Ephedrine plus caffeine causes age-dependent cardiovascular responses in Fischer 344 rats

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Submitted 18 November 2004; accepted in final form 10 January 2005

Ephedrine plus caffeine causes age-dependent cardiovascular responses in Fischer 344 rats. Am J Physiol Heart Circ Physiol 288: H2219–H2224, 2005.—Human consumption of ephedrine and caffeine in dietary supplements has been associated with a number of adverse effects, including changes in the ECG, myocardial infarction, hyperthermia, and, in rare instances, death. The purpose of this study was to investigate the potential mechanisms associated with the cardiotoxicity of combined ephedrine and caffeine ingestion. Seven- and fourteen-week-old Fischer 344 rats treated with ephedrine in combination with caffeine exhibited increases in heart rate (HR), temperature, and corrected QT interval. Of the 14-wk-old rats treated with 25 mg/kg ephedrine plus 30 mg/kg caffeine, 57% died within 3–5 h of treatment, whereas none of the similarly treated 7-wk-old rats nor any of the rats treated with vehicle died. One hour after treatment with this dose of ephedrine plus caffeine, 14-wk-old rats exhibited a larger increase in HR (as % increase over baseline) than 7-wk-old rats. Furthermore, the 14-wk-old rats that died had a higher HR and temperature than the 14-wk-old rats that lived. Histopathological studies suggested interstitial hemorrhage and myofiber necrosis in the 14-wk-old rats treated with the highest concentration of ephedrine and caffeine. This study showed enhanced susceptibility to ephedrine plus caffeine in 14-wk-old rats compared with 7-wk-old rats. The greater mortality in the 14-wk-old rats was associated with increases in body temperature, HR, and myocardial necrosis.

Typical supplements contain ~20 mg of ephedrine and 200 mg of guarana-derived caffeine (4), which correspond to roughly 0.3 mg ephedrine/kg body wt and 3 mg caffeine/kg body wt. Because the adverse effects of ephedrine and caffeine were only observed in a small percentage of those consuming the ephedrine-containing supplement, we examined doses that were 5- to 10-fold higher for caffeine and 10- to 100-fold higher than the recommended dose of ephedrine to enhance our ability to observe effects.

The mechanisms responsible for adverse responses to concurrent ephedrine and caffeine consumption are poorly understood. Therefore, the purpose of this study was to investigate the effects of a combined ephedrine and caffeine dose on ECG, heart rate (HR), temperature, and myocardial histology in inbred Fischer rats. In rats treated with the highest dose of ephedrine plus caffeine, we found a striking difference in mortality in 14-wk-old rats compared with 7-wk-old rats. We used this age-dependent difference to identify parameters associated with mortality.

MATERIALS AND METHODS

Animals

Male 7-wk-old (147.3 ± 2.3 g; n = 40) and 14-wk-old (283.0 ± 4.3 g; n = 40) Fischer 344 rats (Charles River, Raleigh, NC) were used in this study. All rats were housed individually with a 12:12-h light-dark cycle. Food (NIH-31) and water were provided ad libitum. The National Institute of Environmental Health Sciences Care and Use of Laboratory Animals Committee approved all animal protocols.

Experimental Design

Seven- and fourteen-week-old rats were treated with 25 mg/kg ephedrine plus 30 mg/kg caffeine (7 wk, n = 13; 14 wk, n = 14), 12.5 mg/kg ephedrine plus 30 mg/kg caffeine (7 wk, n = 3; 14 wk, n = 4), 2.5 mg/kg ephedrine plus 30 mg/kg caffeine (7 wk, n = 3; 14 wk, n = 4), or 25 mg/kg ephedrine plus 15 mg/kg caffeine (7 wk, n = 4; 14 wk, n = 4) by oral gavage. l-Ephedrine hydrochloride (Sigma-Aldrich, St. Louis, MO) and caffeine (Pfalz and Bauer, Waterbury, CT) were prepared in 0.5% methylcellulose. To verify that the oral gavage of methylcellulose did not influence the end points assessed in this study, 14-wk-old (n = 8) and 7-wk-old (n = 9) rats were dosed with a 0.5% methylcellulose control.

