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Effects of Naloxone on Calcium Turnover in Cows Affected by Milk Fever

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ABSTRACT

Milk fever is a metabolic disorder of calcium homeostasis that affects about 2 to 6% of postpartum cows. Current therapy is based on the administration of calcium gluconate. On the basis of the clinical signs, and given that endorphins increase at parturition, we supposed that endogenous opioid peptides (EOP) could be responsible for this pathology. In this study, cows with milk fever were administered the opiate antagonist, Naloxone (Nx; experiment 1) or Nx with calcium salts (experiment 2). In experiment 1, Nx induced the recovery of affected cows. The effects of Nx therapy, expressed in terms of proportion of recovered cows, of cows recovering in less than 30 min and cows requiring repeated treatments, were not statistically different than those obtained by means of calcium administration (17/17, 100%; 10/17, 59% and 7/17, 41% vs. 33/35, 94%; 22/35, 63% and 11/35, 31%, respectively; NS). In experiment 2, a significantly higher ratio of cows recovered in less than 30 min in the group of animals treated with Nx in association with calcium salts, compared with the group of cows treated with the calcium traditional therapy (106/118, 90% for calcium-Nx treated cows vs. 34/62, 55% for calcium-treated cows). Moreover, in the group of cows treated with calcium-Nx, the number of cows requiring repeated treatments was significantly reduced and no unrecovered cows were observed. The results support the idea that high EOP levels interfere with inward movement of calcium through the cell membrane and with calcium activity. The association of calcium and Nx at low dosage is a safe method to treat milk fever in cows and reduces muscular complications.
(**Key words:** cow, milk fever, hypocalcemia, Naloxone)

Abbreviation key: EOP = endogenous opioid peptides, **Nx** = Naloxone.

INTRODUCTION

Milk fever, also known as periparturient paresis or recumbent hypocalcemia syndrome, occurs in pluriparous dairy cows ages 4 to 10 yr (Goff et al., 1989; Goff et al., 1991; Oetzel et al., 1988; White, 2001; Yamagishi et al., 1999). The pathology is characterized by progressive symptoms and starts with the inability to remain standing followed by, in the final stage, nervous disorders, anorexia, digestive atonia and meteorism, suppressed defecation, unconsciousness, cardiorespiratory difficulties, coma, tetany, and death (Gaines, 1997; Noakes, 1996; White, 2001). This disease is related to the metabolic turnover of calcium and affects the bovine species, which has the highest calcium turnover per kilogram of BW during milk production (Minoia, 1978). Current therapy is based on the i.v. administration of calcium gluconate, thus replacing serum calcium until the homeostatic mechanisms can maintain physiological calcium levels.

In all mammals the highest concentrations of endogenous circulating opioid peptides (**EOP**), namely met-enkephalin, leu-enkephalin, β-endorphin, dynorphin, endomorphin, etc., occur at parturition (Dobrinski et al., 1991; Dondi et al., 1991; Terzic et al., 1995; Weissberg et al., 1992). In human drug addicts, a high level of these substances can influence behavior. Exogenous drug poisoning (morphine, other opioid or related drugs) in human overdose results in many symptoms similar to the clinical signs reported in milk fever in the cow, i.e., nervous disorders, respiratory difficulties, tetany, coma, and death. The elective therapy in opioid addicts is to administer the opioid antagonist, Naloxone (**Nx**), which occupies the opioid receptors without affecting behavior.

The opioid-dependent calcium L-channel block can alter the intracellular calcium, both acutely and chronically, thus affecting changes in the calcium-mediated signals that control metabolic activities. The voltage-independent inhibition is effective on all calcium channel subtypes but predominates on L-type calcium channels (Albillos et al., 1995; Page et al., 1997; Sciorsci et al., 2000).

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The aim of the present study was to evaluate the Nx therapy, alone (experiment 1) or in association with calcium salts (experiment 2), on cows with milk fever.

