EJN fens European Journal of Neuroscience

European Journal of Neuroscience, Vol. 30, pp. 110–115, 2009 doi:10.1111/j.1460-9568.2009.06800.x

BEHAVIORAL NEUROSCIENCE

GABAergic transmission in the rat ventral pallidum mediates a saccharin palatability shift in conditioned taste aversion

Tadashi Inui,[1] Takashi Yamamoto[2] and Tsuyoshi Shimura[1]
[1]Division of Behavioral Physiology, Department of Behavioral Sciences, Graduate School of Human Sciences, Osaka University, 1-2 Yamadaoka, Suita, Osaka 565-0871, Japan
[2]Faculty of Health Science, Kio University, Koryo, Kitakatsuragi, Nara, Japan

Keywords: aversive, free-moving, hedonics, microdialysis, saccharin

Abstract

We previously found that the blockade of γ-aminobutyric acid $(GABA)_A$ receptors in the ventral pallidum (VP) alters the taste palatability of a conditioned stimulus (CS) from aversive to ingestive after the establishment of conditioned taste aversion (CTA). Because these results suggest that GABAergic transmission in the VP mediates decreased palatability of the taste in CTA, the present study aimed to examine the effects of taste stimulation on the extracellular release of GABA in the VP using *in vivo* microdialysis. Initially, rats received a paired presentation of 5 mM saccharin or 0.3 mM quinine solution with an intraperitoneal injection of 0.15 M lithium chloride (S-CTA and Q-CTA groups) or saline (S-control and Q-control groups). After conditioning, microdialysis was carried out before, during and after the presentation of the CS via an intra-oral cannula. We measured the latency of the first aversive orofacial responses to the CS as behavioral indices. In the S-CTA group, which rapidly rejected the CS (within 100 s), the GABA efflux was significantly increased (147%) and was maintained for 2 h. On the other hand, the S-control group expressed no aversive responses and showed no significant alterations in GABA efflux. Although the Q-CTA group immediately expressed aversive responses to the CS (within 30 s), GABA release was not changed by presentation of the CS, which was similar in the Q-control group. These findings suggest that the palatability shift from ingestive to aversive in conditioned aversion to saccharin, but not quinine, is mediated by the change in GABAergic transmission in the VP.

Introduction

When an animal drinks a taste solution, and illness follows, the animal will acquire aversion to the taste, called conditioned taste aversion (CTA) (Garcia *et al.*, 1955). It is generally believed that the presence of CTA changes the perceived palatability of a taste solution from ingestive to aversive, inducing a decrease in the consumption of the taste solution (Spector *et al.*, 1988; Parker, 2003). However, the neural mechanisms underlying the palatability shift are still unknown.

In an attempt to reveal the neural basis of taste palatability, we previously found that the ventral pallidum (VP) is involved in the palatability-related aspects of fluid intake. Microinjections of the γ-aminobutyric acid $(GABA)_A$ receptor antagonist bicuculline into the VP of intact rats increase the consumption of a normally preferred saccharin solution, but not a normally aversive quinine solution and water (Shimura *et al.*, 2006). We also previously examined the involvement of the VP GABAergic system in the perceived palatability of a conditioned aversive taste stimulus (Inui *et al.*, 2007). After saccharin solution had been paired with a malaise, the rats were bilaterally microinjected with bicuculline or vehicle in the VP just before the presentation of the saccharin solution in the taste reactivity (TR) test developed by Grill & Norgren (1978) for the assessment of taste palatability. The bicuculline-injected rats exhibited decreased aversive responses and increased ingestive responses, indicating that the blockade of $GABA_A$ receptors in the VP causes an alteration in the perceived palatability of the saccharin solution from aversive to ingestive.

The previous experiment strongly suggested the participation of the VP in the palatability of the conditioned stimulus (CS) in CTA, but the data did not provide any further clues about the role of the GABAergic system in CTA. Thus, in order to prove that GABAergic transmission in the VP is critical for the palatability shift in CTA, we investigated the relationship between the decrease in the palatability of a CS after the establishment of CTA and changes in GABA release in the VP.

