previously found to inhibit AC maximally (36). The effect of clonidine was assessed in samples in which AC was first stimulated by Iso or forskolin (36). Receptor binding capabilities were assessed by methods described in earlier publications (24, 33). The overall strategy was to examine binding of [¹²⁵I]iodopindolol at a single, subsaturating ligand concentration (67 μM) in preparations from each animal; changes can thereby be detected regardless of whether they result from alterations in receptor dissociation constant (Kₐ) or maximal binding (Bₘₐₓ). This approach was necessitated by the requirement to measure binding in hundreds of membrane preparations in the study. Scatchard analyses were then performed on several additional preparations to confirm whether alterations resulted from changes in Kₐ or Bₘₐₓ, using ligand concentrations ranging from 32 to 1,024 μM. Binding was determined in samples containing ≤200 μg of membrane protein in 250 μl of (in mM) 145 NaCl, 2 MgCl₂, 1 sodium ascorbate, 20 Tris (pH 7.5); samples were incubated for 20 min at ambient temperature, and incubations were stopped by dilution with 3 ml of ice-cold buffer. The labeled membranes were trapped by rapid vacuum filtration onto Whatman GF/C filter, which were then washed with additional buffer and counted by liquid scintillation spectrometry. Nonspecific binding was determined by displacement with 100 μM di-iso and ranged from 15 to 35%, depending on age and tissue. In some experiments, we assessed the β-AR subtypes by displacing 67 μM [¹²⁵I]iodopindolol with the selective β₁-antagonist CGP-20712A in concentrations ranging from 1 μM to 1 mM.

Data analysis. Data are presented as means and SEs. For convenience, some data are presented as the percent change from control values, but statistical differences were always established using the original data. To establish treatment differences in receptor binding or AC activity, a global ANOVA (data log transformed whenever variance was heterosogeneous) was first conducted across the in vivo treatment groups, age, tissue, and, for AC, all in vitro conditions under which AC was determined; the in vitro stimulant conditions were repeated measures, because each membrane preparation was used for the multiple types of AC determinations. As justified by significant interactions of treatment × age, treatment × tissue, and treatment × stimulant (see RESULTS), data were then subdivided to permit testing of individual treatments and AC measures that differed from control values; these were conducted by lower-order ANOVAs, followed, where appropriate, by Fisher’s protected least-significant difference to identify specific ages at which the Ter group differed from the corresponding control. However, in situations where there was no interaction of treatment × age, only...
Table 1. β-Receptor binding and AC activities in control tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Fetus (GD20)</th>
<th>Fetus (GD21)</th>
<th>Adult (Pregnant dam)</th>
<th>Adult (Male)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Receptor binding</td>
<td>10.7 ± 0.2</td>
<td>12.5 ± 0.2*</td>
<td>5.7 ± 0.2</td>
<td>8.8 ± 0.1†</td>
</tr>
<tr>
<td>Basal AC</td>
<td>8.4 ± 0.3</td>
<td>6.3 ± 0.2*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+GTP</td>
<td>12 ± 1</td>
<td>9.4 ± 0.4*</td>
<td>14 ± 1</td>
<td>18 ± 1†</td>
</tr>
<tr>
<td>+GTP + isoproterenol</td>
<td>17 ± 1</td>
<td>25 ± 1*</td>
<td>19 ± 1</td>
<td>35 ± 1†</td>
</tr>
<tr>
<td>+GTP + NaF</td>
<td>30 ± 2</td>
<td>40 ± 2*</td>
<td>53 ± 2</td>
<td>52 ± 2</td>
</tr>
<tr>
<td>+GTP + forskolin</td>
<td>187 ± 18</td>
<td>304 ± 24*</td>
<td>340 ± 12</td>
<td>377 ± 9†</td>
</tr>
<tr>
<td>+GTP + Mn2+</td>
<td>97 ± 4</td>
<td>143 ± 10*</td>
<td>43 ± 1</td>
<td>69 ± 3†</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Receptor binding</td>
<td>21 ± 2</td>
<td>22 ± 1</td>
<td>4.6 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Basal AC</td>
<td>7.0 ± 0.4</td>
<td>9.2 ± 0.2*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+GTP</td>
<td>9.8 ± 0.7</td>
<td>13.9 ± 0.5*</td>
<td>3.9 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>+GTP + isoproterenol</td>
<td>22 ± 1</td>
<td>28 ± 1*</td>
<td>4.5 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>+GTP + NaF</td>
<td>32 ± 2</td>
<td>46 ± 1*</td>
<td>20 ± 1</td>
<td></td>
</tr>
<tr>
<td>+GTP + forskolin</td>
<td>36 ± 3</td>
<td>65 ± 2*</td>
<td>47 ± 3</td>
<td></td>
</tr>
<tr>
<td>+GTP + Mn2+</td>
<td>73 ± 3</td>
<td>94 ± 3*</td>
<td>47 ± 3</td>
<td></td>
</tr>
</tbody>
</table>

