Visceral pain decreases tolerance to blood loss in conscious female but not male rabbits

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Shafford HL, Schadt JC. Visceral pain decreases tolerance to blood loss in conscious female but not male rabbits. Am J Physiol Regul Integr Comp Physiol 293: R721–R728, 2007. First published May 23, 2007; doi:10.1152/ajpregu.00705.2006.—Pain is a component of traumatic blood loss, yet little is known about how pain alters the response to blood loss in conscious animals. We evaluated the effects of colorectal distension on the cardiorespiratory response to blood loss in six male and six female conscious, chronically instrumented New Zealand White rabbits. The goal of these experiments was to test the hypotheses that 1) colorectal distension would increase tolerance to hemorrhage (i.e., increase the blood loss required to decrease mean arterial pressure ≤ 40 mmHg); and 2) the increase in tolerance would be similar in male and female rabbits. For hemorrhage, venous blood was withdrawn until mean arterial pressure decreased to ≤40 mmHg. Conscious rabbits underwent three treatments in a balanced design: a control hemorrhage, hemorrhage with a colorectal balloon present but not inflated (sham CRD), and hemorrhage in the presence of colorectal distension (CRD). Colorectal distension reproducibly increased mean arterial pressure, decreased respiratory rate, and did not change heart rate. There was no difference in control blood loss between males (21.8 ± 0.3 ml/kg) and females (21.6 ± 0.3 ml/kg). However, although CRD blood loss did not change in males (22.8 ± 0.3 ml/kg), it was significantly less than control in females (19.1 ± 0.3 ml/kg; P = 0.004). Thus, in conscious rabbits, colorectal distension alters cardiovascular control during hemorrhage. Furthermore, colorectal distension did not improve tolerance to blood loss in males or females as hypothesized but instead decreased tolerance to blood loss only in females.

hemorrhage; sex differences; colorectal distension

THE CARDIOVASCULAR RESPONSE to blood loss in conscious animals, including humans, can be divided into two phases (40). Initially, during phase 1, mean arterial pressure is well maintained. Continued blood loss leads to an abrupt transition from phase 1 maintenance of arterial pressure to phase 2 hypotension. The blood loss required to produce the transition from phase 1 to phase 2 may be considered an indicator of an animal’s tolerance to hemorrhage. For example, we know that some psychological stressors (e.g., air jet and oscillation stress) interact with the physical stress of hemorrhage and extend the blood loss necessary to precipitate phase 2 (38, 39). Understanding the forces that can alter an animal’s ability to compensate in the face of hemorrhage may ultimately lead to improved treatment of traumatic blood loss.

Painful sensory input may be another example of a psychological stressor (2) capable of altering the response to blood loss. Although pain often accompanies traumatic blood loss, little is known about how this sensory stimulus affects the response to blood loss in conscious animals. This is despite the close interaction between central nervous system regulation of cardiovascular function and nociceptive inputs (25, 34, 47) and numerous studies demonstrating that noxious stimuli alter cardiovascular (1, 5, 6, 31) and respiratory control (11, 32).

We feel that evaluating the cardiovascular and respiratory responses to blood loss in a conscious animal in the presence of pain may begin to approximate the response to clinically significant blood loss. Thus, the aim of this study was to evaluate the effect of a painful visceral stimulus, colorectal distension, on the cardiovascular and respiratory responses to blood loss in conscious, chronically instrumented male and female rabbits. We tested the following hypotheses. First, since other psychological stressors increase tolerance to blood loss (38, 39), we hypothesized that colorectal distension would have a similar effect. Second, although some reports indicate that females are more sensitive to colorectal distension (18, 22), evidence for definitive sex differences in the behavioral and affective response to visceral pain is inconclusive (for review, see Ref. 3). In addition, there appears to be little difference between males and females in the hemodynamic response to hypotensive hemorrhage (14, 44). Therefore, our second hypothesis was that the increase in tolerance to blood loss during colorectal distension would be similar in male and female rabbits. To our knowledge, this is the first study to evaluate the effect of visceral pain on the response to blood loss in conscious animals.

