Prenatal cocaine exposure abolished ischemic preconditioning-induced protection in adult male rat hearts: role of PKCε

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Meyer KD, Zhang H, Zhang L. Prenatal cocaine exposure abolished ischemic preconditioning-induced protection in adult male rat hearts: role of PKCε. Am J Physiol Heart Circ Physiol 296: H1566–H1576, 2009. First published March 13, 2009; doi:10.1152/ajpheart.00898.2008.—Prenatal cocaine exposure in rats resulted in decreased PKCε protein expression in the heart of adult male but not female offspring. The present study determined its functional consequence of inhibiting cardioprotection mediated by ischemic preconditioning. Pregnant Sprague-Dawley rats were administered intraperitoneally saline or cocaine (30 mg·kg⁻¹·day⁻¹) from day 15 to day 21 of gestational age. Hearts were isolated from 3-mo-old offspring and were subjected to ischemia and reperfusion injury in a Langendorff preparation, with or without prior ischemic preconditioning. Preischemic levels of left ventricular function were the same between the saline control and cocaine-treated animals. Ischemic preconditioning of two episodes of 5-min ischemia significantly decreased infarct size and enhanced postischemic functional recovery of the left ventricle in the saline control animals. This ischemic preconditioning was associated with increased phospho-PKCε, but not phospho-PKCa, levels and was blocked by a PKCε translocation inhibitor peptide. Prenatal cocaine treatment abolished the ischemic preconditioning-mediated increase in phospho-PKCε and cardioprotection in the heart of male offspring. In contrast, the cardioprotective effect was fully maintained in female offspring that were exposed to cocaine before birth. The results suggest that prenatal cocaine exposure causes a sex-specific loss of cardioprotection by ischemic preconditioning in adult offspring, which is most likely due to fetal programming of PKCε gene repression, resulting in a downregulation of PKCε function in the heart of adult male offspring.

fetal programming; protein kinase C; ischemia

COCAINE IS A WIDELY ABUSED drug that has significant cardiovascular effects. Cocaine abuse clearly increases the risk of several cardiac disease states in the adult user, most notably myocardial infarction (30). Additionally, exposure to cocaine in utero has a detrimental impact on the developing heart. While the effect of fetal cocaine exposure on birth weight and gross cardiac malformations is still under investigation, recent animal studies in our laboratory have demonstrated that the heart undergoes significant changes at the cellular and genetic levels in response to cocaine exposure (4, 39). Fetal cocaine exposure causes myocardial cell apoptosis, resulting in a decreased number of myocytes at birth and increases the heart’s sensitivity to ischemia and reperfusion injury in adult male offspring in a sex-dependent manner (2, 4). These studies also showed a correlation between a decrease in both total and phosphorylated protein kinase Cε and increased myocardial sensitivity to ischemia in adult male offspring after fetal cocaine exposure (2).

A short period of ischemia has been shown to be both a potent and reproducible method of protecting the heart from subsequent prolonged ischemia and reperfusion injury. This phenomenon, known as ischemic preconditioning (IPC), causes the translocation and activation of the ε-isofrom of PKC (PKCε) (29), which has been shown to be an important component of the IPC pathway (12, 13, 17). Studies in a PKCε knockout (KO) mouse model have demonstrated no significant difference in baseline susceptibility to ischemia and reperfusion injury between the wild-type and PKCε KO hearts (10). However, targeted disruption of the PKCε gene blocked cardioprotection caused by IPC (10), suggesting dichotomy of mechanisms in heart susceptibility to ischemia-reperfusion injury and inducible protection against oxidative stress observed during cardiac preconditioning.

