Relationship of Serum Oxytocin Concentration to Positive Social Behaviors in Cattle

Siyu CHEN[1], Sanggun ROH[2] , Shusuke SATO[3]* and Shin-ichiro OGURA[1]

[1]Laboratory of Land Ecology, Graduate School of Agricultural Science, Tohoku University, Osaki, Miyagi, 989-6711, Japan
[2]Laboratory of Animal Physiology, Graduate School of Agricultural Science, Tohoku University, Miyagi, 981-8555, Japan
[3]Animal science, Faculty of Life & Environmental Science, Teikyo University of Science, Tokyo, 120-0045, Japan

Corresponding Author: Shin-ichiro OGURA, shin-ichiro.ogura.el@tohoku.ac.jp

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Abstract

In order to study the relationship of serum oxytocin concentration to behavior in cows, two types of experiments were used: 1) calves were introduced to new rearing conditions; and 2) an event was created (new access to silage) in a stable herd where cows were familiar with other individuals. For the first experiment, fourteen Japanese Black calves (equal in gender) were used: six calves were named “introduced calves” and the other eight were named “group calves” and were split into two four-calf groups. Introduced calves were individually moved into the four-calf groups, and social behaviors (explorative behavior and affiliative behavior) were observed for the introduced calves from 1000 h to 2000 h (first day of introduction) and 0830 h (start of feeding) to 1030 h by continuous sampling. Blood samples were also collected from the introduced calves before and after the observational period. For the second experi ment, a stable herd of 16 Holstein milking cows was used. Social behaviors were observed for 3 h/day immediately after having access to grass silage for a total of six times. Blood samples were collected three times after finishing the behavioral observation and were used for the analysis of serum oxytocin concentrations. Results showed that serum oxytocin concentration positively correlated with explorative behavior in the calves (n = 6, r = 0.81, P = 0.05), and that serum oxytocin concentration significantly correlated with social affiliative behavior in the herd of Holstein cows (n = 16, r = 0.58, P = 0.02). These results suggest that the higher the serum oxytocin concentration is, the more explorative and social affiliative behavior are observed in cattle. Therefore, increases in serum oxytocin levels may improve the welfare of livestock animals as it enhances positive social behavior.

1. Introduction

Recently, oxytocin has been shown to be of increasing importance in terms of animal welfare (Broom and Fraser, 2007), since it plays an important role in positive social behaviors such as maternal behavior (Da et al., 1996; Williams et al., 2001), affiliative behavior (Winslow et al., 1993; Babygirija et al., 2012), and social grooming behavior in rats (Amico et al., 2004) and monkeys (Winslow and Insel, 1991). It is also known that oxytocin mediates pleasure events and positive interactions (Uvnäs-Moberg, 1998). For example, massage-like stroking of the abdomen lowers blood pressure, and this effect was diminished by intravenous injection of oxytocin antagonist in rats (Kurosawa et al., 1996). The supportive interaction “warm touch” between married couples increased their salivary oxytocin concentrations (Holt-Lunstad et al., 2008). These findings suggest that oxytocin may improve animal welfare through enhancing social grooming behavior, social bond generation, and stabilization of social bonds in herds.

On the other hand, farm animals are often forced to experience changes in rearing conditions (e.g., weaning, grouping of herds) during their lifetimes. These environmental changes affect an animal's behavioral and physiological indicators. Kondo et al. (1984) observed behavioral changes in Holstein calves (i.e., changes in activity, spatial patterns of laying, and aggressive behavior) after grouping. Mench et al. (1990) showed significant increases in serum cortisol concentrations of beef cows after regrouping, suggesting that regrouping causes behavioral and physiological changes in adaptation in calves. Social grooming, a positive social behavior, is thought to have functional significance in the formation and maintenance of social bonds and the stabilization of social relationships in cows (Sato et al., 1991).

However, there is scarce information on oxytocin in cattle. We recently found that serum oxytocin concentrations in calves were different among individuals under the same rearing system (Chen et al., 2014). Hence, it is hypothesized that there is a positive relationship between serum oxytocin concentrations in individuals and positive social behaviors in cattle.

In the present study, we investigated the relationship of serum oxytocin concentration to positive social behaviors in cattle under different rearing conditions. First, we studied the relationship of serum oxytocin concentration to positive social behaviors of calves when they were introduced into a novel herd and faced new mates. Second, we studied the relationship of serum oxytocin concentration to positive social behaviors of cows in a stable herd where they were familiar with other individuals.