Surgery and ECG Analysis

Under sterile conditions a random selection of 7- and 14-wk-old rats were anesthetized with inhaled isoflurane; Carprofen was given...
for analgesia. A 3-cm midline dorsal incision was made in the skin, and a subcutaneous tissue pocket was formed with a blunt instrument. A Data Sciences International (DSI; Arden Hills, MN) ETA-F20 transmitter was placed inside the tissue pocket and sutured to the left latissimus dorsi muscle. The anodal and cathodal leads were tunneled subcutaneously and sutured over the left superficial gluteus and right trapezius muscles, respectively. All incisions were closed with wound clips, and animals were allowed 7 days to recover. HR, corrected QT (QTc) interval (Bazett’s correction), and R-wave amplitude were measured from the ECG waveforms with DSI Physiostat ECG Analysis software version 3.22.

Isolated, Perfused Heart Preparation

Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (25 mg/kg body wt) followed by intravenous administration of 100 U heparin sodium. The heart was excised and arrested in ice-cold Krebs-Henseleit (KH) buffer and connected via the aorta to the perfusion cannula. Retrograde perfusion was initiated under constant pressure of 100 cmH2O as previously described [17]. Hearts were perfused with nonrecirculating KH buffer containing (in mM) 120 NaCl, 4.6 KCl, 1.2 MgSO4, 1.2 KH2PO4, 1.25 CaCl2, 25 NaHCO3, and 11 glucose at pH 7.4. The perfusate was equilibrated with 95% O2-5% CO2 and maintained at a temperature of 37°C. Hemodynamic measurements were taken after 20 min of equilibration. To monitor contractility, a fluid-filled latex balloon was inserted into the left ventricle. The balloon was connected to a Statham pressure transducer and inflated to achieve an end-diastolic pressure of 5–10 cmH2O. A Maclab/2e and Chart version 4.2.2 software (AD Instruments; Colorado Springs, CO) were used to collect and process hemodynamic parameters. Function was expressed as a rate-pressure product (HR × left ventricular developed pressure).

Histology

Randomly selected hearts were fixed in 10% neutral buffered formalin, processed, trimmed, embedded in paraffin, sectioned to a thickness of 4 – 6 μm, and stained with hematoxylin and eosin for microscopic examination. For the 25 mg/kg ephedrine plus 30 mg/kg caffeine treatment, five 7-wk-old and seven 14-wk-old hearts were processed for histology.

Statistical Analysis

Data are expressed as means ± SE. Means were compared by one-way ANOVA, with a post hoc Fisher’s protected least significant difference test applied for multiple comparisons. Differences were considered significant at P < 0.05.

Table 1. Heart rate, ECG parameters, and subcutaneous temperature in 7-wk-old rats dosed concurrently with ephedrine and caffeine

<table>
<thead>
<tr>
<th>Treatment, mg/kg</th>
<th>n</th>
<th>Time Point</th>
<th>HR, beats/min</th>
<th>QTc, ms</th>
<th>R-amp, mV</th>
<th>Temp, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>17</td>
<td>Baseline</td>
<td>421±6</td>
<td>0.111±0.002</td>
<td>0.421±0.017</td>
<td>36.8±0.1</td>
</tr>
<tr>
<td>25 Eph + 15 Caff</td>
<td>4</td>
<td>1 h</td>
<td>501±6*</td>
<td>0.121±0.002*†</td>
<td>0.443±0.026</td>
<td>37.2±0.2†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 h</td>
<td>490±14*</td>
<td>0.121±0.003*†</td>
<td>0.452±0.021</td>
<td>36.7±0.3†</td>
</tr>
<tr>
<td>2.5 Eph + 30 Caff</td>
<td>3</td>
<td>1 h</td>
<td>519±11*</td>
<td>0.122±0.005*†</td>
<td>0.458±0.084</td>
<td>37.5±0.1*†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 h</td>
<td>488±23*</td>
<td>0.114±0.002†</td>
<td>0.447±0.065</td>
<td>37.4±0.1</td>
</tr>
<tr>
<td>12.5 Eph + 30 Caff</td>
<td>3</td>
<td>1 h</td>
<td>517±6*</td>
<td>0.122±0.002*†</td>
<td>0.541±0.038*</td>
<td>37.5±0.3*†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 h</td>
<td>489±14*</td>
<td>0.118±0.001†</td>
<td>0.512±0.048</td>
<td>37.5±0.1*</td>
</tr>
<tr>
<td>25 Eph + 30 Caff</td>
<td>7</td>
<td>1 h</td>
<td>520±7*</td>
<td>0.184±0.003*</td>
<td>0.450±0.018</td>
<td>38.4±0.2*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 h</td>
<td>505±9*</td>
<td>0.190±0.002*</td>
<td>0.519±0.028*</td>
<td>37.6±0.3*</td>
</tr>
</tbody>
</table>

Values are means ± SE for n rats. HR, heart rate; QTc, corrected QT interval; R-amp, R-wave amplitude; Temp, subcutaneous temperature; Eph, ephedrine; Caff, caffeine. *Significantly different from baseline; †significantly different from the 25 Eph + 30 Caff dose at the same time.

