Prenatal Exposure to Carbon Monoxide Affects Postnatal Cellular Electrophysiological Maturation of the Rat Heart

A Potential Substrate for Arrhythmogenesis in Infancy

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Background—Maternal smoking is an independent risk factor for sudden infant death syndrome (SIDS). Carbon monoxide (CO) is a major component of smoke. No information is available about the effect of CO and/or smoking on postnatal maturation of the heart. The aim of this study was to investigate the effect of prenatal exposure to CO on cellular electrophysiological maturation in male Wistar rats.

Methods and Results—The patch-clamp technique was used to measure action potential (AP) and ionic currents (Ica,L) from rat ventricular myocytes. During growth, AP duration measured at −20 and −50 mV (APD−20 and APD−50) decreased progressively in both groups; the process was significantly delayed in rats exposed prenatally to 150 ppm CO: at 4 weeks, APD−20 and APD−50 were 89.5±18.2 and 147.7±24.5 ms in CO (n=13) and 35.6±4.5 and 77.8±8.3 ms in control rats (Ctr; n=14; P<0.01 and P<0.05, respectively) and normalized at 8 weeks. At 4 weeks, the density of Ica,L was significantly higher (21.3±1.6 pA/pF, n=17, versus 15.9±1.6 pA/pF, n=22; P<0.05) and the density of Ina significantly lower (9.6±1.5, n=22, versus 15.2±2.2 pA/pF, n=19; P<0.01) in CO than in Ctr and normalized thereafter.

Conclusions—Prenatal CO exposure affects the physiological shortening of APD in neonatal rats. We speculate that a prolonged myocyte repolarization induced by prenatal exposure to smoke may establish a period of vulnerability for life-threatening arrhythmias in infancy. (Circulation. 2004;109:419-423.)

Key Words: death, sudden, infant • electrophysiology • ion channels • smoking • carbon monoxide

Exposure to smoke during pregnancy is associated with several detrimental outcomes in newborns, including a significantly higher risk for sudden infant death.1 Tobacco smoking is an important source of chronic exposure to carbon monoxide (CO): CO contained in cigarette smoke (≈4%) easily crosses the placental barrier by passive diffusion, causing a 4-fold increase in carboxyhemoglobin levels in the umbilical cord blood.2 The consequent chronic fetal hypoxia may retard fetal growth2 and alter the physiological development of organs and tissues, especially those most susceptible to hypoxia damage, including the brain.3,4 Alterations in the autonomic nervous system in infants born from smoking mothers have been reported.5 Little is known about the effects of prenatal exposure to smoke or to CO on the electrophysiological properties of the heart.

There is a consensus that the cause of sudden infant death syndrome (SIDS) is multifactorial, but despite the many hypotheses proposed, none have been proved. On the basis of a large prospective study, it has been suggested that congenital long-QT syndrome could account for some cases of SIDS.6 Although this hypothesis remains controversial,7,8 some cases of SIDS have been linked to sodium channel gene mutations.9–12 In newborns, the QT interval temporarily lengthens and then physiologically shortens during the first 6 months of life.13 Postnatal electrophysiological remodeling has been documented for both newborn dogs14 and rats15,16: Ventricular repolarization spontaneously undergoes a progressive shortening during the first weeks of life. Neurohumoral and hormonal signals (sympathetic innervation, thyroid hormone) seem to play a key role in these developmental changes, because their deficiency causes an abnormally prolonged repolarization.17,18 To the best of our knowledge, the effect of maternal smoking during pregnancy on postnatal electrophysiological remodeling has never been studied. For these reasons, we aimed to assess whether prenatal exposure to CO (a proxy of maternal smoking) could affect the

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electrophysiological maturation of the ventricular myocytes, eventually predisposing the heart to arrhythmias. Thus, we studied the consequences of chronic prenatal exposure to CO on the cellular electrophysiological maturation (action potential [AP] and ionic currents) of rat hearts from birth to 2 months of age. A CO concentration (150 ppm) was chosen that resulted in blood levels of carboxyhemoglobin comparable to those found in human cigarette smokers.3

**Methods**

**CO Treatment of the Animals**

All the experiments were performed in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC), for experimental animal care. Pregnant primiparous Wistar female rats (Harlan, Udine, Italy) were exposed to 0 or 150 ppm CO mixed with air throughout the time of pregnancy as described previously.1 Within 24 hours of birth, every litter was reduced to 6 male pups, and when necessary, female pups were used to reach the number of 6 pups. From 1 to 60 days of life, 1 control rat and 1 CO-exposed male rat were killed at any experimental time to maintain litter homogeneity. During weaning, male rats were kept apart from females, gathered together into 2 groups (control [Ctr] and CO), which then were reduced to an equal number. All the rats survived until they were killed at the moment of the experiment.