In the present study, we examined the effects of taste stimulation on GABA efflux in the VP using *in vivo* microdialysis. We simultaneously measured the expression of aversive responses to the taste stimulus. Consequently, we could directly compare the stimulus-evoked GABA release with behavioral results. We used a saccharin solution as the CS. If the VP GABAergic system mediates the palatability shift from ingestive to aversive in CTA, we would predict that the changes in VP GABA levels due to the conditioned aversive saccharin solution would be different from those due to the

Correspondence: Dr T. Inui, as above.
E-mail: kenken@hus.osaka-u.ac.jp

Received 27 February 2009, revised 1 May 2009, accepted 6 May 2009

unconditioned preferred saccharin solution. In addition, we used a quinine solution as another CS in order to explore the differences between conditioned and normally aversive taste solutions.

Materials and methods

Animals

Twenty-four male Wistar rats (CLEA, Osaka, Japan) weighing 280–320 g served as subjects. Rats were maintained in a temperature-controlled and humidity-controlled room under a 12-h light/dark cycle. Food and water were available *ad libitum* except where noted. All animals were handled in accordance with the procedures outlined in the Guide for the Care and Use of Laboratory Animals (National Institute of Health Guide), and approval for this study was obtained from the institutional committee on animal research (Animal Research Committee in Osaka University).

Surgical procedures

Rats were anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg, Nembutal; Abbott, North Chicago, IL, USA) and placed in a stereotaxic apparatus (SR-8; Narishige, Tokyo, Japan). The scalp was opened with a midline incision, and the skull was leveled between bregma and lambda by adjusting the bite bar. A small hole was drilled in the skull, and a guide cannula (AG-8; Eicom, Kyoto, Japan) was implanted above the VP. The coordinates were 0.3 mm posterior from bregma, 2.4 mm lateral from the midline, and 7.0 mm ventral from bregma (Paxinos & Watson, 1997). The cannula was secured to the skull with dental cement and anchor screws. In the same operation, all rats were implanted with a unilateral intra-oral cannula (PE-50) for oral fluid infusion. The intra-oral cannula entered the mouth lateral to the first maxillary molar, traveled beneath the zygomatic arch, and exited the dorsal head near the skull screws (Grill & Norgren, 1978). After surgery, the rats were housed individually and allowed to recover for a week before beginning behavioral testing.

Behavioral testing procedures

On training days, subjects were placed in a Plexiglas chamber in a quiet testing room for 6 h before receiving a 10-min oral infusion of distilled water (0.5 mL/min). Three hours after the infusion, the subjects were allowed to drink supplemental water for 1 h in their home cages. The training was conducted for 2 days. On the conditioning session day, the rats received a 10-min oral infusion of 5 mM saccharin or 0.3 mM quinine solution as a CS. Immediately after the oral infusion, the rats were given 0.15 M intraperitoneal LiCl or saline (2% of body weight) as an unconditioned stimulus. To estimate the perceived palatability of the taste stimuli, the latency of the first expression of aversive TRs (gaping or chin rubbing) during the presentation of the CS was measured. In the normal TR test (Grill & Norgren, 1978; Berridge, 2000), the rat was placed in a Plexiglas test chamber, and a view of the rat's face and mouth reflected by a mirror positioned beneath the transparent floor of the chamber was videotaped for subsequent analysis. However, in this study, 5 cm of wood-chip bedding on the floor in the test chamber, which minimized the rat's stress in response to the long-term dialysis sampling, prevented us from recording all of the TRs. Thus, the rat's behaviors were observed from the side of the test chamber and only the latency of the first expression of aversive TRs was measured.

Rats were divided into four groups according to the combination of CS and unconditioned stimulus [saccharin–LiCl (S-CTA); saccharin–saline (S-control); quinine–LiCl (Q-CTA); and quinine–saline (Q-control); $n = 6$ in each group]. Two days after conditioning, the retrieval session was performed to measure the latency of the aversive responses during microdialysis sampling. The CS solution was infused via the intra-oral cannula for 10 min.

