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Cortisol release and heart rate variability in sport horses participating in equestrian competitions

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Abstract Equestrian competitions require both physical activity and mental adaptation in horses. Cortisol, heart rate, and heart rate variability (HRV) are accepted stress parameters and, in this study, have been determined in horses (n = 13) participating in equestrian competitions for up to 3 consecutive days. Participation in competitions caused an increase in salivary cortisol concentrations (e.g., on day 1 from 1.0 ± 0.2 before to 2.2 ± 0.4 ng/mL after the competition, days 1 and 2: $P < 0.001$, day 3: $P < 0.05$) and an increase in heart rate (days 1 and 2: $P < 0.001$, day 3: $P = 0.01$). A consistent decrease in HRV occurred only in response to the final competition on day 3 ($P < 0.01$). When horses competing in dressage and show jumping were compared, cortisol release and HRV did not differ between groups, but after the competition, heart rate was lower in dressage than in show jumping horses ($P < 0.05$). Heart rate increased not only during the actual competition but already when horses were prepared in their stables (e.g., day 1: −60 minutes, 38.6 ± 2; −5 minutes, 77 ± 7; competition, 81 ± 10 beats per minute; $P < 0.01$). In conclusion, participation in equestrian competitions caused an increase in cortisol release and heart rate and a decrease in HRV variables. However, competitions were not a major stressor compared with other anthropogenic challenges such as transport, to which horses are exposed regularly.

Introduction

Although domestic horses have been selected over centuries for characteristics that fit the individual equestrian disciplines, a variety of anthropogenic challenges to which horses are exposed have still to be considered as potential stressors. These include equestrian training (Schmidt et al., 2010b) and competitions (Dybdal et al., 1980; Lange et al., 1997; Cayado et al., 2006, Fazio et al., 2008), transport (Baucus et al., 1990; Clark et al., 1993; Friend, 2000; Schmidt et al., 2010a,d), veterinary examinations (Berghold et al., 2007), and exposure to new groups (Alexander and Irvine, 1998).

The stress response in horses can be analyzed by behavioral and physiological parameters. Behavior has been used to study the response of horses to situations

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such as weaning (McCall et al., 1985) and hot iron branding (Lindegaard et al., 2009) and to compare different handling procedures (Fureix et al., 2009) or housing systems (Visser et al., 2008) under animal welfare aspects. However, ridden horses cannot express behavior in the same way as undisturbed or freely moving horses. Thus, especially in ridden horses, physiological parameters provide valuable information. Cortisol is an accepted physiological stress parameter in animals (Prunier et al., 2005). Recently, techniques to analyze cortisol in equine saliva have become available, avoiding the need of venipuncture for blood sampling (Schmidt et al., 2010d). Additional stress parameters are heart rate and heart rate variability (HRV). HRV, that is, short-term fluctuations in the beat-to-beat (RR) interval reflect the stress response of the autonomic nervous system. Increases in the HRV variables standard deviation of the RR interval (SDRR) and root mean square of successive RR differences (RMSSD) reflect a shift toward parasympathetic dominance, whereas reduced values indicate a shift toward sympathetic dominance. Whereas SDRR represents overall variability, RMSSD reflects primarily high-frequency variation dependent on vagal tone (von Borell et al., 2007).

Most research on stress responses to exercise in horses is focused on sports where horses are competing by speed or stamina, such as racing, trotting, and endurance (Leleu and Haentjens, 2010), and many studies have been conducted under experimental conditions on a treadmill (Jimenez et al., 1998; Kurosawa et al., 1998; Golland et al., 1999). In contrast, the equestrian disciplines dressage and show jumping require mainly agility, obedience, skills, and "understanding" from the horse. Cortisol release, and occasionally heart rate, but not HRV, has been determined during show jumping and dressage competitions, with a more pronounced effect of dressage than show jumping (Cayado et al., 2006). Higher plasma cortisol concentrations were found in inexperienced than in experienced show jumping horses (Clayton, 1989), and well-conditioned show jumpers participating regularly in equestrian competitions were not stressed compared with less experienced horses (Covalesky et al., 1992; Lange et al., 1997). In association with equestrian competitions, horses are usually transported to the competition site. It is thus difficult to determine their response to competition without an influence of potentially confounding factors. Transport to the competition augmented cortisol release of horses in response to a long-distance trekking event (Medica et al., 2010) but not to show jumping (Fazio et al., 2008). In neither study, cardiac activity was determined.

