The effect of diet on cribbing behavior and plasma \( \beta \)-endorphin in horses

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Abstract

Five cribbing horses and six control horses were used in a latin square design dietary study to investigate the effects of different diets on the frequency of cribbing behavior and plasma levels of beta-endorphin in the horse. Feeding grain or sweetened grain rations was found to cause a significant increase in the cribbing frequency whereas alfalfa pelleted hay was without significant effect on the frequency of the behavior. Baseline beta-endorphin levels in cribbing horses were half those of the non-cribbing controls and remained significantly lower during the feeding trials. These results are discussed as they apply to treatment of cribbing horses and in terms of the underlying mechanism of cribbing.

\textit{Keywords:} Horses; Cribbing; Endorphins; Diet

1. Introduction

Endogenous opioids have been linked to many stereotypic behaviors in horses (Dodman et al., 1987), dogs (Dodman et al., 1988\textsuperscript{b}), rats (Broderick et al., 1983), swine (Rushen et al., 1990), and humans (Weizman et al., 1990). A decrease in stereotypic behavior has been reported in horses (Dodman et al., 1987, \* Corresponding author.
1988a), pigs (Rushen et al., 1990), and voles (Kennes et al., 1988) after the administration of opioid antagonists, suggesting that endogenous opioids facilitate stereotypy. In 1987, Dodman et al. noted that cribbing horses fed sweetened grain rations appeared to show an increase in this stereotypic behavior. Consumption of highly palatable foods in other species has been shown to cause an increase in stereotypy and analgesia (Roane and Martin, 1990; Rushen et al., 1990; Kanarek et al., 1991). The stimulatory effect of feeding on stereotypic chain biting in sows was not related to plasma glucose levels (Terlouw et al., 1993). It has been shown that feeding highly palatable foods will produce naloxone-reversible analgesia even in animals that do not exhibit stereotypic behavior (Roane and Martin, 1990; Rushen et al., 1990; Blass and Hoffmeyer, 1991). Dum et al. (1983) reported that palatable food caused release of β-endorphin from the hypothalamus. Levels of circulating β-endorphin were found to increase in humans after the ingestion of chocolate (Melchior et al., 1991).

The present study was designed to investigate the relationship between selected diets and cribbing frequency in horses. The connection between endogenous opioids and cribbing behavior was also examined by measuring plasma β-endorphin levels.

2. Animals, materials and methods

Eleven horses were used in this study. The test group consisted of five cribbing horses. The control group comprised six horses without any stereotypic disorder. The signalment of the horses used in this study is shown in Table 1. Each trial was started at approximately the same time of day (between 12:30 h and 14:30 h). On the morning of a study grain was withheld. An indwelling intravenous

<table>
<thead>
<tr>
<th>Identification (name)</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Breed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cribbers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disco</td>
<td>17</td>
<td>Mare</td>
<td>Thb</td>
</tr>
<tr>
<td>Cajun Spice</td>
<td>5</td>
<td>Mare</td>
<td>Qtr</td>
</tr>
<tr>
<td>Pee Wee</td>
<td>&lt;20</td>
<td>Gelding</td>
<td>Thb</td>
</tr>
<tr>
<td>Mekko</td>
<td>&gt;20</td>
<td>Gelding</td>
<td>Qtr</td>
</tr>
<tr>
<td>Major Fox</td>
<td>23</td>
<td>Gelding</td>
<td>Thb</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vixin</td>
<td>22</td>
<td>Mare</td>
<td>Qtr</td>
</tr>
<tr>
<td>Vedette</td>
<td>11</td>
<td>Mare</td>
<td>Thb</td>
</tr>
<tr>
<td>Drift</td>
<td>10</td>
<td>Mare</td>
<td>Grade</td>
</tr>
<tr>
<td>China</td>
<td>15</td>
<td>Mare</td>
<td>Grade</td>
</tr>
<tr>
<td>Beautiful Bertha</td>
<td>3</td>
<td>Mare</td>
<td>Thb</td>
</tr>
<tr>
<td>Vandergot</td>
<td>3</td>
<td>Gelding</td>
<td>Thb</td>
</tr>
</tbody>
</table>
Table 2
Nutrient composition of individual feedstuffs

<table>
<thead>
<tr>
<th>Feedstuff</th>
<th>Crude protein (CP)</th>
<th>Crude fiber (CF)</th>
<th>Estimated net energy (ENE)</th>
<th>CP to ENE</th>
<th>Calcium (Ca)</th>
<th>Phosphorus (P)</th>
<th>Ca/P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Legume hay</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alfalfa</td>
<td>15.0</td>
<td>&lt;80%</td>
<td>46.1</td>
<td>1:3.1</td>
<td>0.90</td>
<td>0.24</td>
<td>3.7/1</td>
</tr>
<tr>
<td>Sweet feed</td>
<td>12.0</td>
<td>9.0</td>
<td>75.0</td>
<td>1:6.3</td>
<td>0.90</td>
<td>0.70</td>
<td>1.3/1</td>
</tr>
<tr>
<td>Pellet feed</td>
<td>16.0</td>
<td>7.5</td>
<td>75.0</td>
<td>1:4.7</td>
<td>0.90</td>
<td>0.70</td>
<td>1.3/1</td>
</tr>
</tbody>
</table>

A catheter was placed in a jugular vein and flushed with heparinized saline to maintain patency.

The trials consisted of four 30-min phases. In Phase 1, the control phase for each group, cribbing frequency was recorded and a venous blood sample was taken. In Phases 2–4 each horse received one of three feeds in random order (latin square design). Test diets consisted of 200 g of either a sweetened grain ration (sweet feed), a high protein (16%) pellet feed, or a high fiber feed (alfalfa pellets). The nutrient composition of these feeds is shown in Table 2. Cribbing frequency per 5 min interval was recorded during each 30 min test period.