Microdialysis procedure

Seven hours prior to the start of the retrieval session (09:00 h), a microdialysis probe (AI-8-02, active membrane length, 1.5 mm; diameter, 0.22 mm; Eicom) was connected to the microdialysis assembly and flushed with Ringer's solution (FUSO Pharmaceutical Industries, Osaka, Japan). The rats were gently restrained, and the probe slowly inserted into the guide cannula. The dialysis assembly consisted of Teflon tubing (WT-20T; Eicom) connecting the probe to a 2.5-mL gas-tight syringe (Hamilton, Reno, NV, USA) mounted on a syringe pump (ESP-32; Eicom) through a liquid swivel (TCS2-23; Eicom). The rats were then placed in the Plexiglas chamber, and the probe was perfused with Ringer's solution at a flow rate of 2 μL/min. Six hours after probe insertion, three consecutive 20-min dialysis samples (baseline samples 1–3) were collected for the determination of baseline GABA concentrations. After the baseline determination, the retrieval session was conducted for 10 min. Six consecutive samples were collected 20, 40, 60, 80, 100 and 120 min after starting the oral infusion of the CS. Dialysates were recovered using a refrigerated fraction collector (EFC-82; Eicom) and stored at −80°C.

Quantification of GABA

The GABA concentrations were determined using high-performance liquid chromatography with an electrochemical detection system (HTEC-50; Eicom) and sampling injector (M-500; Eicom). The mobile phase consisted of 50 mM sodium phosphate and 50% methanol, adjusted to a pH of 2.8, and was pumped through the system at a flow rate of 500 μL/min. Separation occurred on a 3 × 150 mm column (SC-50DS; Eicom). On the day of analysis, precolumn derivatization was performed by the refrigerated (4°C) sampling injector by adding 10 μL of 20 mM *O*-phtalaldehyde and 0.2% 2-mercaptoethanol in 0.1 M potassium carbonate (pH 9.5) to a 30-μL sample 10 min before injection. The injection volume was 10 μL. The chromatograms were evaluated with the PowerChrom280 system (eDAQ, Nagoya, Japan). The retention time was 15 min, and the limit of the detection was 1.0 nM.

Histology

At the completion of the experiment, subjects were deeply anesthetized with intraperitoneal sodium pentobarbital (100 mg/kg) and perfused through the heart with 0.02 M phosphate-buffered saline, followed by 10% formalin in 0.1 M phosphate buffer. Brains were removed and post-fixed overnight in the same solution, and then immersed in 0.1 M phosphate buffer containing 30% sucrose. Sections (60 μm) cut from the implanted areas using a freezing microtome were stained with Cresyl Violet to verify the anatomical placement of the probe according to the atlas of Paxinos & Watson (1997).

Data analysis

Behavioral data (latency) were expressed as time (s) from the start of the oral infusion of a taste solution. Statistical comparisons of

unpaired data (CTA vs. control) at the retrieval session were performed using the Mann–Whitney *U*-test. The baseline GABA concentrations for each animal were calculated as the average concentration of baseline samples 1–3. Dialysate GABA concentrations were expressed as a percentage of the average concentration. The effects of the CS presentation were analysed using a two-factor (group × time) repeated measures ANOVA. *Post hoc* analysis was performed using Tukey's HSD test.

Results

Histology

The locations of all microdialysis probes are presented in Fig. 1. The 1.5-mm lengths of active membrane were in the VP. The histological placements show that the dialysis probes were located throughout the entire rostral–caudal and medial–lateral extent of the VP. However, there were no regional differences in GABA release within the VP. Rats with a correct probe location were included in Fig. 1 and later data analyses. One of the rats in the S-control group, two in the Q-control group, and one in the Q-CTA group were excluded from the statistical analysis because the cannula was placed outside the VP.

CTA

The mean latencies for displaying the first aversive response to the saccharin solution in the conditioning and retrieval sessions in the S-control and S-CTA groups are shown in Fig. 2A. In the conditioning session, both groups continuously consumed the saccharin solution for 10 min without rejection. In the retrieval session, although the S-control group ingested all of the saccharin solution for 10 min, the S-CTA group rejected the saccharin solution within 100 s after the start of oral infusion. There was a significant difference in the latency during the retrieval session between the S-control and S-CTA groups ($P < 0.01$, Mann–Whitney *U*-test).