METHODS

Animals and instrumentation. All procedures were approved by the University of Missouri Animal Care and Use Committee and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (27a). Six female and six male New Zealand White rabbits weighing 3.2 ± 0.1 and 3.3 ± 0.1 kg (means ± SE), respectively, were chronically instrumented with indwell ing catheters and diaphragmatic electromyographic (dEMG) electrodes. Antibiotics (enrofloxacin, 22.7 mg sc; Baytril, Bayer) were administered the day before surgery. Food but not water was withheld 15–20 h before surgery. Anesthesia was induced and maintained with halothane in oxygen via face mask (5% for induction, 0.5–3.5% for maintenance). The analgesic buprenorphine hydrochloride (0.06 mg sc; Buprenex, Reckitt Benkiser) was administered to all rabbits before and after each surgery. Rabbits were allowed to recover from surgery a minimum of 2 wk before the start of experiments.

Catheters and EMG electrodes. A midline laparotomy was performed for implantation of nonocclusive abdominal venous and arterial catheters (17). The arterial catheter allowed for recording of pulsatile arterial pressure, and the venous catheter was used for blood withdrawal and administration of drugs. EMG electrodes (42) were

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implanted in the diaphragm to record diaphragmatic muscle activity and allow monitoring of respiratory rate. A ground electrode was secured to the abdominal wall. Catheters and EMG wires were tunneled under the skin and exteriorized at the base of the neck.

Colorectal distension. Mechanical distension of the distal colon and rectum was performed by manually inflating a 5-cm balloon with water. A double-lumen balloon catheter allowed monitoring of balloon pressure during inflation. Acute colorectal balloon placement was achieved by placing the rabbits on their backs with their eyes covered until they became relaxed (1–3 min). The well-lubricated balloon was inserted 9 cm into the rectum and gently secured to the tail, and the rabbit was returned to the rabbit box. In the course of a colorectal distension experiment, we first performed a 30-s test inflation (i.e., ≤20 mmHg) to check for the ability to register changes in balloon pressure. Colorectal distension was then performed by manually inflating the balloon until mean arterial pressure increased by 10–15 mmHg or colorectal balloon pressure reached 90 mmHg. This limit of 90 mmHg was used to avoid tissue damage (20).

All rabbits underwent training to accustom them to placement of the colorectal balloon catheter (≤10 min) and being in a rabbit box (33 × 15 × 18 cm) in the laboratory setting. Training sessions were performed several times during the week before initiating experiments and subsequently on each day preceding an experiment. Over a 2-wk period rabbits underwent progressively longer colorectal distension. The initial duration of the distension was 1–3 min and progressed up to 25 min before colorectal distension plus hemorrhage experiments were performed. Colorectal distension experiments were done three times per week if the distension duration was less than 15 min and no more than twice per week if the distension duration was greater than 15 min. To assess stability of the cardiovascular and respiratory changes associated with colorectal distension, we evaluated the response of eight rabbits (2 female) to 25 min of colorectal distension (Fig. 1).

Colorectal distension plus hemorrhage. All experiments were performed on conscious rabbits accustomed to sitting quietly in a rabbit box that restricted their movement. Animals were fasted for 15–20 h before each experiment. On the day of the experiment, rabbits were heparinized (sodium heparin, 2,000 units iv; Elkins-Sinn), the arterial catheter was connected to a pressure transducer, and the EMG electrode wires were connected to custom-built differential amplifiers (10×) placed in close proximity to the rabbit. Heart rate, mean arterial pressure, and dEMG activity were monitored throughout each experiment. Time was allowed for the animal to reach a steady baseline in terms of heart rate and arterial pressure (i.e., equilibration period).

Three treatments (control, sham CRD, and CRD) were performed with hemorrhage in each rabbit in a balanced design. In control experiments, hemorrhage was performed in the absence of a colorectal balloon catheter. For sham CRD and CRD treatments, acute placement of the colorectal balloon catheter was performed. The colorectal balloon was connected to a pressure transducer, and intraballoon pressure was monitored throughout the experiment. The colorectal balloon catheter was present but was not inflated during hemorrhage in the sham CRD treatment. During CRD treatment, the colorectal balloon catheter was inflated 4 min before blood loss.

For hemorrhage, venous blood was withdrawn into sterile syringes at a fixed rate of 8–9 ml/min until 5 ml after mean arterial pressure reached 40 mmHg. No more than 45% of the animal’s calculated blood volume, or 27 ml/kg, was withdrawn. All animals in this study reached hypotension within this limit. In preparation for future experiments evaluating pharmacological interventions aimed to improve recovery from hypotensive blood loss, a control injection (0.2 ml/kg iv of 0.9% saline, followed by a 2-ml saline flush) was performed 1 min after mean arterial pressure reached 40 mmHg.