RESULTS

Age-Dependent Effects of Treatment

Mortality. Three to five hours after treatment with 25 mg/kg ephedrine plus 30 mg/kg caffeine there was a 57% mortality rate in 14-wk-old rats (8 of 14 rats), but no mortality was observed in 7-wk-old rats. No mortality was observed in 7- or 14-wk-old rats treated with lower doses of ephedrine plus caffeine. Furthermore, treatment with the 0.5% methylcellulose vehicle had no effect on the measured parameters.

Heart rate. Mean baseline HR was significantly higher in 7-wk-old rats than in 14-wk-old rats (Tables 1 and 2). One hour after all treatments, HR in 7-wk-old rats remained higher compared with 14-wk-old rats. Three hours after treatments, the HR of the younger rats was still significantly different from the HR of 14-wk-old rats in all groups except rats treated with 2.5 mg/kg ephedrine plus 30 mg/kg caffeine and rats treated with 25 mg/kg ephedrine plus 30 mg/kg caffeine. In both the 7- and 14-wk-old rats, all treatments resulted in a significant increase in HR compared with baseline measurements at both 1 and 3 h. When examined as a percentage over baseline (because of the significantly higher baseline HR in 7-wk-old rats), the increase in HR for the highest dose of ephedrine plus caffeine was higher in 14-wk-old rats (35 ± 1% and 37 ± 5% at 1 and 3 h, respectively) than in 7-wk-old rats (24 ± 2% and 20 ± 2% at 1 and 3 h, respectively).

Electrocardiogram. No baseline differences in mean QTc interval were found between 7- and 14-wk-old rats (Tables 1 and 2). One and three hours after treatment with the highest dose of ephedrine (25 mg/kg) plus caffeine (30 mg/kg), a significant prolongation of QTc interval relative to baseline was observed in both age groups (Tables 1 and 2). However, at this dose of ephedrine plus caffeine, which caused mortality in some 14-wk-old rats, QTc was not significantly different in 7- vs. 14-wk-old rats, nor in 14-wk-old rats that lived compared with those that died (Table 3); these data suggest that changes in QTc are not the primary predictor of mortality in this study.

No differences were found between the two age groups in R-wave amplitude at baseline (Tables 1 and 2). However, 12.5 mg/kg ephedrine plus 30 mg/kg caffeine and 25 mg/kg ephedrine plus 30 mg/kg caffeine treatments were associated with...
significant increases in R-wave amplitude in the 7-wk-old rats. Furthermore, R-wave amplitude in the 14-wk-old rats increased after treatment with 25 mg/kg of ephedrine plus 15 mg/kg of caffeine and 25 mg/kg of ephedrine plus 30 mg/kg caffeine. These data do not show consistent dose- or age-dependent changes in R-wave amplitude.

Subcutaneous temperature. No difference in baseline subcutaneous temperature was found between 7- and 14-wk-old rats (Tables 1 and 2). In 7-wk-old rats, there was a significant increase in temperature compared with baseline at 1 h after treatment with all ephedrine doses plus 30 mg/kg of caffeine (Table 1 and Fig. 1). With the 25 mg/kg ephedrine plus 30 mg/kg caffeine and 12.5 mg/kg ephedrine plus 30 mg/kg caffeine treatments, the temperature remained elevated at the 3-h time point. In 14-wk-old rats, the increase in temperature was significantly elevated compared with baseline only in the rats treated with 25 mg/kg ephedrine plus 30 mg/kg of caffeine at both 1 and 3 h after treatment (Table 2).

Langendorff perfusion of isolated hearts. We were interested in examining whether the treatment with ephedrine plus caffeine resulted in depressed cardiac contractility. To evaluate contractile function independent of innervation and the presence of catecholamine and caffeine in the blood, we used a Langendorff-perfused heart model. Hearts were isolated from 7- and 14-wk-old rats 3–4 h after treatment with 25 mg/kg ephedrine plus 30 mg/kg caffeine or 0.5% methylcellulose vehicle. Hemodynamic measurements were taken after 20 min of perfusion, without added ephedrine and caffeine. The measurements demonstrated that there was no difference in the rate-pressure product (HR × left ventricular developed pressure) of perfused hearts from either 7-wk-old rats (45,021 ± 4,789 for control vs. 36,406 ± 6,240 for ephedrine + caffeine-treated) or 14-wk-old rats (40,053 ± 2,624 for control vs. 39,439 ± 3,703 for ephedrine + caffeine-treated). Ephedrine plus caffeine treatment also did not affect flow rate in the perfused hearts of either 7-wk-old rats (7.2 ± 0.9 ml/min for control vs. 7.5 ± 0.1 ml/min for ephedrine + caffeine treated) or 14-wk-old rats (7.6 ± 0.8 ml/min for control vs. 8.4 ± 1.3 ml/min for ephedrine + caffeine treated).

