Effects of Novel Environmental Stimuli on Neuroendocrine Activity in Thoroughbred Horses

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Emotional stimuli have been shown to suppress vasopressin and facilitate oxytocin release from the posterior pituitary in some mammalian species. However, it is not clear in horses. Here we examined effects of novel environmental stimuli on plasma concentrations of vasopressin, oxytocin, cortisol, adrenaline and noradrenaline in Thoroughbred horses. To impose novel environmental stimuli, horses were introduced to an experimental stall (4.5 × 5.0 m) with side walls of black rubber board, in which a yellow balloon (80 cm in diameter) was hung from the ceiling. Horses were exposed to the novel environmental stimuli for 3 min. Heart rates were significantly increased during the stimuli. Plasma concentrations of adrenaline and noradrenaline in the blood collected immediately after the stimuli were twice as high as those at rest and decreased to the basal levels within 10 min after the stimuli. Plasma cortisol concentration increased after the stimuli and reached the peak 20 min later. However, plasma concentrations of vasopressin and oxytocin did not significantly change throughout the experiments. These data suggest that the novel environmental stimuli do not significantly influence the hypothalamo-neurohypophyseal system while the stimuli activate the sympatho-adrenal medulla and the hypothalamo-pituitary-adrenal cortical axes in horses.

Key words: novel environment, oxytocin, stress, vasopressin

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Emotional stimuli have been shown to activate sympatho-adrenomedullary and pituitary-adrenocortical responses in mammalian species including horses [10, 28]. Emotional stimuli such as isolation of a horse from the herd, exposure to a novel environment or confinement of a horse with dominant residents facilitate ACTH, cortisol, adrenaline or noradrenaline release [3, 4, 14].

On the other hand, vasopressin release from the posterior pituitary has been shown to be suppressed by emotional stimuli in some mammalian species [41]. For example, conditioned fear or novel environmental stimuli have been shown to inhibit vasopressin release from the posterior pituitary in rats [23–27]. In sheep, isolation of an animal from the herd has been also shown to inhibit the vasopressin release [29]. In

horses, however, isolation stress has been reported to increase vasopressin concentrations in the pituitary venous blood [3], indicating that vasopressin release is increased from the median eminence and/or the posterior pituitary by emotional stimuli in horses. It is thus possible that control of vasopressin release after emotional stimuli in horses may be different from that in other mammalian species.

In the case of oxytocin, emotional stimuli have been shown to facilitate oxytocin release from the posterior pituitary in rats [23, 27] in human [38]. In horses, on the other hand, effects of emotional stimuli upon oxytocin concentrations in the peripheral blood have not been reported.

The present experiments aimed to discover the effects of novel environmental stimuli on release of arginine vasopressin and oxytocin from the posterior pituitary in horses. We measured vasopressin and oxytocin concentrations in the peripheral blood of

male Thoroughbred horses before and after novel environmental stimuli. We also measured heart rates and plasma concentrations of cortisol, adrenaline and noradrenaline, since these parameters are known as physiological indexes of acute stress.

Materials and Methods

Horses

Eight healthy male Thoroughbred horses were used (24.8 ± 0.4 months old).

Novel environmental stimuli

The tests were performed between 13:00 and 14:30. An experimental stall (4.5×5.0 m), in which side walls are made of black rubber board, was used. In the experimental stall, a yellow balloon was hung from the ceiling above the horse's back. The stall was about 50 m apart from their home stalls and the horses had never been in it before. The horses were taken into the experimental stall, kept there for 3 min and brought back in their individual home stalls.

Blood sampling

A catheter (14 G, Angiocath, Beckon Dickinson, Sandy, USA) was placed in the left jugular vein at least 90 min before the first blood sampling. Bloods of horses during the experiment were collected via the catheter at rest, immediately before and after the stimuli and 5, 10, 20, 30, 60 and 120 min after the stimuli. The blood samples were immediately put into tubes containing disodium ethylene diamine tetraacetic acid (7.5 mg/5 ml) and kept in ice. Each plasma sample was separated by centrifuge and kept at -80°C until measurements of hormones.

Measurements

A heart rate monitor (VANTAGE XL, POLAR, Kempele, Finland) was attached to the chests of the horses and their heart rates were recorded at a 5-sec interval.

Plasma concentrations of cortisol were measured by radioimmunoassay (RIA) with a specific anti-serum against cortisol (#A907/R1Y, Biogenesis, Poole, England) as described previously [5]. The intra- and inter-assay coefficient of variance was 5 and 9%. Plasma concentrations of adrenaline and noradrenaline were measured by a high-performance liquid

Fig. 1. Mean heart rates before, during and after the novel environmental stimuli. Data indicate mean $\pm$ S.E.M. for 1 min at rest and for 3 min before, during and after the novel environmental stimuli. *: $p < 0.05$ VS the values at rest (Rest) by Fisher's PLSD analysis.

chromatography (C-RA, Shimadzu, Tokyo, Japan) with an electrochemical detector (Nanospace SI-1, Shiseido, Tokyo, Japan) as described previously [18]. The intra- and inter-assay coefficient of variance for adrenaline and noradrenaline was 2%, respectively. Plasma samples were extracted with acetone and diethyl ether for vasopressin measurements. Vasopressin concentrations were measured by RIA with a specific anti-serum [36] as described previously [26]. Plasma concentrations of oxytocin were measured by RIA as described [24]. The intra- and inter-assay coefficient of variance was 6% and 14% for vasopressin, and 4% and 10% for oxytocin RIA.

Plasma osmolality was measured by a freezing point depression method with an osmometer (Fiske one-ten, Fiske, Norwood, USA).