Colorectal distension was maintained throughout the hemorrhage and the 5-min recovery. Pressure was released from the balloon after the recovery period by withdrawing water from the catheter. We reinfused the shed blood at the end of each experiment. Experiments involving hemorrhage were separated by at least 4 days in individual rabbits.

Data acquisition and analysis. Data were acquired at 4 kHz using a personal computer-based data acquisition system (Power Lab; AD Instruments, Colorado Springs, CO). Off-line analysis of all records was performed using Spike2 software (CED, Cambridge, UK). The pulsatile arterial pressure signal was used to determine heart rate. dEMG activity was high-pass filtered (200 Hz) to minimize ECG artifact. The signal baseline was set to zero and then rectified and smoothed (time constant = 50–100 ms). Inspiratory bursts of dEMG activity were used to measure respiratory rate.

Repeated-measures analysis of variance (ANOVA) (SigmaStat v3.1; Systat Software, Richmond, CA; or SAS v9.3.1; SAS Institute, Cary, NC) was used to compare mean arterial pressure, heart rate, respiratory rate, and colorectal balloon pressure during 1) 25-min colorectal distension, 2) hemorrhage, and 3) recovery from hemorrhage. For all analyses, significance was set at P < 0.05. Data are means ± SE. The pooled estimate of the variance from the associated ANOVA was used to calculate the SE.

The response to a 25-min colorectal distension was evaluated by comparing 30-s averages of the four measured parameters at four times: 1) during a stable period before the test inflation (baseline), 2) during the first 2 min of the distension (start), 3) during the last 2 min of the distension (end); and 4) within 2 min of release of the pressure in the balloon (release). Stability of the response to colorectal distension was determined by comparing changes in measured parameters between the start and end of the 25-min colorectal distension experiments. Sex differences were not evaluated. Bonferroni’s multiple comparison test was used to compare individual values.

Any effects of the three treatments and/or sex on cardiovascular and respiratory parameters before hemorrhage (prehemorrhage) were assessed using a two-way ANOVA. Sex and treatment were the independent variables.

Hypotensive blood loss was defined as the amount of blood withdrawn when mean arterial pressure reached 40 mmHg. Cardiovascular and respiratory parameters were compared at three time points [before hemorrhage (prehemorrhage), near the end of phase 1 (phase 1) (23), and when mean arterial pressure reached 40 mmHg (phase 2)] by using a three-way ANOVA with time, treatment, and sex as independent variables. If there were no significant effects of sex in this initial analysis, data from males and females were pooled and reanalyzed using a two-way ANOVA with time and treatment as independent variables. Hypotensive blood loss (ml/kg) and cardiovascular parameters at the end of the 5-min recovery period were evaluated using a two-way ANOVA for repeated measures with sex
and treatment as independent factors. Bonferroni’s multiple comparison test was used for post hoc comparison of individual means.

RESULTS

Response to colorectal distension. Figure 1 is an experimental record illustrating the cardiovascular and respiratory response to a 25-min colorectal distension in a conscious rabbit. Table 1 contains summary data from similar experiments in eight rabbits (2 females). Colorectal distension produced statistically significant increases in mean arterial pressure and decreases in respiratory rate (Table 1). The elevation in mean arterial pressure (−20%) was sustained throughout the 25-min distension and returned to baseline within 2 min following release of the pressure in the colorectal balloon. Respiratory rate decreased dramatically (40%) at the start of colorectal distension and remained below baseline throughout the distension and release. Although there was a significant effect of time on heart rate (P = 0.021), comparisons between individual time points were not significant. Thus, colorectal distension was associated with stable changes in arterial pressure for as long as 25 min. The duration of distension during hemorrhage experiments never exceeded 25 min. Previous experiments demonstrated reproducible physiological responses to colorectal distension (see Supplemental Table).

Treatment effects before hemorrhage. Before treatment (control, sham CRD, and CRD), there were no differences in baseline values for mean arterial pressure, heart rate, or respiratory rate (Table 2). The steady-state effects of each treatment on measured parameters are represented by the prehemorrhage time point (see Fig. 3, 0 time). There were no significant interactions of sex and treatment or significant effects of sex on the prehemorrhage values for any parameter. Therefore, values presented represent males and females pooled. Treatment significantly affected prehemorrhage values for mean arterial pressure (P < 0.001) and heart rate (P < 0.001). Prehemorrhage arterial pressure with CRD (88 ± 2 mmHg) was higher than for control (68 ± 2 mmHg; P < 0.001) or sham CRD (73 ± 2 mmHg; P < 0.001). Likewise, prehemorrhage heart rate was higher with CRD treatment (167 ± 3 beats/min) compared with control (143 ± 3 beats/min; P < 0.001) or sham CRD (153 ± 3 beats/min; P = 0.032). Prehemorrhage values for arterial pressure and heart rate were not different between the sham CRD and control treatments. There were no significant effects of sex and/or treatment for prehemorrhage respiratory rate (152, 171, and 147 ± 17 breaths/min for control, sham CRD, and CRD treatments, respectively).