Data represent means ± SEs obtained from 8–32 determinations in each group at each age in pmol·min⁻¹·mg protein⁻¹, except for binding, which is fmol/mg protein. For every measure, ANOVA across all ages indicates significant differences between fetal and pregnant dam or adult male values (main effect of age). AC, adenylyl cyclase; GD, gestational day. *GD20 and GD21 values differ from each other; †adult males differ from pregnant dams.

RESULTS

Receptors and AC in control rats. In keeping with earlier findings (24, 34), β-ARs were overexpressed in fetal rat heart and liver relative to values in adult males or in the pregnant dam (Table 1). AC activity was efficiently coupled to G proteins in both the fetal tissues, with 40–50% increments on addition of GTP and further 2.5- to 4-fold increases after maximal G protein activation with NaF, a response equivalent to that seen in the adult. β-AR stimulation by Iso evoked a 40% increase in AC activity in the heart on GD20 and more than doubled the activity on GD21; in the liver, Iso also doubled the activity at both fetal ages. Although the receptor-mediated effects in both fetal heart and liver equaled or exceeded the stimulatory responses seen in either adult males or pregnant dams, there were major differences between the two tissues in their responses to direct AC stimulants. In the heart, forskolin elicited a massive increase and Mn2+ was much less effective. In the liver, forskolin was only slightly more effective than NaF and far less effective than Mn2+. The same tissue selectivity for direct AC stimulants was apparent in the adult.

General effects of fetal Ter. Administration of Ter to pregnant rats on GD17–20 did not produce any increase in fetal resorption (data not shown) but did evoke a small (2–4%), albeit statistically significant, impairment of fetal weight (Table 2). Heart and liver weights were not significantly affected, but whereas the liver weight showed a tendency toward decreased values, heart weights did not. Consequently, there was relative cardiac sparing (increased heart-to-body wt ratio) of ~4%. Ter treatment did not elicit a significant change in the membrane protein concentration in either fetal heart or liver.

Effects on fetal β-ARs. Maternal Ter treatment caused a small (<10%), but significant, decrease in cardiac β-AR binding and more robust downregulation (~30%) in the liver (distinguishable from heart, treatment × tissue, P < 0.0001; Fig. 1). Scatchard analyses confirmed a loss of receptor sites (decreased Bmax). We also determined whether the β-AR downregulation in either tissue represented a shift in subtype. Using a β1-selective antagonist, CGP-20712A, to displace [125I]iodopindolol, we found that, in the liver, displacement occurred over a very narrow concentration range in the micromolar range; Ter treatment did not elicit a shift in this pattern. In the heart, the β1-antagonist displaced 60% of the ligand in the nanomolar range and 40% in the micromolar range in both the control and Ter groups. The 3:2 ratio for β1/β2 corresponds to a...
higher proportion of β₂-ARs than found in the neonatal heart (41).

Effects on fetal AC. Across all AC stimulants, both gestational age points, and both tissues, fetal Ter treatment evoked an overall increase in activity (main treatment effect, \( P < 0.006 \)), with selectivity for tissue (treatment \( \times \) tissue, \( P < 0.1 \)), stimulant (treatment \( \times \) stimulant, \( P < 0.0001 \)), and age (treatment \( \times \) stimulant \( \times \) age, \( P < 0.03 \)). Accordingly, we present the data separated by tissue and AC stimulant.

