Supporting Text

We measured heart rate (HR) and heart rate variability (HRV) by ECG telemetry to study autonomic regulation in the α1A/C KO mouse. Hypotension in the KO might have been expected to stimulate a reflex increase in sympathetic activity, and we used autonomic regulation of HR and HRV to test for this. We used ECG telemetry to study HRV (1-4), so that data could be obtained at least one week after anesthesia and surgery, and for the superior frequency response compared with a fluid-filled catheter (5, 6).

Methods

**ECG Telemetry.** Under isoflurane anesthesia, a Data Sciences International biopotential and physical activity transmitter (Minneapolis; Model TA10EA-F20) was placed in the abdomen through a midline incision and was sutured to the abdominal wall. Leads were tunnelled subcutaneous and sutured in place at a lead II position, with the positive lead in the lower left thoracic and the negative lead in the upper right thoracic. Buprenorphine, 0.05 mg/kg intraperitoneal, was provided for 48 h after surgery. One week after surgery, the home cage was placed on a Data Sciences RPC-1 receiver. The experimental protocol is described in the legend to Table 3. Analogue ECG telemetry data were recorded digitally at 1,000 Hz by using Biopac MP 100 data acquisition hardware and AcqKnowledge software and were stored on hard disc.

Raw ECG data were transformed to RR intervals expressed as ms. The RR interval is the time between adjacent QRS signals of the ECG, and each QRS reflects ventricular depolarization. ECG data not transformed correctly to RR interval were replaced using a nearest point method, and recordings that required more than 1 s of data to be replaced were not included in the analysis. RR interval data were resampled at 100 Hz, and 163.84-s segments (approximately 1,000-2,000 beats) were selected for analysis (see legend to Table 3). The mean RR interval over the 163.84-s segment and the standard deviation (SD) of the mean RR interval were calculated. The power spectral density was calculated by squaring the magnitude of the RR interval time domain from Fast Fourier transform analysis, windowed using a Blackman
algorithm. Power density was plotted versus frequency (see Figs. 6 and 7). Values before versus after drug and values in WT versus KO were compared by paired or unpaired $t$ test, and a $P < 0.05$ was considered significant.

Results and Discussion

Heart Rate (HR) and RR Interval. We implanted ECG telemeters in four pairs of male $\alpha_{1A/C}$ KO and WT mice to measure HR and HRV. Mice were studied awake and resting in their home cage, beginning one week after surgery. Each day for four days, one KO and one WT mouse were injected intraperitoneal with a single drug: phenylephrine (PE), isoproterenol, atropine, or propranolol. The ECG was recorded before drug (basal) and after.

The average basal HR over all four days of recording was 11% faster in the KO (Table 3), a difference that was not quite significant ($P = 0.058$). The basal RR interval in the KO was correspondingly shorter.

As shown in Table 3, increasing blood pressure with the $\alpha_{1}$-AR agonist PE reduced HR and lengthened the RR interval significantly in both genotypes. Bradycardia with PE in the KO was less than that in the WT ($86\%, P = 0.11$). The $\beta$-AR agonist isoproterenol increased HR significantly in both genotypes, and to a somewhat faster final HR in the KO. Parasympathetic blockade with atropine increased HR significantly only in the KO ($P < 0.05$). The $\beta$-AR antagonist propranolol reduced HR considerably and significantly in both genotypes.

In summary, ECG telemetry in male mice revealed HR regulation with autonomic drugs given intraperitoneal very similar to that seen with intraarterial catheterization in female mice given intravenous drugs (Figs. 4 and 5). We interpret the greater tachycardia in the KO after atropine (Fig. 5 and Table 3) as evidence for relatively greater sympathetic activity in the KO, unmasked by parasympathetic blockade. We did not measure intrinsic HR after combined sympathetic and parasympathetic blockade, because this combination was not tolerated well.
However, basal HR in the mouse is under predominant sympathetic control (5, 6), and HRs in the KO and WT were almost identical after β-AR blockade (Table 3).

**Heart Rate Variability (HRV).** Prior studies in the mouse used basal HRV as an index of autonomic activity in genetic models. These prior studies report that HRV is decreased in models of increased sympathetic activity (1, 2), and in a model of decreased parasympathetic activity (3). To confirm these findings in our model, we tested the effects on HRV of the autonomic drugs indicated in Table 3. As shown by the plots of power spectral density in Fig. 6, PE increased HRV markedly in both WT and α1A/C KO. Because the bradycardic effect of PE was antagonized completely by atropine (Fig. 5), it could be concluded that the increase in HRV with PE reflected parasympathetic activation. Conversely, parasympathetic blockade with atropine reduced HRV significantly in both genotypes (Fig. 6). Isoproterenol tended to reduce HRV, but this effect was not significant (WT \( P = 0.18 \), KO \( P = 0.056 \)). Propranolol had negligible effect. Our results agree reasonably well with prior studies in mice using analogous drug protocols (4-6). Taken together, our data indicate that decreased HRV in the mouse reflects a shift in autonomic tone toward sympathetic, whereas an increase in HRV indicates a shift toward parasympathetic. This conclusion agrees with prior reports (1-3).

