β-Adrenoceptor control of G protein function in the neonate: determinant of desensitization or sensitization

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Auman, J. T., F. J. Seidler, and T. A. Slotkin. β-Adrenoceptor control of G protein function in the neonate: determinant of desensitization or sensitization. Am J Physiol Regul Integr Comp Physiol 283: R1236–R1244, 2002.—Neonatal β-adrenoceptors (β-ARs) are resistant to agonist-induced desensitization. We examined the functioning of Gi and Gs after repeated administration of β-AR agonists to newborn rats. Isoproterenol (β1/β2 agonist) obtunded Gs function in the heart but not the liver; in contrast, terbutaline, a β2-selective agonist, enhanced Gs function. Isoproterenol, but not terbutaline, increased membrane-associated Gαi, which would enhance receptor function. In addition, isoproterenol increased and terbutaline maintained the proportion of the short-splice (S) variant of Gαs in the membrane fraction; GαsαS is functionally more active than the long-splice variant. Either isoproterenol or terbutaline treatment increased Gai in the cytosolic fraction, a characteristic usually associated with desensitization in the adult. Decreased Gi activity, coupled with increased membrane-associated Gαs concentrations and maintenance or increases in membrane GαsαS, provide strong evidence that unique effects on G protein function underlie the ability of the immature organism to sustain β-AR cell signaling in the face of excessive or prolonged stimulation; these mechanisms also contribute to tissue selectivity of the effects of β-agonists with divergent potencies toward different β-AR subtypes.

Development; heart; isoproterenol; liver; terbutaline

RECEPTOR DESENSITIZATION REPRESENTS the major mode for cellular homeostasis in the presence of continued stimulation (12). In the case of β-adrenoceptors (β-ARs) and their signaling mediated through adenyl cyclase (AC), attenuation of receptor function is especially important: prolonged, excessive β-AR stimulation can lead to cell damage (5, 9, 29, 36). It is therefore critical to note that, in all mammalian species that have been examined, the ability of β-agonists to elicit desensitization is absent in the fetus or neonate and is acquired during postnatal development (34, 35, 42, 44). This anomaly has both physiological and therapeutic implications. The perinatal transition requires a coordinated series of cardiovascular, respiratory, and metabolic adjustments (17). These are triggered by intense catecholaminergic stimulation (17) so that maintenance of β-AR signaling is critical to perinatal survival and indeed to trophic effects on general somatic growth (16, 38). Nevertheless, the deficiency in β-AR desensitization renders developing cells vulnerable to disruption by β-AR agonists (5, 8, 9, 31). These effects are likely to account for a number of adverse consequences noted after fetal exposure to drugs such as terbutaline or ritodrine, β2-AR agonists that are used to arrest preterm labor but that also cross the placenta to stimulate fetal β-ARs (5, 7, 9, 14, 19, 24).

Studies exploring the resistance of immature tissues to β-AR desensitization uncovered a number of unique features. In neonatal rats given repeated injections of either isoproterenol or terbutaline, cardiac or hepatic β-AR/AC signaling is not desensitized, but rather shows agonist-induced sensitization (2, 4, 37). One main factor accounting for the anomalous response is the induction of AC, leading to heterologous sensitization of all signals mediated through this signaling pathway (3, 4, 43, 45); thus administration of β-AR agonists augments the response to glucagon, which shares the same effector, AC (3, 42, 45). In addition, alterations in G protein expression and/or function may also participate in the production of sensitization instead of desensitization. We recently found that repeated β-AR agonist administration decreased neonatal cardiac Gi expression and enhanced Gs function (42, 44), a response pattern opposite to that typically seen in the adult (11, 25, 26).