Despite β-AR downregulation, cardiac AC activity failed to display desensitization of the response to Iso in vitro (Fig. 2); instead, there were small, equivalent increases in basal activity and activity with GTP added and a more robust increase in the response to NaF. These results suggested that any homologous desensitization might be offset by heterologous sensitization of signaling elements downstream from the receptor. Accordingly, we evaluated the response to two direct stimulants of AC, forskolin and Mn\(^{2+}\), and observed significant Ter-induced elevations in both, with a larger effect for forskolin (decrease in the Mn\(^{2+}\)-to-forskolin response ratio). Differential effects on the two stimulants could represent either changes in the AC isoform or alterations in G protein function, because the AC response to forskolin is enhanced by G protein association. We also found a small, but significant, decrease in the Iso-to-NaF response ratio.

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Fig. 1. Effects of maternal terbutaline (Ter) treatment [10 mg/kg sc daily on gestational days (GD) 17–20] on β-adrenoceptor binding in fetal heart and liver. Data represent means and SEs obtained from 14–16 determinations in each treatment group at each age, presented as the percentage change from control values (Table 1). A: binding was assessed in every animal at 67 pM \( [^{125}\text{I}] \)iodopindolol. ANOVA across treatments, ages and tissues appears at top with subdivision for each tissue below A. Separate differences for each age point were not assessed because of the lack of interaction of treatment \( \times \) age. B: representative Scatchard plots. C: displacement of 67 pM \( [^{125}\text{I}] \)iodopindolol by the β₁-selective antagonist CGP-20712A. Con, control.
Although the fetal liver showed much greater Ter-induced \( \beta \)-AR downregulation than did the heart, we did not find desensitization of Iso-stimulated hepatic AC activity (Fig. 3). Differences between liver and heart were apparent for NaF-stimulated responses: the liver showed no significant increase, whereas the response to NaF was sensitized in the heart (treatment \( \times \) tissue, \( P < 0.01 \)). Direct stimulants of hepatic AC, forskolin and Mn\(^{2+}\), again showed elevations in the Ter group, but the magnitude of effect was smaller than that seen in the heart and did not achieve statistical significance for Mn\(^{2+}\). There was little or no change in the proportional increase of hepatic basal activity on addition of GTP (no change in the +GTP-to-basal ratio), but Iso activity was reduced relative to activity in the presence of GTP alone (decreased Iso-to+GTP ratio), suggesting a small degree of homologous desensitization. As in the heart, there was a small decrease in the Iso-to-NaF stimulation ratio and a decrease in the Mn\(^{2+}\)-to-forskolin stimulation ratio.

Changes in the AC isoform or heterologous shifts in G protein-linked AC activity could produce a differential effect on expression or function of \( G_s \) and \( G_i \) (47). We therefore examined the effects of clonidine, an \( \alpha_2 \)-AR agonist that typically inhibits AC through \( G_i \). We studied the liver rather than the heart, because earlier work showed a lack of \( \alpha_2 \)-AR-mediated \( G_i \) inhibition of AC in the neonatal rat heart (23), despite the fact that the immature heart, similar to the liver, overexpresses the \( \alpha_2 \)-receptor relative to tissues in older animals (23, 24). Samples were prepared with Iso or forskolin as a stimulant, with or without 500 \( \mu \)M clonidine (Table 3). On GD20, clonidine failed to inhibit Iso-induced AC activity, but a 20% inhibitory response...
Table 3. Effects of clonidine on hepatic AC

<table>
<thead>
<tr>
<th>Stimulant</th>
<th>+GTP + isoproterenol</th>
<th>+GTP + forskolin</th>
</tr>
</thead>
<tbody>
<tr>
<td>GD20 Control</td>
<td>0 ± 4*</td>
<td>+140 ± 27</td>
</tr>
<tr>
<td>GD20 Terbutaline</td>
<td>−5 ± 3</td>
<td>+133 ± 13</td>
</tr>
<tr>
<td>GD21 Control</td>
<td>−21 ± 1</td>
<td>−22 ± 1</td>
</tr>
<tr>
<td>GD21 Terbutaline</td>
<td>−19 ± 2</td>
<td>−24 ± 1</td>
</tr>
</tbody>
</table>