Response to hemorrhage. The response to control hemorrhage in a conscious rabbit is illustrated in Fig. 2A. Maintenance of arterial pressure and increases in heart rate during phase 1 were followed by an abrupt transition to hypotension accompanied by a relative bradycardia during phase 2. Figure 2B illustrates the cardiovascular and respiratory response to hemorrhage in the presence of colorectal distension (i.e., CRD treatment) in the same rabbit. Colorectal distension increased arterial pressure and decreased respiratory rate before blood withdrawal. However, the biphasic nature of the changes in arterial pressure and heart rate associated with hemorrhage was not altered by colorectal distension.

The cardiopulmonary responses of males and females during progressive blood loss are illustrated in Fig. 3. The patterns of change in arterial pressure, heart rate, and respiratory rate were qualitatively similar for all three treatments in males and females. Hemorrhage produced a biphasic response in arterial pressure with maintenance early in hemorrhage, followed by an abrupt drop in pressure. Heart rate increased steadily during phase 1 before falling during phase 2. Respiratory rate was relatively stable over the course of hemorrhage in females and appeared to increase during phase 2 in males. There were no significant three-way interactions of time (prehemorrhage, phase 1, and phase 2), treatment (control, sham CRD, and CRD), and sex for any parameter. There were significant two-way interactions, and those are detailed below.

Changes in mean arterial pressure during hemorrhage. There were no significant interactions or effects involving sex for mean arterial pressure changes during hemorrhage, so results from both sexes were pooled for analysis and presentation. Whereas CRD treatment increased arterial pressure before hemorrhage, the biphasic response to hemorrhage was preserved with all three treatments. However, treatment altered the arterial pressure response to hemorrhage as indicated by the significant treatment by time interaction (P < 0.001). During phase 1, mean arterial pressure did not change from prehemorrhage values for control (70 ± 2 mmHg) or sham CRD (74 ± 2 mmHg) treatments but decreased for the CRD treatment (80 ± 2 mmHg; P < 0.001). Despite this decrease, arterial

Table 1. Stability of cardiorespiratory changes during a 25-min colorectal distension

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Start</th>
<th>End</th>
<th>Release</th>
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<tr>
<td>MAP, mmHg</td>
<td>72 ±1</td>
<td>86 ±1*</td>
<td>83 ±1*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>152 ±4</td>
<td>167 ±4</td>
<td>163 ±4</td>
</tr>
<tr>
<td>RR, breaths/min</td>
<td>207 ±15</td>
<td>141 ±15*</td>
<td>134 ±15*</td>
</tr>
<tr>
<td>Balloon, mmHg</td>
<td>0 ±3</td>
<td>50 ±3*</td>
<td>38 ±3*</td>
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</table>

Mean arterial pressure (MAP), heart rate (HR), respiratory rate (RR), and colorectal balloon pressure (balloon) were measured during a 25-min colorectal distension experiment in 8 rabbits (2 females). Values (means ± SE) are 30-s averages taken at 4 time points: before colorectal distension (baseline), at the start of the colorectal distension (CRD) experiment, at the end of the distension, and after the release of pressure within the colorectal balloon. There was a significant effect of time for MAP (P < 0.001), HR (P = 0.02), RR (P = 0.006), and balloon pressure (P < 0.001); however, there were no differences between individual points for HR. There was no significant difference in MAP between the start and end of the distension. *P < 0.05, significantly different from baseline.