The current study addresses two key issues in β-AR control of G protein function in the neonate. First, does the β-agonist-induced decrease in Gi expression (44) elicit impairment of the ability of this protein to control AC activity? There is a relative excess of G proteins compared with neurotransmitter or hormone receptors or with AC (23), so that demonstrating a loss of Gi function is essential. Accordingly, we treated neonatal rats with β-AR agonist drugs and then evaluated the ability of pertussis toxin (PTX) to affect AC responses mediated by the β-AR or by forskolin, a direct AC stimulant whose activity is influenced by the association of AC with Gi or Gs (28). The second issue was to determine how neonatal β-agonist treatment elicits an anomaly in AC function during the development of β-AR receptors.
increase in Gs function (42, 44). Overexpression of Gsα protects β-ARs from agonist-induced desensitization (39), so that an increase in the concentration of Gsα could provide a ready explanation for enhanced receptor-Gs coupling. Gsα is in equilibrium between the cell membrane and cytosol (21), and in the mature cell, β-AR activation displaces Gsα from membrane to cytosol, contributing to desensitization (41). Similarly, during development, there are major shifts both in the expression of specific long- and short-splice variants of Gsα (GsαL and GsαS, respectively) and in the relative proportions of each variant in the membrane-bound and cytosolic fractions (21). Accordingly, we evaluated the relative proportions of GsαL and GsαS in membrane and cytosol after neonatal β-agonist administration to determine the potential role of these factors in the ability of the neonate to resist desensitization.

In designing these studies, we used models based on our earlier work that delineated tissue- and β-AR-subtype selectivity for the balance between agonist-induced neonatal sensitization and desensitization (3, 4, 42–45). First, we compared the effects of isoproterenol, a mixed β1/β2-AR agonist, to those of terbutaline, which is more selective for β2-ARs. Second, we contrasted effects on the heart to those in the liver; these two tissues differ both in their relative expression of β-AR subtypes (β1 predominant in heart, β2 in liver) and in their ontogenetic patterns of receptor expression, because the heart acquires β-ARs during neonatal development (18), whereas the liver shows developmental decrements in β-AR expression (13).

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. Timed pregnant female Sprague-Dawley rats were shipped by climate-controlled truck (transit time 12 h) and housed with free access to food and water. The day after birth, pups were randomized and redistributed to the nursing dams with a litter size of 10; randomization was repeated daily and, in addition, dams were reassigned to different litters to distribute any maternal factors equally. Equivalent numbers of males and females were assigned to each treatment group. On postnatal days 2-5, pups were given daily subcutaneous injections of L-isoproterenol hydrochloride (1.25 mg/kg), terbutaline hemisulfate (10 mg/kg), or an equivalent volume (1 ml/kg) of isotonic saline vehicle. These regimens elicit robust β-AR antagonism (Polytron in 9 vol of ice-cold buffer containing 145 mM NaCl, 1 mM EDTA, and 20 mM Tris (pH 7.5), with freshly added protease inhibitor (0.5 mM phenylmethylsulfonyl fluoride). Homogenates were sedimented at 600 g for 5 min, and the supernatant solution was then sedimented at 50,000 g for 30 min to separate cell membranes from the cytosol. Pellets were dispersed with a Polytron in one-half the original volume of buffer and aliquots of supernatants and resuspended pellets were stored at –80°C.