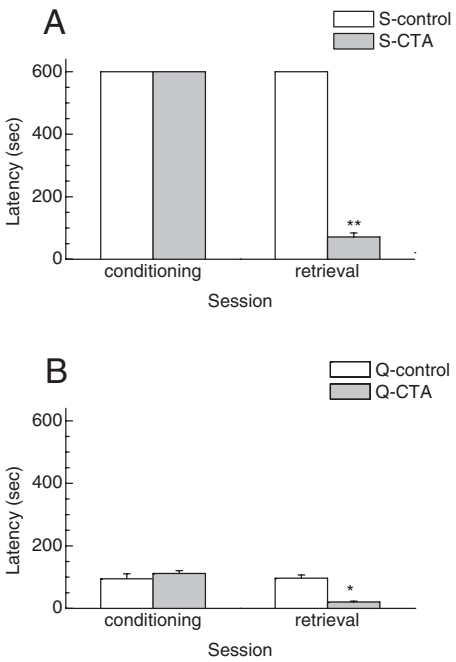

FIG. 2. Mean latencies of the first expression of aversive responses after beginning the oral infusion of saccharin solution (A) or quinine solution (B) in the conditioning and retrieval sessions. Error bars represent standard errors of the mean. $^{**}P < 0.01$ between the saccharin–LiCl (S-CTA) and saccharin–saline (S-control) groups in the retrieval session. $^{*}P < 0.05$ between the quinine–LiCl (Q-CTA) and quinine–saline (Q-control) groups in the retrieval session.

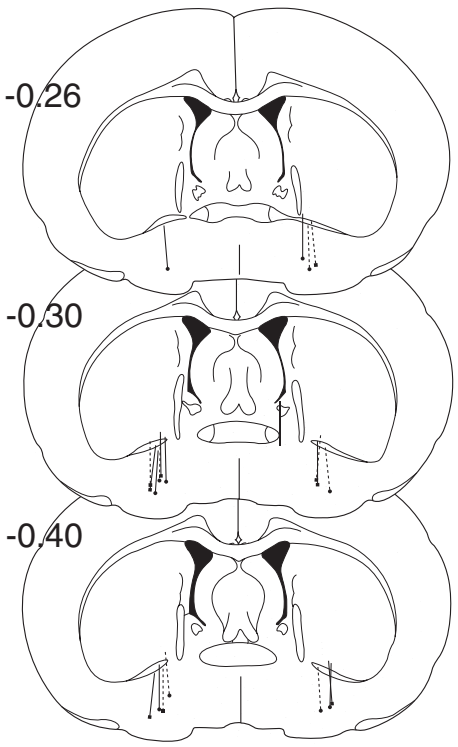

FIG. 1. Location of the 1.5-mm length of active membranes of microdialysis probes in the ventral pallidum in the saccharin–saline (solid line and square), saccharin–LiCl (solid line and circle), quinine–saline (broken line and square) and quinine–LiCl (broken line and circle) groups. The numbers beside each plate correspond to millimeters from bregma. Coronal drawings were modified from Paxinos & Watson (1997).

The mean latencies for the initial expression of aversive responses to the quinine solution in the conditioning and retrieval sessions are shown in Fig. 2B. Both the Q-CTA and Q-control groups exhibited aversive responses to the quinine solution approximately 100 s after the start of oral infusion, but the Q-CTA group displayed aversive responses more quickly (within 30 s) than the Q-control group (100 s) in the retrieval session ($P < 0.05$, Mann–Whitney *U*-test).

Effects of saccharin on GABA efflux in the VP

The effects of the oral infusion of the saccharin solution on GABA efflux in the VP in the S-CTA and S-control groups are shown in Fig. 3. The baseline GABA concentrations prior to oral infusion were 14.26 ± 5.89 nmol/L and 13.44 ± 3.65 nmol/L in the S-CTA and S-control groups, respectively, and did not differ between the groups. After the presentation of the saccharin solution, the GABA level immediately increased (147%) in the S-CTA group, whereas no significant alterations were detected in the S-control group. An ANOVA indicated main effects for group ($F_{1,9} = 22.46$, $P < 0.01$) and time ($F_{8,72} = 3.71$, $P < 0.01$) and a significant interaction ($F_{8,72} = 4.72$, $P < 0.001$). *Post hoc* analysis revealed that there was a significant difference between baseline sample 3 and 20 min ($P < 0.001$), but not between baseline sample 3 and other sampling time points. The comparison between groups indicated that there were significant