HRV has been analyzed in horses during various situations (Clement and Barrey, 1995; Visser et al., 2002; Schmidt et al., 2010a,b,c,d). It decreased during exercise on a treadmill (Thayer et al., 1997) but may be of value to assess autonomic modulation of cardiac activity at low exercise intensities only (Physick-Sheard et al., 2000). Based on HRV in trotters exercised at different intensities, it has been suggested that horses are more relaxed during moderate exercise than when standing still or during anaerobic exercise (Kinnunen et al., 2006). Changes in HRV, to our knowledge, so far have not been determined together with cortisol release in competing horses.

In the present study, we investigated cortisol release, heart rate, and HRV variables in horses participating in show jumping and dressage competitions. Cortisol release reflects adrenocortical function, whereas heart rate and HRV indicate changes in sympathoadrenomedullary activity. We thus hypothesized that both cortisol release and cardiac function change in response to equestrian competition, but the responses of the 2 systems may differ.

Materials and methods

Animals

For the study, 13 German sport horses owned by the Brandenburg State Stud at Neustadt (Dosse), Germany, were available. Mean age of the horses was 6.2 ± 3.8 years, and the group consisted of 2 geldings, 4 mares, and 7 stallions. The horses were kept in individual loose boxes on straw and were fed concentrates 3 times daily (6:00, 12:00, 17:00 hours) and hay twice daily (6:00 and 17:00 hours). Water was available at all times. For at least 6 months before the experiment, horses were exercised on a near-daily basis, that is, 5-6 times per week.

Experimental procedures

All horses in the study participated in equestrian competitions during the Berlin and Brandenburg State Championship in dressage ($n = 7$) and show jumping ($n = 6$) organized at Neustadt (Dosse). Horses thus were not transported to the event and remained in their normal stables between competitions. During the 3-day championship, horses had to qualify themselves for the final competition on the last day. Thus, all 13 horses competed on day 1 (dressage: $n = 7$, show jumping: $n = 6$), whereas 9 horses competed also on day 2 (dressage: $n = 5$, show jumping: $n = 4$), and 4 horses participated in the final competition on day 3 (dressage: $n = 1$, show jumping: $n = 3$). Competitions took place throughout the day between 9:00 and 16:00 hours. The horses were ridden by 7 professional riders (3 female, 4 male) of the Brandenburg State Stud, trained to advanced level.

To determine differences between dressage and show jumping competitions on the parameters studied, for day 1 of the competition, data were analyzed for the group as a whole and also divided by equestrian discipline. Because of the reduced number of horses competing on days 2 and 3, no comparisons between equestrian disciplines were made for these days. Saliva samples were collected repeatedly, beginning 2 days before the competition. For each competition, the RR interval was recorded before, during, and after riding, and heart rate and HRV variables were calculated. The study was approved by the Brandenburg State Ministry for Rural Development, Environment and Consumer Protection (license number 32-2347/5+21-87915/2007).

Salivary cortisol

Saliva samples for determination of basal cortisol concentrations were taken on 2 days before the first competition at 7:00, 7:30, 14:00, 14:30, 20:00, and 20:30 hours. Additional samples were collected on each competition day at 60 minutes and 30 minutes before riding. Further samples were taken immediately after each competition (time 0) and at 5, 15, 30, 60, 90, 120, and 180 minutes thereafter. Horses were made familiar with the procedure of saliva sampling on 2 days before the study. Saliva was collected with cotton rolls (Salivette; Sarstedt, Nümbrecht-Rommelsdorf, Germany) placed loosely onto the tongue of the horse for 1 minute with the help of a surgical arterial clamp until the swab was well soaked. Concentrations of cortisol were determined with a direct enzyme immunoassay without extraction (Palme and Möstl, 1997) validated for equine saliva (Schmidt et al., 2009). Because the antiserum cross-reacts with cortisone and some cortisone metabolites, values have to be interpreted as cortisol immunoreactivity. The intra-assay coefficient of variation was 5.0%, the inter-assay variation was 6.7%, and the minimal detectable concentration was 0.3 pg/well.

