

Short communication

Glutamate in the parabrachial nucleus of rats during conditioned taste aversion

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Accepted 12 September 2000

Abstract

Brain microdialysis combined with HPLC and spectroscopic detection was used to monitor extracellular glutamate in the parabrachial nucleus (PBN) of rats during acquisition of a conditioned taste aversion (CTA). Microdialysis fractions taken every 20 min were used to assess the effects of presentation of the conditioned stimulus alone (CS, consumption of 0.1% saccharin), the unconditioned stimulus alone (US, intraperitoneal injection of 0.15 M LiCl, 2% b.w. induced malaise after water drinking) as well as that of CS–US pairing. After 15 min of saccharin drinking, the glutamate concentration in the eluate (20 μ l/20 min) reached 80% above the baseline but returned to the basal value in the next fraction. LiCl alone (applied 1 h after 15 min drinking of water) increased glutamate only following some delay, i.e. in the second and third post-lithium fraction by 90 and 67%, respectively. However, when LiCl was injected 1 h after the onset of saccharin intake, the glutamate concentration rose significantly (by 95%) already in the first post-LiCl fraction and by 120% in the second one. It appears, therefore, that the ‘saccharin trace’ facilitates the effect of lithium on extracellular concentration of glutamate in PBN during acquisition of CTA. © 2000 Elsevier Science B.V. All rights reserved.

Theme: Neural basis of behaviour

Topic: Learning and memory: pharmacology

Keywords: Conditioned taste aversion; Parabrachial nucleus; Glutamate; Microdialysis; Rats

Conditioned taste aversion (CTA) is a robust type of learning of basic importance for survival. In this behavioral model the animal acquires an aversion to a novel taste which has been paired with digestive malaise [2].

The main central pathways for gustatory information processing (the solitary tract nucleus, parabrachial nucleus, lat. hypothalamus, ventral posteromedial thalamus, amygdala, gustatory area of the insular cortex) are closely linked with those for abdominal visceral information processing. The PBN is of special importance since it has been suggested not only as an association mediating site but also as a site where the CTA memory trace is formed [13,22].

The role of glutamatergic neurotransmission in CTA has been suggested by several studies using antagonists or agonists of the glutamate receptors applied either sys-

temically or directly into some of the regions mentioned above. Thus for instance, application of 2-amino-5-phosphonovaleric acid (AVP) or 3(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP), antagonists of the NMDA receptor, into the insular cortex disrupted acquisition of CTA [7,11,21,23]. Microinjection of MK-801 (Dizocilpine; [5*R*,10*S*]-[1]-5-methyl-10,11-dihydro-5-11-dibenzo[*a,d*]cyclohepten-5,10-imine) into the amygdala attenuated but did not completely disrupt CTA. It was ineffective when injected into the lateral hypothalamus [25]. Ketamine, a noncompetitive NMDA antagonist, has been shown to disrupt CTA [19]. The response to systemically administered drugs depends, however, on both the emetic properties and on the degree of their penetration into the brain. Such drugs may also serve as an unconditioned stimulus in the CTA formation [1,14]. Effective brain concentrations of ketamine prevent induction of CTA [19]. The ketamine effect depends also on the strength of CTA (related to the concentration of LiCl used as US) [20]. The possible involvement of metabotropic

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glutamate receptors has also been suggested. Knockout mice missing the mgluR7 subunit of this receptor failed to associate saccharin intake with LiCl poisoning [18]. The nucleus of the solitary tract possesses metabotropic glutamate receptors but their role in CTA has not yet been explored [10]. D-Cycloserin, a partial agonist of the NMDA receptor, has been reported to enhance acquisition of CTA when applied systemically [16]. The involvement of glutamate in CTA is supported also by the finding that injection of this amino acid into the amygdala induced CTA [25].

Thus glutamatergic transmission in CTA has been demonstrated only indirectly. No attempt has been made as yet to measure glutamate changes occurring during CTA acquisition. The only report, to our knowledge, concerns the glutamate increase established by microdialysis in the amygdala of rats with consolidated CTA (7 days following saccharin–LiCl pairing). No changes have been found in the lateral hypothalamus [25]. In these experiments intraoral infusion of saccharin was used. It has been shown recently, however, that several aspects of CTA differ depending on whether the intraoral method or bottle presentation of the taste was used [4,24]. In spite of the key role of PBN in the CTA formation, no data about glutamatergic transmission in the PBN are available.

The aim of the present communication was to see whether any changes in the extracellular glutamate in the PBN could be elicited by the CS alone, i.e. after drinking saccharin alone, by the US alone, i.e. after LiCl administration alone and particularly by the CS–US pairing, i.e. during acquisition of CTA.