2. Materials and methods

This study was carried out at the Field Science Center, Graduate School of Agricultural Science, at Tohoku University, Japan. Fourteen Japanese Black calves (equal in gender, 3.0 ± 1.1 months old) were used in the first experiment, and 16 Holstein milking cows (47.1 ± 24.1 months old) were used in the second experiment. Both experiments were approved by the president of Tohoku University, Sendai, Japan (Approval number: 2014 agriculture animal-61 and 2014 agriculture animal-65).

2.1.1 Animal management

Six calves (equal in gender) were used as “introduced animals.” These animals would be moved into a stable herd of other calves. The six calves were separated from their dams the day after delivery. Then, they were reared individually under a bucket suckling system in a single pen (1 m × 2 m). The calves were spatially, but not visually or acoustically isolated from each other. They were fed commercial artificial milk powder (All-in-One, Tokyo, Japan) dissolved in warm water (300 g/3 L) twice daily at 0840 h and 1540 h through a suckling bucket with a nipple. Then, after they were seven days old, calves were fed hay and starter (JA group, Miyagi, Japan). Water was provided *ad libitum*.

The other 8 calves were raised with their dams after delivery and allowed to suckle freely in a nursing barn. Two weeks before the beginning of the experiment, two groups of four calves each (i.e., 8 calves in total) were moved to another barn and were reared in a group in a 4 m × 6 m pen. The barn was 500 m from the single pen with the introduced calves; therefore, the introduced calves were completely segregated from the group calves. The amount of the starter was determined so that the calves gained greater than 0.6 kg/d (NARO, 2008) of body weight. Water and salt was provided *ad libitum*.

2.1.2 Behavioral observation and blood collection of the introduced calf

A 75-day-old calf was introduced into each four-calf group by two animal managers familiar with the calves. Calves in the two four-calf groups were 125.1 ± 9.0 days and 127 ± 14 days old (mean ± SD). Three video cameras (KJH-F 690, Samsung Co. Ltd., Seoul, Republic of Korea) connected to one recorder (H210, Panasonic Co. ltd., Tokyo, Japan) were installed one week before the test. It was assumed that behaviors would change after introduction and feeding. Thus, behaviors of the introduced calf were observed (i.e., 1000 h [(the beginning of introduced time)] to 2000 h, and 0830 h [feeding time] to 1030 h) by a continuous sampling method. During these periods, the number of self-grooming behaviors, social affiliative behaviors, received social affiliative behaviors, jumps, agonistic behaviors, received agonistic behaviors, and explorative behaviors were recorded. The above behavior types are described in Table 1.

Blood samples were collected from the introduced calf five days before test commencement and after observations were finished (1100 h). A 15 mL blood sample was collected from the calf in three 5-mL tubes, each containing 250 KIU aprotinin. The tubes were kept on ice until centrifugation (1,600 × *g* for 20

Table 1. Definition of behaviors analyzed in the both tests of calves and cows.

Behavioral type	Definition
Frequency of self-grooming behavior	Licks body or scratches head with a foot by one-self One sequence of several licks or scratches was counted as one
Frequency of social affiliative behavior	Licks or sniffs another's body One sequence of several licks or sniffs was counted as one
Frequency of received social affiliative behavior	Receives Lick or sniff from another One sequence of several licks or sniffs was counted as one
Frequency of jumping	Jumps with 4 legs off the floor
Frequency of agonistic behavior	Threatens another using head
Frequency of received agonistic behavior	Receives head threat from another
Frequency of explorative behavior	Sniffs or touches wall or floor with nose or tongue
Frequency of social affiliative behavior	Licks or sniffs another's body One sequence of several licks or sniffs was counted as one
Duration of self-grooming behavior	Duration of body licking or head scratching with a foot by one-self
Duration of social affiliative behavior	Duration of licking or sniffing another's body

min). After centrifugation, serum samples were stored at -80°C until oxytocin analysis was performed.

2.2.1 Management of Holstein milking herd

The Holstein milking herd consisted of 16 milking cows and was milked twice a day at 0840 h and 1600 h. The barn consisted of an indoor free lying area and an outdoor sport area. The indoor lying area was covered with rice hulls and was parted into independent 1.4 m × 1.9 m beds. The outdoor sport area had a concrete floor, in the middle of which there was a feeder (3 m × 4 m). The cows had free access to grass silage in the feeder. Concentrates were also fed to cows, at about 1/3 of the milk yield, as specified by NARO (2006). Water and salt were always available to the cows.