RR interval and HRV

The RR interval was recorded with a mobile recording system (S810i; Polar, Kempele, Finland), as described previously (Schmidt et al., 2010a,d). Recordings were made for 1 hour directly before riding, continuously during riding, and for 1 hour thereafter. Horses were prepared for the recordings by putting on a girth with a nonactivated recording device for 1-2 hours on 2 days before the experiment. HRV was analyzed with the Kubios HRV software (Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland). To remove trend components, data were detrended and, in addition, an artifact correction was made (Schmidt et al., 2010b,d) following established procedures (Tarvainen et al., 2002). In our study, heart rate, SDRR, and RMSSD were calculated. The means for variables were determined for subsequent periods of 5 minutes each.

Statistical analysis

Statistical analysis was done with the SPSS statistics package (version 17.0; SPSS, Chicago, IL), and nonparametric tests were used throughout. Changes in salivary cortisol concentrations, heart rate, and HRV variables over time were analyzed by Friedman test, and comparisons between equestrian disciplines for each time point were compared by Mann–Whitney U test. A $P < 0.05$ was considered significant. All data given are means ± standard error of mean (SEM).

Results

Salivary cortisol

On the 2 days preceding the competitions, cortisol concentrations decreased throughout the day on both days (day −2: $\chi^2 = 36.5$, $df = 5$, $P < 0.001$; day −1: $\chi^2 = 23.6$, $df = 5$, $P < 0.001$; Figure 1). Cortisol concentrations

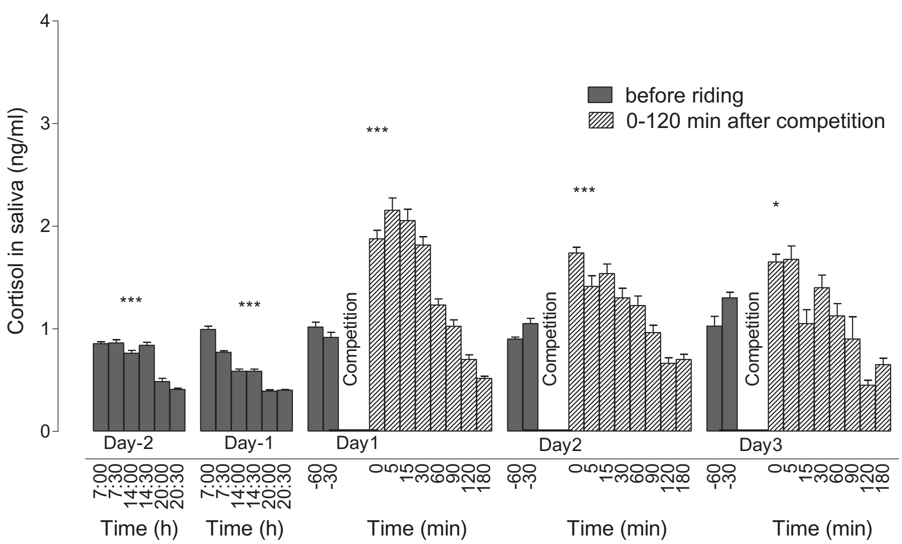

Figure 1 Salivary cortisol concentrations in horses in response to equestrian competitions (day 1: n = 13, day 2: n = 9, day 3: n = 4). Asterisks indicate significant differences over time for respective days (*$P < 0.05$, ***$P < 0.001$). Values are means ± standard error of mean (SEM).