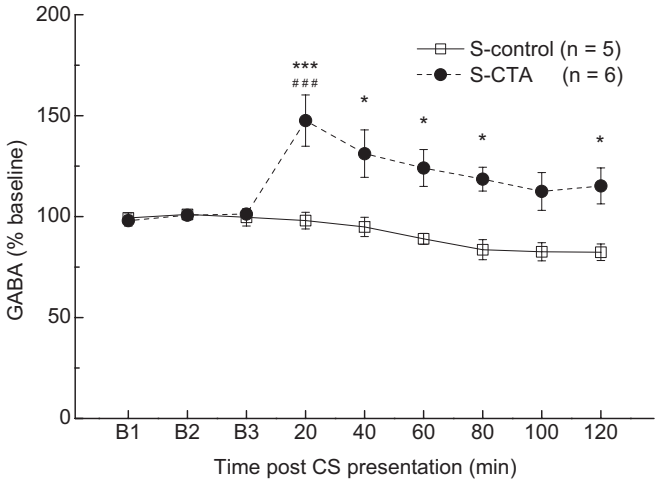

FIG. 3. γ-Aminobutyric acid (GABA) concentrations in the ventral pallidum (VP) evoked by the presentation of the saccharin conditioned stimulus (CS) in the saccharin–LiCl (S-CTA; broken line and filled circle) and saccharin–saline (S-control; solid line and open square) groups. The saccharin solutions were intraorally infused for 10 min. The presentation of the CS increased GABA levels in the VP. Values are expressed as the mean percentage change from baseline ± standard error of the mean. ***$P < 0.001$ and *$P < 0.05$ between the S-CTA and S-control groups. ###$P < 0.001$ between baseline sample 3 and 20 min.

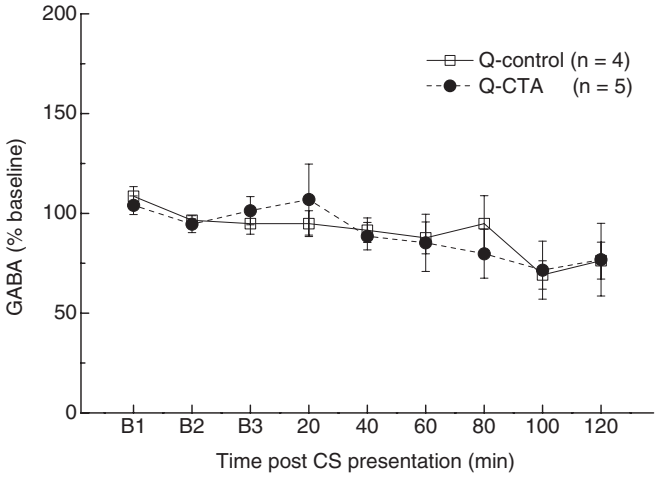

FIG. 4. γ-Aminobutyric acid (GABA) concentrations in the VP evoked by the presentation of the quinine conditioned stimulus (CS) in the quinine–LiCl (Q-CTA; broken line and filled circle) and quinine–saline (Q-control; solid line and open square) groups. The quinine solutions were intra-orally infused for 10 min. The CS presentation did not change GABA levels in the VP. Values are expressed as the mean percentage change from baseline ± standard error of the mean.

differences in the GABA level at all time points except for 100 min (20 min, $P < 0.001$; others, $P < 0.05$). Although the GABA level in the S-control group tended to decrease after oral infusion, there were no significant differences in the GABA level among all time points.

Effects of quinine on GABA efflux in the VP

The effects of the oral infusion of the quinine solution on GABA efflux in the VP in the Q-CTA and Q-control groups are shown in Fig. 4. The baseline GABA concentrations prior to oral infusion were 13.50 ± 6.45 nmol/L and 14.47 ± 5.85 nmol/L in the Q-CTA and Q-control groups, respectively, and did not differ between the groups. After the presentation of the quinine solution, no significant changes in the GABA level were detected in either group. However, because an ANOVA indicated a main effect for time ($F_{8,56} = 3.80$, $P < 0.01$), the results suggest that the GABA level gradually decreased throughout the microdialysis sampling.

Discussion

The S-CTA group quickly displayed aversive responses in the retrieval session as compared with the S-control group, suggesting that the pairing of the saccharin solution with the intraperitoneal injection of 0.15 M LiCl induced the palatability shift for the saccharin solution from ingestive to aversive. The observation that the latency of the S-CTA group was the same as that of the Q-control group (approximately 100 s) implies that the strength of aversion to the conditioned saccharin solution was identical to aversion to the unconditioned quinine solution. The most aversive solution was the conditioned quinine solution in the Q-CTA group, because this group rejected it immediately (within 30 s) after presentation.