Data represent means ± SEs obtained from 14–16 determinations in each group. Across both ages and both measures, ANOVA indicates a change in the clonidine response with age (main effect of age, P < 0.0001) that was different for the effect on isoproterenol-stimulated activity compared with forskolin-stimulated activity (interaction of age × measure, P < 0.0001). The increase between GD20 and GD21 in the inhibitory response to clonidine for isoproterenol-stimulated activity was statistically significant (main effect of age, P < 0.0001). The switch from stimulation of the forskolin response by clonidine on GD20 to inhibition on GD21 was also significant (main effect of age, P < 0.0001). Terbutaline treatment had no significant effect on the clonidine response (no main effect of terbutaline and no interaction of terbutaline × other variables). *Percent change from the corresponding values without clonidine.

emerged by GD21; Ter treatment did not alter the clonidine response. To our surprise, clonidine augmented the response to forskolin on GD20, rather than evoking inhibition. By GD21, the inhibitory response emerged, again constituting an ~20% reduction in AC activity, without any change evoked by Ter treatment.

Ter in the adult. The lack of fetal β-AR desensitization and the presence of homologous sensitization might reflect the major differences in hormonal milieu accompanying pregnancy. Accordingly, we next examined whether Ter treatment elicits the same pattern of effects in the pregnant dam, which shares at least some of its hormonal changes with the fetus. As a positive control, we also treated adult males with Ter or Iso, using a regimen known to elicit cardiac β-AR down-regulation and desensitization of AC (13, 45, 48).

In the pregnant dams, Ter did not affect the ratio of heart to body weight, nor were there effects on the membrane protein concentration (Table 4). However, in adult males, Ter evoked significant cardiac hypertrophy, with a significant increase in the heart-to-body weight ratio. Iso had a larger effect on the ratio, with an increase of >35%, consequently decreasing the cardiac membrane protein concentration, reflecting the diminution of cell surface/volume accompanying cellular enlargement. Thus, for Iso effects on the heart, we will point out where determinations per gram of tissue gave different results from values assessed per milligram of membrane protein. No decrease was seen for hepatic membrane proteins (control, 40 ± 1; Ter, 40 ± 1; Iso 39 ± 1 mg/g).

Administration of Ter to pregnant dams produced robust downregulation of cardiac β-ARs (Fig. 4); the magnitude of effect (25% decrease) was readily distinguishable from the much smaller effect seen in the fetus (<10%, P < 0.005 vs. dam). Downregulation in the dam was also significantly greater than in adult males given the same Ter treatment (P < 0.04). In the adult males receiving Iso instead of Ter, we found cardiac β-AR downregulation approximately equivalent to that obtained with Ter; however, because Iso evoked a significant decrement in membrane protein concentrations, the downregulation for the Iso group was correspondingly greater per gram tissue (41 ± 2% decrease) than for Ter (18 ± 3% decrease, P < 0.0001 vs. Iso). On this basis, the effect of Iso in male rats was greater than that of Ter in the pregnant dams (30 ± 3% decrease, P < 0.01 vs. Iso); however, if we just compare the effects of Ter in adult males and pregnant dams, the effect remained greater than in adult males even when determined as binding per gram tissue (P < 0.02), because Ter did not elicit a fall in membrane protein. Scatchard analyses again confirmed that the receptor decrements represented a reduction in Bmax.

In contrast to the results for cardiac β-ARs, hepatic β-ARs did not show a larger decrement in the adult compared with the fetus (Fig. 4). Either Ter or Iso treatment of adult males produced a 30% decrease in hepatic β-AR binding, the same as with fetal Ter treatment (no interaction of treatment × age group).

Somewhat surprisingly, administration of Ter to pregnant dams did not cause significant desensitization of Iso-induced AC (Fig. 5). The treatment did not augment the response to NaF or forskolin, although increased responsiveness to Mn2+ was detected. Ter also failed to desensitize cardiac AC in adult males (Fig. 6). In contrast, Iso administration caused robust decreases in G protein-dependent components of AC regulation (stimulation by GTP and NaF); although the response to Iso was decreased, the effect did not reach statistical significance. Evaluating AC activity per gram of tissue to correct for the lowering of membrane proteins by Iso resulted in significant (P < 0.0001) decrements for all measures except Mn2+: +GTP, −41 ± 2%; +GTP+Iso, −34 ± 3%.