MATERIALS AND METHODS

Drugs

Two commercial preparations of calcium were used: calcium pH (446 m*M* calcium gluconate, 18 m*M* sodium toldimfos, 16 m*M* magnesium chloride; Fatro, Ozzano Emilia, Italy) and calcium borogluconate (446 m*M* calcium gluconate, 15.4 m*M* magnesium cloride and 646.9 m*M* boric acid; Nuova ICC-UPJOHN S.p.A., Aprilia-LT, Italy). Naloxone hydrochloride (Diosynth B.V.; 5340 BH OSS, The Netherlands) was used as the opiate antagonist.

Animals

The research was conducted on 53 (experiment 1) and 180 (experiment 2) mixed breed cows affected by milk fever, aging from 5 to 10 yr (mean ± SD = 7.8 ± 1.6). The animals came from several farms in Southern Italy. The pathology was clinically evident from 2 to 20 h after normal parturition. Many of these cows had developed milk fever during previous parturitions and had been successfully treated by means of calcium gluconate.

Milk fever diagnosis was based on clinical signs. The cows admitted to the study were recumbent and depressed; body temperature was normal to decreased; respiration was shallow and irregular; heart rate was irregular and rapid (about 90 pulses/min); rumen motility was decreased; extremities were cool to the touch. Diagnosis was confirmed, in the laboratory, by serum calcium values. Blood samples were collected from all cows immediately before treatment. The results of the serum calcium tests were not known until after treatment. Calcium values were measured with a DU640 Beckman Spectrophotometer, by using a Boehringer-Mannheim kit (n. 1553953, Boehringer-Mannheim, Monza, Italy). Samples with calcium levels lower than 5.5 mg/dl were considered hypocalcemic (Goff et al., 1989; Goff et al., 1991b; Oetzel et al., 1988). Milk from treated cows was marketed 5 d following treatment, even though Nx has a brief half-life of 15 to 20 min (Panerai, 1998).

Experimental design

Except for the cases indicated in the Results, the cows were treated as soon as possible and in less than 2 h from the onset of recumbency for progressive and ingravescent hypocalcemia. They were randomly assigned to experimental groups as follows:

Experiment 1. Group 1: 35 cows were treated with the traditional therapy, consisting of 2 ml of 20% calcium pH (n = 29 cows) or calcium borogluconate (n = 6 cows) per kg of BW. Group 2: 17 cows were injected intravenously with 0.01 mg of Nx hydrochloride per kg of BW dissolved in 2 ml of physiological saline (0.9% wt:vol NaCl).

Experiment 2. Group 1: 62 cows were treated with the traditional therapy, consisting of 2 ml of 20% calcium pH (n = 33 cows) or calcium borogluconate (n = 29 cows) per kg of BW. Group 2: 118 cows were injected intravenously with 0.01 mg of Nx per kg of BW plus 0.2 ml per kg of BW of 20% calcium pH (n = 70 cows) or calcium borogluconate (n = 48 cows).

Therapy outcome was recorded in terms of number of recovered/treated cows, number of cows recovered in less than 30 min/treated cows; and number of cows requiring repeated treatments/treated cows. Treatment was repeated if cows showed no signs of recovery within 120 min.

Statistical Analysis

The proportions of recovered cows, of cows recovered in less than 30 min, and of cows requiring repeated treatments were compared between groups of treatment by chi-square test with the Yates correction for continuity. Fisher's exact test was used when a value of less than 5 was expected in any cell. Values with $P < 0.05$ were considered significant.

RESULTS

Experiment 1

The results of calcium or Nx alone treatment are compared in Table 1. There was no statistical difference in response to calcium treatment due to calcium source (calcium pH or calcium borogluconate), so data in the table were pooled. Overall, calcium treatment resulted in recovery of 33 out of the 35 cows (94.3%) (Table 1). All six cows (100%) treated with calcium borogluconate, recovered. Four of them (67%) recovered in less that 30 min, and 2 cows (33%) needed repeated treatments. Twenty-seven of the calcium pH group (93%) recovered. Eighteen of them (62%) recovered in less than 30 min, and nine cows (31%) needed repeated treatments. In seven of these nine cows, the serum calcium tests showed severe hypocalcemia with levels lower than 5 mg/dl. Three cows of this group were treated 9, 13, and 14 h following recumbency. Two cows in this experimental group did not recover (6%) and were slaughtered after 1 wk. There were no relationships between the three cows treated late and the two cows that did not recover. One of the cows that did not recover was treated

Table 1. Effects of therapy with calcium or naloxone in cows affected by milk fever.