In the microdialysis test, both the Q-control and Q-CTA groups exhibited no alteration in the GABA levels. These results suggest that the VP GABAergic system is not involved in the establishment of quinine taste aversion. On the other hand, the S-CTA group exhibited a significant increase in GABA efflux after the presentation of the CS, and the GABA levels in the S-control group did not change. There are several explanations that may account for the differences in GABA release in the VP between the S-CTA and S-control groups.

First, it is plausible that the taste information for the solution activates the GABAergic projection neurons to the VP, which receives dense GABAergic inputs from the nucleus accumbens (NAc) (Zahm *et al.*, 1985). Electrophysiological studies have shown that the neurons of the VP and NAc respond to sucrose and quinine solutions (Roitman *et al.*, 2005; Tindell *et al.*, 2006). In the present study, however, the S-control and Q-control groups exhibited no changes in GABA levels in the VP. In addition, the oral infusion of sucrose or quinine solutions induces the rare expression of Fos-like immunoreactivities in the NAc (Yasoshima *et al.*, 2006). These data indicate that the VP GABAergic system does not receive the inputs from taste stimuli. Other neurotransmitters may be involved in the transfer of sensory information between the VP and NAc for a taste stimulus.

Second, the presentation of the saccharin solution to the S-CTA group could induce the retrieval of aversive taste memory. GABAergic transmission in the VP may play a role in the retrieval process. However, the Q-CTA group exhibited no alterations in GABA efflux in the VP upon the presentation of the quinine solution. Thus, these data suggest that the retrieved memory of conditioned aversive taste does not affect the VP GABA levels. Because the lesions of the VP do not disrupt the acquisition of CTA (Cromwell & Berridge, 1993), the VP GABAergic system may not play a role in CTA memory.

Third, the orally infused conditioned saccharin solution might produce aversive orofacial responses as behavioral outcomes. Microinjection of the $GABA_A$ receptor antagonist bicuculline into the VP produces elevated ingestive responses to the normally preferred (Shimura *et al.*, 2006) and conditioned aversive saccharin solutions during the TR test (Inui *et al.*, 2007). Bicuculline injections into the VP also increase food intake (Stratford *et al.*, 1999). Thus, it has been assumed that the VP GABAergic system is involved in the orofacial and somatic responses related to consumption behaviors. Although the S-CTA group exhibited increased GABA levels in the VP, the Q-control and Q-CTA groups, which also displayed aversive

responses, did not demonstrate alterations in GABA efflux in the VP. There were no significant changes in the GABA level in the S-control group, which showed only ingestive responses. Therefore, the changes in GABA levels in the VP are not directly related to the consumption behaviors.

The behavioral results indicate that the S-CTA group, but not the S-control group, showed an aversion to the saccharin solution. We previously reported that the microinjection of the $GABA_A$ receptor agonist muscimol into the VP produced aversive responses to orally infused fluids (Shimura *et al.*, 2006). Furthermore, it was found that the muscimol injections induced the expression of Fos-like immunoreactivities in the intermediate and caudal parts of the nucleus of solitary tract (Inui, T. *et al.*, unpublished observation), which receive the nausea-like aversive visceral cues (Sakai & Yamamoto, 1997). We also observed the occurrences of chin rubbing in response to the fluids after the VP microinjections of the $GABA_A$ receptor agonist muscimol (Shimura *et al.*, 2006). These data suggest that the increased GABA release in the VP caused by the presentation of the conditioned saccharin solution might elicit visceral discomfort and aversive TR responses. Thus, it is highly possible that the palatability shift for the conditioned saccharin solution induced the increase in GABA efflux in the S-CTA group.

The reduced GABA levels in the S-CTA group were maintained for 120 min, even after completion of the 10-min oral infusion of the saccharin solution. Mark *et al.* (1995) reported that the 20-min oral infusion (0.25 mL/min) of the conditioned aversive saccharin solution (2.5 mM) produces a significant elevation in extracellular acetylcholine (ACh) in the NAc, and the high level of ACh is maintained for 120 min. Because the accumbens ACh excites the GABAergic output neurons (Kaneko *et al.*, 2000), the enhancement of VP GABA release by the presentation of the saccharin solution may be due to the augmentation of ACh levels in the NAc.