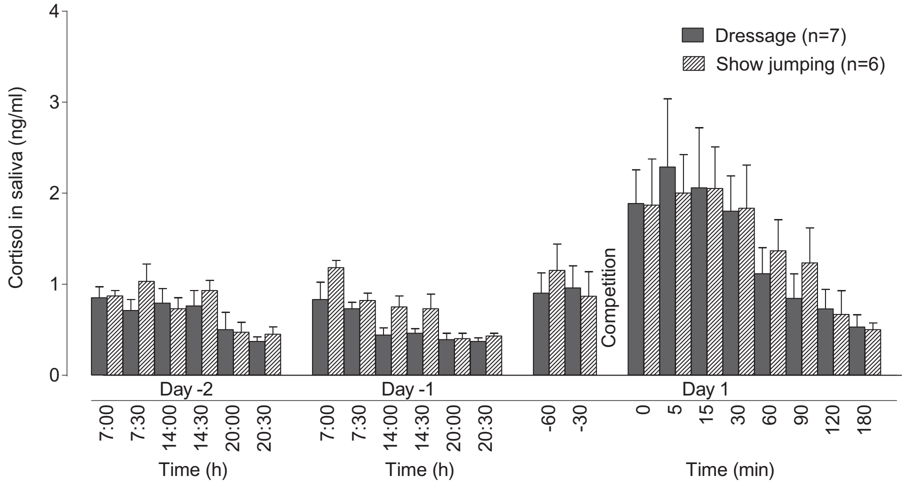

Figure 2 Salivary cortisol concentrations in horses participating in the equestrian disciplines of dressage (n = 7) and show jumping (n = 6). No significant differences between groups were found. Values are means ± SEM.

increased significantly in response to each of the competitions (day 1: $\chi^2 = 69.5$, $df = 9$, $P < 0.001$; day 2: $\chi^2 = 31.1$, $df = 9$, $P < 0.001$; day 3: $\chi^2 = 20.0$, $df = 9$, $P < 0.05$). This response tended to be highest for the first competition (2.2 ± 0.4, second competition: 1.7 ± 0.2, third competition: 1.7 ± 0.3 ng/mL, Figure 1) but did not differ significantly. Baseline values were reached again within approximately 1 hour after the competition. When the competing horses were divided by equestrian discipline (day 1), cortisol concentrations did not differ significantly between groups (Figure 2).

Heart rate and HRV

Heart rate increased significantly in response to the competitions on all 3 days (day 1: $\chi^2 = 50.1$; $df = 8$, $P < 0.001$; day 2: $\chi^2 = 33.6$, $df = 8$, $P < 0.001$; day 3: $\chi^2 = 20.2$, $df = 8$, $P = 0.01$, Figure 3A). A pronounced increase in heart rate occurred already in the stable during preparation of the horses (grooming and saddling) at 5 minutes before a rider was mounting and thus before any physical activity of the horse (comparison of the 3 basal values only: day 1: $\chi^2 = 10.4$, $df = 2$, $P < 0.01$; day 2: $\chi^2 = 16.0$, $df = 2$, $P < 0.001$; day 3: $\chi^2 = 6.0$, $df = 2$, $P = 0.05$). Heart rate remained at this increased level during the competition and decreased to precompetition baseline values within 30 minutes thereafter. Baseline heart rate on day 1 at 60 minutes before the competition was 38.6 ± 2 beats per minute (bpm), and highest values reached on days 1, 2, and 3 during the competition were 81 ± 10, 94 ± 6, and 92 ± 9 bpm, respectively. When horses competing in dressage and show jumping were compared (day 1 only), differences between equestrian disciplines could be demonstrated. Heart rate was lower in dressage horses than in show jumpers at the end of the competition (46 ± 3 vs. 81 ± 9 bpm; $z = -2.9$, $P < 0.05$) and immediately thereafter (43 ± 4 vs. 53 ± 12 bpm; $z = -2.6$, $P < 0.05$; Figure 4A).

The HRV variable SDRR only tended to decrease in response to competition, but this decrease at no time reached statistical significance. For RMSSD, a significant decrease was found during preparation of the horses for competition in the stable and during the actual competition on day 2 ($\chi^2 = 19.4$, $df = 8$, $P < 0.05$) and was nearly reached on day 3 ($\chi^2 = 15.2$, $df = 8$, $P = 0.055$; Figure 3C). None of the HRV variables differed significantly between horses competing in dressage and show jumping.

Discussion

Participation of horses in equestrian competitions caused an activation of hypothalamo-adrenocortical function, reflected by a transient increase in cortisol release, and an increase in sympathoadrenal activity, indicated by a rise in heart rate. This response during riding is not surprising and at least in part caused by the physical activity requested from the animals. However, heart rate already increased in the stable during preparation for the competition, that is, grooming and saddling of the horses. The animals apparently correlated being groomed and saddled with riding already before any physical activity was requested from them and reacted with increased sympathetic activity. Sympathoadrenal activity was thus not only induced by physical activity of the animals but also by emotional factors in the horses before and most likely also during the competition. An anticipatory stress response is well known in humans (e.g., Starcke et al., 2008; Edwards and Kurlander, 2010; Preuss et al., 2010). It has been found in infants as young as 6 months (Haley et al., 2011) and also in adult horses anticipating to be loaded onto a transport vehicle (Schmidt et al., 2010b). In a preliminary study available in abstract form only, heart rate in the horse and its rider was significantly correlated during the warm-up phase of a dressage competition, suggesting a certain synchrony in heart rate between the horse and its rider (Bridgeman et al., 2006).

