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Is vasopressin a "stress hormone" in the horse?

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Summary

The aim of this study was to compare the effects of stress caused by exercise and dehydration and stress induced by restraint and use of a nasogastric tube on the plasma arginine vasopressin (AVP) concentration in the horse. Two experiments were performed. In the first experiment, four horses were studied during three different fluid status (normohydrated, dehydrated and hyperhydrated) when performing an incremental exercise test on a treadmill. In contrast to our expectations, the exercise-induced increase in AVP after hyperhydration was much greater than when the horses were exercised during normo- and dehydration. We hypothesised that the high level of AVP in the hyperhydrated horses was a "stress reaction" caused by the naso-gastric administration of fluid. Therefore, in the second experiment, the effects of the use of a naso-gastric tube, combined with different methods of restraint and fluid administration, on plasma AVP concentrations were studied in four horses. Dependant on the method of restraint, AVP increased to different levels when the naso-gastric tube was used. AVP decreased immediately when the tube was withdrawn. In our study the combined use of a naso-gastric tube and twitch induced a much greater AVP response than exercise even following dehydration. Our results suggest a role of AVP in mediating stress responses in the horse. The significance of AVP during exercise and the possible effects of high levels on the haemodynamics in the exercising horse needs further investigations.

Keywords: horse, exercise, fluid status, naso-gastric tube, restraint, stress

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Ist Vasopressin ein Streßhormon beim Pferd ?

Ziel dieser Arbeit ist der Vergleich der Auswirkungen von Streß durch Körperbelastung und Dehydratation des Pferdes mit der Streßinduktion durch Anwendung verschiedener Zwangsmaßnahmen in Kombination mit der Verabreichung einer Nasenschlundsonde bezogen auf die Plasmakonzentration von Arginin-Vasopressin (AVP).

Zu diesem Zweck wurden 2 Experimente durchgeführt. Im ersten Versuch wurde 4 Pferden auf einem Laufband belastet, wobei die Tiere sich jeweils in verschiedenen Hydratationszuständen befanden (normo-, hyper- und dehydriert). Entgegen aller Vermutungen stieg die AVP-Konzentration im Plasma nach Beendigung der Belastung bei den hyperhydrierten Pferden viel stärker an als im normo- oder dehydrierten Zustand. Die Autorinnen vermuten, daß der hohe AVP-Spiegel bei hyperhydrierten Pferden als Streßreaktion auf die Verabreichung der zusätzlichen Flüssigkeit über die Nasenschlundsonde zu werten ist.

Im zweiten Versuch wurde den Pferden unter verschiedenen Zwangsmaßnahmen eine Nasenschlundsonde geschoben und Flüssigkeit in den Magen geleitet. Dabei wurden die Vasopressin-Werte im Plasma ermittelt. Der Umfang des Anstiegs der AVP-Konzentration hing von der Art der Zwangsmaßnahme ab. Es wurden unterschiedliche Level erreicht, obwohl in allen Fälle eine Nasenschlundsonde geschoben wurde. Nach Herausziehen der Sonde fiel die Vasopressinkonzentration im Blut rapide ab.

Der höchste Anstieg des AVP-Spiegels wurde bei der Kombination von Nasenbremse und Schlundsonde verzeichnet. Der gemessene Wert lag weit über den Vasopressinkonzentrationen nach der Laufbandarbeit. Die Pferde schütteten selbst im dehydrierten Zustand nach der Laufbandbelastung weniger Vasopressin aus als nach der kombinierten Anwendung von Bremse und Schlundsonde.

Die Ergebnisse dieser Studie zeigen, daß Arginin-Vasopressin eine wichtige Rolle bei der unmittelbaren Streßreaktion des Pferdes auf bestimmte Bedingungen hin spielt. Die Bedeutung des AVP-Spiegels während Belastung sollte ebenso in weiteren Studien erforscht werden wie die Auswirkungen hoher Vasopressinkonzentrationen auf die Hämodynamik von Pferden bei Belastung.