Three-month-old male hooded rats (Long-Evans strain) were obtained from the breeding colony of the Institute of Physiology, Academy of Sciences, Prague, Czech Republic. The animals were housed five to a cage in an animal room with constant temperature (20–22°C) and maintained on a 12 h light–dark cycle starting at 06:00 a.m.

After 2 days of water deprivation, the rats were habituated to receive their daily ration of water limited by a 15-min stay in a drinking box. It was equipped with an array of ten calibrated pipettes each containing 2 ml of water. After 2 days of habituation the animals were anaesthetized with an i.p. injection of ketamine–xylazine mixture (87 mg/kg and 13 mg/kg, respectively) and fixed in the stereotaxic apparatus. The skull was exposed and a trephine opening (1.5 mm diameter) was drilled above the target point. Concentric microdialysis probes with a membrane of 0.22 mm O.D. (assembled from Partially assembled M-SS probes from Applied Neuroscience, UK) were inserted through the openings to a point with the stereotaxic coordinates AP8, L1.5, V7 (lateral part of PBN) according to the atlas by Fifkova and Marsala [9]. Scheme of the probe location is shown on Fig. 1. The probes were secured by dental acrylate and anchoring screws to the skull. A scheme of the probe location is shown in Fig. 1.

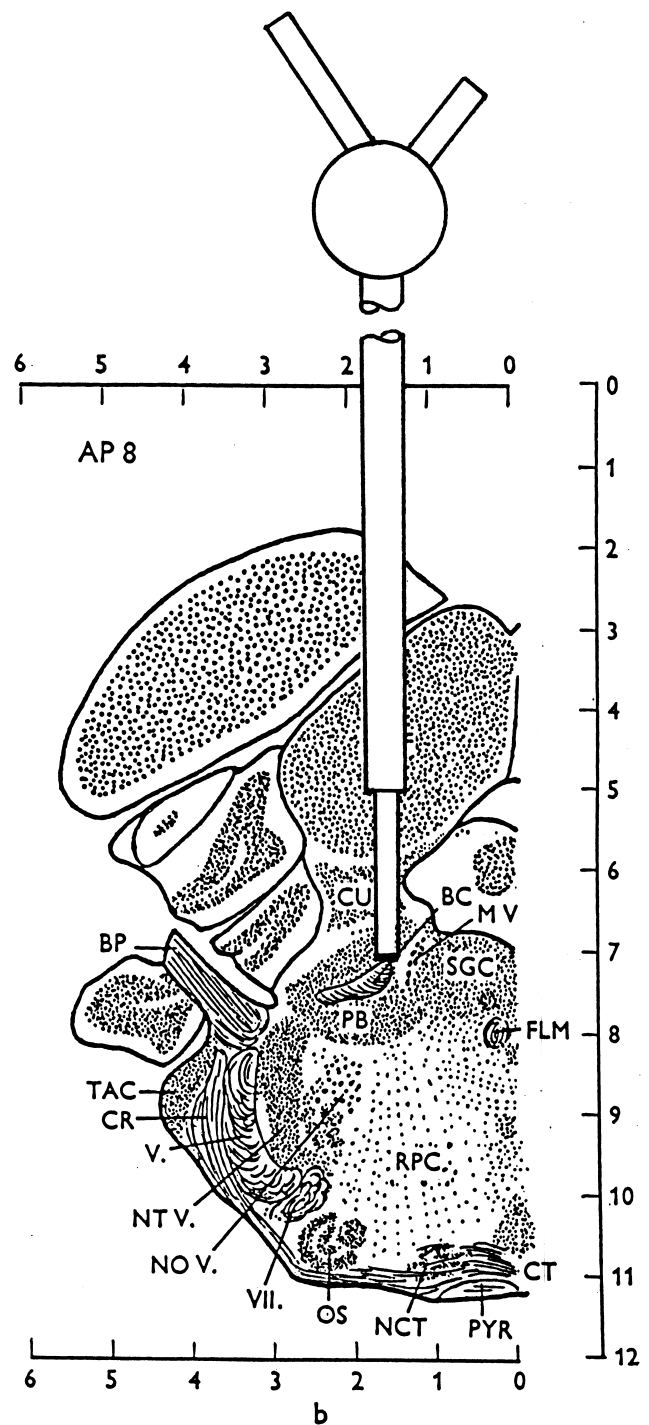


Fig. 1. The scheme of location of the microdialysis probe in PBN. The histological picture is from Ref. [9].