**Cell Isolation**

Single left ventricular myocytes were isolated from Ctr or CO-exposed rats according to a protocol based on previously described procedures19,20 and used within the day.

**Electrophysiological Recordings**

The experimental setup for patch-clamp (whole-cell) recording and data acquisition was similar to that described previously.19 Experiments were performed simultaneously in 2 patch-clamp setups. The patch-clamped cell was superperfused by means of a temperature-controlled microsuperfusor (36°C to 37°C), which allowed rapid changes of the solution bathing the cell, with normal or modified Tyrode’s solutions. Patch-clamp pipettes had a resistance of 1.5 to 2.5 MΩ.

Cell membrane capacitance (Cm) and AP parameters were determined as previously described.19 L-type calcium current (I\(\text{Ca,L}\)) and the transient outward current (I\(\text{O transient}\)) were recorded according to previously described protocols.20 The availability of Ca current was characterized by measuring peak I\(\text{Ca,L}\) during a test pulse to 0 mV after application of 500-ms conditioning pulses to different potentials (from −70 to 0 mV).

**Data Analysis and Statistics**

Offline data analysis was performed with pClamp (version 6.0, Axon Instruments Inc) and Origin 4.1 (MicroCal Software Inc). All data are expressed as mean±SEM. Statistical analysis was performed by means of the Graph Pad Instat program, using the Kruskal-Wallis test (nonparametric test) followed by Dunn’s test for comparing membrane capacitance values and Student’s t test for grouped data or ANOVA followed by Tukey’s test for all other values. A probability value of P<0.05 was considered significant.

**Results**

**Effect of CO Treatment on Cell Size**

Figure 1 shows the distribution of membrane capacitance, a widely used index of cell size, measured in single myocytes isolated from the ventricles of the heart of rats exposed during fetal life to normal air (Ctr) or to 150 ppm CO (CO). Animals of different ages were used: from 5 to 17 days of age (indicated as 2 weeks), from 21 to 35 days (4 weeks), and from 45 to 65 days (8 weeks). As expected, aging is associated with an increase in cell membrane capacitance, a process referred to as postnatal hypertrophic growth of the heart.19,21 Cells from Ctr and CO rats show a similar increase in membrane capacitance throughout the age range tested. The mean±SEM values for Cm measured at 2, 4, and 8 weeks were 20.7±1.1, 54.9±2.8, and 125.8±5.2 pF and 18.7±0.9, 62.8±2.7, and 137.4±6.2 pF, respectively, in Ctr and CO rats, without any significant difference caused by CO exposure.

**Effect of CO Treatment on AP Duration**

Figure 2 shows the profiles of typical APs recorded from ventricular cardiomyocytes. Recordings were obtained from cells isolated from Ctr or age-matched CO-exposed rats at

![Figure 1](http://circ.ahajournals.org/)

**Figure 1.** Membrane capacitance during postnatal development in Ctr (○) and CO-exposed (●) neonatal rats.

![Figure 2](http://circ.ahajournals.org/)

**Figure 2.** Typical APs recorded from (A) 2-, (B) 4-, and (C) 8-week-old rats.
different times after birth: newborn (2 weeks) (A), 4 weeks (B), and 8 weeks (C). It is apparent that at 2 weeks after birth, the AP profile from Ctr is nearly identical to that recorded from CO-exposed rats (A). The AP duration (APD), as expected, is quite long; in these cells, the phase of rapid repolarization appears to be slowed down, and the plateau phase is long. With development (4 weeks old), the AP from Ctr myocytes shows the expected decrease in duration and a prominent rapid repolarization phase; conversely, the AP from CO myocytes remains long (B). After 8 weeks, the APs from both groups are similarly shortened and almost superimposable (C).

The mean values of the AP parameters are reported in Table 1. Maximum diastolic potential and overshoot are similar in the 3 groups of age-matched Ctr and CO-exposed rats. Conversely, mean values of APD measured at −20 mV (APD_{−20}) and at −50 mV (APD_{−50}) are similar 2 weeks after birth but are significantly different at 4 weeks. APD_{−20} and APD_{−50} from Ctr rats show the usual clear-cut decrease, whereas no shortening of APD is observed in age-matched CO rats. However, at 8 weeks after birth, the CO-exposed cells also exhibit a shortening of APD, with APD_{−50} remaining longer, although not statistically significantly so (P = 0.07), than in Ctr cells.