Gsα isoforms were determined by Western immunoblot analysis essentially as described previously (44). Aliquots containing 40 μg of protein were diluted in buffer containing 2% sodium dodecyl sulfate, 10% glycerol, 0.1% bromphenol blue, 100 mM dithiothreitol, and 50 mM Tris (pH 6.8) and denatured for 5 min at 65°C. Samples were then separated by electrophoresis, after which proteins were transferred from the gels to nitrocellulose membranes at 100 V for 1.5 h. The membranes were shaken for 1 h at room temperature in blocking solution, consisting of 200 mM CaCl2, 800 mM NaCl, 0.0025% sodium azide, 0.2% NP-40, 5% nonfat dry milk, and 200 mM Tris (pH 7.7). Antibody specific to Gsα (diluted 1:10,000) was then added for a further 1 h incubation, after which there were three 10-min washes with blocking solution. The membranes were incubated with goat anti-rabbit IgG (Fc) alkaline phosphatase conjugate (1:7,500) for 1 h, followed by three washes in blocking solution, two rinses in 200 mM CaCl2, 800 mM NaCl, and 200 mM Tris (pH 7.7), and three 2-min washes in 150 mM NaCl, 0.05% Tween 20, and 50 mM Tris (pH 7.7). The blots were developed in 100
mM NaCl, 5 mM MgCl$_2$, 100 mM Tris, 0.17 mg/ml 5-bromo-4-chloro-3-indolyl phosphate, and 0.33 mg/ml nitroblue tetrazolium (pH 9.5), and images were digitized and quantitated.

As we evaluated subcellular fractions containing different populations of proteins, it was not feasible to standardize the preparations against a housekeeping protein such as β-actin, especially as the study involved drugs that specifically alter cardiac contractile proteins. Accordingly, we ensured standardization of the Western blots in several ways. First, protein concentrations were measured before blotting to ensure that exactly the same amount of protein was applied to each lane. Second, in addition to the samples, a standard preparation from the same adult heart was run on every blot to enable normalization of values between blots. Furthermore, a sample of authentic G$_\text{mL}$ and G$_\text{mS}$ was included both to identify the bands and to standardize the hybridization of these specific proteins from blot to blot. Finally, each blot contained a protein ladder to verify molecular weights of the G$_\text{m}$ bands. Values were calculated in relative units by dividing the reading for each band by the value of the standard preparation run on the same blot. Thus, although the actual measurement units are arbitrary, the values maintained their relative proportions and could be contrasted among ages, treatments, and tissues.

Data analysis. Data are presented as means and SEs, with intergroup differences established by ANOVA (data log-transformed whenever variance was heterogeneous), incorporating all relevant variables: treatment, specific agonist, and tissue; for AC studies, values with vs. without PTX; for G$_\text{m}$ distribution studies, G$_\text{mL}$ vs. G$_\text{mS}$, and cytosol vs. membrane. Fisher's protected least significant difference was used post hoc to establish differences among individual treatments for each variable; this was carried out only where the global test indicated an interaction between treatment and the other variables; in the absence of significant interactions, only main treatment effects were compiled. Significance was assumed at the level of $P < 0.05$ for main effects; however, for interactions at $P < 0.1$, we also examined whether lower-order main effects were detectable after subdivision of the interactive variables (33).

Materials. Rats were obtained from Zivic Laboratories (Pittsburgh, PA). cAMP radioimmunoassay kits were purchased from Amersham Pharmacia Biotech (Piscataway, NJ). G$_\text{mL}$, G$_\text{mS}$, G$_\text{mL}$, and G$_\text{mS}$ antibody were gifts from Dr. P. J. Casey (Duke University, Durham, NC) and goat anti-rabbit IgG (Fc) alkaline phosphatase conjugate was purchased from Promega (Madison, WI). All other reagents were obtained from Sigma Chemical (St. Louis, MO).

RESULTS

Before evaluating G$_\text{s}$ function with PTX, we assessed the effect of preincubation of the cardiac and hepatic membranes with the reagents required for PTX-induced ADP ribosylation, but conducted in the absence of PTX itself. The preincubation led to a loss of ~50% of basal AC activity (pmol·min$^{-1}$·g tissue$^{-1}$) in control preparations: heart, 432 ± 17 without preincubation, 196 ± 9 with preincubation ($n = 12, P < 0.0001$); liver, 311 ± 13 without preincubation, 160 ± 9 with preincubation ($n = 12, P < 0.0001$). However, the specific β-AR-mediated cardiac response (isoproterenol-stimulated/basal AC) was not reduced and was actually increased over the unincubated condition (2.43 ± 0.08 without preincubation, 3.80 ± 0.21 with preincubation, $n = 12, P < 0.0001$). In the liver, the preincubation led to the loss of about one-third of the net β-AR response, but robust stimulation was still evident (2.57 ± 0.05 without preincubation, 1.93 ± 0.18 with preincubation, $n = 12, P < 0.005$). Similarly, the forskolin response (forskolin-stimulated/basal AC) remained robust despite the preincubation: heart, 44 ± 2 without preincubation, 49 ± 3 with preincubation ($n = 12$, not significant); liver, 16.1 ± 1.0 without preincubation, 12.2 ± 0.6 with preincubation ($n = 12, P < 0.005$). The loss of AC activity entailed by the preincubation required for ADP ribosylation agrees with an earlier report (1).