Previous studies have suggested that the palatability shift in CTA is based on alterations in the response profiles of the neurons in the gustatory relay nuclei, including the nucleus of solitary tract (Chang & Scott, 1984), parabrachial nucleus (Tokita *et al.*, 2007), central and basolateral nuclei of the amygdala (Yasoshima *et al.*, 1995), and insular cortex (Yasoshima & Yamamoto, 1998). Because the NAc, which sends GABAergic projections to the VP, receives afferent projections from the nucleus of solitary tract (Wang *et al.*, 1992), basolateral nucleus of the amygdala (Brog *et al.*, 1993) and insular cortex (Wright & Groenewegen, 1996), it is likely that the neural activities of the gustatory relay nuclei upon stimulation with a conditioned aversive saccharin solution excite the NAc and activate the GABAergic output neurons in the NAc, inducing an increase in the extracellular release of GABA in the VP.

As we previously showed that the bicuculline injections into the VP increased the palatability of normally preferred saccharin (Shimura *et al.*, 2006), we predicted changes in the GABA level in the S-control group. In the present study, however, we found that the stimulation involving the conditioned aversive saccharin (S-CTA group), but not of the normally preferred saccharin (S-control group), elevated the GABA efflux in the VP. In addition, another study demonstrated that the bicuculline injections into the VP had no effect on taste palatability (Smith & Berridge, 2005). Thus, the GABAergic transmission in the VP might play a specific role in a learned shift in palatability rather than a global role in taste palatability.

The present study has shown that the presentation of the conditioned aversive saccharin solution increased the GABA efflux in the VP. Although we used only saccharin as a palatable taste stimulus, a conditioned palatability shift may occur for other preferred tastes, such as salt and umami. An electrophysiological study favors the notion. Neurons in the ventral pallidum were shown to participate in brain representations that encode the positive hedonic impact of salty taste rewards (Tindell *et al.*, 2006).

In conclusion, the present results suggest that the palatability shift from ingestive to aversive in conditioned aversion to a normally preferred saccharin solution, but not to a normally aversive quinine solution, is mediated by a change in GABAergic transmission in the VP.

Acknowledgements

This work was supported in part by grants from SKYLARK Food Science Institute and Nestle Nutrition Council, Japan to T. Inui, and from 21th Century COE Program and Ajinomoto Co. Ltd to T. Yamamoto.

Abbreviations

ACh, acetylcholine; CS, conditioned stimulus; CTA, conditioned taste aversion; GABA, γ-aminobutyric acid; NAc, nucleus accumbens; Q-control, quinine–saline; Q-CTA, quinine–LiCl; S-control, saccharin–saline; S-CTA, saccharin–LiCl; TR, taste reactivity; VP, ventral pallidum.

References

Berridge, K.C. (2000) Measuring hedonic impact in animals and infants: microstructure of affective taste reactivity patterns. *Neurosci. Biobehav. Rev.*, **24**, 173–198.

Brog, J.S., Salyapongse, A., Deutch, A.Y. & Zahm, D.S. (1993) The patterns of afferent innervation of the core and shell in the 'accumbens' part of the rat ventral striatum: immunohistochemical detection of retrogradely transported fluoro-gold. *J. Comp. Neurol.*, **338**, 255–278.

Chang, F.C. & Scott, T.R. (1984) Conditioned taste aversions modify neural responses in the rat nucleus *tractus solitarius*. *J. Neurosci.*, **4**, 1850–1862.

Cromwell, H.C. & Berridge, K.C. (1993) Where does damage lead to enhanced food aversion: the ventral pallidum/substantia innominata or lateral hypothalamus? *Brain Res.*, **624**, 1–10.

Garcia, J., Kimeldorf, D.J. & Koelling, R.A. (1955) Conditioned aversion to saccharin resulting from exposure to gamma radiation. *Science*, **122**, 157–158.

Grill, H.J. & Norgren, R. (1978) The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurologically normal rats. *Brain Res.*, **143**, 263–279.

Inui, T., Shimura, T. & Yamamoto, T. (2007) The role of the ventral pallidum GABAergic system in conditioned taste aversion: effects of microinjections of a GABAA receptor antagonist on taste palatability of a conditioned stimulus. *Brain Res.*, **1164**, 117–124.

