Catecholamines act via a β-adrenergic receptor to maintain fetal heart rate and survival

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Porthbury, Andrea L., Rashmi Chandra, Marybeth Groelle, Michael K. McMillian, Alana Elias, James R. Herlong, Maribel Rios, Suzanne Roffler-Tarlov, and Dona M. Chikaraishi. Catecholamines act via a β-adrenergic receptor to maintain fetal heart rate and survival. Am J Physiol Heart Circ Physiol 284: H2069–H2077, 2003. First published February 6, 2003; 10.1152/ajpheart.00588.2002.—Mice lacking catecholamines die before birth, some with cardiovascular abnormalities. To investigate the role of catecholamines in development, embryonic day 12.5 (E12.5) fetuses were cultured and heart rate monitored. Under optimal oxygenation, wild-type and catecholamine-deficient fetuses had the same initial heart rate (200–220 beats/min), which decreased by 15% in wild-type fetuses during 50 min of culture. During the same culture period, catecholamine-deficient fetuses dropped their heart rate by 35%. Hypoxia reduced heart rate of wild-type fetuses by 35–40% in culture and by 20% in utero, assessed by echocardiography. However, catecholamine-deficient fetuses exhibited greater hypoxia-induced bradycardia, reducing their heart rate by 70–75% in culture. Isoproterenol, a β-adrenergic receptor (β-AR) agonist, reversed this extreme bradycardia, restoring the rate of catecholamine-deficient fetuses to that of nonmutant siblings. Moreover, isoproterenol rescued 100% of catecholamine-deficient pups to birth in a dose-dependent, stereospecific manner when administered in the dam’s drinking water. An α-AR agonist was without effect. When wild-type fetuses were cultured with adrenergic receptor antagonists to create pharmacological nulls, blockade of α-ARs with 10 μM phentolamine or β-ARs with 10 μM bupranolol alone or in combination did not reduce heart rate under optimal oxygenation. However, when combined with hypoxia, β-AR blockade reduced heart rate by 35%. In contrast, the muscarinic blocker atropine and the α-AR antagonist phentolamine had no effect. These data suggest that β-ARs mediate survival in vivo and regulate heart rate in culture. We hypothesize that norepinephrine, acting through β-ARs, maintains fetal heart rate during periods of transient hypoxia that occur throughout gestation, and that catecholamine-deficient fetuses die because they cannot withstand hypoxia-induced bradycardia.

tyrosine hydroxylase; dopamine β-hydroxylase; norepinephrine; hypoxia; adrenergic receptor

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DESPITE THE FACT THAT IN RODENTS catecholamines and their adrenergic receptors (AR) are present as early as embryonic day 8 and 9 (E8–9) (4, 33), pharmacological experiments suggested that β-AR activation by norepinephrine and epinephrine was not essential in the fetus. Pregnant rat dams treated with a nonselective β-AR antagonist had pups with modest developmental deficiencies that resolved soon after birth. In addition, β-AR blockade did not affect litter size, birth weight, or the number of stillbirths or resorptions (20). Furthermore, direct application of a β-AR antagonist to E10–12.5 rat fetuses in culture failed to decrease cardiovascular function (27). These findings, along with the fact that fetal catecholamine neurons lack efferent and afferent connections and mature synaptic machinery (31), supported the view that catecholamines were not necessary during development.

It was therefore a surprise to learn that tyrosine hydroxylase knockout mice (Th−/−) die in utero. Depending on the particular null allele and genetic background, between 75 and 100% of catecholamine-deficient animals die before birth, with the majority of lethality occurring during midgestation (E11.5–E14.5), long before synaptic transmission is established, and equivalent to the first trimester in the human fetus. Deficient mice that make it to birth exhibited bradycardia (19, 40) and some had cardiovascular abnormalities (40). Th−/− mice (19, 26, 40) that lack dopamine, norepinephrine, and epinephrine, and dopamine-β-hydroxylase knockout (Dbh−/−) mice (33) that lack norepinephrine and epinephrine, had the same pattern of lethality, suggesting that norepinephrine and/or epinephrine mediate survival. Mutant mice lacking the homeodomain transcription factor Phox2b, which fail to develop sympathetic ganglia and therefore lack peripheral sources of norepinephrine, also exhibit the same fetal lethality as Th−/− and Dbh−/− mice (24, 25). In contrast, mice that lack dopamine, but not norepinephrine and epinephrine, are viable (39), ruling out a critical role for dopamine during fetal development.

