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The Effect of Calcium-Naloxone Treatment on Blood Calcium, β-Endorphin, and Acetylcholine in Milk Fever

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ABSTRACT

Milk fever is a postpartum syndrome of cows characterized by acute hypocalcemia, which reduces the release of acetylcholine (ACH), inducing flaccid paralysis and recumbency. Our aim was to evaluate the effect of calcium (Ca^{2+}) combined with naloxone (Nx, an opioid antagonist; Ca^{2+}-Nx) on plasma concentrations of ACH, ß-endorphin (ßE), and Ca^{2+} just before treatment (T0) and at 15, 30, and 90 min after treatment (T15, T30, and T90, respectively). Thirty cows were divided into 3 groups of 10 cows each. In group A1, cows affected by milk fever were treated (i.v.) with a combination of 0.2 mL/kg of body weight (BW) of Ca^{2+} borogluconate (20%) and 0.01 mg/kg of BW of Nx hydrochloride dihydrate. In group A2, cows affected by milk fever were treated (i.v.) with 2 mL/kg of BW of Ca^{2+} borogluconate (20%). In group C, healthy cows were treated (i.v.) with a combination of 0.2 mL/kg of BW of Ca^{2+} borogluconate (20%) and 0.01 mg/kg of BW of Nx hydrochloride dihydrate. Cows underwent treatments within 24 h of calving. Blood samples were collected at T0 and at T15, T30, and T90 for quantitative determination of ACH, ßE, and Ca^{2+}. The cows in groups A1 and A2 recovered within a mean of 20 ± 10 min, although 4 cows in group A2 underwent a relapse. Blood Ca^{2+} concentrations in group C increased slightly at T30 and at T90 (T30: 8.8 ± 0.6 mg/dL; T90: 8.7 ± 0.6 mg/dL) after treatment, whereas the response in groups affected by milk fever was similar, even though Ca^{2+} concentrations showed a sharp increase (A1: 8.9 ± 0.8 mg/dL; A2: 6.0 ± 0.7 mg/dL), particularly at T15 in group A1. Concentrations of ßE showed a similar pattern in groups A1 and C, with an increase at T15 (A1: 8.2 ± 1.0 ng/mL; C: 2.7 ± 0.4 ng/mL) and a subsequent decrease until T90 (A1: 1.4 ± 0.3 ng/mL; C: 1.4 ± 0.4 ng/mL), whereas ßE remained constant throughout in group A2. Concentrations of ACH in group A1 decreased significantly between T0 and T15, T30, and T90 (T0: 7.2 ± 1.1 nmol/L; T15: 4.2 ± 1.2 nmol/L; T30: 2.9 ± 0.8 nmol/L; T90: 3.1 ± 0.3 nmol/L), whereas in group A2, it did not change. In group C, concentrations of ACH decreased at T15 and increased again at T30 (T15: 1.1 ± 0.3 nmol/L; T30: 3.2 ± 0.7 nmol/L). Our results suggest that administration of Ca^{2+}-Nx, which restored the physiological Ca^{2+} concentrations, might have an effect on nicotinic receptors by restoring the normal neuromuscular transmission at the motor endplate.

Key words: milk fever, acetylcholine, ß-endorphin, calcium-naloxone

INTRODUCTION

Milk fever is one of the most common metabolic disorders in dairy cattle, with an incidence of 5 to 10% per lactation. In milk fever, the homeostatic mechanisms fail to maintain normal plasma calcium (Ca^{2+}) concentrations (9 to 10 mg/dL; Goff et al., 1995). Plasma Ca^{2+} concentrations are controlled by the coordinated actions of calcitropic hormones, such as the parathyroid hormone and 1,25-dihydroxyvitamin D_3, the concentration of which increases in response to hypocalcemia and acts to augment the pool of plasma Ca^{2+} (Horst and Reinhardt, 1983). Milk fever is associated with the sudden onset of lactation and usually occurs within 72 h of calving (Sorensen et al., 2002).

At parturition, cows have an increased Ca^{2+} requirement, and if they are unable to respond quickly to this demand, hypocalcemia develops. In most of the mammalian species, this condition is associated with a progressive increase in endogenous opioid peptides (**EOP**), which occurs during parturition (Petraglia et al., 1985; Sciorsci et al., 2001). These EOP, particularly ß-endorphins (**βE**), activate their specific receptors, giving rise to many cellular responses, including blockage of the voltage-gated Ca^{2+} channels and opening of the K^+ channels, which act synergistically, thereby inhibiting the release of acetylcholine (**ACH**) from the presynaptic membrane (Kim et al., 2005). This inhibition impairs normal neuromuscular transmission and muscle contraction (Sciorsci et al., 2000; Minoia and Sciorsci, 2001; Mayerhofer and Fritz, 2002).