Effect of CO Treatment on Calcium and Potassium Currents
To investigate the ionic basis of the previously described delay in the time course of AP shortening in CO-exposed rats,

<p>| TABLE 1. Action Potential Characteristics of Ventricular Myocytes From Control and CO-Exposed Rats |
|-------------------|-----|-----|-----|-----|-----|</p>
<table>
<thead>
<tr>
<th>Age, wk</th>
<th>CO</th>
<th>n</th>
<th>MDP, mV</th>
<th>OS, mV</th>
<th>APD_{−20}, ms</th>
<th>APD_{−50}, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctr</td>
<td>2</td>
<td>25</td>
<td>−74.9 ± 1.1</td>
<td>31.6 ± 2.5</td>
<td>50.7 ± 5.3</td>
<td>108.7 ± 12.9</td>
</tr>
<tr>
<td>CO</td>
<td>4</td>
<td>14</td>
<td>−73.3 ± 1.5</td>
<td>25.8 ± 1.5</td>
<td>35.6 ± 4.5</td>
<td>77.8 ± 8.3</td>
</tr>
<tr>
<td>Ctr</td>
<td>8</td>
<td>10</td>
<td>−71.8 ± 1.4</td>
<td>29.1 ± 3.3</td>
<td>28.3 ± 7.2</td>
<td>66.8 ± 6.8</td>
</tr>
<tr>
<td>CO</td>
<td>8</td>
<td>10</td>
<td>−71.0 ± 1.7</td>
<td>20.0 ± 1.3</td>
<td>33.1 ± 7.9</td>
<td>91.7 ± 11.5</td>
</tr>
</tbody>
</table>

MDP indicates maximum diastolic potential; OS, overshoot; and Ctr, control. Values represent mean ± SEM. n is the number of cells.

I_{\text{Ca,L}} and I_{\text{K,A}} (ie, the 2 ionic conductances that largely control the APD in the rat heart) were measured. Table 2 summarizes the properties of I_{\text{Ca,L}} in cells isolated from 2-, 4-, and 8-week-old rats. The density of peak current is relatively constant in Ctr rats at the different ages; the only consequence of aging appears to be a shift in the voltage of half-maximal activation (V_{\text{hl}}) toward more negative values: V_{\text{hl}} is significantly more negative at 8 weeks than at 2 or 4 weeks, in agreement with previous studies.22

In CO-exposed rats, I_{\text{Ca,L}} density was not different from that in Ctr rats at 2 and 8 weeks; however, it was significantly larger at 4 weeks (ie, the age at which the APD was prolonged) compared with Ctr rats. The voltage dependences of activation and inactivation (V_{\text{vl}}, V_{\text{hl}}) were similar to those in Ctr. Figure 3 shows typical I_{\text{Ca,L}} recordings obtained in 4-week-old Ctr and CO-exposed rats (A and B, respectively) and average I-V relationships (C). The data clearly show that I_{\text{Ca,L}} is larger at any potential in CO-exposed rats and strongly indicate that, at this age, the contribution of calcium current to APD is significantly higher in myocytes from CO-exposed than from Ctr rats.

It is well known that postnatal cell growth is accompanied by a progressive increase in I_{\text{K}} density, largely responsible for the APD shortening that occurs during the first weeks of life in the rat heart.15,18 An age-dependent increase of peak I_{\text{K}} density was clearly detected in Ctr rats, as shown in Figure 4C: Current density measured at +60 mV increased from 5.4 ± 0.6 to 13.1 ± 1.9 and 13.7 ± 2.0 pA/pF at 4 and 8 weeks of age, respectively (P < 0.01); maximal current density, evaluated by fitting I-V relationships to a Boltzmann function, increased to a similar extent (Table 3).

I_{\text{K}} increased during aging in rats that had been exposed prenatally to CO, but to a lesser extent; in fact, the peak current density measured at +60 mV was not statistically different at 2 and 4 weeks (P = 0.07). Furthermore, at 4 weeks, I_{\text{K}} maximal density was significantly smaller in CO-exposed rats than in age-matched Ctr (9.6 ± 1.5 versus 15.2 ± 2.2 pA/pF, P < 0.01). I_{\text{K}} remained markedly smaller at 8 weeks in CO-exposed rats, even if the difference did not reach statistical significance (10.4 ± 2.3 versus 15.5 ± 2.0 pA/pF, P = 0.06) (Figure 4C). Typical recordings obtained in 4-week-old control (A) and CO-exposed (B) rats are shown in Figure 4. Similar results were obtained with respect to maximal current density, which was reduced significantly in 4-week-old CO versus age-matched Ctr (Table 3). The midpoint of