Table 2. Baseline values for males and females in control, sham CRD, and CRD treatments

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Sham CRD</th>
<th>CRD</th>
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<tbody>
<tr>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>70 ±1</td>
<td>69 ±1</td>
<td>73 ±1</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>143 ±4</td>
<td>154 ±4</td>
<td>146 ±4</td>
</tr>
<tr>
<td>RR, breaths/min</td>
<td>193 ±27</td>
<td>196 ±27</td>
<td>172 ±27</td>
</tr>
<tr>
<td>Balloon, mmHg</td>
<td>NA</td>
<td>−2 ±1</td>
<td>2 ±1</td>
</tr>
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</table>

Values (means ± SE) are 20-s averages at baseline during hemorrhage experiments in males (M; n = 6) and females (F; n = 6) during control (no colorectal balloon catheter), sham CRD (acute placement but no inflation of colorectal balloon catheter), and CRD (acute placement and inflation of colorectal balloon catheter) treatments. SE are from pooled estimates by ANOVA as described in Table 1. There was no significant interaction of sex and treatment or any significant effect of sex or treatment for MAP, HR, RR, and balloon pressure, NA, not applicable.
pressure remained higher near the end of phase 1 for CRD than for control ($P < 0.001$) or sham CRD ($P = 0.003$) treatments. During phase 2, mean arterial pressure decreased ($P < 0.001$) to a similar level (39 ± 2 mmHg) for all three treatments.

**Changes in heart rate during hemorrhage.** Compared with control, the increase in heart rate during phase 1 was not altered by sham CRD for either sex or by CRD in female rabbits. However, the phase 1 increase in heart rate was greater with the CRD treatment in male rabbits. Analysis of heart rate changes during hemorrhage demonstrated significant interactions between time and sex ($P = 0.001$) and treatment and sex ($P = 0.043$), as well as a tendency for an interaction of time and treatment ($P = 0.056$). Heart rate increased as expected during phase 1 in all treatments ($P < 0.001$). At the end of phase 1, heart rate was similar for control (238 and 226 ± 8 beats/min) and sham CRD (241 and 226 ± 8 beats/min) in males and females, respectively. In male rabbits, phase 1 heart rate was higher during CRD (290 ± 8 beats/min) than during control ($P < 0.001$) or sham CRD ($P < 0.001$). Phase 1 heart rates in female rabbits during CRD (250 ± 8 beats/min) were not different from control or sham CRD. Heart rate measured during phase 2 was not different from that during phase 1 for control (249 and 239 ± 8 beats/min), sham CRD (264 and 243 ± 8 beats/min), or CRD treatments (288 and 248 ± 8 beats/min), and the values within a treatment were statistically similar for males and females, respectively. Thus the ANOVA results were consistent with a differential effect of treatment on heart rate in males versus females.

The greater phase 1 increase in heart rate in males during colorectal distension plus hemorrhage could reflect a CRD-induced change in the heart rate baroreflex or simply the greater blood loss required to produce hypotension in males during CRD treatment. To indirectly evaluate the heart rate baroreflex, we divided the increase in heart rate during phase 1 by the blood loss (normalized to the rabbit’s weight) during the same period. The resulting ratio should reflect, to some degree, heart rate baroreflex sensitivity. There were no significant effects of sex on this ratio, so the male and female data were pooled and analyzed using one-way ANOVA. Treatment had a significant effect ($P = 0.038$) on this ratio (5.1, 4.8, and 6.2 ± 0.4 beats·min⁻¹·ml⁻¹·kg for control, sham CRD, and CRD, respectively).

**Changes in respiratory rate during hemorrhage.** Colorectal distension did not affect the respiratory rate response to hemorrhage. Respiratory rate changes during hemorrhage were significantly affected by the interaction of time and sex ($P = 0.046$). This result reflects the increase in respiratory rate seen during phase 2 in males but not females (see Fig. 3 and Ref. 45).

**Colorectal balloon pressure.** Colorectal balloon pressure decreased slightly but significantly over the course of the hemorrhage in both males and females. There were no significant interactions or main effects of sex for changes in colorectal balloon pressure. Thus results from males and females were pooled for analysis. Colorectal balloon pressure was affected by time ($P < 0.001$). Specifically, balloon pressures were significantly higher at prehemorrhage (53 ± 2 mmHg) than phase 1 (44 ± 2 mmHg) or phase 2 (45 ± 2 mmHg).

**Sex differences in tolerance to blood loss.** Hypotensive blood loss (the blood loss required to decrease mean arterial pressure ≤40 mmHg) in males and females is shown in Fig. 4. There was a significant interaction of sex and treatment for hypotensive blood loss ($P = 0.006$), reflecting the fact that treatment affected the response to blood loss differently in males and females. Post hoc analysis revealed no difference in hypotensive blood loss between males (21.8 ± 0.3 ml/kg) and females (21.6 ± 0.3 ml/kg) in the control treatment. The difference in blood loss between males (22.1 ± 0.3 ml/kg) and females (20.0 ± 0.3 ml/kg) during sham CRD approached, but did not reach, significance ($P = 0.083$). During CRD, females (19.1 ± 0.3 ml/kg) required significantly less blood removal than males (22.8 ± 0.3 ml/kg; $P = 0.005$) to become hypotensive. Within each sex, differences among treatments were compared. In females, hypotensive blood loss was significantly less than control during CRD ($P = 0.004$), tended to be less than control during sham CRD ($P = 0.072$), and was not different between sham CRD and CRD. By contrast, in males there were no differences in hypotensive blood loss between any of the three treatments.