The present study investigated the effect of fetal cocaine exposure and IPC in the heart of the adult offspring. Our previous studies suggest a possible link between an increased sensitivity to ischemia and decreased levels of activated (phosphorylated) PKCε (2). Because IPC is known to activate PKCε in the myocardium to induce protection from ischemia-reperfusion injury, we investigated the possibility that IPC may rescue the myocardium from the increased ischemic sensitivity induced by fetal cocaine exposure. As IPC is a powerful activator of PKCε, we hypothesized that it would temporarily restore the level of active PKCε, although the effect may be less dramatic than in controls due to lower total PKCε expression. The goal of this study was to gain additional insight into the physiological effect of fetal cocaine exposure on the myocardium and increased understanding of the mechanism of increased ischemic sensitivity previously observed in this model.

MATERIALS AND METHODS

Experimental animals and cocaine treatment. All experiments and procedures used in this study adhered to the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Loma Linda University. Time-dated pregnant Sprague-Dawley rats were purchased from Charles River Laboratories (Portage, MI). As described previously, the animals were randomly divided into two groups: 1) saline control, and 2) 15 mg/kg cocaine administered intraperitoneally twice daily at 10:00 AM and 4:00 PM from day 15 to 21 of gestational age, the dose that produces plasma cocaine levels in the range of those reported in human cocaine users (2). The animals were allowed to give birth naturally, and no fetal loss in the control and cocaine-treated groups was observed. Pups were weaned and separated by sex 21 days after birth. The offspring were
given food and water ad libitum and were subjected to no further treatment before death.

**Langendorff preparation and IPC protocol.** At age of 3 mo old, offspring from both cocaine- and saline-treated dams were anesthetized with 75 mg/kg ketamine and 5 mg/kg xylazine injected intramuscularly. The heart was rapidly excised and transferred to ice cold Krebs-Heinsleit buffer. After which it was perfused via the aorta in a modified Langendorff apparatus under constant pressure (70 mmHg) with gassed (95% O₂, 5% CO₂) Krebs-Heinsleit buffer at 37°C, as previously described (22). A latex balloon attached to a pressure transducer was inserted into the left ventricle and inflated to obtain a left ventricular end-diastolic pressure (LVEDP) of ~5 mmHg.

Hearts either were treated with IPC before ischemia, or underwent continuous perfusion before ischemia. IPC was achieved by two cycles of 5 min of global ischemia with 5-min recovery between cycles, after which the heart was recovered for 20 min and then was subjected to 20 min of ischemia, followed by 40 min of reperfusion.

Values are means ± SE; n, no. of animals. HR, heart rate; LVDP, left ventricular developed pressure; LVEDP, left ventricular end-diastolic pressure; dP/dt max, maximal rate of contraction; dP/dt min, maximal rate of relaxation; CF, coronary flow; TIP, PKCε translocation inhibitory peptide.

### Table 1. Preischemic left ventricular functional parameters of 3-mo-old offspring of prenatal saline control and cocaine treatments

