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REVIEW
Central Regulation of Stress Response in Cattle

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Abstract

Useful and biologically meaningful determinants of stress of farm animals at the field level do not exist at present, though quantitative measurement of stress is essential for the farm animals' well-being. At laboratory level using rodents, physiological and behavioral changes induced by stressors and its regulation by the central nervous system have been elucidated in considerable detail. However, it remains unclear whether we could apply that knowledge to species other than rodents. Therefore, we studied several neurotransmitters involved in the regulation of stress response in cattle. Examination of the effects of intracerebroventricularly administered neurotransmitter candidates revealed which component of stress response was induced or reduced. Thus, we identified several stress-inducing neurotransmitters and one stress-reducing neurotransmitter in cattle, and suggested that the regulatory roles of the neurotransmitters on stress response differ from those of other species, as well exemplified by rodents. Furthermore, for the stress-reducing neurotransmitter, oxytocin, we showed for the first time the association between individual differences in behavioral and hypothalamic-pituitary-adrenal (HPA) axis reactivity to acute stressors and basal levels of plasma oxytocin concentration and its reactivity to the stressors. This suggests that oxytocin may be a neurobiological candidate involved in individual differences for stress susceptibility in cattle. Currently, we are examining the association between polymorphism of oxytocin-related genes and individual differences in stress susceptibility. This knowledge will help improve animal welfare by allowing genetic selection for adaptability to the environment.

Discipline: Animal health
Additional key words: arginine vasopressin, corticotropin-releasing hormone, individual differences, oxytocin, stress susceptibility

Introduction

Animal welfare, to ensure the well-being of animals under human care, is one of the key global issues of animal husbandry in the 21st century. The EU conducted a Welfare Quality project that aimed to accommodate societal concerns and market demands as well as develop reliable on-farm monitoring systems, product information systems, and practical species-specific strategies to improve animal welfare[9]. Furthermore, the International Epizootic Office is developing standards and recommendations in several areas important to animal welfare[14]. With this in mind, the quantitative analysis of stress in farm animals is essential to evaluate the animal well-being. However, useful and biologically meaningful determinants of stress at the field level do not currently exist. At the laboratory level, various evaluative approaches have been attempted such as monitoring changes in behavioral functions like suppression of normal behavioral repertoire, the occurrence of conflict and/or abnormal behavior; monitoring changes in endocrine functions like changes in plasma adrenocorticotropic and cortisol concentrations; and monitoring changes in autonomic nervous functions like heart rate and body temperature[1]. However, several discrepancies are present among these indicators depending on the animal as well as the type and intensity of the stressor, which makes the quantitative analysis of animal stress non-cross-sectional.

The present review focuses on recent studies conducted on the central mechanisms regulating stress responses, and their application in elucidating the neurobiological basis of individual differences in stress susceptibility in cattle. To facilitate the objective measurement of animal stress, we identified the neurotransmitter candidates involved in the central regulation of be-

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havioral and physiological stress responses in cattle. Furthermore, to help improve animal welfare by genetic selection for adaptability to the environment, we are currently conducting studies on the neurobiological basis of individual differences in stress susceptibility in cattle.

Inducers of stress response

1. Corticotropin-releasing hormone and arginine vasopressin

The neuropeptides corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) are released within the brain to regulate behavioral and physiological stress responses[8,21]. In rodents, increased locomotor activity, hypothalamic-pituitary-adrenal (HPA) axis activity, and sympathetic nervous activity were simultaneously observed in an integrated manner following a central injection of CRH[6,31]. CRH was a more potent stimulator of the HPA axis than AVP in rodents[45]. A central injection of AVP also induced stereotypical anxiety-like behavior such as scratching and self-grooming[26], and increased blood pressure and heart rate in rats[42]. However, few studies[7,18,32] on farm animals have evaluated the effects of central CRH and AVP on these functions in an integrated whole animal model. Although many of the CRH- and AVP- mediated responses observed in rodents may be expressed in other species, there may also be some species-specific characteristics. For example, in comparison to fight or flight responses or intensive vocalization in ruminants, rats show a tendency to freeze in response to situations of fear or shock. Initially, we showed in cattle that bovine CRH (bCRH) and arginine vasopressin (AVP)