Table 4. Effects of terbutaline or isoproterenol on body and tissue weights and on membrane protein concentration in adults

<table>
<thead>
<tr>
<th>Pregnant Dams</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>422 ± 11</td>
</tr>
<tr>
<td>Heart wt, mg</td>
<td>820 ± 23</td>
</tr>
<tr>
<td>%Heart/body</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>Heart membrane protein, mg/g</td>
<td>47 ± 2</td>
</tr>
</tbody>
</table>

Data represent means ± SEs obtained from 8–16 determinations in each group. *Individual values differ from the corresponding control, evaluated by Fisher’s protected least-significant difference.
Neither Ter nor Iso evoked desensitization of hepatic AC, and Iso actually evoked an overall stimulation of activity.

**DISCUSSION**

Receptor downregulation and desensitization represent major homeostatic mechanisms that offset prolonged or excessive β-adrenergic stimulation. In the current study, Iso, a mixed β₁/β₂-agonist, elicited a decrease in β-AR binding in both the adult heart (predominantly β₁-ARs) and liver (predominantly β₂-ARs). In both tissues, receptor binding was selectively decreased compared with other membrane proteins (homologous downregulation). However, in the heart, Iso elicited cardiac hypertrophy, and the resultant decrease in cell surface-to-volume ratio produced a corre-

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**Fig. 5.** Effects of Ter treatment (10 mg/kg sc daily for 4 days) on cardiac AC activity in pregnant dams. Data represent means and SEs obtained from 8 dams in each group, presented as the percentage change from control values (Table 1). *AC activity measure for which the terbutaline group differs from the controls.

**Fig. 6.** Effects of Ter treatment (10 mg/kg sc daily for 4 days) or Iso treatment (1.25 mg/kg sc daily for 4 days) on cardiac (A) and hepatic (B) AC activity in adult males. Data represent means and SEs obtained from 12–16 determinations in each treatment group, presented as the percentage change from control values (Table 1). ANOVA across both treatment groups and all stimulants appears at top of A and subdivision by tissue appears at bottom of A. A representative Scatchard plot of cardiac β-receptor binding in pregnant dams appears in B.

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**Table 1.**

- GTP + NaF: -43 ± 4%
- GTP + forskolin: -25 ± 3%
- GTP + Mn²⁺: -6 ± 8%

Neither Ter nor Iso evoked desensitization of hepatic AC, and Iso actually evoked an overall stimulation of activity.
sponding fall in membrane proteins, including β-ARs, so there was a greater decrement in binding per gram tissue (heterologous downregulation). The liver, which did not show changes in membrane proteins, displayed only the homologous component. Thus the mature heart is protected from excessive β-adrenergic stimulation by both a decrease in specific receptor concentrations and a reduction in the cell surface-to-volume ratio. In contrast to the effects of Iso, treatment of adults with Ter evoked only the homologous component of β-AR downregulation, but Ter was as effective as Iso in eliciting this effect, despite the fact that the mature heart contains far fewer β2-ARs compared with the β1-subtype. In pregnant dams, Ter was more effective in producing cardiac β-AR downregulation than in adult males given either Ter or Iso; this may reflect hormonal changes associated with pregnancy (6, 30).

Accordingly, when we turn to β-AR regulation in the fetus, there are a number of reasons why we might expect to see even greater susceptibility to Ter-induced downregulation: sharing of pregnancy-related changes in hormonal status, higher β-AR concentrations (34), and a higher proportion of cardiac β2-ARs than in the adult. Instead, we found a barely detectable reduction; this did not reflect a failure of Ter to penetrate to the fetus, because fetal and adult hepatic β-ARs showed equivalent downregulation. Indeed, resistance to receptor downregulation persists into the neonatal period (37). Certainly, one factor hindering downregulation is the reduced ability of β-agonists, even including Iso, to elicit cardiac hypertrophy in the immature heart (48), thus eliminating the possibility of heterologous downregulation. Another is the unique ability of fetal β-AR stimulation to decrease Gi expression (47), whereas Gs is increased by stimulation in mature cells (31). Additional developmental differences may exist at the level of receptor recycling. We already know that, in the neonatal heart, G protein-receptor kinase activity is actually higher than in the adult (45), but there have been no studies to date that follow the functional steps of internalization and recycling of β-ARs in the fetus.