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Based on this knowledge, Sciorsci et al. (2001) demonstrated that administration of Ca^{2+} and naloxone (**Nx**), an opioid antagonist, together (Ca^{2+}-Nx) resulted in a faster and better recovery from milk fever than that achievable from administration of either Nx or Ca^{2+} alone. Thus, our aim was to evaluate the effects of Ca^{2+}-Nx on plasma concentrations of ACH, ßE, and Ca^{2+}.

MATERIALS AND METHODS

Experimental Animals

The study was performed between June 2005 and April 2006 on 30 Friesian cows (5 to 8 yr old). All the cows delivered their calves no more than 24 h before the treatments, with normal delivery and without placental retention. The animals had a mean BW of 600 kg (range 560 to 650 kg) and were maintained on farms in the south of Italy (Bari, Apulia).The animals were between the third and the fifth lactation, with average milk production ranging from 8,300 to 8,500 kg per lactation. Cows were restrained in tie stalls and fed hay, concentrate, and minerals, with access to water ad libitum. All cows underwent a clinical examination resulting in the diagnosis of milk fever (n = 20) within 24 h of calving. The diagnosis was performed by reference to the anamnesis, the evaluation of clinical symptoms (anorexia, recumbency, absence of rumination, tachycardia, tachypnea, and muscular spasms followed by flaccid paralysis) and plasma Ca^{2+} concentrations <6 mg/dL (Lindsay and Pethick, 1983). The cows were divided into 3 groups: groups A1 and A2, made up of 10 cows each that were affected by milk fever; and group C, made up of 10 healthy cows. In the first week after parturition, all the cows underwent a daily general health examination to evaluate their clinical status. All the procedures were carried out in accordance with the Italian Legislation on animal care (DL 116/92).

Treatment and Blood Collection

Groups A1 and C received the same treatment, consisting of a single i.v. infusion of 0.2 mL/kg of BW of Ca^{2+} borogluconate (20%; Fatro, Ozzano Emilia, Italy) and 0.01 mg/kg of BW of Nx hydrochloride dihydrate (Sigma, Milano, Italy) administered soon after the clinical examination. Group A2 was treated with a single i.v. administration of 2 mL/kg of BW of Ca^{2+} borogluconate (20%; Fatro; Sciorsci et al., 2001).

Blood samples were collected before (**T0**) and at 15 (**T15**), 30 (**T30**), and 90 (**T90**) min after treatments for the analysis of ACH, ßE, and Ca^{2+} concentrations. All the blood samples were collected by jugular venipuncture with refrigerated Vacutainer tubes and were maintained at 4°C until taken to the laboratory within 2 h.

ACH Determination

The blood samples for ACH determination were collected in Vacutainer tubes containing lithium-heparin, with the addition of 2.5 mmol of physostigmine (Sigma). They were centrifuged at 1,620 × *g* for 10 min at 4°C. The plasma was stored at −20°C in Eppendorf tubes (Eppendorf, Milan, Italy) with the addition of 0.25 mmol of physostigmine, until analysis. The analysis was performed by using HPLC-electrospray ionization mass spectrometry, with an 8 mpos dead-end path column (Superchrom, Milan, Italy). The HPLC method was a modified isocratic reversed-phase ion-pairing procedure. The mobile phase was prepared by adding 1 mL of heptafluorobutyric acid to 980 mL of water, followed by 20 mL of methanol. Luna C18 HPLC columns were used (3-µm particles, 2.0 × 150 mm; Phenomenex, Torrence, CA). The column was eluted isocratically at a flow rate of 0.3 mL/min and was maintained at 60°C. The mass spectrometer was operated either in the selected ion-monitoring mode or in the selected reaction-monitoring mode. The selected reaction-monitoring experiments monitored a collision-induced dissociation transition for each of the compounds. The transition producing the most abundant ion fragments was selected for the analysis ($146^+/87^+$ for ACH). Detection was performed by using a Thermo mass spectrometer (Thermo Finnigan, San Jose, CA) equipped with the manufacturer's heated capillary atmospheric pressure ionization interface operating in the electrospray ionization mode. The method detected ACH and its primary degradation product, choline, at the 10-fmol level, with an analysis time of less than 6 min. The intraassay CV was <2%.

βE Determination

The blood samples for ßE determination were collected by using Vacutainer tubes containing EDTA and aprotinin (500 kIU; Sigma). The samples were centrifuged at 1,620 × *g* for 10 min at 4°C and plasma was stored at −20°C in Eppendorf tubes containing 50 kIU of aprotinin until analysis. ß-Endorphin determination was performed by using the Basic Robot Immunoassay Operator 4.54 (Radim, Pomezia, Italy), which relied on an immunoenzymatic method, and a Peninsula kit (Peninsula Laboratories Inc., San Carlos, CA) specific for bovine species (sensitivity 0.03 to 0.06 ng/mL). The intraassay CV was <5%.