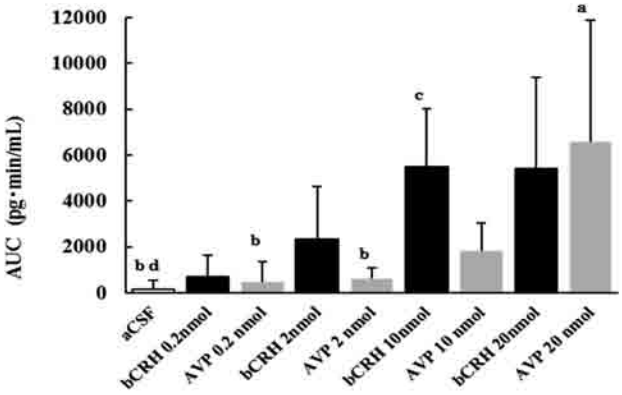

Fig. 1. The effects of intracerebroventricular infusions of artificial cerebrospinal fluid (aCSF) and 0.2, 2, 10 and 20 nmol of bovine corticotropin-releasing hormone (bCRH) or arginine vasopressin (AVP) on plasma ACTH concentrations in steers

Values are expressed as the mean area under the curve of the plasma ACTH concentration from 0 to 120 min after the onset of infusion (+ SD, n = 4). Different superscript letters indicate statistical differences ($p < 0.05$: between a and b, $p<0.1$: between c and d).

CRH was more potent ACTH secretagogue than AVP. (Modified from Yayou et al., 2008b)

Table 1. Effects of intracerebroventricular infusions of artificial cerebrospinal fluid (aCSF), bovine corticotropin-releasing hormone (bCRH), or arginine vasopressin (AVP) on behavior over 120 min after the onset of infusions

		aCSF	bCRH 0.2 nmol	bCRH 2 nmol	bCRH 10 nmol	bCRH 20 nmol	AVP 0.2 nmol	AVP 2 nmol	AVP 10 nmol	AVP 20 nmol	χ^2	p
Total Incidents of Head Shaking	Mean	0.75	3.0	2.8	12.0	6.3	2.3	5.5	9.8 †	12.3 †	22.79	0.004
	SD	0.5	2.6	2.9	5.6	5.3	2.1	4.1	5.9	7.4		
	Number of Animals exhibiting the behavior	(3/4)	(3/4)	(4/4)	(4/4)	(4/4)	(3/4)	(4/4)	(4/4)	(4/4)		
Total Incidents of Head Rubbing	Mean	6.3	5.5	5.3	13.5	5.5 [a]	5.3	10.8	41.5 [b]	40.3 [b]	23.93	0.002
	SD	2.9	3.8	2.6	8.1	2.5	3.3	3.3	24.5	7.8		
	Number of Animals exhibiting the behavior	(4/4)	(4/4)	(4/4)	(4/4)	(4/4)	(4/4)	(4/4)	(4/4)	(4/4)		
Total Incidents of Tongue Playing	Mean	26.3	40.3	36.5	117.0	83.8	34.8 [a]	72.5	151.0 * [b]	151.8 †	27.18	6.58E^{-4}
	SD	11.6	21.7	7.3	28.4	35.0	15.9	6.1	39.2	42.2		
	Number of Animals exhibiting the behavior	(4/4)	(4/4)	(4/4)	(4/4)	(4/4)	(4/4)	(4/4)	(4/4)	(4/4)		
Total Incidents of Vocalization	Mean	0.8	0.8	0.3	32.8	34.8	0	0	0	0	26.84	7.54E^{-4}
	SD	1.0	1.5	0.5	40.2	19.9	0	0	0	0		
	Number of Animals exhibiting the behavior	(2/4)	(1/4)	(1/4)	(4/4)	(4/4)	(0/4)	(0/4)	(0/4)	(0/4)		

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: $p < 0.1$).
a, b: Tended to differ between different superscript (Nemenyi multiple comparison: $p < 0.1$).
(Modified from Yayou et al. 2008b)

could activate the HPA axis in steers when infused intracerebroventricularly and that bCRH was more potent at stimulating the HPA axis than AVP at equimolar concentrations (Fig. 1)[54]. These results resembled those in rodents but differed from those in sheep, which show that ovine CRH (oCRH) and AVP have comparable potential to stimulate cortisol release into the systemic circulation when infused intracerebroventricularly[51,52]. We have also shown that high dosages of both peptides induced stereotyped behaviors and that the types of stereotyped behaviors induced differed between bCRH and AVP (Table 1)[54]. These results were comparable to those in sheep that showed oCRH-induced stereotypical bleating[51] and AVP-induced oral stereotyped behaviors[52]. This suggests that the CRH and AVP system within the brain may regulate HPA axis activation during stress, with CRH being a more potent stimulator than AVP, and that the two systems may work differently to regulate different behavioral responses to stress in cattle.