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development. Taken together, the survival pattern of these four types of mutant mice provides support for an essential role for norepinephrine in the fetus. Although low levels of epinephrine have been detected at midgestation and are synthesized in the embryonic heart (10, 11), biosynthesis of epinephrine does not become substantial until after the peak window of death, suggesting that the essential catecholamine is norepinephrine (33). Because both norepinephrine and epinephrine act at the same receptors, these data show that activation of adrenergic receptors is essential for fetal viability.

The mechanism by which catecholamines maintain fetal survival is unknown. Although norepinephrine and epinephrine could serve a trophic role during development (40) (analogous to the role played by another biogenic amine, serotonin, in cardiac development) (38), we investigated whether norepinephrine and epinephrine might serve an acute physiological function in the fetus. In both Th−/− and Dbh−/− mice, the severity of morphological abnormalities varied from animal to animal, suggesting that the primary defect might be physiological rather than anatomic in nature. In this work, we hypothesize that fetuses release catecholamines to sustain cardiovascular function in response to stress in utero, suggesting that the physiological role for catecholamines in the fetus is analogous to their role in postnatal animals.

METHODS

Drugs. Bupranolol was a gift of Schwarz-Mann (Liestal, Switzerland). Other drugs and chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

Animals. For experiments examining the in vivo rescue of Th−/− pups to birth, pups were genotyped on the day of birth from matings between albino ICR Th−/− mice, in which the Th null allele had been back-crossed for six generations onto C57bl/6J for six generations. Similar results were obtained from albino ICR Th−/− matings (data not shown). The presence of a vaginal plug was taken as E0.5 and all fetuses were used at E12.5. Animals were genotyped with PCR using primers in exon 6 (5′-GAGTGCAATCCCCAC-3′) and a reverse primer in Th exon 8 (5′-TGAGTCAGTGAGGAGG-3′) and a reverse primer in the neomycin gene from the null allele (5′-GATACCGGAATAGCCTGAG-3′). Animals were used in accordance with approved protocols under Institutional Animal Care and Use Committee review.

Rescue of Th−/− pups via maternal drinking water. Multiparous Th−/− ICR females were mated with Th−/− ICR males and treated with ascobic acid at 2.5 mg/ml, isoproterenol HCl (1–100 μg/ml), and/or l-phenylephrine HCl (10 μg/ml) in ascobic acid from E8.5 until parturition. Drugs were administered in the drinking water, which was changed daily and protected from light.

Tissue preparation for histological examination. E13.5 fetuses were immersion fixed in 10% buffered formalin (Baxter Diagnostic; Deerfield, IL) and embedded in paraffin. The sections (15 μm) were stained with hematoxylin and eosin.

Fetal culture. Fetal culture was adapted from previously published protocols. Pregnant female mice were anesthetized with isoflurane (IsoFlo, Abbott Laboratories, North Chicago, IL) and euthanized by cervical dislocation. Both uterine horns were removed into a petri dish containing room temperature W3 buffer composed of (in mM) 120 NaCl, 5 KCl, 1 NaH2PO4, 20 HEPES, and 2 glucose (pH 7.3) that was supplemented with 10 mM MgSO4, and the fetal masses were gently removed. The fetuses, with their visceral yolk sacs intact, were placed in fresh W3 medium supplemented with 1 mM MgSO4 and 2 mM CaCl2, which was the same medium used for culturing. The fetuses were freed of their surrounding membranes but left attached to the yolk sac via the vitelline stalk. Each litter was divided between control and drug treatment groups. Three to four fetuses per group were placed in a 25-ml Falcon T-flask and partially submerged in 4 ml of prewarmed W3 buffer preequilibrated with 95% O2-5% CO2. Flasks were then placed on a heated rocker platform that maintained the buffer at 37°C. Flasks were rocked such that the buffer washed over the fetuses 12 times/min without submerging them completely. A constant infusion at 20 ml/min of either 95% O2 to create a normoxic condition or 55% O2 to create a hypoxic condition was bubbled into the buffer via a gas line embedded in the lid of the flask.

Heart rate determination in vitro. Heart rate for each fetus was counted in the culture flask under a dissecting microscope for 30 s at 10-min intervals. Culture flasks were kept on a heated stage at all times to maintain buffer temperature at 37°C, because heart rate was very dependent on incubation temperature. Two control counts at 95% O2-5% CO2 were taken before the commencement of hypoxia or addition of drugs into the media. Hypoxia was induced by infusing reduced 55% O2-5% CO2-balance N2. Drugs were added directly into the buffer surrounding the fetuses. Except where indicated, antagonists were used at 10 μM: phentolamine (9, 14, 15) should block all subtypes of α-ARs, bupranolol (1, 12, 18, 36) should block all subtypes of β-ARs, and atropine should block all five classes of cholinergic muscarinic receptors (2).