2.2.2 Behavioral observation and blood collection of cows

Grass silage was provided in the feeder at 1100 h twice a week (Monday and Thursday). All the cows were released into the outdoor sport area immediately after the new grass silage was provided. They were kept in the outdoor area until the end of behavioral observations. Behavioral observation was carried out for 3 h/day, for a total of 18 h over six days in three successive weeks (twice a week). In order to record cow behavior, four cameras (Sony DCR-SR 220, Sony Co. ltd., Tokyo, Japan) were installed on the wall one week before test commencement. The frequency and duration of self-grooming behavior, social affiliative behavior, and the frequency of agonistic behavior and received agonistic behavior were recorded. The above behavior types are described in Table 1.

Blood samples were collected once a week (a total of 3 times) from the jugular vein of the cows after the end of the behavioral observations at 1430 h. Serum samples were obtained according to the previously described method.

2.3. Measurement of serum oxytocin concentration

Serum samples were used for the analysis of oxytocin concentrations. Oxytocin concentrations were measured using an enzyme-linked immunosorbent assay (ELISA). Rabbit anti-oxytocin purified IgG (G-051-01, Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA) was diluted to 57 ng/mL in coating buffer (0.015 M Na_2CO_3 and 0.034 M $NaHCO_3$: pH 9.6). Then, 100 µL of rabbit anti-oxytocin purified IgG was placed into a 96-well plate (445101, Thermo Scientific Nunc™, Denmark). After 24 h incubation at 4°C, the 96-well plate was washed with washing buffer (0.05% Tween 80) twice, and 250 µL blocking buffer (0.04 M Na_2HPO_4, 0.145 M NaCl, 0.1% BSA:

pH 7.2) was added to each well. After 2 h at room temperature, the plate was washed with washing buffer twice. Oxytocin standards and serum samples (100 μL) were added to the wells. After 24 h of incubation at 4°C, the plate was washed with washing buffer twice. Biotin-labeled oxytocin (AnaSpec Inc., San Jose, CA, USA) was diluted to 1 μg/mL in assay buffer (0.042 M Na_2HPO_4, 0.008 M KH_2PO_4, 0.02 M NaCl, 0.0048 M EDTA 1.79 g, 0.05% BSA: pH 7.5). Then, 100 μL of diluted biotin-labeled oxytocin was added to each well. After 24 h incubation at 4°C, the plate was washed twice with washing buffer. Streptavidin peroxidase (100 ng/well, SAP) (S5512, Sigma-Aldrich) diluted in assay buffer was added to each well, and the plate was stored at 4°C for 1 h. After that, the plate was washed with washing buffer twice, 100 μL TMB Soluble Reagent (High Sensitivity 23141, ScyTek Laboratories, Inc., Logan, UT, USA) was added to each well, and the plate was stored at 37°C for 30 min. The reaction was stopped by adding 25 μL of 2N HCl to each well. The absorbency at 450 nm was measured directly through the bottom of the plates with an ELISA Reader (International Reagents Corporation, Kobe, Japan). All other reagents were purchased from Wako Chemical Ltd. (Osaka, Japan).

2.4. Statistical analysis

In this study, the correlation coefficient was calculated to examine the relationship of serum oxytocin concentration to all the behaviors recorded. Probability values ≤ 0.05 were regarded as statistically significant and values ≤ 0.1 were considered to show a significant tendency. All the data were analyzed using SYSTAT 13 statistical software (SYSTAT Software, Inc., San Jose, CA, USA).

3. Results

3.1. Relationship between serum oxytocin concentration and behaviors in the introduced calves

Serum oxytocin concentrations in the introduced calves were 4.5 ± 1.3, 5.6 ± 0.5, 13.3 ± 5.2, 9.4 ± 3.0, 6.7 ± 1.8 and 7.9 ± 2.2 pg/ml (mean ± SD). Since serum oxytocin concentrations were not different between before and after the experimental period ($P > 0.05$), the mean value of the two samples was used for correlation analysis.

Serum oxytocin concentration positively correlated with explorative behavior in calves, but did not significantly correlate with other behaviors (r = 0.81, $P = 0.05$; n = 6). (Table 2)

3.2. Relationship between serum oxytocin concentration and behaviors in milking cows

Since serum oxytocin concentration was not different among the three sampling occasions ($P > 0.05$), the mean value of the three samples was regarded as a basal concentration and was used for correlation analysis.

Serum oxytocin concentration positively correlated with social affiliative behavior in milking cows (r = 0.58, $P = 0.02$; n = 16) (Table 3), but did not significantly correlate with other behaviors.