**Recovery.** Summary data for the three treatments at the end of the 5-min recovery period are contained in Table 3. Animals experiencing visceral pain had higher blood pressure following recovery from blood loss. Although there was no difference...
between treatments in arterial pressure at the end of hemorrhage, there was a significant difference between treatments ($P = 0.016$) at the end of the recovery period. Mean arterial pressure in the CRD treatment was significantly higher than control ($P = 0.025$) and tended to be higher than sham CRD ($P = 0.053$). There was not a significant interaction of sex and treatment or a significant effect of sex for mean arterial pressure at the end of recovery. Similarly, there were no significant effects of treatment and/or sex for respiratory rate. However, there was a significant interaction of sex and treatment for heart rate ($P = 0.037$). Heart rate was higher in males compared with females in the sham CRD ($P = 0.01$) and CRD treatments ($P = 0.009$), respectively, and not different between the sexes in the control treatment at the end of recovery. In these experiments, colorectal distension was sustained throughout the recovery period in the CRD treatment. Balloon pressures were higher during recovery with CRD than with sham CRD treatment and higher in males than in females as indicated by the significant main effects of treatment ($P < 0.001$) and sex ($P = 0.007$), respectively. However, there was not a significant interaction of sex and treatment for colorectal balloon pressure.

Fig. 3. Average changes in cardiovascular and respiratory parameters for males (A) and females (B) during hemorrhage with 3 treatments: control (open circles, solid lines), sham CRD (filled circles, dashed line), and CRD (asterisks, shaded line). Blood loss adjusted to body weight is shown on the abscissa. Symbols represent data points at prehemorrhage, the end of phase 1, and during phase 2 (i.e., when MAP reached 40 mmHg). Error bars (SE) are displaced from the y-axis for clarity; $n = 6$ females and 6 males.
Fig. 4. Blood loss required to produce hypotension in females (filled bars) and males (open bars) in 3 treatments: control, sham CRD, and CRD. Two-way ANOVA revealed a statistically significant interaction between sex and treatment ($P = 0.006$). There was no difference between females and males during control. Placement of the colorectal balloon catheter (sham CRD) tended to reduce tolerance to blood loss in females compared with control ($P = 0.07$). Females tolerated significantly less blood loss during CRD compared with control ($P = 0.004$). Within the CRD treatment, blood loss was significantly less in females compared with males ($P = 0.005$). *$P < 0.05$, significantly different from control. †$P < 0.05$, significantly different from males within CRD, $n = 6$ females and 6 males.

**DISCUSSION**

Traumatic blood loss is the leading cause of death in battlefield conditions (4) and contributes significantly to mortality in the civilian population as well (30, 36, 41). Improving the management of trauma patients suffering from blood loss requires a better understanding of how the cardiovascular and respiratory response to hemorrhage is altered in the presence of painful stimuli. To our knowledge, this is the first study to assess the effect of a painful visceral stimulus on the tolerance to blood loss in conscious animals. The important new finding of this study is that although visceral pain increased blood pressure, it did not increase tolerance to blood loss as predicted. In fact, in females, colorectal distension decreased the blood loss required to produce hypotension.

**Pain and blood loss.** Several earlier studies have tried to assess the impact of painful stimuli and/or injury on the response to blood loss. For example, Kirkman and colleagues [Sawdon et al. (37)] demonstrated that thoracic blast injury in anesthetized rats blunted the tachycardia associated with phase 1 of hemorrhage, and decreased the blood loss required to reduce arterial pressure to 40 mmHg. From this study, it appears that traumatic injury alters the cardiovascular response to hemorrhage and reduces an animal’s tolerance to blood loss. Kirkman, Little, and their colleagues have also used afferent nerve stimulation as a model of somatic pain and/or tissue injury. In acutely prepared, anesthetized pigs, brachial nerve stimulation was associated with lower blood pressure following a fixed-volume hemorrhage (40% blood volume) compared with control (33). The authors concluded that pain compromised maintenance of blood pressure during hemorrhage. However, details related to cardiovascular changes during blood loss (i.e., the blood loss required to produce hypotension) were not provided. Indeed, in a later study, during brachial nerve stimulation, anesthetized pigs were better able to maintain blood pressure in the face of 30% blood volume loss compared with control (16, 27). In contrast to control animals in these studies, “injured” animals did not become hypotensive (mean arterial pressure <65 mmHg), suggesting that injury may increase tolerance to hemorrhage. Similarly, ischemic muscle injury (i.e., tourniquet application) plus hemorrhage in conscious, acutely instrumented rats increased blood loss required to produce hypotension (24) and, thus, increased tolerance to blood loss.