Statistical analysis. Statistical analysis for fetal culture experiments was performed with Statview software (SAS Institute, Cary, NC) using one-way ANOVA, followed by Fisher’s protected least-significant difference post hoc test where appropriate. Heart rate in each treatment group was averaged and the means ± SE calculated. Statistical significance for Figs. 2C and 5 was determined by comparing the averaged heart rate at each time point after the addition of drugs, with the average heart rate of sibling fetuses without drugs. Data from C57bl/6J and ICR fetuses were statistically identical and were pooled for Figs. 2C and 5. Statistical significance for Fig. 4 was determined by comparing Th−/− fetuses with Th+/− sibling fetuses in the same flasks. For Fig. 3B, fetal heart rate was measured for 60 min during exposure to various concentrations of O2 and the average of the 40-, 50-, and 60-min heart rate was calculated. This value was then expressed as a percentage of the hypoxic heart rate. Statistical analysis for Fig. 6 was performed with the use of x2 analysis.

Doppler echocardiography. Doppler echocardiography (Sonos 500, Hewlett-Packard Instruments; Palo Alto, CA) was performed essentially as described by Gui et al. (13) with the use of a 5- to 12-MHz probe and a commercial standoff. The

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pregnant dam was anesthetized with pentobarbital sodium (50 mg/kg Nembutal; Abbott Laboratories). Abdominal fur was removed with depilatory cream.

RESULTS

Catecholamine-deficient fetuses show cardiovascular abnormalities. Th−/− fetuses are deficient in all catecholamines and die in utero starting as early as E9.5 (28). At E12.5–E13.5 about one-half of the Th−/− fetuses show markedly dilated blood vessels and pooling of the blood in the liver, heart (compare Fig. 1, A and C, with Fig. 1, B and D), and vasculature (arrows, Fig. 1B). In the heart, the ventricular septum and walls are thinner and show greater cellular disorganization in Th−/− fetuses (compare Fig. 1, E and F). Atria in the mutant animals are often enlarged with thinner walls compared with Th+/+ or Th+/− siblings (compare Fig. 1, G and H).

AR blockade has no effect on wild-type fetal heart rate under control conditions in culture. To examine the role of norepinephrine in fetal cardiovascular function, we cultured wild-type E12.5 fetuses in the presence of various adrenoreceptor antagonists to block the action of endogenous catecholamines and pharmacologically mimic a catecholamine-deficient phenotype. For these experiments, we developed an acute fetal culture assay by modifying existing techniques in two ways. First, we infused a constant supply of oxygen into the culture vessels and pooled of the blood in the liver, heart (compare Fig. 1, A and C). With existing techniques, the generation of free radicals and oxygen radicals (28). At E12.5 to 180 beats/min. It fell 20% within 3 min of switching the dam to 6% O2 (Fig. 3C). After 5 min, heart rate recovered, but not quite to prehypoxic levels. Taken together, these data show that hypoxia induces bradycardia in culture and in utero.

Catecholamine-deficient fetuses cannot maintain heart rate during hypoxia. If catecholamines regulate heart rate in response to hypoxic stress, catecholamine-deficient fetuses should show a greater decrease in heart rate than Th+/− fetuses. To test this, Th−/− mice were mated and the fetuses cultured under hypoxic conditions without drugs. Fetal heart rate was monitored for 50 min, and, at the termination of the experiment, fetuses were collected and genotyped. Th−/− fetuses slowed their heart rate similarly to wild-type sibling fetuses in response to hypoxia (Fig. 4A). In the same flask, however, the heart rate of Th+/− fetuses was one-half that of Th+/+ or Th+/− siblings, dropping to 25–35% of prehypoxic levels, which occurred primarily at later time points (30, 40, and 50 min) (Fig. 4B). The decrease in the heart rate of Th−/− fetuses was reversed by isoproterenol, a β-AR agonist, which restored heart rate to ~80% of prehypoxic levels (Fig. 4B). Hence, the extreme bradycardia induced in catecholamine-deficient fetuses is due to the lack of norepinephrine rather than to anatomic or physiologic inability of Th−/− fetuses to respond to β-AR activation. Th−/− fetuses in the same flask restored their heart rate to the same level as Th+/+ siblings (Fig. 4B), demonstrating that the difference between Th+/− and Th+/+ fetuses is eliminated in the presence of a β-AR agonist. Even while under 95% O2, Th−/− fetuses developed a slower heart rate than their Th+/+ and Th+/− siblings (Fig. 4C). However, the drop in normoxic Th−/− fetuses (33% reduction compared with Th+/− fetuses) was less than in hypoxic Th−/− fetuses (55% reduction compared with Th+/− fetuses), supporting the idea that catecholamines are more important in regulating chronotropy in response to hypoxia than