Next, we determined the effects of the neonatal β-agonist treatments on AC in the membrane preparations preincubated for ADP ribosylation but without addition of PTX (Table 1). Neither isoproterenol nor terbutaline administration had any significant effect on basal AC activity in heart and liver. In accord with earlier results (3, 4, 45), the β-AR-mediated AC response did not exhibit pronounced desensitization in animals treated with either of the β-agonists. Animals given isoproterenol displayed sensitization (10–15% increase). Without PTX, the isoproterenol-induced response was not significantly affected by any of the β-agonist treatments.

Table 1. Effects of neonatal terbutaline or isoproterenol treatment on cardiac and hepatic adenyl cyclase measured in vitro

<table>
<thead>
<tr>
<th>Animal Treatments</th>
<th>Heart</th>
<th>Liver</th>
</tr>
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<tbody>
<tr>
<td>Basal activity, (pmol·min$^{-1}$·g tissue$^{-1}$)</td>
<td>Con</td>
<td>Iso</td>
</tr>
<tr>
<td>3.81 ± 0.08</td>
<td>4.28 ± 0.16</td>
<td>3.68 ± 0.09</td>
</tr>
<tr>
<td>Forskolin response (forskolin/basal)</td>
<td>48.8 ± 0.8</td>
<td>53.1 ± 2.1</td>
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Data represent means and SEs obtained from 6–12 determinations in each group, using the membranes preincubated for ADP ribosylation, but without addition of pertussis toxin (PTX). Animals were treated with saline (control, Con), isoproterenol (Iso), or terbutaline (Ter) on postnatal days (PN)2–5 and assays were conducted on PN6. Differences in basal activity were not significant. For the β-adrenoceptor (AR) response, ANOVA across both tissues indicates a significant main effect of treatment ($P < 0.0001$), with a reduction in the animals receiving Ter treatment ($P < 0.03$) and an augmentation in those given Iso treatment ($P < 0.0001$). For the forskolin response, ANOVA across both tissues indicates a significant main effect of treatment ($P < 0.0005$), with an increase in the group treated with Iso ($P < 0.0002$). Separate tests were not carried out for each tissue because of the absence of a treatment × tissue interaction for any of the variables.
increase), whereas those given terbutaline showed a small (5–10%) decrement in the response. Similarly, the response to forskolin showed significant enhancement in the animals given isoproterenol (10% increase in the heart, 25% increase in the liver) but was unchanged by neonatal terbutaline treatment.

**β-AR agonists affect Gs signaling.** Before examining effects of PTX on individual components of AC signaling, we performed a global ANOVA incorporating all treatments, both tissues, and the three different AC measures (basal, β-AR response, forskolin response). This initial test indicated a significant overall increase in AC activity evoked by PTX (main effect, \( P < 0.0001 \)) and significant interactions of treatment \( \times \) tissue \( \times \) PTX (\( P < 0.0009 \)) and treatment \( \times \) tissue \( \times \) PTX (\( P < 0.0004 \)). Accordingly, we evaluated the three AC measures separately across the two different tissues. For basal activity, PTX failed to cause an overall stimulation of AC (Fig. 1). Although a tissue-selective effect was seen (treatment \( \times \) tissue interaction for the response to PTX), the only individually significant change was a small (5%) increment in the effect of PTX in the liver of isoproterenol-treated animals; other differences of similar magnitude were inconsistent and did not achieve statistical significance.

