FULL PAPER *Ethology*

Effects of Intracerebroventricularly Administered Carbetocin on Social Behavior in Holstein Steers

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ABSTRACT. To shed light on the role of central oxytocin (OXT) in regulating social behavior in cattle, the impact of intracerebroventricularly administered OXT agonist, carbetocin (CBT), on the social behavior of a group of familiar steers was investigated. In the first experiment, we determined the dose response of intracerebroventricularly administered CBT (0.5, 5 or 50 nmol) on plasma cortisol level and behavior using 7 steers aged from 6 to 10 months. Five of the steers were assigned to the second experiment. CBT (50 or 200 nmol/200μl) in artificial cerebrospinal fluid (aCSF) or aCSF (200 μl) was injected into the third ventricle. Immediately after the injection, the animal and two peers were taken outside to the adjacent paddock. Thirty minutes later, maintenance and social behaviors of the animal were observed for 2 hr. CBT had no effect either on the basal cortisol level or on the maintenance and the abnormal behavior in steers with their movement restricted by a stanchion stall in the first experiment. However, in the same steers with no movement restrictions in the second experiment, CBT facilitated lying, probably because of its sedative effect via OXT receptor activation, which disturbed some aspects of social behavior. These results suggest that central OXT receptor activation might not affect social behavior itself among "familiar members", because the stimulation of the central OXT system by intracerebroventricular administration of CBT did not facilitate social behavior between familiar steers.

KEY WORDS: carbetocin, oxytocin agonist, sedative effect, social behavior, steer.

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In modern cattle management systems, steers are generally kept in groups. Many social factors, such as the scramble for food and space, the introduction of new members into the group and some other restraints of behavior, lead to the reduction of production efficiency. A number of studies have tried to solve this problem by mainly studying their social behavior from a herd perspective [17] and optimizing the facility and management system according to their socio-behavioral characteristics. Through the knowledge of the central mechanisms of social behavior, more efficient management techniques can be developed to allow a balance to be struck between production efficiency and animal welfare.

Oxytocin (OXT) is a neuropeptide in the central nervous system; besides its well-known role in parturition and lactation, it acts to regulate social cognition and behavior [9, 19, 24, 26]. Examining the effect of intracerebroventricularly administered OXT or its agonist on social behavior can be helpful in confirming whether the central OXT system modulates social behavior in cattle as well. For that purpose, continuous stimulation of central OXT receptors is necessary. The half-life of OXT itself, however, is less than 20 min in cerebrospinal fluid [18]. Moreover, high doses of intracerebroventricularly administered OXT have been shown to induce stress-like responses, such as an increase in plasma cortisol (CORT) concentration and abnormal stereotypical behavior, in steers [27].

Deamino-1-monocarba-(2-O-methyltyrosine)-oxytocin (carbetocin; CBT), an OXT analog, has been synthesized to be protected from aminopeptidase and disulfidase cleavage and has prolonged uterotonic activity [2, 5, 8]. Both the peripheral and central administration of CBT induces anxiolytic-like behavioral effects through OXT receptor activation [3, 11–13, 15]; unlike OXT, these effects are dose-dependent and last longer [5, 12, 13]. Though the role of OXT in the regulation of social behavior is widely recognized, the effect of CBT on social cognition and behavior has not been investigated.

The aim of the present study was to investigate the impact of the intracerebroventricular administration of CBT on the social behavior of a group of familiar steers to shed light on the role of central OXT in regulating social behavior in cattle.

MATERIALS AND METHODS

Experimental procedures and care of the animals were approved by the Institute Committee for Animal Use and Care

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Animals and surgery: Seven steers (6 to 10 months old, 197–268 kg, at the start of the experiment) were used in the first experiment, and five of the steers were used in the second experiment. They were castrated at least two months before the first experiment. After stereotaxic surgery, animals were individually reared in a stanchion stall in an experimental room in groups of three. The animals were fed 2 kg of concentrate feed and chopped timothy hay twice a day at 09:00 and 16:00 hr. The amount of hay differed according to the body weight of the steers to maintain 0.9 kg daily body weight gain. Water was available *ad libitum*.

At least one month before the first experiment, an 18–G stainless cannula (Eicom, Kyoto, Japan) was stereotaxically implanted into the third cerebral ventricle of each steer placed in a stereotaxic apparatus meant for calves [21] under isoflurane anesthesia according to a procedure reported earlier [10].

During the pre-experimental period, steers were continually tamed and allowed to adapt to their environment to minimize the impact of the stress of handling associated with intracerebroventricular infusions and blood sampling. On the day prior to the first experiment, each animal was fitted with an indwelling jugular catheter (Terufusion IVH catheter kit, Terumo, Tokyo, Japan) to collect blood samples.