Inspection of power spectral density plots suggested a trend toward reduced HRV in the α1A/C KO compared with WT after PE, atropine, and isoproterenol, but these differences were not significant statistically (Fig. 6). However, calculation of another index of HRV, the SD of the HR or RR interval (Table 3), showed a significant reduction in HRV in the KO compared with WT after PE and atropine (Table 3). In addition, in the KO but not the WT, the SD of the HR or RR interval was significantly smaller than basal after isoproterenol (Table 3). The smaller SD of the HR or RR interval indicated less HRV after drugs in the KO. Furthermore, combined analysis of HRV for all power spectral density recordings revealed a significant reduction in basal HRV in the KO over the total frequency range 0-5 Hz (Fig. 7).
In summary, two main findings suggested that sympathetic activity was relatively increased in the α1A/C KO, a faster HR after atropine in the KO but not the WT, and reduced HRV in the KO versus the WT. Plausibly this relative increase in sympathetic activity in the KO reflected a response to hypotension caused by deletion of the α1A/C subtype.


Table 3. Autonomic regulation of heart rate and heart rate variability in α1A/C KO mice

<table>
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<tr>
<th></th>
<th>Basal</th>
<th>Phenylephrine (3 mg/kg)</th>
<th>Isoproterenol (20 µg/kg)</th>
<th>Atropine (1 mg/kg)</th>
<th>Propranolol (20 mg/kg)</th>
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<tr>
<td></td>
<td>N (mice)</td>
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<tr>
<td>WT</td>
<td>4</td>
<td>4</td>
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<tr>
<td>KO</td>
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<thead>
<tr>
<th></th>
<th>HR (bpm)</th>
<th>SD HR</th>
<th>RR Interval (ms)</th>
<th>SD RR</th>
<th>HR (bpm)</th>
<th>SD HR</th>
<th>RR (ms)</th>
<th>SD RR</th>
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<tbody>
<tr>
<td></td>
<td>509 ± 89</td>
<td>30 ± 20</td>
<td>118 ± 21</td>
<td>7 ± 5</td>
<td>305 ± 35B</td>
<td>85 ± 25B</td>
<td>197 ± 22B</td>
<td>54 ± 16B</td>
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<tr>
<td>KO</td>
<td>567 ± 51E</td>
<td>27 ± 12</td>
<td>106 ± 10</td>
<td>5 ± 2</td>
<td>355 ± 41B</td>
<td>68 ± 15BC</td>
<td>169 ± 20B</td>
<td>32 ± 7BC</td>
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ECG telemeters were implanted in four pairs of male α1A/C KO and WT mice aged 12 weeks. On four successive days beginning one week after surgery, between 1 and 4 P.M., a basal ECG was recorded, and then one of the drugs indicated was injected intraperitoneal. A 163.84-s recording was analyzed before (Basal) and after drug, when the effect had stabilized as follows: phenylephrine (75 s after injection); isoproterenol (300 s); atropine (240 s); and propranolol (300 s). The complete protocol with all four drugs over four days was successful for two of four mice of each genotype. The values shown are the mean HR (or RR interval) and the mean SD of the HR (or RR interval) over 163.84 s, and are mean ± SD for the number of mice given in the Table. Basal vs. drug: A P < 0.05; B P < 0.01. WT vs. KO: C P < 0.05; D P < 0.01; E P = 0.058.
Fig. 6. Autonomic regulation of HRV in α1A/C KO and WT mice. HRV was quantified in α1A/C KO and WT mice using the protocol described in Methods and the legend to Table 3. The average power spectral density, an index of HRV, is plotted versus frequency before the indicated drugs (basal, dark lines) and after (light lines). Each line is the average of two to four ECG recordings of RR interval, as in Table 3. The P values are for drug versus basal.
Fig. 7. Decreased HRV in α1A/C KO mice. HRV is plotted for all basal recordings from α1A/C KO (light line) and WT mice (dark line) (13 recordings in four mice of each genotype). Total spectral density in the KO is 86% of that in the WT, indicating significantly reduced HRV ($P < 0.05$).