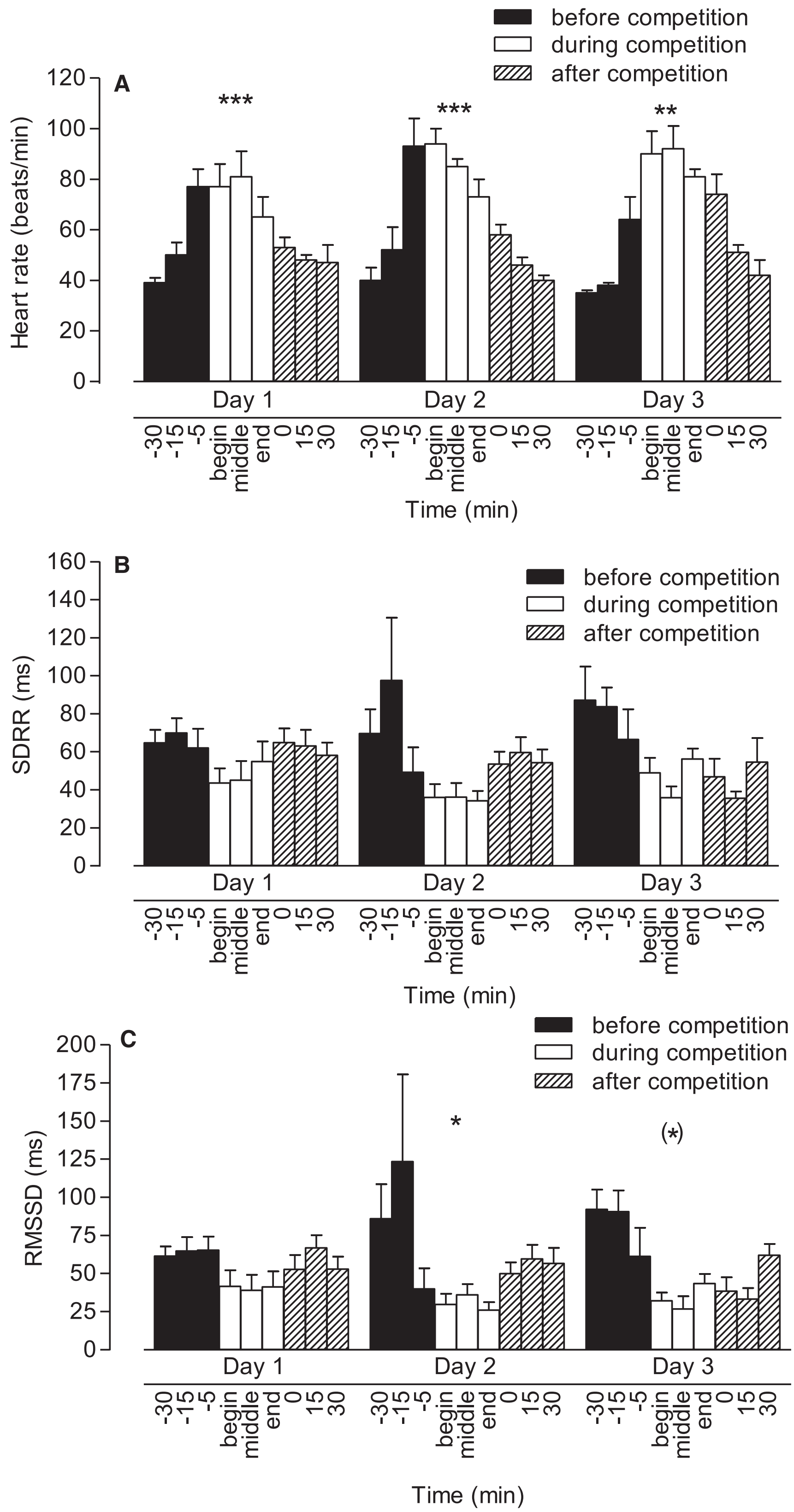

Figure 3 (A) Heart rate and HRV variables (B) SDRR and (C) RMSSD in horses participating in equestrian competitions (day 1: n = 13, day 2: n = 9, day 3: n = 4). Asterisks indicate significant differences over time for respective days (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, $^{(*)}P = 0.055$). Values are means ± SEM. HRV, heart rate variability; SDRR, standard deviation of the RR interval; RMSSD, root mean square of successive RR differences.