Mortality in 14-Week-Old Rats

Heart rate. No significant differences in baseline HR were found between 14-wk-old rats treated with 25 mg/kg ephedrine plus 30 mg/kg caffeine that died (D) compared with those that lived (L; Table 3). One hour after treatment with 25 mg/kg ephedrine plus 30 mg/kg caffeine, the HR of both groups (L and D rats) had significantly increased by 132 and 154 beats/min, respectively. At 3 h after treatment, the HR of the L rats remained elevated relative to baseline but was not significantly different compared with 1 h after treatment (Table 3). However, the mean HR of D rats was increased significantly (40 beats/min) at the 3-h time point compared with 1 h after treatment (Table 3).

Electrocardiogram. A representative ECG record from a 14-wk-old rat treated with 25 mg/kg ephedrine plus 30 mg/kg

<table>
<thead>
<tr>
<th>Table 3. Effect of ephedrine plus caffeine on heart rate, ECG parameters, and subcutaneous temperature in 14-wk-old rats dosed concurrently with 25 mg/kg ephedrine and 30 mg/kg caffeine that died vs. those that did not die</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality Response</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Lived</td>
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<td></td>
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<tr>
<td>Died</td>
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</tbody>
</table>

Values are means ± SE for n rats. *Significantly different at same time point from animals that died; †significantly different from baseline within group; ‡significant difference between the 1- and 3-h time points within group.
caffeine is shown in Fig. 2. This record shows an example of reduced R-wave amplitude from an animal that later died.

Ephedrine (25 mg/kg) plus caffeine (30 mg/kg) induced a prolongation of QTc interval in 7- and 14-wk-old rats, but no significant differences in QTc interval were found between the D and L rats (Table 3). No significant difference in R-wave amplitude between the D and L groups was observed at baseline. However, the R-wave amplitude in the D rats was significantly lower compared with the L rats 3 h after treatment with the highest dose of ephedrine plus caffeine (Table 3).

Subcutaneous temperature. Mean subcutaneous temperature in the D rats was significantly higher than in the L rats 1 h after 25 mg/kg ephedrine plus 30 mg/kg caffeine treatment (Table 3 and Fig. 1), which occurred before any visible signs of distress. Three hours after treatment with this dose of ephedrine plus caffeine, subcutaneous temperature returned to baseline levels in the L rats but remained significantly elevated in the D rats.

Histological changes. In the hearts of rats that died or were euthanized 4–5 h after treatment with 25 mg/kg ephedrine plus 30 mg/kg caffeine, massive interstitial hemorrhage was observed, especially in the subendocardial myocardium. Furthermore, generalized multifocal myofiber degeneration, the presence of hyperbasophilic fragments of myocardial nuclei, and myofiber loss were found. The most noteworthy histological change (Fig. 3) was hemorrhage in the myocardial papillary muscle and focal myofiber necrosis. The inset in Fig. 3 shows higher magnification of the necrotic focus.

DISCUSSION

Dietary supplements containing ephedrine plus caffeine have been reported to induce a number of serious adverse cardiovascular effects, including myocardial infarction and prolonged QT interval, which have prompted the Food and Drug Administration to ban these products (2, 4, 6, 7, 10, 11, 14). Consumption of ephedra- and caffeine-containing supplements appears to have serious consequences in a subset of the population. The mechanisms responsible for this variable response are poorly understood. The aim of this study was to evaluate the effect of age and to investigate potential mechanisms responsible for these adverse effects in Fischer 344 rats treated with ephedrine plus caffeine. Significant differences between the 7- and 14-wk-old rats in responses to treatment were found. Specifically, there was a significant level of mortality in 14-wk-old rats treated with ephedrine (25 mg/kg) plus caffeine (30 mg/kg), but not in the 7-wk-old rats. Rats at 7 and 14 wk of age are both considered young adults, and therefore it was somewhat surprising that such large age-related differences were observed in response to ephedrine plus caffeine treatment. We used the age-dependent difference in mortality to determine which parameters correlated with death.