2. Neuromedin U and neuromedin S

The neuropeptides neuromedin U (NMU) and neuromedin S (NMS) were identified as endogenous ligands for two orphan G protein-coupled receptors, FM-3/GPR66 and FM-4/TGR-1[27,30], currently identified as NMU type-1 and -2 receptors, respectively[10,13,36]. Since NMU type-2 receptors are highly expressed in the central nervous system, the physiological roles of these neuropeptides in the central nervous system have been investigated, mainly in rodents. Both neuropeptides activated the HPA axis and induced grooming through the CRH system[11,16], but only NMU increased locomotor activity[11,49]. Although we showed that CRH was a more potent stimulator of the HPA axis than AVP, as in rodents, behavioral data suggest species differences between cattle and rodents in the central roles of CRH and AVP in regulating behavioral responses under stressful conditions[54]. Since NMU and NMS may play important roles in regulating stress responses through CRH and/or AVP, we elucidated the central roles of these neuropeptides as part of our study on the central regulation of stress responses in cattle[55]. We confirmed that intracerebroventricular administration of either NMU or NMS activated the HPA axis in steers and triggered marked cortisol responses, suggesting that both NMU and NMS are involved in the control of the HPA axis also in this species. We also demonstrated that intracerebroventricular administration of NMU induced an increase in body temperature, suggesting that this peptide may be involved in thermoregulation in cattle as in the case in rodents[13]. A high dose of NMS tended to shorten the duration of lying and increased the amount of head shaking, suggesting that it may induce restlessness in this species. In rats, NMS activated grooming[16] and NMU activated grooming and increased overall locomotor activity[11,49], which are reported to be CRH-mediated[11,16]. In our previous study in cattle, however, intracerebroventricularly administered CRH did not induce either stereotypical grooming or behavioral activation; rather intracerebroventricularly administered AVP induced restlessness including head shaking[54]. There might be species-specific differences in the downstream regulation of behavioral responses by central NMS. Although further studies are required to determine the exact roles of central NMS in regulating restlessness in cattle, we speculate at this point that central NMS might regulate restlessness by modulating the activity of AVP neurons unlike rodents. Further research using specific antagonists for CRH or AVP is needed to confirm downstream neuroendocrine targets of NMU type-2 receptors.

3. Prolactin-releasing peptide

Prolactin-releasing peptide (PrRP) has been identified as an endogenous ligand for an orphan G-protein-coupled receptor in bovine hypothalamus, GPR 10/hGR3[12]. The highly expressed PrRP receptor in the paraventricular nucleus of the hypothalamus[37], increased c-fos expression in the PrRP neuron after foot shock and restraint stressors[29,57]and increased plasma adrenocorticotropic hormone and corticosterone concentrations in rats after the intracerebroventricular administration of PrRP[24], indicates the possible role of this receptor in the regulation of the HPA axis. The PrRP receptor also regulates energy expenditure via central CRH receptors[22] and stimulates the sympathetic nervous system through CRH neurons to increase body temperature, heart rate, and blood pressure[22,50]. To date, little data have been published regarding the role of central PrRP in ruminants. As Mogi el al[28] reported that intracerebroventricular administration of PrRP did not increase plasma cortisol concentrations in sheep, there may be some species-specific differences between rodents and ruminants. We recently confirmed that intracerebroventricular administration of bovine PrRP activated the HPA axis in steers, evoking a dose-dependent cortisol response, and suggesting that PrRP is involved in controlling the HPA axis also in this species[20]. Although the intracerebroventricular administration of PrRP affected body temperature, the hypothermia followed by long-lasting hyperthermia reported in rats[22] was not observed in steers[20]. Like other species, central PrRP is unlikely to be involved in controlling stress-related behavior in cattle since it did not induce stress-related behavior[20]. Although many studies in rodents indicated that PrRP stimulate the CRH neuron

in the paraventricular nucleus of the hypothalamus[29,37], and intracerebroventricularly administered CRH can mimic stress-related behaviors, such as an increase in ambulatory activity and the induction of abnormal behaviors[6,31], no reports to our knowledge have reported details of stress-related behaviors induced by intracerebroventricularly administered PrRP.