Treatment solutions: The infusion vehicle was artificial cerebrospinal fluid (aCSF: NaCl, 125 mM; KCl, 2.5 mM; NaH_2PO_4, 0.5 mM; Na_2HPO_4, 1.2 mM; $CaCl_2$, 1.2 mM; $MgCl_2$, 1.0 mM; $NaHCO_3$, 27 mM) [7]. The pH of the medium was adjusted to 7.4. For the first experiment, CBT (Bachem AG, Bubendorf, Switzerland) was dissolved in aCSF at dilutions of 2.5, 25 and 250 nmol/1 m*l*, and stored at −20°C before use. For the second experiment, CBT dissolved in the aCSF solution at dilutions of 250 and 1,000 nmol/1 m*l* was used.

Experimental procedure: *Experiment 1*. Seven animals were assigned to the following 4 treatments randomly at intervals of at least 2 days. Experiments were performed between 13:30 and 15:30 hr. At 14:00 hr (point 0 of the experiment), the animal was lightly restrained with a rope and 0.5, 5 and 50 nmol of CBT in 200 μl aCSF or 200 μl of aCSF was injected into the third ventricle via the implanted cannula at a rate of 200 μl/20 sec. Serial blood samples for measurements of plasma CORT concentrations were collected via indwelling jugular catheters at –30, 0, 10, 20, 30, 40, 50, 60 and 90 min. The maintenance and abnormal behaviors of the animal were observed from 0 to 90 min.

Experiment 2. Five of the animals used in the first experiment were assigned to the following 3 treatments randomly at intervals of at least 2 days. Experiments were performed between 13:30 and 16:00 hr. At 13:30 hr, the animal was lightly restrained with a rope, and 50 or 200 nmol of CBT in 200 μl aCSF or 200 μl of aCSF was injected into the third ventricle via the implanted cannula at a rate of 200 μl/20 sec. Immediately after the injection, the animal and 2 peers were taken outside to the adjacent paddock (18 m × 20 m). The maintenance and social behaviors of the animal were observed from 14:00 to 16:00 hr. Before the experiment began, animals had spent at least 1 week getting accustomed to being taken outside to the paddock in a group of three around the same time.

Data analysis. Blood samples were taken into prechilled tubes containing EDTA sodium and stored on ice until the end of the experiment. After the experiment, the tubes were centrifuged, and plasma samples were stored at −20°C until assay. The plasma CORT concentrations were obtained by enzyme immunoassay [22] using peroxidase-labeled CORT (FKA403, 1:40,000 final dilution; Cosmo Bio Co., Ltd., Tokyo, Japan) and anti-CORT serum (FKA404E, 1:70,000 final dilution; Cosmo Bio Co.). The standard curve ranged from 0.16 to 160 *n*g/m*l*, and the ED_{50} of the assay was 3.2 *n*g/m*l*. The intra-assay coefficient of variation (CV) was 3.5% at 24 *n*g/m*l*. The inter-assay CV was 8.6% at 24 *n*g/m*l*. The areas under the CORT concentration curve between 0 and 90 min after the injection were calculated for the first experiment.

Continuous behavior sampling was performed for 90 min after injection using the videotaped record in the first experiment and for 2 hr from 30 min after injection by visual observation in the second experiment. In the first experiment, we observed maintenance behavior, such as self-grooming and water access, and abnormal behavior, such as rubbing, abnormal licking and tongue playing. The percentages of time spent lying or ruminating for 90 min after the injection were calculated. The social behaviors observed in the second experiment are listed in Table 1. We also observed maintenance behaviors, such as the duration of standing, lying and ruminating, latency to the first lying and frequency of self-grooming.

Statistical analysis. In the first experiment, the effects of time and treatments on CORT were analyzed using two-way analysis of variance with treatment group as the between-subject factor and time as the within-subject repeated measure. The differences in variation with time depend on whether the time-treatment interaction was significant or not ($P<0.05$). Additionally, the area under the CORT curve (AUC) was calculated from 0 to 90 min with basal concentrations subtracted during each treatment. The AUCs were analyzed using one-way analysis of variance with treatment group as the between-subject factor. Behavioral data were statistically assessed by Friedman's test followed by a Nemenyi multiple comparison, if there was a significant effect of treatment. In the second experiment, behavioral data were statistically assessed by Friedman's test followed by a Nemenyi multiple comparison, if there was a significant effect of time.