The concentration of β-ARs is not the only determinant of β-adrenergic function. In the current study, Iso and Ter had distinctly different effects on AC signaling in the mature heart, despite similar effects on β-ARs. Chronic treatment of adult male rats with Iso desensitized cardiac AC through heterologous mechanisms, indicated by parallel reductions in basal activity, β-AR-mediated stimulation, and G protein activation by NaF; a downward shift in the Gs-to-Gi ratio also explains the rise in the ratio of AC response to Mn2+ relative to forskolin (47, 48). Just as with β-ARs, the decrease in membrane proteins from cell enlargement elicits heterologous desensitization in Iso-treated adult heart. In contrast, Ter treatment of adult males failed to elicit desensitization of cardiac AC and instead increased the response to Mn2+. Ter treatment of pregnant dams elicited the same pattern despite a greater β-AR downregulation than in adult males. Further disparities between effects on AC signaling vs. β-AR regulation were apparent in the adult liver, where neither Iso nor Ter elicited desensitization; instead, we found heterologous sensitization of all AC measures. As even the response to direct AC stimulants (forskolin, Mn2+) was increased, the sensitization likely reflects induction of AC itself, a response that, as discussed below, is also characteristic of fetal heart and liver.

With fetal Ter treatment, there was no hypertrophy-related component for heterologous AC desensitization. Similarly, given the hormonal changes of pregnancy, Ter should, as in the dam, fail to cause either homologous or heterologous desensitization of AC responses at the levels of β-ARs or G proteins. However, in addition to the absence of desensitization, fetal Ter exposure produced heterologous sensitization: increases in AC activity with GTP, with maximal G protein activation (NaF), or with direct stimulation of AC itself (forskolin, Mn2+). Because forskolin and Mn2+ act on different epitopes of the AC molecule and exhibit disparate effects of G proteins (47), the augmented response to both stimulants implies an increase in expression/activity of AC. Superimposed on that basic effect there was a preferential increase in the forskolin response, indicating either a change in Gs/Gi or a shift in the AC isoform (46, 47). Our data tend to support the latter mechanism. First, we did not see a larger enhancement of the response to Iso compared with basal activity, a finding that might be expected from increased Gs function. Second, a Gs stimulant, 100 μM carbachol (48), showed no reduction in its ability to inhibit AC (data not shown). Third, the Mn2+/forskolin response ratio was unaffected. Fourth, we found a fall in the Mn2+-to-forskolin response ratio, a characteristic of the ontogenetic shift in AC isoform (46). Thus, even if there are changes in G proteins, these are masked by more robust changes in total AC activity and AC catalytic properties. In any case, Ter induction of fetal AC and the isoform shift are clearly distinguishable from effects in the adult male or pregnant dam. A modest amount of homologous desensitization may actually be present in the fetal heart after Ter administration, evidenced by a fall in the Iso-to-NaF activity ratio, but it is masked by heterologous sensitization from AC induction. The difference in the ratio was small (~10% reduction), the same magnitude as β-AR downregulation.

One key question is whether the unique AC responses to prenatal β-agonist exposure are selective for the heart or are shared by other fetal tissues that overexpress β-ARs. The liver provides an ideal comparison, because Ter produced marked hepatic β-AR downregulation and thus might be expected to cause desensitization as well. However, when we examined the effects of Ter on AC signaling in the fetal liver, we again obtained AC induction and a reduction in the Mn2+-to-forskolin response ratio, effects similar to, albeit smaller than, those seen in the heart. Homologous desensitization did tend to be larger in the fetal liver, as evidenced by a fall in the Iso-to-GTP and Iso-to-NaF activity ratios, reflecting in part the greater β-AR
downregulation. Nevertheless, the main point is still that, despite receptor downregulation, fetal exposure to Ter did not desensitize the net hepatic AC response to β-AR stimulation.