<table>
<thead>
<tr>
<th></th>
<th>HR, beats/min</th>
<th>LVDP, mmHg</th>
<th>LVEDP, mmHg</th>
<th>dP/dt max, mmHg/s</th>
<th>dP/dt min, mmHg/s</th>
<th>CF, ml/min</th>
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<tbody>
<tr>
<td><strong>Male</strong></td>
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<tr>
<td>Control (n = 15)</td>
<td>264 ± 8</td>
<td>118 ± 8</td>
<td>5 ± 0</td>
<td>3,832 ± 193</td>
<td>2,491 ± 103</td>
<td>11 ± 1</td>
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<tr>
<td>Cocaine (n = 15)</td>
<td>255 ± 6</td>
<td>119 ± 7</td>
<td>6 ± 0</td>
<td>3,745 ± 209</td>
<td>2,518 ± 162</td>
<td>12 ± 1</td>
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<tr>
<td>TIP control (n = 10)</td>
<td>265 ± 9</td>
<td>120 ± 5</td>
<td>6 ± 0</td>
<td>3,789 ± 212</td>
<td>2,459 ± 120</td>
<td>11 ± 1</td>
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<tr>
<td>TIP cocaine (n = 10)</td>
<td>272 ± 8</td>
<td>116 ± 9</td>
<td>5 ± 0</td>
<td>3,822 ± 225</td>
<td>2,386 ± 154</td>
<td>11 ± 1</td>
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<tr>
<td><strong>Female</strong></td>
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<tr>
<td>Control (n = 10)</td>
<td>269 ± 12</td>
<td>109 ± 9</td>
<td>6 ± 0</td>
<td>3,307 ± 204</td>
<td>2,260 ± 140</td>
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<tr>
<td>Cocaine (n = 10)</td>
<td>255 ± 6</td>
<td>110 ± 10</td>
<td>5 ± 0</td>
<td>3,197 ± 301</td>
<td>2,207 ± 122</td>
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<td>255 ± 10</td>
<td>107 ± 10</td>
<td>5 ± 0</td>
<td>3,241 ± 474</td>
<td>2,014 ± 280</td>
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<td>TIP cocaine (n = 10)</td>
<td>261 ± 14</td>
<td>104 ± 11</td>
<td>5 ± 0</td>
<td>3,184 ± 238</td>
<td>2,193 ± 185</td>
<td>10 ± 1</td>
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Values are means ± SE; n, no. of animals. HR, heart rate; LVDP, left ventricular developed pressure; LVEDP, left ventricular end-diastolic pressure; dP/dt max, maximal rate of contraction; dP/dt min, maximal rate of relaxation; CF, coronary flow; TIP, PKCε translocation inhibitory peptide.
ANOVA followed by a Tukey post hoc analysis, or t-test, as appropriate for the data sets.

RESULTS

Body weight and baseline cardiac function. Both male and female offspring had no significant difference in body mass between saline control animals and those exposed to cocaine before birth (data not shown). The baseline cardiac function parameters in the Langendorff preparation also showed no significant difference between saline- and cocaine-exposed rats (Table 1).

IPC in hearts of saline control animals. We first determined the IPC of two cycles of short ischemia with 5 min each in cardioprotection in the heart of offspring of saline control animals. In both males and females, IPC had no significant effect on the baseline function of the left ventricle before the prolonged ischemia of 20 min, but resulted in a significant increase in postischemic recovery of LVDP, dP/dt max, and dP/dt min during reperfusion after prolonged ischemia (Figs. 1 and 2, left). Additionally, the IPC-mediated increases in postischemic recovery of dP/dt max and dP/dt min were significantly greater in females than males (P < 0.05; Figs. 1 and 2, left). IPC also caused a significant reduction in the increased LVEDP seen after prolonged ischemia in both males (Fig. 3A) and females (Fig. 3D). Female hearts showed significantly greater reduction in LVEDP in the presence of IPC than did the male hearts (P < 0.05, Fig. 3, A and D). Consistent with the improved LVEDP, there was a significant decrease in infarct size of the left ventricle in both males (Fig. 3C) and females (Fig. 3F) after the IPC treatment.

IPC in hearts of cocaine-treated animals. In contrast to the findings in the hearts of saline control animals, IPC failed to produce cardioprotection and did not improve postischemia recovery of LVDP, dP/dt max, and dP/dt min during reperfusion after prolonged ischemia in the heart of male offspring.