Kaneko, S., Hikida, T., Watanabe, D., Ichinose, H., Nagatsu, T., Kreitman, R.J., Pastan, I. & Nakanishi, S. (2000) Synaptic integration mediated by striatal cholinergic interneurons in basal ganglia function. *Science*, **289**, 633–637.

Mark, G.P., Weinberg, J.B., Rada, P.V. & Hoebel, B.G. (1995) Extracellular acetylcholine is increased in the nucleus accumbens following the presentation of an aversively conditioned taste stimulus. *Brain Res.*, **688**, 184–188.

Parker, L.A. (2003) Taste avoidance and taste aversion: evidence for two different processes. *Learn. Behav.*, **31**, 165–172.

Paxinos, G. & Watson, C. (1997) *The Rat Brain in Stereotaxic Coordinates*. Academic Press, San Diego.

Roitman, M.F., Wheeler, R.A. & Carelli, R.M. (2005) Nucleus accumbens neurons are innately tuned for rewarding and aversive taste stimuli, encode their predictors, and are linked to motor output. *Neuron*, **45**, 587–597.

Sakai, N. & Yamamoto, T. (1997) Conditioned taste aversion and c-fos expression in the rat brainstem after administration of various USs. *Neuroreport*, **8**, 2215–2220.

Shimura, T., Imaoka, H. & Yamamoto, T. (2006) Neurochemical modulation of ingestive behavior in the ventral pallidum. *Eur. J. Neurosci.*, **23**, 1596–1604.

Smith, K.S. & Berridge, K.C. (2005) The ventral pallidum and hedonic reward: neurochemical maps of sucrose 'liking' and food intake. *J. Neurosci.*, **25**, 8637–8649.

Spector, A.C., Breslin, P. & Grill, H.J. (1988) Taste reactivity as a dependent measure of the rapid formation of conditioned taste aversion: a tool for the neural analysis of taste–visceral associations. *Behav. Neurosci.*, **102**, 942–952.

Stratford, T.R., Kelley, A.E. & Simansky, K.J. (1999) Blockade of GABAA receptors in the medial ventral pallidum elicits feeding in satiated rats. *Brain Res.*, **825**, 199–203.

Tindell, A.J., Smith, K.S., Pecina, S., Berridge, K.C. & Aldridge, J.W. (2006) Ventral pallidum firing codes hedonic reward: when a bad taste turns good. *J. Neurophysiol.*, **96**, 2399–2409.

Tokita, K., Shimura, T., Nakamura, S., Inoue, T. & Yamamoto, T. (2007) Involvement of forebrain in parabrachial neuronal activation induced by aversively conditioned taste stimuli in the rat. *Brain Res.*, **1141**, 188–196.

Wang, Z.J., Rao, Z.R. & Shi, J.W. (1992) Tyrosine hydroxylase-, neurotensin-, or cholecystokinin-containing neurons in the nucleus tractus solitarii send projection fibers to the nucleus accumbens in the rat. *Brain Res.*, **578**, 347–350.

Wright, C.I. & Groenewegen, H.J. (1996) Patterns of overlap and segregation between insular cortical, intermediodorsal thalamic and basal amygdaloid afferents in the nucleus accumbens of the rat. *Neuroscience*, **73**, 359–373.

Yasoshima, Y. & Yamamoto, T. (1998) Short-term and long-term excitability changes of the insular cortical neurons after the acquisition of taste aversion learning in behaving rats. *Neuroscience*, **84**, 1–5.

Yasoshima, Y., Shimura, T. & Yamamoto, T. (1995) Single unit responses of the amygdala after conditioned taste aversion in conscious rats. *Neuroreport*, **6**, 2424–2428.

Yasoshima, Y., Scott, T.R. & Yamamoto, T. (2006) Memory-dependent c-Fos expression in the nucleus accumbens and extended amygdala following the expression of a conditioned taste aversive in the rat. *Neuroscience*, **141**, 35–45.

Zahm, D.S., Zaborszky, L., Alones, V.E. & Heimer, L. (1985) Evidence for the coexistence of glutamate decarboxylase and Met-enkephalin immunoreactivities in axon terminals of rat ventral pallidum. *Brain Res.*, **325**, 317–321.