Although the heart and the liver share heterologous sensitization at the level of AC itself, our results nevertheless indicate pronounced disparities between the tissues at the level of effects on individual signaling molecules involved in the response. First, if the induction of AC is a response dictated by the absolute concentration of β-ARs or by the magnitude of receptor stimulation, then the increase should have been greater in the liver (higher receptor number, predominantly β2-ARs, administration of a β2-agonist) than in the heart, whereas the opposite was the case. Thus, if the induction of AC involves a selective β-AR subtype, it would appear that β1-AR stimulation is more effective than β2-ARs. Second, the reduction in the Mn2+ -to-forskolin response ratio tended to be larger in the heart, indicating a more pronounced AC isoform shift. In fact, even the control values indicate a profound difference in AC catalytic profiles between the two tissues: forskolin is far more effective than Mn2+ in the fetal heart, whereas Mn2+ is more effective than forskolin in the liver. The expression of individual AC isoforms, rather than the β-AR subtype, thus appears to govern the ability of β-agonists to cause heterologous sensitization in fetal tissues. A third tissue difference was apparent when we tested hepatic AC responses to a receptor agonist operating through Gα in this case clonidine, an α2-agonist. On GD20, clonidine actually synergized with forskolin to produce massive AC stimulation, whereas by GD21, the expected inhibitory effect was seen. Although Ter did not alter the clonidine response pattern, the results for normal development suggest that, in the liver, some types of adrenergic receptors that ordinarily are linked to inhibition of AC, instead exhibit stimulatory properties. This change may represent a subpopulation of transiently expressed α2-ARs (24) or stimulation of AC through G protein βγ-subunits or even Gα (10, 12). In light of the fact that the fetal heart also transiently overexpresses α2-ARs (23), further work needs to be performed delineating how differential expression or function of these receptors might interact with β-AR subtypes and AC isoforms to influence the net effect of β-agonists on AC signaling.

The heterologous sensitization elicited by prenatal Ter treatment has important implications in the therapy of preterm labor. Overexpression of β-adrenergic receptors in fetal tissues and their effective linkage to the AC signaling pathway (34), combined with higher proportions of the β2-subtype in fetal heart compared with adult heart, superimposed on the deficiency of fetal cardiac β-AR downregulation and the heterologous sensitization downstream from the receptor, all will produce a more profound stimulation that will intensify, rather than subside, with prolonged agonist treatment. Catecholamine actions at β-ARs are essential for the cardiovascular, respiratory, and metabolic events that mark the transition from intrauterine to extraterine life (19), and the lack of desensitization plays an important role in the maintenance of adrenergic effect during this period (13, 16, 17, 26). Accordingly, Ter can be expected to elicit, and to sustain, the same types of physiological adjustments that would ordinarily occur at full-term parturition. However, the same cellular events are likely to contribute to adverse fetal effects of Ter. In the short term, newborns from women receiving β2-agonists demonstrate elevated heart rate (5), a likely consequence of sensitization of the AC signaling pathway; at the same time, hyperinsulinism in this population (5) may result from sensitization of hepatic AC signaling, which would lead to abnormally high rates of gluconeogenesis. Sensitization of fetal AC signaling by β-AR stimulation has even more sinister implications in light of the role of cAMP in cell differentiation and fate. β-Adrenergic input plays a key role initially in the maintenance of cardiac cell replication and differentiation (35, 49) and subsequently in terminating cell replication (7, 39); excessive β-AR stimulation induces cardiac cell apoptosis (8). Cardiac anomalies, including apoptosis, have indeed been noted after fetal Ter treatment (20, 28).

Equally important, the developing brain exhibits the same trophic role for β-adrenergic input in the control of cell replication and differentiation (1, 9, 14, 18, 21, 25, 40, 44) and thus is likely to provide a similar target for adverse effects of prenatal Ter treatment. Although only a few papers have appeared on this topic (11, 25, 32, 38), they all point toward Ter-induced disruption of cell differentiation and synaptic function. A recent study indicates that tocolytic therapy with β2-agonists increases the subsequent incidence of cognitive dysfunction and psychiatric disorders in the offspring (27). It thus would be important to pursue the issue of the regulation of β-ARs, AC signaling, and their control of cell development in the fetal brain.

In conclusion, we found that prenatal Ter exposure results in only a small degree of fetal cardiac β-AR downregulation, which is more than offset by a unique effect on AC signaling: heterologous sensitization at the level of AC expression. Although agonist-induced sensitization provides for the maintenance of β-adrenergic function in the perinatal period, the very same factors may serve to produce cardiac cell damage, hepatic malfunction, or lasting effects on brain development in the offspring after tocolysis with β2-agonists.

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REFERENCES