Fig. 1. Effect of ischemic preconditioning (IPC) on postischemic recovery of left ventricular function in male offspring. Hearts were isolated from 3-month-old male offspring of prenatal saline (left) or cocaine (right) treatments and were treated without (−) or with (+) IPC, followed by ischemia-reperfusion (20/40 min). Postischemic recovery of left ventricular function during reperfusion was measured relative to the preischemic values. LVDP, left ventricular developed pressure; dP/dt max, maximal rate of contraction; dP/dt min, maximal rate of relaxation. Values are means ± SE; n = 5. *P < 0.05 vs. no IPC for the entire curve.
with prenatal cocaine treatment (Fig. 1, right). Additionally, the IPC-mediated reduction of LVEDP and infarct size of the left ventricle after prolonged ischemia was abolished in the hearts of cocaine-treated male offspring (Fig. 3, B and C). Unlike that in the male offspring, the IPC-mediated cardioprotection was fully preserved in the female offspring with prenatal cocaine treatment (Fig. 2, right, Fig. 3, E and F). Compared with the saline control females, the cocaine-treated females showed significantly greater IPC-induced improvement of postischemia recovery of LVDP (77 ± 2 vs. 56 ± 4%, P < 0.05) and dP/dt max (74 ± 1 vs. 59 ± 2%, P < 0.05) in the early phase of recovery at 10 min of reperfusion. Additionally, LVEDP at 10 min of reperfusion was significantly lower in the heart of cocaine-treated females than that of saline control females in the presence of IPC (27.6 ± 3.8 vs. 39.4 ± 3.3 mmHg, P < 0.05). These differences were not seen at the end of reperfusion at 40 min.

**Fig. 2. Effect of IPC on postischemic recovery of left ventricular function in female offspring.** Hearts were isolated from 3-mo-old female offspring of prenatal saline (left) or cocaine (right) treatments and were treated without or with IPC followed by ischemia-reperfusion (20/40 min). Postischemic recovery of left ventricular function during reperfusion was measured relative to the preischemic values. Values are means ± SE; n = 5. *P < 0.05 vs. no IPC for the entire curve.

**Protein and mRNA abundance of PKCe and PKCb in the heart.** To determine whether prenatal cocaine exposure alters PKCe and PKCb expression in adult hearts, protein and mRNA levels of PKCe and PKCb in the left ventricle were measured by Western blot analyses and quantitative real-time RT-PCR, respectively. As shown in Fig. 4A, prenatal cocaine exposure resulted in significant decreases in both mRNA and protein abundance of PKCe in the left ventricle of male, but not female, offspring. Unlike PKCe, there was no significant difference in PKCb mRNA and protein abundance in the left ventricle of adult offspring between saline control and cocaine-treated animals, in either males or females (Fig. 4A).

**IPC activates PKCe.** To determine a role of PKCe in the cocaine-mediated loss of IPC-induced protection in the heart of male offspring, phospho-PKCeSer729 levels were determined in the left ventricle in the absence or presence of IPC. Phosphorylation plays a key role in converting nascent PKC isozenes
The active form of phospho-PKCε has been identified in cardiomyocytes (27, 33). IPC in the heart of saline control animals resulted in a significant increase in phospho-PKCε levels (Fig. 4B), suggesting a role for PKCε activation in the IPC-mediated cardioprotection in the control hearts. In contrast, IPC had no significant effect on phospho-PKCε levels in the heart of male offspring that had been exposed to cocaine before birth (Fig. 4E), consistent with the lack of IPC-induced protection in the heart of cocaine-treated male offspring (Fig. 1, right; Fig. 3, B and C). Unlike PKCε, IPC had no significant effect on phospho-PKCδ levels in the left ventricle of either saline control or cocaine-treated offspring (Fig. 4B).

Effect of PKCε-TIP on IPC. To further demonstrate the cause-and-effect relation between PKCε activation and the IPC-mediated protection in the heart, we determined the effect of selective inhibition of PKCε on the IPC-induced cardioprotection using a selective PKCε-TIP. Hearts were treated with 5 μM PKCε-TIP before and during IPC. The dosage was chosen based on a previous study that showed 5 μM of PKCε-TIP inhibited PKCε translocation in the heart of adult male rat in the Langendorff preparation (28). Treatment of the heart with PKCε-TIP did not significantly alter the baseline left ventricular function before ischemia (Table 1). In both male and female control animals, comparison of the effects of IPC in the absence or presence of PKCε-TIP showed that the inhibition of PKCε completely reversed the IPC-mediated protection in postischemic functional recovery of the left ventricle (data in Figs. 5 and 6, left, compared with those in Figs. 1 and 2, left). This reversal in the IPC-mediated protection was also demonstrated in the cocaine-treated female offspring (data in Fig. 6, right, compared with those in the Fig. 2, right). The inhibition of PKCε in the cocaine-treated male offspring had no signifi-
Fig. 4. Effect of prenatal cocaine on PKCε expression and IPC-induced PKC activation in the heart of adult offspring. A: left ventricles were obtained from 3-mo-old male and female offspring of prenatal saline and cocaine treatments. PKCε and PKCδ mRNA and protein abundance were determined with qPCR and Western blot analyses, respectively. Values are means ± SE; n = 5. *P < 0.05 vs. no IPC.