<p>| TABLE 2. Properties of I_{\text{Ca,L}} Recorded in Ventricular Myocytes From Control and CO-Exposed Rats |
|-------------------|-----|-----|-----|-----|</p>
<table>
<thead>
<tr>
<th>Age, wk</th>
<th>CO</th>
<th>n</th>
<th>Peak I_{\text{Ca,L}}, pA/pF</th>
<th>V_{\text{hl}}, mV</th>
<th>V_{\text{hl}}, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctr</td>
<td>2</td>
<td>11</td>
<td>15.4 ± 2.9</td>
<td>−13.2 ± 1.6</td>
<td>−35.3 ± 3.0</td>
</tr>
<tr>
<td>CO</td>
<td>2</td>
<td>10</td>
<td>16.8 ± 3.8</td>
<td>−17.9 ± 3.6</td>
<td>−41.7 ± 3.3</td>
</tr>
<tr>
<td>Ctr</td>
<td>4</td>
<td>22</td>
<td>15.9 ± 1.6</td>
<td>−14.9 ± 2.0</td>
<td>−40.1 ± 2.6</td>
</tr>
<tr>
<td>CO</td>
<td>4</td>
<td>17</td>
<td>21.3 ± 1.6*</td>
<td>−16.4 ± 3.6</td>
<td>−39.2 ± 2.3</td>
</tr>
<tr>
<td>Ctr</td>
<td>8</td>
<td>28</td>
<td>15.2 ± 1.0</td>
<td>−25.9 ± 1.1†</td>
<td>−37.9 ± 1.4</td>
</tr>
<tr>
<td>CO</td>
<td>8</td>
<td>18</td>
<td>14 ± 1.1</td>
<td>−26.6 ± 1.4†</td>
<td>−39.4 ± 2.2</td>
</tr>
</tbody>
</table>

V_{\text{hl}} indicates voltage of half-maximal steady-state activation; V_{\text{hl}}, voltage of half-maximal steady-state inactivation; and Ctr, control. Values represent mean ± SEM. n is the number of cells.

*P < 0.05 vs age-matched control rats; †P < 0.01 vs younger rats of the same group.
the $I_{K}$-voltage relationship activation ($V_{0}$) and the slope factor (k) were not different in all groups (Table 3), suggesting that the properties of the channel are not modified.

No differences were observed between Ctr and CO-exposed rats in steady-state outward currents ($I_{m}$), recorded at the end of a depolarizing step to +60 mV (Figure 4D). This steady-state outward component is determined by several K$^{+}$ currents, including the delayed rectifiers, the inward rectifier, and probably the pH-sensitive TASK-1 currents. The ultrarapid delayed rectifier and the inward rectifier currents are reported to decrease with postnatal maturation, and this may account for the age-related decrease in $I_{m}$ shown in Figure 4D. However, the lack of difference between $I_{m}$ from Ctr and CO-exposed rats prevented us from investigating this matter further.

**Discussion**

The present study is the first to demonstrate an effect of prenatal exposure to CO on postnatal electrophysiological maturation of rat ventricular myocytes. The most prominent effect is a delay in the age-related shortening of APD measured at −20 and at −50 mV. In a limited temporal range, ie, at ≈4 weeks of age, ventricular myocytes from CO-exposed rats exhibit significantly longer APDs than Ctr. The effect occurs in concert with a significantly lower density of $I_{K}$ and a significantly higher density of $I_{Ca,L}$.

**Molecular Effects of Prenatal CO Exposure**

Rat ventricular repolarization is regulated primarily by the balance of $I_{m}$, $I_{Ca,L}$, and $I_{Ca,C}$. and changes in the number and/or properties of these channels deeply affect the shape and duration of AP. $I_{m}$ channel density increases steeply from birth to 1 month of life in the rat, as shown by our data (Table 3) and in agreement with the literature. At variance with $I_{m}$, we found that $I_{Ca,C}$ density is not affected by postnatal growth (Table 2), because it remains unaltered during development, as reported by Katsube et al and in contrast to Gomez et al. Hormonal systems play a major role in controlling the maturation of rat cardiac myocyte functional properties. Importantly, the densities of $I_{m}$ and $I_{Ca,L}$ are regulated by thyroid hormones in the rat cardiac ventricle. Moreover, postnatal evolution of sympathetic innervation parallels ventricular APD shortening in the rat and contributes to the developmental differences in $I_{m}$. Likewise, an abnormal sympathetic innervation in neonatal rats prolongs the ECG QT interval, which is an indicator of a prolonged ventricular myocyte repolarization. Thus, the delay in the age-related shortening of APDs as well as in the increase of $I_{m}$ determined by prenatal CO exposure may depend on an imbalance of the controlling systems, ie, sympathetic innervation. This possibility relies on some experimental evidence. First, infants born from mothers who smoke during pregnancy have an altered autonomic control of the cardiovascular system. Moreover, impaired sympathetic development may be caused by the toxic effects of CO exposure, which result from the combination of tissue hypoxia and direct damage at the cellular level. The fetus is