Schlüsselwörter: Pferd, Belastung, Flüssigkeitsvolumen, Nasenschlundsonde, Zwangsmaßnahmen, Stress

Introduction

Arginine vasopressin (AVP) is the main water saving hormone in the body. The primary stimuli for its release are an increased plasma osmolality or a decreased plasma volume. Apart from the antidiuretic effect of AVP at low levels, higher concentrations of AVP result in vasoconstriction of arterioles and thereby an increase of the arterial pressure. The plasma concentration of AVP increases during exercise in horses (*Alexander* et al., 1991), but its role during physical activity is not fully understood. It has been shown that AVP also can be released in stressful situations in rats (*Husain* et al., 1979) and during isolation stress in horses (*Alexander* et al., 1988). Recently it has been suggested that AVP is a more potent adrenocorticotropic hormone (ACTH) secretagogue than corticotropin-releasing factor (CRF) in the sheep (*Liu* et al., 1990, *Katoh* et al., 1994) and in the horse (*Alexander* et al., 1993). Fur-

thermore, injections with AVP in both sheep and cattle caused increased plasma levels of ACTH, cortisol and glucose (*Senn* et al., 1994). The aim of this study was to compare the effects of stress caused by exercise and dehydration and stress induced by restraint and use of a naso-gastric tube on the plasma AVP concentration in the horse.

Materials and methods

Animals and experimental procedure

Two experiments were performed, both approved by the Uppsala Local Ethics Committee.

In Experiment I, four trained Standardbred horses (three geldings and one stallion), 5–13 years old, were used. The horses were investigated during exercise at three different fluid status; normohydrated (free access to drinking water), dehydrated (when water had been withheld for 24 h) and hyperhydrated. To hyperhydrate the horses, an upper lip twitch (rope) was applied, a nasogastric-tube inserted and 12 l of 38°C water was administrated into the stomach during a time span of 10 min. After water administration the horses were allowed 30 min of rest before the exercise test. The first blood sample was drawn from a jugular vein catheter, with the horse standing still on the treadmill and after a two min warm-up walk, the horses performed an incremental exercise test at 6.25% incline with speeds increasing from 6, 7, 8 to 9 m/sec with two min trotting at each speed. Blood samples were drawn after 6 and 10 min of exercise, immediately after exercise and at 5, 15, 60 and 120 min post-exercise. After completion of exercise the horses were kept walking on the treadmill until 15 min post-exercise. Before the horses were brought back to their boxes, they were washed with body warm water. After a blood sample was drawn at 60 min post-exercise the horses had free access to water from buckets.

In Experiment II, four Standardbred geldings, 4–9 years old, were used. The horses had free access to drinking water from automatic waterers in their boxes. All horses underwent three treatments at random order, one treatment a day during a four day period. A naso-gastric tube was placed in position during;

1) restraint by holding of the ears
2) restraint by an upper lip twitch (rope)
3) restraint by an upper lip twitch and administration of 10 l of body warm (38°C) saline-solution (9 g NaCl/l).

The same persons handled the horses in all treatments. All horses were well accustomed to handling and experiments before the study, but one horse was more easily excited than the other three. We did not manage to insert the tube in this horse without using the twitch and therefore only three horses participated in the treatment restraint by ear holding. In the morning of the experimental day, a catheter was inserted into one of the jugular veins under local anaesthesia with lidocain (Xylocain®, Astra, Sweden) and the first blood sample was taken at 08.00 h in the box, immediately before feeding. After two hours, when the horses had finished their morning feed, the first horse was taken to a stock in a room next to their stable. A second blood sample was drawn and the horse underwent one of the three treatments. All treatments lasted for 10 min, i.e. the naso-gastric tube was inserted for this time. Blood samples were drawn 2, 4, 6, 10, 15, 20 and 30 min after the treatment had started. After 30 min the horse was taken back to its box, where blood samples were drawn 60, 90 and 120 min after the treatment had started. The horses were treated in the same order on all experimental days, starting between 10.00 h and 14.00 h.

Analyses

Blood was collected into pre-chilled K_3-EDTA tubes containing aprotinin (Trasylol®, Bayer, Leverkusen, Germany) for AVP analysis and into Li-Heparin tubes for plasma osmolality analysis. The plasma from the K_3-EDTA tubes was stored at –20°C for 24 h and thereafter at –80°C. Before AVP was analysed the plasma was extracted with ethanol. Analysis were made by a commercially available radioimmunoassay (RIA) kit (Vasopressin Rapid RIA, Bühlmann Lab AG, Switzerland). According to the manufacturer the specificity for AVP is 100%. The RIA was validated for horse plasma in our laboratory and the spiking recovery was 91.5%, the intra-assay precision 8% and inter-assay precision 15%. The plasma from the Li-Heparin tubes was analysed for plasma osmolality by freezing point depression technique (Advanced Osmometer Inc, 3 W Wide Range Osmometer, Roebling, Germany).