B: hearts were isolated from 3-mo-old male offspring of prenatal saline and cocaine treatments and were treated without or with IPC. Protein abundance of phospho-PKCε (p-PKCε) and phospho-PKCδ (p-PKCδ) in the left ventricle was determined with Western blot analysis and was normalized to α-sarclo-meric actin. Values are means ± SE; n = 5. *P < 0.05 vs. no IPC.

DISCUSSION

Multiple studies have suggested that PKCε plays a key role in the IPC pathway. It has been demonstrated that IPC induces PKCε activation (29), and several cellular models have shown that the inhibition of PKCε activation blocks the protective effect of IPC (23). The role of PKCε in the IPC protection is thought to be activation of the mitochondrial ATP-sensitive K⁺ channels resulting in a protection of cardiomyocytes from apoptosis (13). Additionally, PKCε-dependent activation of the sphingosine kinase and production of endogenous sphingosine-1-phosphate also play a role in the IPC protection in the heart (14). The present finding of the IPC-mediated protection in the lack of a significant effect on postischemic coronary flow supports the theory that the target mechanisms reside in cardiomyocytes rather than the coronary vasculature.

The present study demonstrated that selective inhibition of PKCε with a PKCε-TIP abolished the IPC-mediated protection of left ventricular recovery in both male and female offspring and thus provided the cause-and-effect evidence of the functional importance of PKCε in the IPC-mediated cardioprotection in the intact heart. PKCε-TIP inhibits activation of PKCε at the intracellular concentration of 3–10 nM and has been widely used to study the role of PKCε in cardiac function (26, 31, 41). A previous study has shown that 5 μM of PKCε-TIP inhibits PKCε translocation in the heart of adult male rats in a Langendorff preparation (28). Consistent with the previous studies (4, 28), the present study showed that PKCε-TIP had no significant effects on left ventricular function at the baseline levels. This is in agreement with the findings obtained in a PKCε KO mouse model, demonstrating that PKCε expression is not required for normal cardiac function under physiological conditions, but PKCε activation is necessary and sufficient for acute cardioprotection caused by IPC (10). Similar findings were obtained in intact rat hearts showing that inhibition of PKCε blocked ouabain-triggered and isoflurane-induced preconditioning (24, 28).

The present finding that IPC-mediated cardioprotection was significantly greater in female hearts than male hearts is intriguing and suggests a sex dimorphism in the IPC pathway. In contrast to the present finding, previous studies in mice showed that two cycles of 2-min ischemic episodes followed by 5-min reperfusion periods preconditioned male, but not female, hearts (34). Given that cardiomyocytes from female hearts have been shown to be more resistant to ischemia and reperfusion injury compared with male cardiomyocytes (4, 32), it is possible that female hearts may require a higher injury threshold for preconditioning to occur. Indeed, a temporal threshold for preconditioning the myocardium has been demonstrated (1, 37). In the present study, we have demonstrated that two cycles of 5-min ischemic episodes followed by a 20-min reperfusion period produced a marked protection in female hearts. Consistent with this finding, myocardial protection from IPC has been demonstrated in canine females (20). While the mechanisms for the increased IPC-mediated protection in female hearts are not clear at present, it has been demonstrated in rats that female hearts have higher levels of the active form of phospho-PKCε and that reperfusion increases phospho-PKCε more in female than male hearts (3).