Considering this earlier work, although some studies suggest that somatic pain and/or injury decreases tolerance to blood loss (33, 37), others have reported that injury enhances tolerance to blood loss (16, 24, 27). Interpretation of the results from these studies, as well as direct comparisons with the work reported presently, is complicated by anesthesia (16, 27, 33, 37), limited time for recovery (24), and different painful stimuli. Conscious, chronically instrumented animals were used in our study to avoid the effects of anesthesia and acute surgical preparation. In addition, we performed all three hemorrhage treatments in each rabbit in a balanced design to minimize any effect of the order of the different treatments.

**Colorectal distension and the response to blood loss.** Colorectal distension is a model of visceral pain in human volunteers (35), as well as conscious rabbits (13, 20, 29), rodents (21, 22, 28), and horses (43). Some consider distension of the colon to be both a psychological stressor and a painful stimulus (26). We know that colorectal distension increases stress hormones in humans [e.g., adrenocorticotropic hormone (ACTH) and cortisol (26)], and conscious rats [e.g., corticosterone (15)]. In addition, colorectal distension activates brain regions also stimulated by other psychological stressors (46). Thus we predicted that colorectal distension would alter the response to blood loss similarly to other acute psychological stressors such as air jet and oscillation stress (38, 39).

A variety of stimuli that increase arterial pressure before hemorrhage (e.g., air jet stress, oscillation stress, infusions of phenylephrine and nitric oxide synthase inhibitors) increase tolerance to blood loss in conscious rabbits (23, 38, 39). In contrast, term pregnancy, a physiological state associated with decreased arterial pressure, decreases tolerance to blood loss in conscious rabbits (8, 12). For these reasons, it was surprising to find that colorectal distension, a painful stimulus associated with increased arterial pressure, did not increase blood loss required to produce hypotension. Consequently, it appears that colorectal distension alters cardiovascular control during blood loss.

**Table 3. Recovery values for males and females in control, sham CRD, and CRD treatments**

<table>
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<th>Control</th>
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<tbody>
<tr>
<td></td>
<td>M</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>56±2</td>
<td>56±2</td>
<td>54±2</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>231±4</td>
<td>221±4</td>
<td>248±4*</td>
</tr>
<tr>
<td>RR, breaths/min</td>
<td>271±11</td>
<td>259±11</td>
<td>249±11</td>
</tr>
<tr>
<td>Balloon, mmHg</td>
<td>NA</td>
<td>NA</td>
<td>4±1</td>
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Values (mean ± SE) are 20-s averages at the end of the 5-min recovery period following hypotensive hemorrhage in males (n = 6) and females (n = 6) in control, sham CRD, and CRD treatments. SE are from pooled estimates by ANOVA as described in Table 1. *$P < 0.05$, significantly different from females.
loss in a way differently than other psychological stressors or infusions of pressor agents. Although traumatic injury is often associated with damage to skin, muscle, or bone and somatic pain, it is not uncommon for hemorrhage to occur in combination with obstetrical or gastrointestinal pain. Thus colorectal distension is a clinically relevant model of pain during blood loss. We chose a model of visceral pain that was not associated with overt tissue damage to perform repeated experiments in individual animals. A strength of this investigation is the fact that despite use of a painful stimulus that is not associated with tissue damage, we were able to demonstrate that visceral pain altered the response to blood loss in females.

Sex differences. A novel finding of this study was that sex differences existed in the effects of visceral pain on the response to hemorrhage. Specifically, colorectal distension decreased tolerance to blood loss in females but not in males. Since this sex difference was not observed in the control experiments reported presently or in previous, unpublished studies in our laboratory, we assume volume or rate of blood withdrawal did not account for the difference. The occurrence of sex differences during CRD treatment points to the potential involvement of sex hormones. Rabbits are classified as induced ovulators (also referred to as "reflex ovulators") and do not have a spontaneous estrous cycle (10). For this reason, we did not control for estrous cycle in our female rabbits.