Therapy	No. of treated cows	Recovery < 30 min no. (%)	No. (%) of cows requiring repeated treatments	Total no.(%) of recovered cows	No. (%) of unrecovered cows
Calcium borogluconate	6	4 (67)	2 (33)	6 (100)	0 (0)
Calcium pH	29	18 (62)	9 (31)	27 (93)	2 (6)
Total calcium	35	22 (62.8)	11 (31.4)	33 (94.3)	2 (6)
Naloxone	17	10 (59)	7 (41)	17 (100)	0 (0)

less than 2 h after the recumbency, and the other was treated 13 h after recumbency.

Naloxone treatment resulted in the recovery of all 17 treated cows (100%; Table 1). Ten of them recovered in less than 30 min (58.8%); in fact, recovery appeared complete within 20 min. In seven cows, it was necessary to repeat the Nx treatment (41.2%). Two cows received treatment after 10 and 15 h, respectively, and the regression of clinical symptoms among these cows was less rapid. In these cows, the serum calcium tests values before treatment were lower than 5 mg/dl. The number and the rate of recovery among cows and the number of cows that needed repeated therapy did not differ between treatments ($P > 0.05$). Thus, Nx appears to be as effective as calcium therapy in the treatment of milk fever.

Experiment 2

The results of calcium or calcium-Nx treatments, in relation to two different calcium sources of Calcium pH and Calcium borogluconate, are compared in Table 2. No statistical difference in response to calcium treatment due to calcium source was observed. A significantly higher rate of cows recovered in less than 30 min was observed in the group of animals treated with Nx in association with calcium salts, compared with the group of cows treated with the calcium traditional therapy (106/118, 90% for calcium-Nx treated cows vs. 34/62, 55% for calcium-treated cows). Moreover, in the group of cows treated with calcium-Nx the number of cows requiring repeated treatments was significantly reduced and no unrecovered cows were observed.

DISCUSSION

In this study, the traditional therapy with calcium gluconate was effective and observed results were similar to those previously described in the literature (Gaines et al., 1997; Noakes, 1996). The administration of Nx resulted in recovery from this syndrome. All animals recovered, except one that had been recumbent for 5 d and that was not included in Table1. In this cow, Nx chlorhydrate treatment was administered on d 5, and it was preceded by traditional therapy with calcium gluconate on d 1, 2, and 3 without positive results. Intravenous Nx treatment on d 5 abruptly induced standing, tetanic syndrome, and immediate death. After this outcome, the treatment with Nx alone was interrupted. Recovery was obtained in all the animals not presenting severe hypocalcemia (5.5 to 5.0 mg/dl).

The therapeutical efficacy of Nx indirectly demonstrates an involvement of EOP in calcium homeostasis, and, thus, it strongly supports the idea that the opioidergic system is involved in the etiopathogenesis of this syndrome. The high and selective affinity of Nx for opioid receptors suggests that the results obtained here may have been mediated through an opioid displacement. Throughout the pregnancy, there is a progressive increase of the opioidergic tone, i.e., a marked and progressive increase of EOP plasma concentrations, with

Table 2. Effects of naloxone (Nx) and calcium therapy in cows affected by milk fever.