Statistics

Data was examined by analysis of variance using the General Linear Model procedure of Statistical Analysis Systems (*Anon*, 1987). For testing significances within treatment paired t-test was used. For testing differences between treatments the variation between horses within treatment was used as an error term. Significance was set at $P<0.05$. All data are presented as mean values (±SE).

Results

Experiment I

The plasma osmolality was increased in the dehydrated group prior to and following exercise until the horses had drunk. Exercise resulted in an increase in plasma osmolality in all treatments (Fig. 1a). The plasma osmolality was similar during normohydration and hyperhydration throughout the exercise phase and until 15 min post-exercise. At 60 and 120 min post-exercise the hyperhydrated group had a significantly lower plasma osmolality than the normohydrated and dehydrated groups.

The hyperhydrated group had a tendency to have a higher pre-exercise plasma AVP concentration, 4.0±2.0 pmol/l, compared to 1.2±0.1 pmol/l and 1.9±0.1 pmol/l in the normo- and dehydrated group, respectively (Fig. 1b). The AVP level increased in all groups during exercise. The peak values at the end of exercise were 7.3±2.6 pmol/l when the horses were normohydrated and 9.5±1.9 pmol/l when they were dehydrated. Hyperhydration caused a significantly greater peak value of 26.3±14.2 pmol/l (Fig. 1b).

Experiment II

The horses responded to the combined use of the twitch and a naso-gastric tube with a great increase in plasma AVP concentration, both with and without fluid administration (Fig. 2). The plasma AVP concentration had a faster increase, from 1.1±0.3 pmol/l to a peak value of 42.5±21.3 pmol/l, when the horses were both restrained by the twitch and given fluid. When the horses were restrained by the twitch AVP increased from 1.1±0.1 pmol/l to 35.5±16.8 pmol/l. Ear-holding and use of the naso-gastric tube caused a slight increase in AVP from 1.1±0.3 pmol/l to 6.6±3.8 pmol/l. The AVP concentrations decreased when the tube had been withdrawn in all treatments.

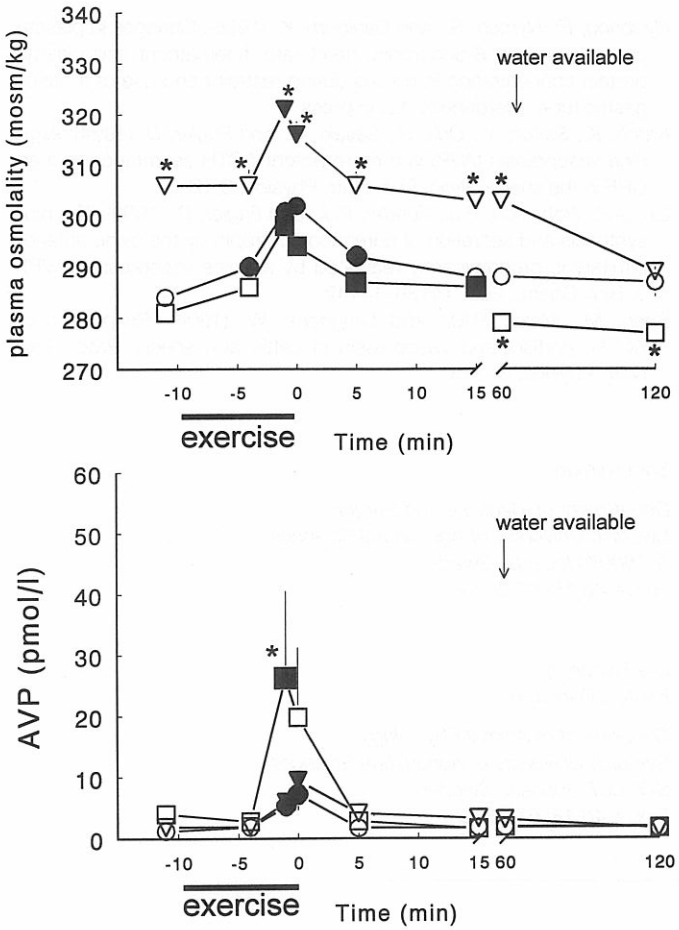

Fig. 1: Plasma osmolality (1a) and plasma AVP concentration (1b) in four horses before, during and after an incremental exercise test on a treadmill at 6.25% incline with speeds increasing from 6, 7, 8 to 9 m/sec with 2 min trotting at each speed. A 2 min warm-up walk preceeded the test. ○= normohydrated, ∇= dehydrated, □=hyperhydrated. Mean±SE. Filled symbols are significantly different from first value within treatment. * significantly different from normohydrated horses. P<0.05.