There are data in the literature showing age-dependent adverse effects of ephedrine plus caffeine (4), but there are no clear estimates for the age-dependent consumption (i.e., the denominator). In the absence of
reliable information regarding consumption by different ages, one can only speculate about age-related susceptibility in humans. The age dependence of adverse effect appears to be reasonably distributed across age groups (7% for <18 yr old, 24% for 18–29 yr old, 32% for 30–45 yr old, 23% for >45 yr old) (4). However, of the three deaths in patients that were definitely or probably related to the use of ephedra-containing supplements, the ages were 37, 38, and 43 yr, and there was also an acute myocardial infarction in a 59 yr old (4). These data are consistent with enhanced susceptibility to mortality in the middle-aged group.

We found greater percentage increases in the HR of 14-wk-old rats that died compared with 7-wk-old rats and 14-wk-old rats that lived after treatment with ephedrine (25 mg/kg) plus caffeine (30 mg/kg). Increases in HR after treatment with ephedrine have been reported previously (18). However, McBride et al. (7) did not report any significant change in HR after a single dose of a supplement containing both ephedra and caffeine. In their study, HR was assessed at baseline and 5 h after treatment. In the present study, significant changes in HR were apparent 1 h after treatment, suggesting an acute HR response.

In addition to changes in HR, other changes were observed in cardiac function. High doses of ephedrine in combination with caffeine caused QTc interval prolongation, which was consistent with previous studies with human subjects (7). The increase in QTc interval was similar in both 7- and 14-wk-old rats treated with 25 mg/kg ephedrine plus 30 mg/kg caffeine, despite the differences in mortality between these two age groups. Furthermore, there was no difference in QTc between the 14-wk-old rats that lived and those that died. Hence, although an increase in QTc interval is associated with a greater risk of arrhythmias (5), these data would suggest that the prolongation of the QTc interval was not the primary cause of mortality in this study.

The most reliable indicator of death in this study was the early rise in temperature to above 40°C. This rise in temperature occurred 1 h after treatment with ephedrine plus caffeine in the D rats. In experiments with another stimulant, (±)-3,4-methylenedioxyamphetamine hydrochloride (nicknamed “ecstasy”), a role for hyperthermia and uncoupling proteins (UCPs) in toxicity was demonstrated (9).

A sustained increase in β1-adrenergic receptor stimulation has been shown to lead to ischemia resulting from an oxygen supply-and-demand imbalance (8, 13). Therefore, a high concentration of catecholamines can induce ischemic injury. An increase in temperature would exacerbate this ischemia, as it is well known that higher body temperatures increase metabolic rate and enhance the risk of ischemic injury. Because an increase in β-adrenergic stimulation may also elevate UCP-2 and -3, this could also induce an increase in body temperature. Hypoxia and ischemia can lead to the release of factors stimulating both vasoconstriction (endothelin) and vasodilatation (adenosine). However, caffeine is a well-known antagonist to adenosine receptor binding (3). Thus, in the presence of caffeine, ischemia coupled with sympathetic stimulation might be expected to produce vasoconstriction. Aadenosine also antagonizes the effects of catecholamines on the myocytes (12, 15). Hence, ischemia with concurrent caffeine-induced sympathetic stimulation may lead to vasoconstriction in the coronary vasculature. Furthermore, the hyperthermic response in the D rats may have enhanced ischemic injury of the myocardium.

In summary, we found a greater increase in body temperature and a greater percent increase in HR over baseline in 14-wk-old rats treated with ephedrine (25 mg/kg) plus caffeine (30 mg/kg) compared with 7-wk-old rats. These differences were particularly notable in the 14-wk-old rats that died compared with either the 14-wk-old rats that lived or the 7-wk-old rats. The combination of ephedrine and caffeine may increase the risk of myocardial ischemic injury due to an increase in body temperature coupled with myocardial ischemia due to an oxygen supply-demand imbalance and/or vasospasm. The occurrence of ischemic injury, coupled with an exacerbated hyperthermic response to ephedrine plus caffeine, may have been the primary causes of death in some of the 14-wk-old rats.

ACKNOWLEDGMENTS

The authors thank Martha Harris, Eric Haskins, and Louise Harris for the oral gavage dosing of the rats. We also thank Brad Collins and Michael Veselica of Research Triangle Institute for formulating the ephedrine and caffeine, Beth Gladen of the National Institute of Environmental Health Sciences for assistance with the statistical analysis, and Dr. Ken Yamamura for assistance with ECG analysis.

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