A 20 ml venous blood sample was taken 5 min after the start of each test period. The blood samples were collected into two 10-ml citrated vacuum tubes and were centrifuged immediately at 3000 rev. min⁻¹ for 4 min; 4.5 ml of plasma were transferred to two polypropylene tubes, each containing 0.5 ml of glacial acetic acid. The samples were stored at −80°C until the study was complete.

Plasma was assayed for β-endorphin as follows. Samples were thawed and precipitated protein was removed by centrifugation at 3000 rev. min⁻¹. The supernatant was passed through a Waters C-18 Sepak column. The column was washed with 10 ml of water followed by 10 ml of 10% acetonitrile containing 0.1% trifluoroacetic acid (TFA). Peptides were eluted with 4 ml of 70% acetonitrile containing 0.1% TFA. The eluate was extracted with an equal volume of chloroform to remove acetonitrile. The upper layer, containing the peptides, was lyophilized. Samples were reconstituted in 800 µl of radioimmunoassay (RIA) buffer. The RIA was performed according to Kumar et al. (1990). Recovery of radiolabeled β-endorphin in these procedures was over 90%.

3. Results

The cribbing frequency was found to change in response to different feeds. Cribbing horses receiving no feed were found to have a low basal cribbing rate. Feeding alfalfa pellets caused an increase in cribbing frequency after 10 min (Fig. 1). However, statistical analysis showed no significant difference from the baseline over the 30 min period (P>0.05 by Newman–Keul's range test). When sweet
feed or unsweetened grain were given a marked (14- to 16-fold) increase in cribbing frequency was observed after 10 min. Cribbing rates returned close to baseline after 30 min. Analysis by the Newman–Keul's range test of the rate of change over the entire 30 min period showed that the effects of sweet feed and unsweetened grain were significantly different from baseline and alfalfa (P<0.001) but indistinguishable from each other (P>0.05). A three-way analysis of variance of cribbing rate shows significant effects of all main variables — time, diet and horse (all P<0.001). The interaction between time and diet is not significant (P=0.058) suggesting that the effects of the different diets are similar over time. 

Mean baseline β-endorphin levels in cribbing horses were half those of the non-cribbing controls when analysed by a two-way repeated measure analysis of variance (P<0.002) (Fig. 2). A two-way repeated measures analysis of variance of
endorphin levels, with diet and group as factors, shows a significant difference between the two groups of animals ($P<0.002$), but no difference between diets ($P=0.481$). Additionally, there was a borderline interaction effect ($P<0.045$) indicating that $\beta$-endorphin levels of horses fed alfalfa moved in different directions (higher for controls and lower for the cribbing animals).

4. Discussion

The behavioral component of this study demonstrated significant increases in the rate of cribbing subsequent to feeding small aliquots of standard equine rations. The greatest and most sustained increase in cribbing frequency was seen following the two grain-based rations. We believe that this response is related to the palatability of these rations, presumably by the release of endogenous opioids. Palatable rations cause elevation of the pain threshold and increases in morphine potency (Roane and Martin, 1990; Kanarek et al., 1991). Presumably the rising $\beta$-endorphin (BE) levels activate dopamine pathways thereby enhancing stereotypy (Goodman et al., 1983).

The second part of the study looked at plasma $\beta$-endorphin levels in cribbing horses and non-cribbing controls. Although it was anticipated that cribbing horses would have higher levels of plasma BE than controls, this turned out not to be the case. The control horses had mean plasma BE levels significantly higher than the cribbers by a factor of two ($P<0.02$). Interestingly, the plasma level of BE has been documented to be low in human subjects with obsessive compulsive disorders (Weizman et al., 1990). There were no significant changes in plasma BE in either group when the different rations were fed. To explain the consistent difference in plasma BE between the control and cribbing horses, it is proposed that cribbing horses may have impaired release of BE resulting in an upshift in opioid receptor sensitivity. Stimuli which increase the release of endogenous opioids would then be expected to produce an exaggerated response. Opioid receptor density has been shown to increase in sows displaying stereotypes (Zanella et al., 1991). In addition, opioid receptor supersensitivity has been demonstrated to be associated with stereotypy in other studies (Broderick et al., 1983). Small changes in the central endorphin release may be responsible for the increase in cribbing seen in this study. The magnitude of these changes may not be sufficient to increase plasma levels of BE. This study defines a population of horses with low basal levels of BE that perform a stereotypic behavior presumably due to an exaggerated response to the central release of BE. Some workers believe that cribbing is an equine equivalent of human obsessive–compulsive disorder (Luescher et al., 1991). Obsessive–compulsive disorder in humans is associated with low levels of BE and has a strong genetic component. The low levels of BE in the cribbing horses of this study may provide support for a genetic component in cribbing (Hosoda, 1950; Vecchiotti and Galanti, 1987; McClure et al., 1992). However, as in obsessive compulsive disorder, there are probably strong environmental influences as well.
The clinical implications of this study are that decreasing the amount of grain component fed to cribbing horses may reduce the rate of cribbing and possibly other stereotypies. Recently, equine self-mutilation syndrome has been reported to be reduced in some horses by reducing the grain component of the ration (McClure et al., 1992). A diet based on high quality hay (alfalfa) rations may reduce cribbing directly by minimizing central opioid release. Additional benefits may be achieved with such a regimen by increasing the time the horse spends eating. Such benefits have been documented in sows displaying stereotypies fed high fiber diets (Brouns et al., 1991).

References