As our previous studies have shown that the hearts of male offspring born to cocaine-treated dams are more sensitive to...
ischemia (2), we hypothesized that our preconditioning protocol would have an effect equal to or greater than the protection in control males. To our surprise, the protective effect of IPC was entirely abolished. IPC did not rescue the heart from the increased sensitivity to ischemia or provide any increase in functional recovery or protect from infarction. Our data suggest that fetal cocaine exposure not only decreases the expression of PKCε, but also results in a defect in the activation of PKCε. This was verified by protein analysis that showed that IPC significantly increased the level of phospho-PKCε in control hearts, but resulted in no increase in the hearts taken from cocaine exposed animals.

The sex dichotomy has been demonstrated further in fetal programming of adult hearts in response to IPC. Prenatal cocaine treatment abolished the IPC-mediated protection in the heart of adult male offspring. Whereas inhibition of PKCε with PKCε-TIP mimicked the effect of cocaine and blocked the IPC-mediated protection in the control males, PKCε-TIP had no further effect on the postischemic functional recovery, as IPC remained ineffective in the cocaine-treated males. In contrast, the IPC-mediated protection of the heart in the cocaine-treated females was fully preserved. Additional studies showed that PKCε remained functional in the IPC-mediated protection in the cocaine-treated female offspring, as it was demonstrated that, in both control and cocaine-treated females, the inhibition of PKCε with PKCε-TIP abolished equally well the IPC-mediated protection of left ventricular recovery. Taken together, these findings indicate that the sex difference in IPC-induced cardioprotection after fetal cocaine exposure is due to a cocaine-mediated downregulation in PKCε functioning that is specific to males rather than a sex-specific difference in the IPC pathway. Human epidemiological studies have demonstrated a link between adverse intrauterine environments and an increased risk of ischemic heart disease in adulthood (5). Recent animal studies demonstrated that fetal exposure to hypoxia (22, 38), glucocorticoids (8), cocaine (2), and nicotine

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**Fig. 5.** Effect of PKCε-translocation inhibitor peptide (TIP) on IPC-induced protection in the cardiac function in male offspring. Hearts were isolated from 3-mo-old control (left) and cocaine-treated (right) male offspring and were treated with 5 μM PKCε-TIP before and during IPC, followed by ischemia-reperfusion (20/40 min). Postischemic recovery of left ventricular function during reperfusion was measured relative to the preischemic values. Values are means ± SE; n = 5.
caused an epigenetic programming in the heart, resulting in an increased heart susceptibility to ischemia and reperfusion injury in adult offspring. The sex dichotomy in fetal programming of adult disease has been demonstrated in several animal models. Although the results are conflicting, it has been shown that female offspring are generally less sensitive in manifestation of cardiovascular disease caused by adverse prenatal stimuli (7). Sex differences in adult offspring, with males often being more susceptible than females on a diversity of measures to the effects of prenatal cocaine exposure in the rat, have been demonstrated consistently (2, 11, 25, 35, 36). Whereas the stage of the estrous cycle of the female rats at the time of experiments was not determined, future studies are needed to investigate further the mechanisms of the sex dichotomy observed in the present study and to test for a role of sex steroid hormones in male and female gonadectomized rats.

Consistent with the finding of a loss of IPC in male but not in female hearts, prenatal cocaine exposure caused a sex-dependent decrease in PKCe mRNA and protein abundance in the heart of male offspring. Our recent study demonstrated a sex-dependent epigenetic mechanism of DNA methylation in programming of cardiac PKCe gene repression, linking fetal cocaine exposure and pathophysiological consequences in the heart of adult male offspring (39). Previous studies in several animal models of fetal stress demonstrated a correlation between a decrease in PKCe expression and increased heart vulnerability to ischemia injury in adult offspring (2, 19, 21). These findings suggest a common mechanism of PKCe in cardiac programming in response to intrauterine adverse stimuli. Unlike fetal cocaine, PKCe protein expression was decreased in the heart of both male and female adult offspring after prenatal nicotine treatment, which corresponded to the decreased postischemic recovery of left ventricular function in both male and female hearts (19). These findings suggest a stimuli specificity of sex-dependent programming of PKCe gene expression pattern in the heart and reinforce a key role for PKCe in programming of heart vulnerability to ischemia and reperfusion injury in adult offspring. In our laboratory’s pre-