In contrast to the relatively minor effect of PTX on basal AC, the β-AR-mediated response showed robust overall enhancement when the membranes were preincubated with PTX (Fig. 1). Treatment of neonates with β-agonists had a significant effect on the PTX response (treatment \( \times \) PTX interaction) that differed between the two tissues (treatment \( \times \) tissue \( \times \) PTX interaction). In the heart, PTX elicited an increase in β-AR-mediated AC stimulation in controls, whereas the response to PTX was completely absent in animals given isoproterenol treatment; in contrast, terbutaline treatment elicited a significant increase in the PTX response. In the liver, PTX elicited a small overall enhancement of the β-AR-mediated response, with little or no alteration evoked by isoproterenol or terbutaline treatment.

Preincubation of cardiac and hepatic membranes with PTX also increased the AC response to forskolin (forskolin/basal AC activity; main effect of PTX, \( P < 0.03 \)). However, neither isoproterenol nor terbutaline treatment evoked any significant alterations in the PTX effect. Values for the ratio of forskolin response with/without PTX were heart: control 1.02 ± 0.02, isoproterenol 1.02 ± 0.02, terbutaline 0.99 ± 0.03; liver: 1.05 ± 0.03, 1.11 ± 0.04, and 1.02 ± 0.06, respectively.

**β-AR agonists affect Gs subcellular distribution and splice variants.** Western blot analysis of Gs detected both GsL (52 kDa) and GsS (45 kDa) isoforms in the membrane and cytosolic fractions of neonatal tissues (Fig. 2). Quantitation was conducted on a relative basis because of incompatibilities in measuring the absolute quantities present in the membrane vs. cytosolic fractions: determinations were conducted relative to a fixed amount of protein loaded onto the gel, but absolute concentrations of membrane and cytosolic proteins in intact cells are not equivalent; additionally, the
membrane fraction required solubilization and attendant recovery corrections, factors that do not operate for the cytosolic fraction. We did, however, include standards to ensure blot-to-blot comparability (see METHODS).

Neonatal isoproterenol treatment increased the levels of membrane-associated Gsα, evaluated as the total of Gsα-L and Gsα-S, with a prominent effect in the liver (≈2.5-fold above control values) and a more modest effect (≈30% increase) in the heart (Fig. 3A). In contrast, treatment with terbutaline did not affect membrane Gsα levels. Changes in cytosolic Gsα were quantitatively and qualitatively different from those in the membrane fraction (Fig. 3B). Isoproterenol evoked a marked increase (≈2.5-fold) that was equivalent for both the heart and liver; terbutaline also caused significant elevations of cytosolic Gsα. Selectivity of the shift toward cytosolic Gsα was readily evident from the ratio of cytosol/membrane values (Fig. 3C): terbutaline preferentially and significantly increased cytosolic Gsα, whereas isoproterenol was much less effective (not statistically significant). In addition, the liver showed a much higher relative pool of cytosolic Gsα than the heart (note different scales for Fig. 3C).

Different Gsα splice variants also influence the effectiveness of β-AR signal transduction (6). In the neonatal heart, GsαS was a minor species of the membrane fraction, accounting for only ≈5% of membrane Gsα (Fig. 4A). In the neonatal liver, however, GsαS represented nearly 40% of membrane-associated Gsα. Isoproterenol treatment, but not terbutaline treatment, substantially increased the proportion of GsαS in both tissues. In contrast to the membrane fractions, nearly all of the cytosolic fraction was GsαS (Fig. 4B). Isoproterenol treatment had little or no effect on the proportion of cytosolic GsαS in the heart but evoked a significant decrement in the liver. Terbutaline treatment decreased the proportion of GsαS in the cardiac cytosol; although the hepatic effect was not significant compared with control values, it also could not be distinguished from the effect in the heart (treatment × tissue interaction was not significant for terbutaline), and the main effect of terbutaline was significant (P < 0.02) when compiled across both tissues.