**Table 3. Properties of $I_{m}$ Recorded in Ventricular Myocytes From Control and CO Rats**

<table>
<thead>
<tr>
<th>Age, wk</th>
<th>n</th>
<th>$I_{m,max}, \mu A/\mu F$</th>
<th>$V_{m}, \text{mV}$</th>
<th>k, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctr 2</td>
<td>30</td>
<td>9.1±0.9</td>
<td>29.6±2.7</td>
<td>14.1±0.8</td>
</tr>
<tr>
<td>CO 2</td>
<td>25</td>
<td>8.6±1.2</td>
<td>30.9±2.1</td>
<td>15.9±0.8</td>
</tr>
<tr>
<td>Ctr 4</td>
<td>19</td>
<td>15.2±2.2</td>
<td>28.0±2.6</td>
<td>14.5±0.7</td>
</tr>
<tr>
<td>CO 4</td>
<td>22</td>
<td>9.6±1.5*</td>
<td>29.0±2.3</td>
<td>15.3±0.7</td>
</tr>
<tr>
<td>Ctr 8</td>
<td>11</td>
<td>15.5±2.0</td>
<td>31.0±3.8</td>
<td>16.0±0.7</td>
</tr>
<tr>
<td>CO 8</td>
<td>14</td>
<td>10.4±1.3</td>
<td>28.3±2.7</td>
<td>15.3±0.8</td>
</tr>
</tbody>
</table>

$V_{m}$ indicates voltage of half-maximal steady-state activation; k, slope factor; and Ctr, control. Values represent mean±SEM. n is the number of cells.

*P<0.01 vs age-matched control rats.

**Figure 3.** Typical $I_{m}$ traces elicited by steps to −45/±55 mV (holding potential, −70 mV) from 4-week-old Ctr (A) and CO-exposed (B) rats and average I-V relationships (C). *P<0.05, **P<0.01, ***P<0.001.

**Figure 4.** Typical $I_{m}$ traces elicited by steps to −40/±70 mV (holding potential, −70 mV) from 4-week-old Ctr (A) and CO-exposed (B) rats. Peak $I_{m}$ currents and $I_{m}$ in Ctr and CO-exposed rats at different ages are shown in C and D, respectively. **P<0.05, †P<0.01 vs 2-week-old rats.
extremely sensitive to the hypoxic effects, because carboxyhemoglobin levels significantly exceed the levels in the mother, thus causing less oxygen to be released to fetal tissues. In this perspective, it is worth noting that prenatal hypoxia has been reported to modify sympathoadrenal system development in the rat. Moreover, the existence of an interesting temporal relation is relevant: The effects of prenatal exposure to CO become manifest in the first month of life, when several electrophysiological developmental changes of the rat heart occur and sympathetic innervation completes its maturation.

So far, we have considered the potential effect of lower oxygen availability on the factors regulating channel protein expression and/or functions. However, we cannot exclude the direct effects of reduced oxygen availability at the cardiomyocyte level. A common cellular mechanism responsible for oxygen homeostasis involves the induction of a variety of genes through activation of hypoxia-inducible factor-1. The relevance of this pathway in controlling ion channel genes through activation of hypoxia-inducible factor-1 is completely unknown. However, it is worth noting that mild hypoxia produces a delay in the maturation of functional properties of cultured rat neonatal myocytes.

Possible Relevance of Prenatal CO Exposure to SIDS

APD is a major determinant for membrane electrical stability in isolated myocytes, because the longer the AP, the higher the propensity to give rise to abnormal activity. Neither arrhythmogenic electrophysiological alterations nor sudden deaths were observed in CO-exposed rats. However, the prolonged APD of ventricular myocytes from 4-week-old rats exposed prenatally to CO suggests that one component of smoke temporarily destabilizes the electrical properties of ventricular myocytes: This may render the ventricular muscle more susceptible to developing arrhythmias, especially in the presence of triggering factors. In a similar context, perhaps prolonged myocyte repolarization induced by prenatal exposure to smoke may establish a period of vulnerability for life-threatening arrhythmias.

Acknowledgments

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