Reducer of stress response, oxytocin

Besides having major functions in ensuring successful parturition as well as nutrition and care of the offspring, oxytocin is released intracerebrally to regulate pituitary–adrenocortical and behavioral stress responses in response to various stressors[35]. Recent studies in rodents have shown that oxytocin suppresses the HPA response to stressors[34,35,47] and has anxiolytic properties[25,46]. However, few studies have examined the anti-stress effect of central oxytocin in animals other than rodents. In sheep, oxytocin infusions directly into the posterior pituitary, but not into the paraventricular nucleus of the hypothalamus, suppressed cortisol responsiveness to an acute psychological stressor, a barking dog[4]. Recently, Ludwig *et al.*[23] reviewed evidence that oxytocin is released from dendrites and diffuses to distant targets to exert long-lasting changes in behavior. We attempted to mimic this phenomenon by the intracerebroventricular administration of oxytocin. The anti-stress effect of central oxytocin has not been elucidated in cattle. We speculated that the role of CRH or AVP in regulating stress responses in cattle may differ from that in sheep and rodents[51,52,54]. Furthermore, oxytocin may suppress the activity of the neuropeptides that induce stress responses, meaning the central role of oxytocin could differ in cattle. Low doses of oxytocin significantly attenuated isolation-induced

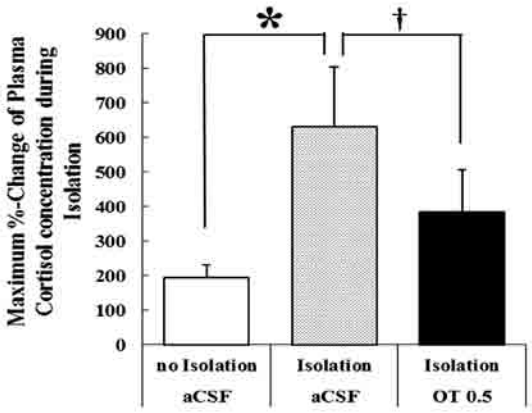

Fig. 2. The effects of intracerebroventricular injections of 200 µl of artificial cerebrospinal fluid (aCSF) and 0.5 µg of oxytocin in 200 µl aCSF on isolation-induced temporal changes in the maximum percentage change of plasma cortisol concentration from pre-injection values during isolation

* indicates statistical difference between the treatments ($p<0.05$).

† indicates tendency to differ between the treatments ($p<0.1$).

(Modified from Yayou et al., 2008a)

Table 2. Percentage of time spent lying and occurrence of conflict behaviors exhibited for the 60 min isolation period

		artificial cerebrospinal fluid (aCSF) no isolation	aCSF isolation	Oxytocin 0.5 µg Isolation	χ^2	p
Lying (%)	Mean	59.5	11.6 *	21.8	8.40	0.024
	SD	20.9	10.8	21.4		
Vocalization (No.)	Mean	0	62.8 *	53 †	7.60	0.040
	SD	0	30.7	41.1		
	Number of Animals exhibiting the behavior	(0/5)	(5/5)	(5/5)		
Body Orientation Change (No.)	Mean	4.2	25 †	31.2 *	7.60	0.040
	SD	1.6	11.4	9.8		
	Number of Animals exhibiting the behavior	(5/5)	(5/5)	(5/5)		

p: P-values obtained by Friedman's test

*: Significantly different from aCSF no isolation (Nemenyi multiple comparison: $p < 0.05$)

†: Tended to differ from aCSF no isolation (Nemenyi multiple comparison: $p < 0.1$)

(Modified from Yayou et al. 2008a)

cortisol increase in steers (Fig. 2)[53]. Conversely, low doses of oxytocin did not affect isolation-induced behavioral responses, i.e. excessive vocalizations, frequent changes in body orientation, and a relative decrease in lying (Table 2)[53]. These results suggest that the oxytocin system within the brain regulates HPA axis inhibition during stress; however, the system may not function in regulating behavioral responses to stress under these experimental conditions. Central oxytocin activity to attenuate anxiety response to a mild stressor might be subject to the strong regulation of gonadal steroids in mice and rats[48], suggesting a strong relation between oxytocin and gonadal steroids. The lack of inhibition in the behavioral response to isolation by oxytocin in our study may also have been attributable to the lower estrogen influence in steers castrated before sexual maturation than that in females. The effect of gonadal steroids on these functions of oxytocin is to be elucidated in future study.