Hypoxia reduces fetal heart rate in vitro and in vivo. Because there was no effect of adrenergic blockade under control conditions, we asked whether the requirement for catecholamines would be unmasked by stress, analogous to the requirement for adrenergic activation in the mature animal. The most likely stressor in utero is hypoxia because periodic contractions of the uterus occur during gestation and transiently reduce fetal oxygenation (PO2) (37). We first assessed whether midgestation fetuses are sensitive to hypoxia by culturing wild-type fetuses under reduced O2 (55%). Hypoxia decreased heart rate to 65–70% of control prehypoxic levels (Fig. 3A). This level was maintained unless fetuses were reoxygenated, in which case heart rate was restored to 90% of prehypoxic levels (Fig. 3A). Fetal heart rate decreased in a stepwise manner with decreasing O2 content; 70% O2 caused a 25% drop in heart rate, whereas extreme hypoxia (20% O2) caused fetal death within a few minutes (Fig. 3B).

To determine whether E12.5 fetuses also responded to hypoxia in utero, we made pregnant dams hypoxic by reducing their inspired O2 from 20.8% (ambient) to 6% O2 and assessed fetal heart rate by Doppler echocardiography. Maternal hypoxia has been shown to reduce fetal PO2 in larger mammals under analogous conditions (16). At ambient O2, fetal heart rate was ~180 beats/min. It fell 20% within 3 min of switching the dam to 6% O2 (Fig. 3C). After 5 min, heart rate recovered, but not quite to prehypoxic levels. Taken together, these data show that hypoxia induces bradycardia in culture and in utero.
under normoxic conditions. These data support the contention that, as in the mature animal, catecholamines limit the extent of fetal bradycardia in response to hypoxia and play a modest role in maintaining heart rate under normoxic conditions.

β-AR blockade causes significant decrease in wild-type fetal heart rate during hypoxia. To determine whether AR activation was responsible for mediating the effects of catecholamines in wild-type animals, we cultured wild-type C57bl/6J and ICR fetuses under normoxic conditions. These data support the contention that, as in the mature animal, catecholamines limit the extent of fetal bradycardia in response to hypoxia and play a modest role in maintaining heart rate under normoxic conditions.
hypoxic conditions and examined the effect of various AR antagonists on heart rate. Phentolamine (at 10 μM), an antagonist of all subtypes of α-ARs, had no effect on heart rate (Fig. 5A), suggesting that α-ARs are not involved in regulating fetal heart rate. In contrast, bupranolol decreased heart rate by 35% compared with sibling fetuses incubated under hypoxia alone in both strains of mice (Fig. 5A). Figure 5B shows that the IC50 for bupranolol is ~10 nM, a concentration at which bupranolol is specific for β-ARs. Because bradycardia is often reversed by the blockade of muscarinic acetylcholine receptors in mature animals, we blocked muscarinic receptors with 10 μM atropine (Fig. 5A). Atropine had no effect on heart rate, suggesting that cholinergic activation does not contribute to hypoxia-induced bradycardia. These data suggest that endogenous norepinephrine and epinephrine are likely to act at β-ARs, rather than α-ARs or muscarinic receptors, to maintain fetal heart rate during hypoxia.
We observed that the bradycardia seen in wild-type fetuses during combined hypoxia and H9252-AR blockade often resulted in cardiovascular changes similar to those seen in Th/H11002/H11002 fetuses in vivo. Blood clots formed in the large cerebral vessels as well as in other large vessels throughout the fetus. Edema built up around the heart and the strength of cardiac contraction weakened until eventually the heart stopped beating altogether. Taken together, these data show that without H9252-AR activation, hypoxia-induced bradycardia reduced cardiovascular function to an extent that was often insufficient to maintain adequate blood flow.