RESULTS

The mean (± SD) dairy body weight gain of the seven experimental animals during the whole experimental period was 0.93 ± 0.17 kg (0.80–1.21 kg).

Experiment 1: The time-treatments interaction was not significant in CORT (Fig. 1A). There was no significant main effect of treatment on the AUC (Fig. 1B).

Table 2 summarizes data for maintenance and abnormal

Table 1. Behaviors analyzed in the second experiment

Measure	Definition
Frequency of social investigation for peers (no.)	Sniffing or touching a peer's body with nose or tongue
Frequency of social investigation from peers (no.)	Sniffing or touching the steer's body with a peer's nose or tongue
Frequency of affiliative behavior for peers (no.)	Snuggling up to a peer to have body contact; making nose to nose contact with a peer; rubbing their face, head, and neck on a peer's body; licking a peer's body
Frequency of affiliative behavior from peers (no.)	Snuggled up to for body contact by a peer; made nose to nose contact with; rubbed face, head, and neck on the steer's body; licked body
Frequency of attacking behavior for peers (no.)	Head–butting for a peer
Frequency of attacking behavior from peers (no.)	Head–butted from a peer
Frequency of head-to-head contact (no.)	Fighting or mock fighting with head-to-head contact
Frequency of escape behavior (no.)	Escaping from peer after being threaten by head throw, attacked from peer, and defeat in fighting
Frequency of foraging behavior within 50 cm of a peer after approaching the peer (no.)	Approaching and foraging near a peer when the distance between their muzzles was within 50 cm
Mean individual distance (body length)	Mean value of the distance to the nearest neighbor as multiples of the steer's body length recorded at 10-min intervals for 2 hr

Table 2. Percentage of time spent lying or ruminating and occurrence of maintenance and abnormal behavior for 90 min after the intracerebroventricular injection of carbetocin

		aCSF	Carbetocin 0.5 *nmol*	Carbetocin 5 *nmol*	Carbetocin 50 *nmol*	χ^2	*P*
Lying (%)	Mean	44.8	49.8	41.7	49.1	2.83	0.42
	SD	15.5	19.6	19.3	13.7		
Ruminating (%)	Mean	33.1	29.4	24.2	22.0	5.57	0.13
	SD	11.0	11.3	10.6	7.9		
Self-grooming (No.)	Mean	8.4	5.6	7.7	8.3	1.93	0.59
	SD	9.1	5.9	6.7	7.1		
	Number of Animals exhibiting the behavior	(7/7)	(5/7)	(7/7)	(7/7)		
Water Access (No.)	Mean	3.7	3.3	3.7	4.4	0.81	0.85
	SD	3.0	3.1	3.3	3.1		
	Number of Animals exhibiting the behavior	(5/7)	(4/7)	(5/7)	(6/7)		
Rubbing (No.)	Mean	6.6	5.9	7.7	13.9	4.20	0.24
	SD	4.8	5.5	6.3	8.6		
	Number of Animals exhibiting the behavior	(7/7)	(5/7)	(6/7)	(7/7)		
Abnormal Licking (No.)	Mean	3.4	3.0	3.6	3.7	0.56	0.91
	SD	3.9	2.7	2.7	2.4		
	Number of Animals exhibiting the behavior	(6/7)	(5/7)	(7/7)	(7/7)		
Tongue Playing (No.)	Mean	2.3	0.9	2.1	2.4	3.30	0.35
	SD	4.4	1.9	4.4	4.7		
	Number of Animals exhibiting the behavior	(3/7)	(2/7)	(4/7)	(4/7)		

P: *P* -values obtained by Friedman's test.

behaviors exhibited for 90 min after injection. There was no significant difference among the 4 treatments.

Experiment 2: Table 3 summarizes data for social behaviors exhibited for the observed 2 hr. There were differences among the three treatments in the frequency of affiliative behavior from peers (Friedman's test, χ^2=6.1, *P*=0.05) and in the frequency of foraging behavior within 50 cm of the peer after approaching the peer (Friedman's test, χ^2=5.2, *P*=0.07). The frequency of affiliative behavior from peers tended to be less in those treated with 50 nmol CBT than those treated with aCSF (*P*<0.1). The frequency of foraging behavior within 50 cm of the peer after approaching the peer tended to be less in those treated with 200 nmol CBT than those treated with aCSF (*P*<0.1). There was a significant effect of treatment in the total duration of lying (Friedman's test, χ^2=7.6, *P*=0.02), and there tended to be differences among the three treatments in the latency to the first lying (Friedman's test, χ^2=4.8, *P*=0.09; Table 4). Compared with those treated with