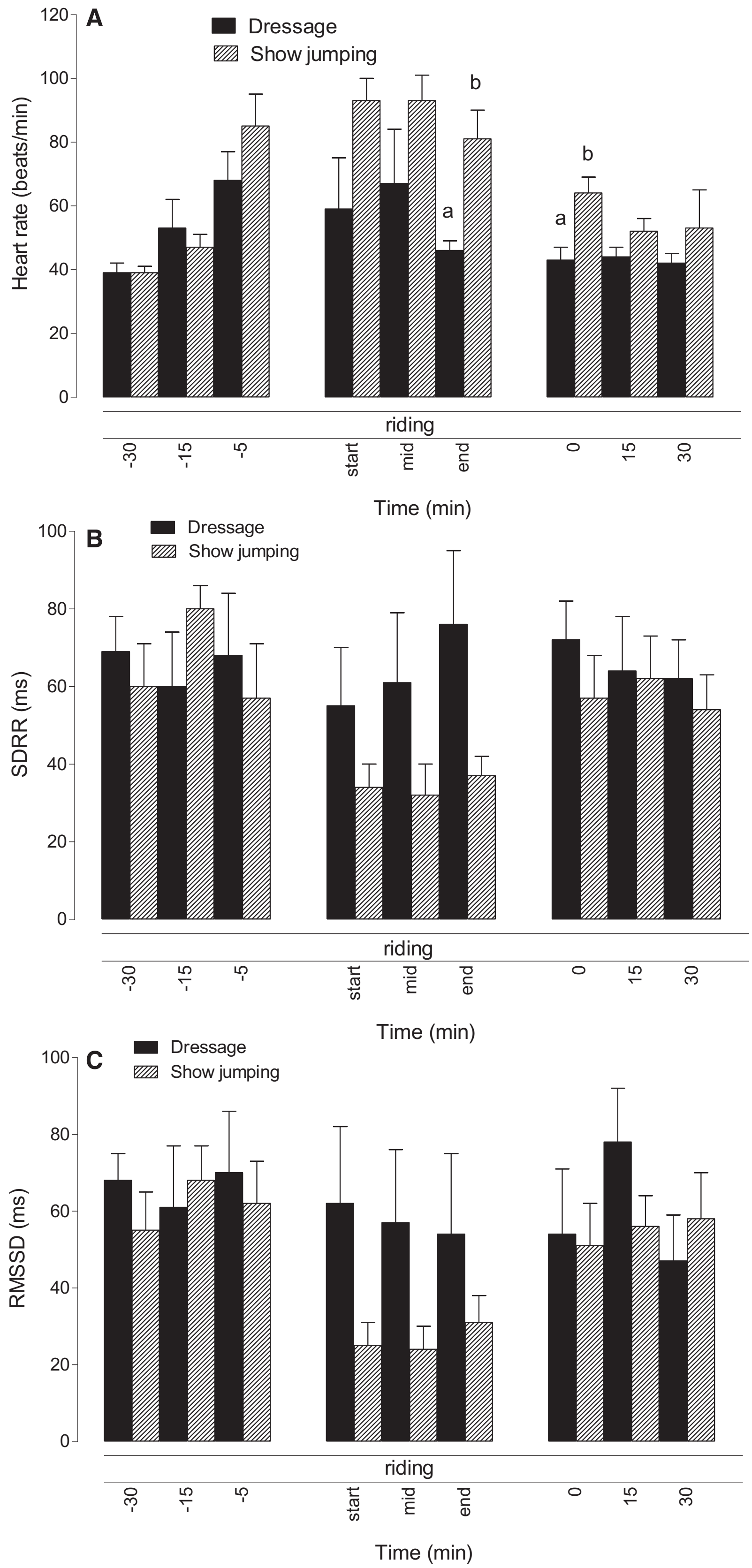

Figure 4 (A) Heart rate and HRV variables, (B) SDRR, and (C) RMSSD in horses participating in the equestrian disciplines of dressage (n = 7) and show jumping (n = 6) before, during, and after riding; (a, b) values with different letters at the same time point differ significantly between groups ($P < 0.05$). Values are means ± SEM.