Isoproterenol, a H9252-AR agonist, rescues Th/H11002/H11002 mice to birth. Our data suggest that H9252-AR activation restores heart rate in response to hypoxia in cultured catecholamine-deficient fetuses and that β-AR blockade exacerbates hypoxia-induced bradycardia in wild-type animals. Are the cardiovascular effects observed in cultured fetuses related to survival in vivo? If β-AR activation also sustains the survival of fetuses in utero, Th⁻/⁻ mice might be rescued to birth by β-AR activation. To test this, Th⁻/+ females were mated with Th⁺/⁻ males and treated from E8.5 to parturition with various concentrations of isoproterenol, a non-subtype-selective β-AR agonist, via the drinking water. Without treatment, only 5% of the Th⁻/⁻ pups survived to birth (Fig. 6). The vehicle ascorbic acid, included as an antioxidant in the isoproterenol and phenylephrine solutions, by itself increased survival from 5% to 18%. Although it is unclear how ascorbic acid mediates increased survival, it may reduce antiadrenergic effects of oxyradicals. Isoproterenol increased survival in a dose-dependent manner to 100%. The inactive (+) enantiomer did not increase survival over vehicle alone. Isoproterenol is not normally administered orally because it is rapidly degraded in the liver (8). Nevertheless, with the use of HPLC we detected isoproterenol in E12.5 fetuses of all genotypes at levels 3–40 times that of norepinephrine (data not shown), demonstrating that isoproterenol can pass the placenta and accumu-
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Physiological role of catecholamines in fetal survival. Catecholamines are essential for fetal survival, as illustrated by the death in utero of various lines of catecholamine-deficient mice. However, the exact role that catecholamines play, as well as their mechanism of action, has not been investigated. Our finding that a specific β-AR agonist, isoproterenol, increases survival of catecholamine-deficient fetuses to 100% in a dose-dependent and stereo-specific manner (Fig. 6) suggests that survival requires β-AR activation. Rescued pups resemble the small number of Th−/− pups that are born without drug treatment and die by postnatal day 21 because they fail to eat or drink (39).

What might β-AR activation do in the fetus? Both in utero and in culture, hypoxia induced bradycardia in midgestation mouse fetuses. In culture, heart rate decreased to 65–70% of prehypoxic levels when E12.5 fetuses were shifted from 95% O2 to 55% O2 (Fig. 3A). Our in vitro culture experiments suggest that catecholamines, specifically norepinephrine, act to limit the extent of hypoxia-induced bradycardia. Two lines of evidence suggest that norepinephrine (and/or epinephrine), acting at β-ARs, maintains heart rate during hypoxic stress and prevents it from decreasing further. First, hypoxia induced a greater bradycardia in catecholamine-deficient fetuses compared with wild-type or heterozygous fetuses (Fig. 4A). Second, a β-AR antagonist induced greater bradycardia than hypoxia alone, suggesting that β-AR blockade mimics a catecholamine-deficient condition. In addition, β-AR blockade often generated a phenotype similar to that of catecholamine-deficient fetuses in vivo (Fig. 1A), with pooling of blood in the major vessels and organs. Interestingly, in the absence of hypoxia (Fig. 2C), β-AR blockers had no effect on heart rate, implying that β-AR activation is only essential during hypoxia or some other form of fetal stress.

How might β-AR activation increase heart rate? Using receptor binding, Chen et al. (5) demonstrated the presence of β-ARs on midgestation mouse hearts at 15% the level of adult hearts. Hence, it is possible that β-ARs on the heart itself (on the myocardium or developing sinoatrial node) could respond directly to β-AR activation by increasing beat frequency and contractility through enhanced calcium influx and excitation-contraction coupling. Because β-ARs, rather than α-ARs, mediate chronotropy and inotropy in postnatal heart, the fact that our data implicate β-ARs as opposed to α-ARs is not surprising.

Our model (Fig. 7) posits that norepinephrine is released in response to fetal stress such as hypoxia. In utero, hypoxia is likely to be induced by the spontaneous increases in myometrial tone, known as contractions, which occur regularly throughout gestation (34). Hypoxia reduces heart rate, which, in wild-type fetuses, is partially counteracted by norepinephrine activation of β-ARs. In either genetically or pharmacologically deficient fetuses, is partially counteracted by norepinephrine activation of β-ARs. In either genetically or pharmacologically deficient fetuses, is partially counteracted by norepinephrine activation of β-ARs.
logically engineered norepinephrine-deficient fetuses, however, heart rate is so severely suppressed that death ensues. In vivo, stimulation of β-ARs with orally administered isoprenalol replaces endogenous norepinephrine and rescues norepinephrine-deficient fetuses to birth (Fig. 6). In culture, direct stimulation of β-ARs restores heart rate (Fig. 4B) in catecholamine-deficient fetuses, whereas blockade of β-ARs with a β-AR antagonist prevents β-AR activation and exacerbates bradycardia in wild-type fetuses (Fig. 5).