Finally, we evaluated whether the effects of neonatal β-agonist treatment on Gsα distribution and isoforms were unique to development. Adult male rats (275 g body wt, 6 animals per treatment group) were given the same isoproterenol regimen as that used in neonates. Twenty-four hours after the last dose, we evaluated the characteristics of hepatic Gsα. Membrane Gsα increased ≈35% after isoproterenol exposure (control, 2.5 ± 0.3 units; isoproterenol, 3.4 ± 0.5), a much smaller effect than had been seen in the neonate (treatment × age, P < 0.04). In the adult, isoproterenol treatment did not produce a significant increase in cytosolic Gsα (control, 2.0 ± 0.2 units; isoproterenol, 2.3 ± 0.2), and again this was statistically distinguishable from the increase seen in the neonate (treatment × age, P < 0.05). The proportion of membrane Gsα-S was unaffected by isoproterenol treatment in the adult liver (control, 55 ± 2%; isoproterenol, 52 ± 2%), whereas the same treatment evoked a robust increase in the neonate (treatment × age, P < 0.03). Finally, in the adult, isoproterenol treatment did not alter the proportion of cytosolic Gsα representing the short-splice variant (control, 84 ± 2%; isoproterenol, 79 ±
Repeated isoproterenol administration to neonatal rats increased the AC response to β-AR stimulation in both the heart and liver, instead of uncoupling receptors from the signaling pathway. In previous work, we showed that induction of AC is responsible, in part, for agonist-induced sensitization in the neonate (43). In addition, unique adaptations at the level of G proteins have been hypothesized to contribute to the response pattern: isoproterenol administration reduces the concentration of Gᵢ, and enhances β-AR coupling to Gₛ (42, 44). Results obtained here indicate that the isoproterenol-induced reduction in Gᵢ produces a decrement in the function of this inhibitory G protein: PTX increased the cardiac AC response to β-AR stimulation in membrane preparations from control animals but failed to do so in membranes from isoproterenol-treated animals. In contrast, in mature cardiac cells, isoproterenol increases Gᵢ expression and activity, contributing to desensitization (26). A decrease in Gᵢ function thus helps produce the opposite response, heterologous sensitization of β-AR signaling, seen in the neonate.

Isoproterenol treatment elicited an ~30% reduction in the concentration of Gᵢ, yet the inhibitory contribution of Gᵢ to the net β-AR signal was completely lost. Thus, although the G proteins are in stoichiometric excess compared with β-ARs or AC (23), loss of a relatively minor proportion nevertheless is sufficient to compromise the response. Recent evidence indicates that β-AR function is determined by restriction of signaling elements to caveolae containing the receptor juxtaposed to its target proteins (22) and our results suggest that the loss of Gᵢ is likely to involve decrements in protein colocalized with β-ARs. Furthermore, the isoproterenol-induced decrement in Gᵢ signaling may contribute ultimately to adverse effects on neonatal cardiac function. Vagal parasympathetic control of heart rate and contractility involve cholinergic receptors operating through Gᵢ, and these are only weakly established in the neonatal period (20). The same isoproterenol treatment found here to interfere with Gᵢ function, elicits a decrement in cardiac m₂-cholinergic receptor expression (10), so that the combination of downregulation of the Gᵢ-linked m₂-receptor, downregulation of Gᵢ, and loss of Gᵢ function can cumulate to produce impairment of vagal cardiac signaling.