Fig. 6. Effect of PKCe-TIP on IPC-induced protection in the cardiac function in female offspring. Hearts were isolated from 3-mo-old control (left) and cocaine-treated (right) female offspring and were treated with 5 μM PKCe-TIP before and during IPC, followed by ischemia-reperfusion (20/40 min). Postischemic recovery of left ventricular function during reperfusion was measured relative to the preischemic values. Values are means ± SE; n = 5.
vious study, we demonstrated that prenatal cocaine exposure significantly decreased not only PKCε protein abundance by 59% but also phospho-PKCε levels by 75% in the left ventricle of male offspring (2). The finding of a greater decrease in phospho-PKCε levels than PKCε protein abundance suggests that, in addition to programming of PKCε gene repression, prenatal cocaine exposure also results in inhibition of PKCε activation in the heart. In the present study, the physiological modulation of PKCε activation by IPC was examined in the heart. Whereas the heart of saline control animals showed a significant increase in phospho-PKCε by IPC, in the heart of cocaine-treated animals, the IPC-induced phosphorylation of PKCε was abolished. This is consistent with the lack of IPC-mediated cardioprotection in the heart of cocaine-treated animals, providing clear evidence that inhibition of PKCε activation plays a key role in the cocaine-mediated loss of IPC-induced protection in the heart of male offspring.

Unlike PKCε, the role of PKCδ in ischemia and reperfusion injury is less clear and is somewhat controversial. Inhibition of PKCδ during reperfusion has been shown to decrease reperfusion-induced injury (26). Other studies demonstrated the cardioprotective effects of PKCδ (6, 15, 40). In contrast to the findings of PKCε, neither mRNA nor protein abundance of PKCδ in the heart of both male and female offspring were altered after prenatal cocaine exposure. Additionally, the lack of increase in phospho-PKCδ with IPC seen in the present study suggests that the activation of PKCδ may not be involved in the IPC-mediated cardioprotection. This is in agreement with the finding in human myocardium showing that PKCε but not PKCδ is essential for the IPC-mediated myocardial protection (12).
Our investigation has demonstrated that prenatal cocaine exposure causes a loss of IPC-mediated cardioprotection in adult male offspring in a sex-dependent manner and provided evidence of a relationship between cocaine-induced inhibition of PKCε and increased myocardial sensitivity to ischemia. In addition to demonstrating that blocking PKCε activation results in a loss of IPC-induced cardioprotection, we demonstrated that IPC failed to activate PKCε in the hearts of cocaine-exposed males. Together, our findings strongly suggest a causal relationship between alterations to PKCε and increased myocardial sensitivity to ischemia in the adult animal with prenatal cocaine exposure. These findings could have significant clinical importance, a contention supported by the observations that preinfarction angina and exercise protect the heart from ischemic injury via the IPC pathway, as well as other evidence that IPC is an important physiological protective mechanism (9, 16, 18). Given that cocaine abuse in pregnant women is a significant problem, especially in urban areas, the present finding suggests that intrauterine cocaine exposure may result in significant long-term changes to the myocardicytes and be a risk factor for morbidity and mortality secondary to myocardial ischemia in adult male offspring. As is often the case with novel findings, the present study may raise more questions than it answers. For instance, whether and to what extent does the impaired cardioprotective signaling pass to the next generation? Is there a critical window in the fetal development that can be isolated during which exposure to the drug can program the heart with pathophysiological consequences in the offspring? Furthermore, does the treatment before conception have a similar effect on the offspring? Undoubtedly, these questions warrant continuing investigations.

REFERENCES