Statistics

Data were expressed as mean $\pm$ S.E.M. For statistical analysis, two-way ANOVA and Fisher's PLSD analysis were employed.

Results

When the horse was brought to the experimental stall, heart rates were significantly higher than those at rest (Fig. 1). The data indicate that the horses were in an excited state before entering the stall (a novel environment). When the horses were introduced into the experimental stall, they showed restless movements,

Fig. 2. Plasma concentrations of A) adrenaline, noradrenaline, B) cortisol, C) vasopressin and oxytocin before, during and after the novel environmental stimuli. Hatched boxes indicate the period of the novel environmental stimuli. *: $p < 0.05$ VS the values at rest (Rest) by Fisher's PLSD analysis.

neighing and hyperventilating. Heart rates were further increased in the stall and remained at the high level.

Plasma concentrations of adrenaline and noradrenaline immediately after the novel

Fig. 3. Plasma osmolality before, during and after the novel environmental stimuli. A hatched box indicates the period of the novel environmental stimuli.

environmental stimuli increased twice as high as those at rest and returned to the basal levels within 10 min after the stimuli (Fig. 2-A).

Plasma concentration of cortisol began to increase after the stimuli and reached the peak 20 min later (Fig. 2-B). Then it decreased gradually and returned to the basal level within 60 min after the stimuli and further went down to a sub-basal level.

Plasma concentrations of vasopressin and oxytocin were not significantly changed throughout the experiments (Fig. 2-C). We also measured plasma osmolality, since vasopressin release from the posterior pituitary depends upon body fluid osmolality [31]. Plasma osmolality was not significantly changed (Fig. 3).

Discussion

A variety of stressful stimuli have been shown to facilitate releases of ACTH, cortisol, adrenaline and noradrenaline in mammalian species [10, 28] including horses [2-4, 6, 7, 12, 13, 15-19, 22, 37, 39, 40]. In the present study, exposure of horses to the novel environmental stimuli increased heart rates and plasma concentrations of cortisol, adrenaline and noradrenaline. These data, therefore, indicate that the environmental stimuli excited the horses emotionally. However, the stimuli did not significantly change plasma concentrations of vasopressin or oxytocin. Thus the results suggest that novel environmental stimuli do not significantly change release of vasopressin and oxytocin in horses. Consistent with this idea, it has been shown that isolation of a horse from

the herd does not change plasma vasopressin concentrations in the jugular venous blood [3].

On the other hand, isolation stress in horses has been shown to increase vasopressin concentration in the pituitary venous blood [3]. The apparent discrepancies in vasopressin data between the jugular and pituitary venous blood may be related to the fact that vasopressin is released both from the median eminence and the posterior pituitary. While vasopressin concentrations in the pituitary venous blood reflect the sum of vasopressin released from the median eminence and the pituitary, those in the peripheral blood are considered to mainly reflect the amount released from the posterior pituitary, since neurohypophysectomy has been shown to induce severe diabetes insipidus, a symptom of vasopressin deficiency in the peripheral plasma [11], and to almost completely abolish an increase of vasopressin after hypotension [30]. Vasopressin release from the median eminence has been shown to increase after emotional stress in rats [8, 32, 33]. It is thus possible that emotional stimuli facilitate vasopressin release from the median eminence but not from the posterior pituitary. Another possibility, however, is not excluded that novel environmental stimuli in the present study or isolation stress in the previous study [3] were not sufficiently strong for inducing a significant change in vasopressin release from the posterior pituitary in horses.

Plasma vasopressin concentration in the peripheral blood has been shown to decrease after emotional stimuli in rats [23–27] and sheep [29]. These results contradict the present data in horses. Causes of this discrepancy are unclear at present. However, two explanations are possible. One may be the difference in experimental designs (osmotic conditions of the body fluid and emotional stimuli used), and the other may be the existence of species difference. Vasopressin plays an important role in maintaining blood volume and blood pressure, and increasing blood flow in muscles [21]. So that, it is tempting to speculate that keeping plasma vasopressin concentrations high and, as a result, maintaining sufficient blood flow in the muscles during emotional stress may have a survival value in horses, since large-sized animals such as horses ought to flee rather than stay still and hide themselves when they come across their predators. In this context, it has been demonstrated in horses that the physical exercise facilitates vasopressin release from the posterior pituitary [2, 20].

The present study demonstrated, for the first time, in horses that novel environmental stimuli do not significantly change plasma oxytocin concentrations in the peripheral blood. In rats, oxytocin concentration in the peripheral blood has been shown to increase [27, 42] or unchanged [9, 15, 25] after novel environmental stimuli. It is of interest to note the study showing that emotional stimuli facilitate oxytocin release from the median eminence in rats [33, 34]. In horses, isolation stress has been claimed to increase oxytocin concentrations in the pituitary blood of horses [1]. The data appear to suggest that emotional stimuli facilitate release of oxytocin as well as vasopressin from the median eminence. Oxytocin released from the median eminence may contribute to ACTH release from the pituitary in horses, as suggested in rodents [35].

In conclusion, we found that novel environmental stimuli do not significantly change vasopressin and oxytocin release from the posterior pituitary in horses, although the stimuli facilitated release of cortisol, adrenaline and noradrenaline. Thus, it is suggested that in horses unlike in rodents or the other mammalian species studied previously, vasopressin release from the posterior pituitary is not suppressed after emotional stimuli. The present study, however, does not assess vasopressin release from the median eminence. Measurements of hormone concentrations in the pituitary venous blood as well as in the peripheral blood remains to be taken for clarification of vasopressin release from the median eminence and from the posterior pituitary.

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