Our results for effects of isoproterenol in the liver and for terbutaline in both heart and liver provide a third corollary: loss of the PTX-related component of AC signaling was not seen in the liver after neonatal isoproterenol treatment, nor in either tissue when the β₂-selective agonist terbutaline was substituted for isoproterenol. Given the predominance of β₁-ARs in the heart and β₂-ARs in the liver (2, 32), these results suggest that the suppression of Gᵢ expression and function are specifically related to stimulation of the β₂-AR subtype. In fact, terbutaline tended to increase the inhibitory actions mediated by Gᵢ, as evidenced by an augmented cardiac AC response to treatment of the membranes with PTX; this resembles the homeostatic response that is seen ordinarily in mature cells (26). The importance of Gᵢ in determining the net response, heterologous sensitization vs. desensitization, is illustrated by the fact that terbutaline, unlike isoproterenol, did not sensitize the AC response to β-AR stimulation.

Although our results indicate that Gᵢ-mediated signaling responds differently to β-AR stimulation in neonates compared with adults, this factor cannot totally explain why agonist administration elicits sensitization instead of desensitization, as the PTX-sensitive component of AC activity represented no more than 15% of the total AC signal. Accordingly, we also examined effects on Gₛ. Earlier work indicated that cardiac β-AR coupling to Gₛ was enhanced after neonatal isoproterenol administration, instead of exhibiting the uncoupling typical of the mature cell (42). In the current study, we found a modest (30%) increase in the Gₛ concentration in cardiac membranes but a massive (2.5-fold) increase in hepatic membranes; because Gₛ...
overexpression is known to protect cells from β-AR
desensitization (39), our findings provide a ready expla-
nation for the ability of hepatic cells to maintain
their signaling capabilities despite the fact that they
did not display a loss of Gs function. Again, this was
seen with isoproterenol treatment but not with ter-
butaline. Nevertheless, we found increased Gsα expres-
sion in the cytosolic fractions of both cardiac and he-
patic cells after neonatal treatment with either of the
β-agonist drugs. In the mature cell, β-AR-mediated heterologous desensitization involves a shift of Gsα
from the membrane to the cytosol, where it is incapable
coupling to the membrane-associated β-ARs (41).
Our findings indicate that this component of desensi-
tization is intact in immature cells. However, as iso-
proterenol induced Gsα by the same proportion in both
the membrane and cytosolic fractions, the removal of
Gsα from the membrane was offset, so that membrane
signaling was sustained. With terbutaline administra-
tion, the membrane component was maintained (but
not enhanced), whereas the cytosolic fraction showed
the increase characteristic of desensitization; accord-
ingly, β-AR/AC signaling was preserved with the ter-
butaline model, but did not show the enhancement that
was characteristic of the isoproterenol treatment par-
digm. For these effects, relative contributions of β1-
ARs and β2-ARs cannot explain the differences in effects between isoproterenol and terbutaline. Isopro-
terol had a much greater proportional effect on mem-
brane-associated Gsα in the liver, which expresses the
β2-subtype, than in the heart, which has a β1-AR ma-
jority (2, 32). If receptor subtype dictated the tissue
difference, then terbutaline should have been even
more efficacious, whereas it actually had a smaller
effect. It is thus likely that the differences in respon-
siveness with the two treatments reflect another fac-
tor; as terbutaline is longer lasting than isoproterenol,
it is possible that episodic stimulation of β-ARs elicits
greater Gsα induction or, alternatively, that continuous
stimulation by terbutaline provides for neonatal up-
regulation of Gsα, but combined with the internalization
that is characteristic of adult-type desensitization. The
greater contribution of desensitization components to
the terbutaline response is likely to explain why het-
 eroslogous sensitization of the β-AR/AC pathway is less
notable after terbutaline than after isoproterenol (4,
37). Regardless of the differences in details of the
effects of isoproterenol and terbutaline, our findings for
the effects on the expression and subcellular distribu-
tion of Gsα indicate an additional unique mechanism not
present in the adult that contributes to the resistance
of immature cells to agonist-induced desensitization.