Table 3. Coefficients of correlation between selected variables

		Basal oxytocin concentration (n = 9)	%-change in plasma oxytocin concentration (n = 9)
Principal component score	1st Principal Component; Curiosity	– 0.683 *	– 0.383
	2nd Principal Component; General Activity	– 0.500	– 0.717 *
	3rd Principal Component; Fearfulness	0.583 †	0.233
	4th Principal Component; Dependence on Human	0.350	0.433
Open-field test	Latency time to enter the open-field	– 0.122	0.357
	Total time in section 1 or 2	0.217	– 0.517
	Total section crossed	– 0.767 *	– 0.25
	Frequency of exploratory behavior	– 0.883	– 0.133
	Frequency of jumping	0.147	0.138
	Frequency of kicking	– 0.422	0.388
	Frequency of tail wagging	0.05	– 0.067
	Frequency of grooming	– 0.211	0.211
	Frequency of vocalization	0.477	0.624 †
Surprise test	Latency time to approach the bucket	0.785 *	0.475
	Frequency of approaching the bucket	– 0.783 *	– 0.540
	Total time in section 1 or 2	0.367	– 0.283
	Total section crossed	– 0.527	– 0.41
	Frequency of exploratory behavior	– 0.517	– 0.767 *
	Frequency of tail wagging	0.583 †	– 0.267
	Frequency of defecation	0.207	– 0.414
	Frequency of urination	– 0.365	– 0.548
	Frequency of grooming	– 0.16	– 0.228
	Frequency of vocalization	–	–
	%-change in plasma cortisol concentration	0.617 †	0.517

∗: The coefficients are significant ($p < 0.05$).
† : The coefficients have trends ($p < 0.1$).
(Modified from Yayou et al. 2010)

Individual variation in stress susceptibility and oxytocin

As shown in rodents[38], oxytocin may be involved in the regulation of neural stress responses in cattle. Some authors have linked emotional reactivity and impulsivity with basal levels or stressor-induced changes in the blood level of this peptide[39,41], since a parallel release of oxytocin into the brain and circulation has been recorded in certain physiological situations[33,40]. In cattle, there is accumulated knowledge concerning individual differences in behavioral and HPA axis responses observed in challenging situations[2,5,15,19,43,44]. The neurobiological basis of these individual differences, however, has not been investigated in domestic animals and to do this in more detail, we examined the association between behavioral and HPA axis reactivity for acute stressors and basal levels and reactivity for the stressors of oxytocin in the blood. The associations of basal and the percentage change in oxytocin with behavioral responses to a novel environment (open-field test) and startling stimulation (surprise test) as well as the percentage change in cortisol were analyzed using principal component analysis and Spearman rank correlations (Table 3)[56]. Among four principal components which explained 56.1% of the overall variation, curiosity was inversely correlated with basal oxytocin level and general activity was inversely correlated with oxytocin reactivity for the novel environment. Fearfulness tended to correlate positively with the basal oxytocin level. In rats, as the blood-brain barrier is immature at an early postnatal stage[17], peripherally injected oxytocin or peripheral blood oxytocin are considered to pass the blood-brain barrier and influence brain development, and behavioral and endocrine characteristics in adults[3]. That is why we currently speculate that oxytocin may be a neurobiological candidate involved in individual differences for stress susceptibility in cattle.

Conclusion and future perspectives

This study identified for the first time, several stress-inducing neurotransmitters and one stress-reducing neurotransmitter in cattle, and possible species differences from rodents and sheep. In particular, for the stress-reducing neurotransmitter, oxytocin, we showed for the first time an association between individual differences in behavioral and HPA axis reactivity to acute stressors and basal levels and reactivity to the stressors of oxytocin in the blood. This result suggests that oxytocin may be a neurobiological candidate involved in individual differences in stress susceptibility in cattle. Furthermore, we are also examining the association between polymorphism of oxytocin-related genes and individual differences in stress susceptibility. If quantitative trait loci for stress susceptibility are found, genetic selection for adaptability to the environment will be possible, thereby offering an opportunity to improve animal welfare. Further research to identify candidate genes for stress susceptibility is needed, considering stress-inducing neurotransmitters.

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