Therapy	No. of treated cows	Recovery < 30 min no. (%)	No. (%) of cows requiring repeated treatments	Total no. (%) of recovered cows	No. (%) of unrecovered cows
Calcium pH	33	19 (58)	11 (33)	30 (91)	3 (9)
Calcium borogluconate	29	15 (52)	9 (31)	24 (83)	5 (17)
Total calcium	62	34 (55)[a]	20 (32)[a]	54 (87)	8 (13)
Nx + calcium pH	70	63 90)	7 (10)	70 (100)	0 (0)
Nx + calcium borogluconate	48	42 (87)	6 (12)	48 (100)	0 (0)
Total Nx + calcium	118	106 (90)[b]	12 (10)[b]	118 (100)	0 (0)

[a,b]$P < 0.001$, within columns, numbers with different superscripts are significantly different.

a peak at parturition and the highest level of EOP is dependent by placental and hypophyseal production (Genazzani et al., 1981; Nakai et al., 1978; Petraglia et al., 1985). Moreover, it may be suggested that the opioid increase at parturition, if not naturally regress to normal levels, is responsible for milk fever.

One of the treated cows with milk fever that was severely hypocalcemic developed tetany after intravenous infusion of Nx and died soon after. Tetanic movements and death naturally occur in highly hypocalcemic cows (Goff et al., 1991). In our opinion, in this subject, the Nx treatment may have induced a displacement of opioids from their receptor sites, calcium channels opened and calcium moved according to its gradient. In the presence of a severe hypocalcemia, cytoplasmic calcium levels may have been altered, thus inducing tetany. Tetany and death after Nx treatment was also described in human stroke, but the tetanic symptomatology was not identified and not ascribed to calcium deficiency (Andree, 1980; Smith and Pinnok, 1985).

Naloxone is commonly used, both in human and veterinary medicine, to counteract anesthesia and opioid overdosage in humans. As previously reported, Nx shows a wide range of affinity to all opioids' receptors: mu, delta, and kappa with respective decreasing affinity. At the low doses we used, Nx probably reacts only with mu receptors, whereas at higher doses it interacts with all other receptors resulting in the modulation of different functions. All these need to be considered in addition to normal pharmakokinetics (Sciorsci et al., 2000).

Another aspect of the Nx administration is related to its rapid hepatic degradation. The half-life of Nx is estimated as 15 to 20 min (Panerai, 1998). Experimental doses of Nx range from 0.006 mg/k g to 1 mg/kg of BW either as single or repeated hourly doses. The LD_{50} (lethal dose 50) of Nx has been estimated in rats and in mice (640 mg/kg and 286 mg/kg of BW respectively; Genazzani et al., 1986). In the proposed therapy, we used the dose of 0.01 mg/kg of BW. In humans, in tests for LH stimulations, a dose of 1 mg/kg of BW was used (Martin-Del-Campo et al., 1998; Tay et al., 1993; Tulandi et al., 1985). Our dose is comparable to the starting dose for overdose therapy in addicts and is absolutely safe if related to the dosage, its half-life, and LD_{50}.

To our knowledge, a relationship between EOP activity and calcium balance has not been reported in an animal model in vivo, except in previous studies from our unit (Di Sole et al., 2001; Minoia and Sciorsci, 1995, 2000; Minoia et al., 2000; Sciorsci and Minoia, 1994; Sciorsci et al., 2000), and this is the first full research paper reporting data on the interaction between calcium homeostasis and opioidergic tone in the etiopatogenesis of milk fever. The hypothesized interaction between opioids and calcium and the results of the treatment provided us with greater insight into the periparturient hypocalcemia as the disease was resolved in cows without severe hypocalcemia by administering only Nx. This experience presents new perspectives for the management of diseases involving changes in tissue-linked opioid levels.

Based on the data obtained, it can be suggested that many diseases controlled by calcium-related functions may be influenced by altered levels of endogenous opioids. Our results demonstrate that endogenous opioids are related to a progressive disease that may culminate in death through an interaction with calcium homeostasis and calcium turnover. This agrees with the demonstrated role of opioids on the disruption of calcium homeostasis in mouse astrocytes in vitro (Hauser et al., 1998). We believe that endogenous opioids linked to the tissues involved in parturition and in the postpartum, such as muscles, particularly miocardium and nervous system, influence the balance between extracellular and intracellular calcium with a block of L-calcium gated channels.

In conclusion, Nx appears to interact with calcium ions exchanges in milk fever cows and in association with calcium salts is an effective and safe therapy.

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