Little is known about the effect of and responses to hypoxia in early and midgestation fetuses. However, physiological responses to hypoxia and anoxia in mature (near term) fetuses and in neonates have been studied. In late-term fetal sheep, hypoxia induces an initial bradycardia, followed by a recovery phase, which coincides with a rise in circulating catecholamines (17). This pattern is similar to that of the midgestation mouse fetuses assessed by Doppler echocardiography in Fig. 3C. Parturition, which is an intrinsically hypoxic event, induces a massive release of norepinephrine and epinephrine from the adrenal medulla. Interference with this release is detrimental to the neonate (21, 30). Because the autonomic nervous system is not functional at birth in small mammals, catecholamine release is through a direct, nonneurogenic process: low P02 closes an O2-sensitive potassium channel, which depolarizes the catecholaminergic cell and induces release of norepinephrine and epinephrine (29, 32). When innervation of sympathetic ganglia and the adrenal medulla matures, the ability for direct sensing of hypoxia by chromaffin cells is lost and neurogenic control of catecholamine release ensues. In the fetus, it is likely that immature sympathoadrenal cells resemble neonatal chromaffin cells and detect low P02 directly, depolarize, and release catecholamines into the fetal circulation.

The phenotypic abnormalities in the cardiovascular system of catecholamine-deficient mice could result from continual episodes of cardiovascular stress, which generate morphological changes similar to those observed in chronic heart failure. The variability in the morphological abnormalities seen from one animal to another may reflect differences in the number or severity of the hypoxic episodes that an individual fetus encounters. Alternatively, differences could result from varying levels of maternal catecholamines that pass the placenta and accumulate to different extents in individual fetuses. Low levels of maternal catecholamines are probably responsible for the small percentage of Th<sup>−/−</sup> pups that survive to birth without drug treatment (33). It is also possible that high levels of octopamine may be present in Th<sup>−/−</sup> fetuses that could activate β-ARs to a limited degree. Finally, catecholamines could also serve a trophic role for heart development, distinct from their physiological function in maintaining heart rate.

Our data do not address which subtype of β-AR is responsible for regulating heart rate. Subtypes of β-ARs on midgestation fetal hearts have not been reported, but our reverse transcriptase-polymerase chain reaction data show that RNAs for β<sub>1</sub>, β<sub>2</sub>, and β<sub>3</sub>-AR are present on E12.5 mouse heart (data not shown). Additionally, by receptor autoradiography, we detect β<sub>1</sub>- and β<sub>2</sub>-AR protein on E12.5 mouse heart and liver, respectively (data not shown). Bupranolol reduced heart rate with an IC<sub>50</sub> of 10 nM (Fig. 5B). Assuming that bupranolol is acting at one of the known β-ARs and that the concentration of norepinephrine in the fetus is between 0.2 and 0.6 μM (19) (authors’ unpublished data) this is equivalent to an inhibition constant (K<sub>i</sub>) of 6–10 nM (6). Because the K<sub>i</sub> for bupranolol is 1–15 nM at β<sub>1</sub>-AR and β<sub>2</sub>-AR and 12–50 nM at β<sub>3</sub>-AR (1, 18, 36), a K<sub>i</sub> of 6–10 nM is consistent with action at any of the three β-ARs. Hence, delineation of the subtype of β-AR responsible for fetal cardiovascular effects will require further pharmacological characterization.

Clinical use of β-AR antagonists during human pregnancy. β-AR antagonists are commonly used as second-line antihypertensives during pregnancy. Despite the fact that most antagonists pass the placenta, their effect on the fetus in the first trimester is largely unknown. Our data in the mouse suggest that β-AR blockade could be deleterious to the fetus by exacerbating ischemia during transient hypoxic periods in utero. In clinical studies (35), mothers treated with antihypertensive drugs during pregnancy had newborns that were small for gestational age. Two randomized, controlled studies (3) showed that atenolol, a β<sub>1</sub>-AR selective antagonist, significantly reduced birth weights by 26% when administered at the end of the first trimester. If our data can be extrapolated to humans, they might explain these clinical findings by suggesting that β-AR blockade is detrimental because it exacerbates transient hypoxic episodes, leading to ischemic damage.

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