Table 2. Behaviors analyzed in introduced calf tests, and coefficient of behaviors with serum oxytocin concentration in calf response to new mates in new rearing conditions.

Behavioral type	No./cow/h (total 12 h) (mean ± SD)	Correlation to serum oxytocin concentration (n = 6)	
		r	*P*
Frequency of self-grooming behavior (No.)	4.9 ± 4.6	-0.03	0.91
Frequency of affiliative behavior (No.)	5.3 ± 5.2	0.51	0.30
Frequency of received affiliative behavior (No.)	3.2 ± 3.5	-0.01	0.99
Frequency of jumping (No.)	2.2 ± 2.1	0.25	0.64
Frequency of agonistic behavior (No.)	0.1 ± 0.1	-0.26	0.62
Frequency of received agonistic behavior (No.)	7.3 ± 7.5	0.35	0.46
Frequency of explorative behavior (No.)	6.7 ± 7.4	0.82	0.05

Table 3. Behaviors analyzed in the test of cows, and coefficient of behaviors with serum oxytocin concentration in a stable Holstein cows.

Behavioral type	No. or s/cow/h (Total 18 h) (mean ± SD)	Correlation to serum oxytocin concentration (n = 16)	
		r	*P*
Frequency of self-grooming behavior (No.)	0.7 ± 0.4	-0.17	0.53
Duration of self-grooming behavior (sec)	4.3 ± 2.4	-0.11	0.68
Frequency of social affiliative behavior (No.)	0.3 ± 0.2	0.58	0.02
Duration of social affiliative behavior (sec)	6.5 ± 0.2	0.09	0.73
Frequency of agonistic behavior (No.)	2.5 ± 1.8	0.19	0.47
Frequency of received agonistic behavior (No.)	2.5 ± 1.5	0.11	0.684

4. Discussion

4.1. Testing in calves

All domestic animals are strongly motivated to explore and investigate new environments. On some occasions, animals are placed in a novel situation that makes them fearful, and it has been stated that this fear may be connected to explorative motivation (Broom and Fraser, 2007). In the present study, the performed explorative behavior might be in response to the new environment and facing new mates. The positive correlation between serum oxytocin concentration and explorative behavior suggests that the higher the serum oxytocin concentration in a calf is, the less fearful the calf is when facing unfamiliar mates and a new environment. This result agrees with the finding that rats that have received oxytocin injections perform more normal behaviors after pro-conflict tests compared to rats in the control group, indicating that oxytocin increased the normal behavior pattern against stress (Svanidze et al., 2012). In addition, our previous study also supports this result that we found: the higher oxytocin concentration of calve is, the more social contacts with a decoy of Holstein calf in the open-field arena are observed (Chen et al., 2015).

4.2. Testing in cows

It has been reported that oxytocin induces affiliative behavior in rats (Babygirija et al., 2012) and voles (Rose and Young, 2009). This is in agreement with Dhakar et al. (2012), who showed that oxytocin receptor knockout rats performed more aggressive behaviors than the control rats. Dunbar (2008) reviewed that social grooming plays a particularly important role in social bonding and has a major impact on an individual's lifetime reproductive fitness. Furthermore, social grooming is thought to have functional significance for the formation and maintenance of social bonds and the stabilization of social relationships in cows (Sato et al., 1991). In the present study, we found that the higher the serum oxytocin concentration was, the more social affiliative behaviors were present in a stable herd of cows. However, it is hard to conclude that oxytocin plays an important role in the bonding and stability of a herd, because ages, body weights, and other factors may affect bonding and stability. Oxytocin did have a correlation with social affiliative behavior that promotes the establishment and stabilization of social bonds.

Da et al. (1996) mentioned central oxytocin induced maternal behavior in sheep and suggested that oxytocin has a positive feedback loop, in which oxytocin induced maternal behavior facilitates oxytocin release in both the brain and the blood. In addition, Neumann et al. (1994) reported that oxytocin acts in a positive feedback loop during suckling, suggesting that natural suckling may increase basal serum oxytocin concentrations in rats. This means that oxytocin concentration may induce positive social behavior, which in turn increases oxytocin concentration. Similarly, higher oxytocin concentrations may be beneficial to animals as they increase animals' stress tolerance, which may be explained by the mechanism in the review of Uvnäs-Moberg (1998).

In conclusion, it is suggested that a higher serum oxytocin concentration induces explorative and social affiliative behaviors in cattle as has been demonstrated for rats and humans. Since positive social behavior connects with improved welfare in cattle, studies on oxytocin in domestic animals may contribute greatly to improved animal management.

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