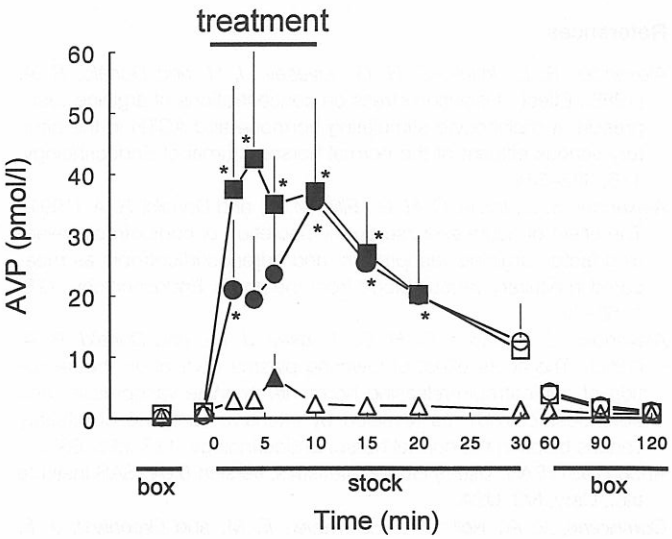

Fig. 2: Plasma AVP concentration in four horses using different methods of restraint during naso-gastric tubing in the horse. △=naso-gastric tube and ear holding (n=3), ○=naso-gastric tube and upper lip twitching (n=4), □=naso-gastric tube, upper lip twitching and administration of 10 l, body warm (38°C) NaCl-solution (9 g/l) (n=4). Means±SE. Filled symbols are significantly different from second sample within treatment. * significantly different from ear held horses. P<0.05.

Discussion

The plasma AVP concentration is correlated to changes in plasma osmolality. In our study 24 h of dehydration was not enough to cause a significant increase in jugular plasma AVP although plasma osmolality was increased 20 mosm/kg (*Dahlborn* et al. 1994). Plasma AVP concentration increases during exercise in man (*Convertino* et al., 1981) and in the horse (*Alexander* et al., 1991). In man these increments in AVP are closely related to changes in plasma osmolality (*Convertino* et al., 1981). In our study exercise caused an increased plasma osmolality and elevated plasma AVP concentrations in all groups. In contrast to our expectations, the exercise-induced increase in AVP after hyperhydration was much greater than when the horses were exercised during normo- and dehydration. We hypothesised that the high level of AVP in the hyperhydrated horses was a "stress reaction" caused by the naso-gastric administration of fluid (*Dahlborn* et al. 1994).

The results from Experiment II showed that horses responded to the combined use of a twitch and a naso-gastric tube with an even greater increase in plasma AVP concentrations than during exercise. The pre-exercise value of AVP, 30 min after hyperhydration in Experiment I, was significantly higher than in the other groups. This is consistent with the results from Experiment II where the plasma AVP values had not returned to pre-treatment values 30 min after the treatment restraint by upper lip twitch and fluid administration had started. This supports the idea that the AVP response seen during exercise in the hyperhydrated horses could have been triggered by the handling during the fluid administration prior to exercise. The elevated plasma AVP might also have been an effect of having 12 l of fluid placed in the upper gastro-intestinal tract during intensive exercise. At such high levels of AVP as seen in both our experiments, AVP can act as a vasoconstrictor. The significance of AVP during exercise and the possible effects of high levels on haemodynamics in the exercising horse needs further investigations.

According to the behaviour of the horses they were more upset when they were restrained by ear holding than during the two treatments when the twitch was used (*Hydbring* et al., 1996). However, the increase in AVP was much lower in this treatment. Plasma levels of β-endorphins and cortisol measured in the same horses during this experiment also had a tendency to be comparatively lower in the ear holding treatment (*Hydbring* et al., 1996). The reason for the difference in plasma AVP concentrations between the two methods of restraint, ear holding and twitching, is not clear but indicates a role of AVP in mediating stress responses in the horse.

Conclusions

In our study the combined use of a naso-gastric tube and a twitch resulted in much greater plasma AVP concentrations than exercise even following dehydration. Our results suggest a role of AVP in mediating stress responses in the horse.

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