The fact that in females there was no significant difference in hypotensive blood loss between sham CRD and CRD treatments suggests that sham CRD and CRD may alter total blood loss through a similar mechanism (see Fig. 4). It is reasonable to propose that colorectal balloon catheter placement and not inflation accounted for at least part of the decreased tolerance to hemorrhage. The tendency for sham CRD to increase arterial pressure (see Fig. 3 and Table 2) is also consistent with some effect of placement of the colorectal balloon catheter. Since mechanical stimulation of the vagina, mounting by other rabbits, and excitement are all reported to induce ovulation in rabbits (10), the possibility exists that placement of the colorectal balloon catheter stimulated ovulation and subsequent neurohumoral changes. Hormonal changes associated with ovulation in rabbits occur on the order of hours to days (10) or, in other words, on a time scale that might be expected to alter cardiovascular function over the course of multiple hemorrhage experiments performed in this study. As part of the training for this study, rabbits underwent brief colorectal balloon placement the day before experiments regardless of treatment (see METHODS), and importantly, a CRD experiment was performed within 4 days of all but one of the control experiments. Because of the balanced experimental design, if neurohumoral changes associated with ovulation accounted for the observed sex differences, we would have expected to see that blood loss in females was decreased during control experiments as well. Others have reported that tolerance to blood loss decreases in conscious, term-pregnant rabbits (8). Although the mechanisms are not fully understood, a change in baroreflex sensitivity may be responsible, in part, for the decreased tolerance to hemorrhage during pregnancy (7).

It is possible that the greater heart rate increase during colorectal distension plus hemorrhage in males reflected the greater blood loss required to produce hypotension rather than a sex-dependent change in the heart rate baroreflex. If so, the change in heart rate normalized for blood loss should have been similar in males and females. This appears to have been the case, because the ratio reflecting heart rate baroreflex sensitivity was affected by treatment but not by sex. These results suggest that although there may have been a change in the heart rate baroreflex as a result of treatment, this change was similar in males and females. In contrast, the effects of CRD on blood loss varied between males and females, suggesting that changes in baroreflex function do not account for the observed sex differences in tolerance to hypotensive blood loss. However, since baroreflex control of heart rate and the vasculature may vary independently, it is possible that baroreflex control of the vasculature is differentially altered in males and females during CRD. Such an alteration could contribute to the observed difference in tolerance to blood loss. Further evaluation of sex hormone levels is warranted to investigate their potential role in acutely altering the response to blood loss in female rabbits during colorectal distension.

It is also possible that an acute neurohumoral change, not related to sex hormones, was associated with placement of the colorectal balloon catheter and that this change decreased tolerance to blood loss in females. Because these animals had undergone colorectal distension experiments before the hemorrhage series, the presence of the balloon catheter may have been associated with anticipation of visceral pain. Consequently, placement of the balloon catheter may have been an acute anticipatory stressor that differentially altered the response to blood loss in males and females. One piece of evidence suggests that anticipation of a painful stimulus is a stressor capable of acutely altering neurohumoral function. Specifically, anticipation of colorectal distension is associated with increased plasma ACTH and cortisol in human subjects (26). A second piece of evidence suggests that sex differences exist in the response to anticipation of pain. In a recent study, women had increased neuronal activity compared with men in regions of the brain associated with fear and anxiety during anticipation of a painful electrical shock (9). Potential sex differences in the neurohumoral response to anticipation of visceral pain might account, in part, for the decreased tolerance to blood loss seen in females in the current study.

Recovery from blood loss. Colorectal distension was associated with improved recovery of arterial blood pressure after hypotensive hemorrhage. Distension of the colon may have resulted in release of neurohumoral agents that aid in recovery of blood pressure and/or plasma volume after blood loss. This appears to be the case in anesthetized rats, where vasopressin levels increased following repeated colorectal distension (18). Thus enhanced recovery of arterial pressure following CRD may have been due to pain-induced release of vasopressin or another vasoactive compound.

In conclusion, colorectal distension increases prehemorrhage blood pressure but does not change the biphasic nature of the response to blood loss. Unlike other stressors that increase arterial pressure, colorectal distension does not improve tolerance to blood loss. In contrast, colorectal distension decreases tolerance to blood loss in females and does not change tolerance to blood loss in males.

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