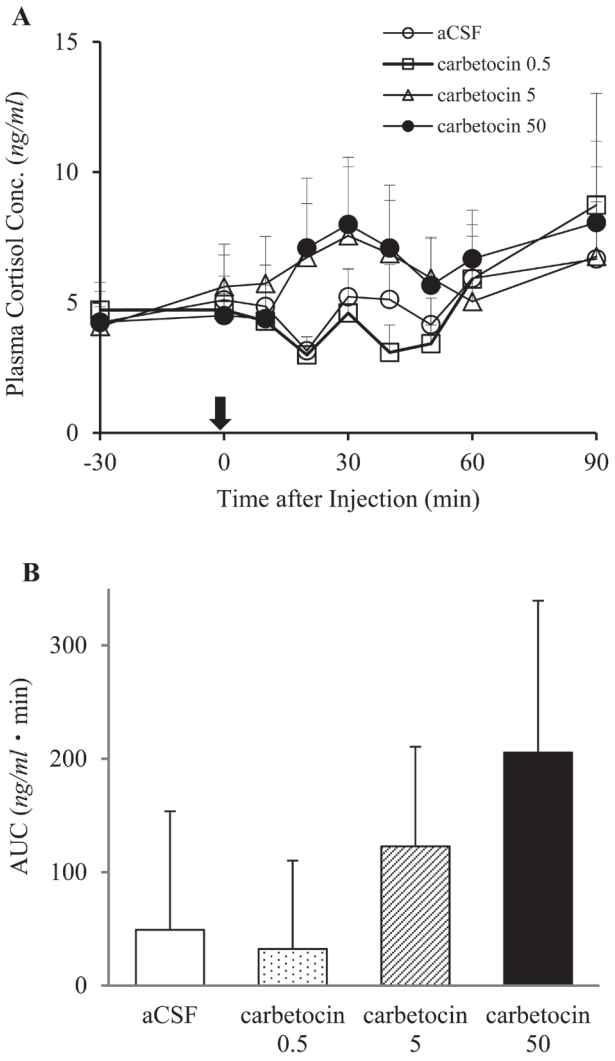

Fig. 1. The effects of intracerebroventricular injections of 200 μl of aCSF and 0.5, 5 and 50 *nmol*/200 μl of carbetocin (CBT) on temporal changes in mean (+ SEM) plasma cortisol (CORT) concentrations in steers (A) and the mean (+ SD) area under the plasma CORT concentration curve from 0 to 90 min after the injection (B). No statistically significant difference was found among the four treatments.

aCSF, the total duration of lying was significantly less in those treated with 200 nmol CBT ($P<0.05$), and it tended to be lesser in those treated with 50 nmol CBT ($P<0.1$).

DISCUSSION

In the present study, intracerebroventricularly administered CBT had no effect either on the basal CORT level or on the maintenance and the abnormal behavior in steers whose movement was restricted by a stanchion stall. However, in the same steers with no restriction of movement, a higher dose of CBT facilitated lying and disturbed some aspects of social behavior.

High doses of CBT (50 nmol) did not induce stress-like responses in the present study. In steers, intracerebroventricular administration of a comparable molecular dose of OXT (50 μg: 49.6 nmol) activated the hypothalamo-pituitary-adrenal axis and induced stereotypical behaviors [27]. Because the closely related nonapeptide, arginine vasopressin (AVP), induced stress-like responses in steers [28], we had speculated that an excessive amount of OXT would stimulate AVP V1b receptors [27]. Schlosser *et al.* also reported that the OXT-elicited increase in adrenocorticotropic hormone release is mediated via AVP V1b receptors in rats, whereas OXT affinity for the V1b receptor is one order of magnitude less than AVP [20, 23]. In contrast, CBT has lower affinity for AVP V1b receptors [4, 5, 16]. Therefore, in the second experiment, we used a highest dose of CBT used in the first experiment (50 nmol) which did not induce stress-like responses, and higher dose of CBT (200 nmol) with a view to allowing prolonged activation of central OXT receptors.

Intracerebroventricularly administered CBT might diffuse and stimulate OXT receptors widely distributed within the brain [1]. Only in the second experiment with no movement restrictions did the latency to first lying decrease and the total duration of lying increase following CBT treatment. In the rat, a low dose of intracerebroventricularly administered OXT has been shown to have an anxiolytic effect, and a high dose has been shown to have a sedative effect [11, 13, 25]. Although we could not show why the same dose of CBT (50 nmol/animal) affected behavior in the free-moving condition, but not under behavioral constraints, a high dose of CBT might induce sedation through the prolonged activation of central OXT receptors.