Based on heart rate and HRV, we could recently demonstrate a stress response independent from physical activity when young horses were mounted for the first time by a rider (Schmidt et al., 2010a). In the current study, changes in HRV were less pronounced than in three-year-old horses, mounted for the first time by a rider (Schmidt et al., 2010a), and decreases in the HRV variable RMSSD occurred only during the second and the third competition. With the demands of the competition increasing each day, this was potentially the most pronounced challenge for the animals. Whereas SDRR reflects long-term overall variability and is influenced by both sympathetic and parasympathetic activity, RMSSD is used to estimate high-frequency beat-to-beat variations that represent mainly parasympathetic activity (von Borell et al., 2007). The decrease in RMSSD thus indicates decreased parasympathetic (vagal) activity.

Heart rate did not only change over time but remained also higher in horses participating in show jumping compared with dressage for day 1 of the competitions. No such differences between equestrian disciplines were found for the HRV variables SDRR and RMSSD. The difference in heart rate most likely reflects different physical demands in dressage and show jumping, whereas the less pronounced changes in HRV and lack of significant HRV differences between groups may indicate that the presentations were not perceived as major stressors by the horses.

Salivary cortisol concentrations in horses participating in equestrian events were lower than concentrations determined with the same analytical techniques in horses during road transport (Schmidt et al., 2010b,c,d) or in foals at weaning (Erber et al., 2012b), but in the competing horses, were in the same range as in young horses during initial equestrian training (Schmidt et al., 2010a). Peak cortisol concentrations in response to transport were 4- to 5-fold higher (Schmidt et al., 2010b,d) than in the current study. Thus, although competitions were associated with an increase in hypothalamo-adrenocortical activity, they cannot be considered a major stressor compared with transport of horses. This is in agreement with previous studies including experienced show jumping horses (Covalesky et al., 1992; Lange et al., 1997). Horses in the present study did not have to be transported to the competition. Even in transported horses, cortisol concentrations rapidly return to baseline values after unloading (Schmidt et al., 2010b,c,d), explaining also why before equestrian competitions, cortisol concentrations did not differ between transported and nontransported horses (Fazio et al., 2008).

In agreement with previous reports, baseline salivary cortisol concentrations determined on 2 days before the championship showed a diurnal rhythm, with highest concentrations in the morning and a decrease throughout the day (Becker-Birck et al., 2012). Salivary cortisol thus mirrors the well-known diurnal rhythm in plasma cortisol concentrations in the horse (Hoffsis et al., 1970; Bottoms et al., 1972). Although the equestrian competitions were distributed throughout the day, the diurnal changes determined on 2 days before were clearly less pronounced than the increase induced by the equestrian events. Thus, a potential influence of the time of day, although it may exist, had no major effects on cortisol release during competitions.

As hypothesized, changes in cortisol release did not exactly parallel the changes in heart rate and HRV. Stressful stimuli initiate both a hypothalamo-pituitary-adrenocortical response and an adrenomedullary-sympathetic nervous system response, but the time course of these responses differs. The most immediate stress response is an increase in adrenomedullary and sympathetic nervous activity, followed with some delay by a longer-lasting increase in cortisol release. Cortisol improves the animal's fitness by energy mobilization (Raynaert et al., 1976) and changes in behavior (Korte et al., 1993). The acute stress of hot iron branding in foals elicited an immediate increase in heart rate, lasting <5 seconds, whereas salivary cortisol concentrations increased within approximately 30 minutes but remained elevated for several hours (Erber et al., 2012a). Comparable differences between changes in cortisol release and heart rate were to be expected in competing horses.

In conclusion, horses responded to equestrian competition with a transiently increased cortisol release. Although clearly evident, cortisol release in response to equestrian events was lower than in horses exposed to transport (Schmidt et al., 2010b,d,c). Heart rate increased already before the actual competition, indicating an anticipatory stress response. Changes in cortisol release were not clearly paralleled by changes in heart rate and HRV.

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