We also evaluated the effects of neonatal β-agonist
administration on splice variants of Gsα. The lower
molecular weight subtype, Gsα,S, has greater functional
activity than the longer splice variant, Gsα,L (6, 40).
Neonatal isoproterenol treatment increased membrane
Gsα,S in both the heart and liver but by differing me-
chanisms. In the heart, the increase in membrane-asso-
ciated Gsα,S occurred without a corresponding decrease
in the cytosolic fraction, thus representing net induc-
tion of the protein. In fact, neonatal isoproterenol
treatment shifted the proportion of the Gsα,S splice
variant in neonates to approximate the higher value in
the normal adult heart (41) or liver (this study), sug-
gesting that neonatal isoproterenol treatment acceler-
ates the maturational profile of Gsα splice variants.
This actually matches the functional effect, which is to
shift β-AR association with G proteins from the lower
efficacy of the neonate to the higher coupling charac-
teristic of the adult (42). In the liver, the increase in
membrane-associated Gsα,S was juxtaposed to a de-
crase in the cytosolic fraction, thus implying redistrib-
ution rather than (or in addition to) induction. Reg-
ardless of the mechanism, induction or redistribution,
either effect would contribute to preservation or en-
hancement of β-AR/AC signaling. Furthermore, both
effects represent actions that are unique to develop-
ment: no such changes were seen in adult hepatic cells
in the present study, nor in earlier work with mature
heart or liver (39). As before, the response to terbutaline
differed from isoproterenol and resembled those asso-
ciated with desensitization (i.e., no change in mem-
brane-associated Gsα,S but a decrease in the cytosolic
fraction), effects that are likely to offset agonist-in-
duced sensitization of AC as seen with terbutaline
(2–4).

Superimposed on the disparities in effects between
isoproterenol and terbutaline, some of the differences
in G protein expression or function may reflect selec-
tivities dictated by the types of cells or tissues over and
above any contribution from different β-AR subtypes.
Although both express β-ARs and G proteins,
cardiac and hepatic cells obviously bear little resem-
biance to each other, either in their repertoires of other
proteins or in their differentiation characteristics. As
just one example, cardiac cells undergo terminal dif-
ferration and lose the ability to replicate, whereas
hepatic cells maintain mitotic capabilities into adult-
hood (27). Thus terbutaline treatment evoked an in-
crease in Gi function in the heart (augmented β-AR
stimulation in PTX-treated membranes) but not in the
liver, despite the fact that hepatic cells express a
higher proportion of β2-ARs. Similar tissue disparities
are likely to contribute to differential effects of β-ago-
nists on G protein splice variants and their subcellular
distributions. Indeed, recent studies demonstrated dis-
parate patterns of terbutaline-induced sensitization
vs. desensitization in different brain regions, with the
outcome dictated by the maturational timetable for
each region (30). Factors dictated by the cellular milieu
may thus contribute to some of the differences in the
effects of isoproterenol and terbutaline on β-AR-medi-
ated responses in cardiac and hepatic cells, but obvi-
ously, future work with other tissues will be needed to
clarify this issue.

In conclusion, we found changes in G protein concen-
trations, subcellular distribution, and functional activ-
ity that provide mechanistic explanations for the resis-
tance of neonatal β-ARs to desensitization: loss of
inhibitory actions mediated through Gi, enhancement of
membrane-associated Gsα,S and a shift to expression
of the more active, short-splice G\textsubscript{max} variant. Differences in the relative contributions of each of these factors explain the disparities of effects seen for neonatal isoproterenol vs. terbutaline treatments on \beta-AR/AC signaling in the heart and liver. However, for either treatment, the net effect of these mechanisms, superimposed on induction of AC (43) and resistance to agonist-induced \beta-AR downregulation (2, 4, 37), combines to preserve and enhance cell signaling mediated by \beta-ARs during the critical period of the perinatal transition (17).

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