The partial decrease in social behavior following CBT treatment, i.e., the decrease in the frequency of foraging behavior within 50 cm of the peer after approaching the peer and affiliative behavior from peers, was probably due to reduced opportunities for social contact by increased resting behavior. In the control treatment in the second experiment, lying had not been observed for more than 80 min after the observation. Conversely, in the CBT treatment, animals started lying after 30 to 40 min, and the total duration of lying was about 8 times more than in the control, though this was not statistically significant. In rats, a high dose of OXT administered subcutaneously decreases spontaneous motor activity in an unstressed condition [25]. Though CBT has been reported to increase spontaneous locomotor activity in their "unfamiliar" "novel" environment [11–13, 15], it may reduce spontaneous activity and increase resting under "familiar" environment like OXT.

Whether the social relationships among the experimental animals were established or not is another point of discussion. The tests were conducted after at least 7 days of social encounters in the experimental paddock. Moreover, the animals had been reared together for at least 2 months, though their movements were restricted by stanchions. Thus, we consider that this treatment of the experimental animals before the experiment was enough to establish social bond-

Table 3. Social behavior for 2 hr in the paddock 30 min after the intracerebroventricular injection of carbetocin

		aCSF	carbetocin 50 *nmol*	carbetocin 200 *nmol*	χ^2	*P*
Frequency of social investigation for peers	Mean	7.4	8.6	8.4	0.7	0.70
	SD	4.2	4.4	6.5		
Frequency of social investigation from peers	Mean	5.2	5.2	7.6	1.9	0.39
	SD	4.1	1.6	3.4		
Frequency of affiliative behavior for peers	Mean	11.5	8.8	9.5	0.1	0.95
	SD	9.1	8.7	8.9		
Frequency of affiliative behavior from peers	Mean	14.3	7.0 †	14.8	6.1	0.05
	SD	9.7	7.4	6.9		
Frequency of attacking behavior for peers	Mean	8.25	9.8	9.5	1.6	0.45
	SD	7.6	14.9	12.6		
Frequency of attacking behavior from peers	Mean	10.75	6.5	11.0	1.3	0.52
	SD	11.5	5.5	11.0		
Frequency of head-to-head contact	Mean	11.3	8.8	10.3	0.4	0.82
	SD	11.4	5.1	10.8		
Frequency of escape behavior	Mean	4.0	4.3	6.3	1.6	0.45
	SD	4.1	4.0	5.9		
Frequency of foraging behavior within 50 cm of the peer after approaching the peer	Mean	6.6	6.0	3.4 †	5.2	0.07
	SD	2.9	2.5	3.0		
Mean individual distance (body length)	Mean	3.0	4.1	3.9	1.6	0.45
	SD	1.0	2.1	1.8		

P: *P*-values obtained by Friedman's test.
†: Tended to differ from aCSF (Nemenyi multiple comparison: $P<0.1$).

Table 4. Maintenance behavior for 2 hr in the paddock 30 min after the intracerebroventricular injection of carbetocin

		aCSF	carbetocin 50 *nmol*	carbetocin 200 *nmol*	χ^2	*P*
Total duration of standing (min)	Mean	114.6	99.4	100.1	2.8	0.25
	SD	5.8	11.7	17.4		
Total duration of lying (min)	Mean	2.4	16.9 †	15.1 *	7.6	0.02
	SD	3.6	9.0	14.5		
Total duration of ruminating (min)	Mean	2.9	3.6	4.8	1.6	0.45
	SD	6.0	4.7	5.3		
Latency to the first lying (sec)	Mean	82.2	33.6	39.6	4.8	0.09
	SD	21.3	12.0	34.9		
Frequency of self-grooming	Mean	56.5	55.8	64.5	2.8	0.25
	SD	9.9	16.1	11.1		

P: *P*-values obtained by Friedman's test.
*: Significantly different from aCSF (Nemenyi multiple comparison: $P<0.05$).
†: Tended to differ from aCSF (Nemenyi multiple comparison: $P<0.1$).

ing among peers [6, 14].

In conclusion, prolonged activation of central OXT receptors might not affect social behavior itself "among familiar members", because the stimulation of the central OXT system by the intracerebroventricular administration of CBT did not facilitate social behavior between familiar steers. The effects of CBT in other social contexts, i.e., the cognitive responses for unfamiliar individual and agonistic interaction over limited resources, should be investigated in future. Since centrally administered CBT, unlike OXT, does not induce stress-like responses, CBT could be a more suitable tool than OXT itself for experimentally studying the central role of OXT in regulating social behavior.

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