After 24 h of recovery the microdialysis began with a degassed artificial cerebrospinal fluid (in mM): NaCl, 140; KCl, 3.9; CaCl₂, 1.26; MgCl₂, 1.15; NaH₂PO₄, 0.30; Na₂HPO₄, 1.35; (pH 7.2) delivered at a flow-rate of 1 µl/min. Three samples were collected every 20 min from ten animals to get baseline values. The animals were then divided into two groups of five animals. The first one was tested for the effect of LiCl alone. They drank water in a

drinking box for 15 min. The 15-min period of water intake was maintained up to the day of the microdialysis experiments, which was the 5th day of water deprivation. After collection of three samples following liquid intake (1 h), the animals received 0.15 M LiCl (2 ml/100 g i.p.) and the microdialysis continued until another five samples had been collected. When the same protocol was used for another five rats that drank saccharin instead of water, both the effects of saccharin alone and of its pairing with lithium chloride could be assessed. This experimental setup, namely the length of water deprivation, habituation to the drinking box, intake of the liquids and injection of lithium chloride have been used in this laboratory for years and provide highly reproducible and well developed CTA.

For the histological inspection of the probe location the animals were deeply anaesthetized with thiopental, the probes were removed and the rats were perfused transcardially with saline followed by formol–saline. The brains were embedded in paraffin and cut at 40 μ m. The sections were stained with cresyl violet. The trace of the probe in PBN tightly followed the dimension of the membrane without additional damage. More importantly, this was confirmed functionally. Bilateral implantation of the probes did not disturb formation of CTA. Thus the PBN remained fully active after implantation of the probe. This is described in the section discussing the results and in Fig. 2. Although only a minor part of the membrane is in contact with the PBN, the amount of glutamate changes

could be measured with good reproducibility. Since the non-PBN tissue being in contact with the membrane has not got any gustatory or visceral connections, the obtained data obviously reflect only the changes confined to NPB. The results from the brains with inappropriate location of the probe have been discarded. Thus eleven rats (six controls and five experimental) were used for evaluation.

The method used for HPLC separation and UV detection of glutamate has already been described [12]. Briefly, perfusates were derivatized with phenylisothiocyanate. The derivatives of both the samples and standards (phenylthiocarbamylglutamate) were separated by reversed-phase high-performance liquid chromatography. A gradient system with sodium acetate, pH 6.4, and acetonitrile was used. The derivatized glutamate was detected spectrophotometrically at 254 nm.

The results are expressed in pmol glutamate/20 μ l fraction and statistically evaluated by the Student's *t*-test. The data are related to the mean of the baseline samples common to both the water and saccharin groups.

In preliminary experiments we tested the possible interference of probe implantation alone with CTA acquisition. The probes were bilaterally implanted to the water deprived rats (see above). After 24 h of recovery the rats drank saccharin for 15 min followed after 60 min by LiCl administration. After 2 days the rats were offered the choice between water and saccharin drinking. As demonstrated in Fig. 2, CTA was well developed. This indicates that the PBN function was not disrupted by implantation of the microdialysis probes alone. These control experiments were performed separately since for the blockade of CTA bilateral disruption of PBN is necessary. In our experiments the microdialysis was made only on one side, so that disruption of CTA after microdialysis could not be expected. In fact, a group of three animals was tested for acquisition of CTA 48 h after the microdialysis experiments. The saccharin preference after CS–US pairing: $3.6 \pm 2.8\%$ (two rats practically did not drink saccharin); controls, where the saline was injected instead of LiCl: $61.7 \pm 5.9\%$.

The mean baseline of the three pooled fractions preceding the intake of liquids is 2.87 ± 0.27 . As shown in Fig. 3, consumption of saccharin alone resulted in the elevation of glutamate up to 180% of the basal value (5.22 ± 0.48 vs. 2.87 ± 0.27 ; $t_{(9)} = 4.20$; $N = 11$; $P < 0.01$). Thus the effect of saccharin intake alone is highly expressed despite the rather long intervals of collection that were used. The lithium alone (following water drinking) induced, after some latency needed for the development of sickness, an 89% increase of glutamate (5.46 ± 0.98 vs. 2.87 ± 0.27 ; $t_{(9)} = 2.54$; $N = 11$; $P < 0.05$) in the second post-lithium fraction. The enhancement (66%) is still apparent also in the third fraction, albeit this was not statistically significant (4.8 ± 0.8 vs. 2.87 ± 0.27 ; $t_{(9)} = 2.29$; $N = 11$; $P > 0.05$). Lithium administration following saccharin consumption, however, resulted in the enhancement of glutamate release

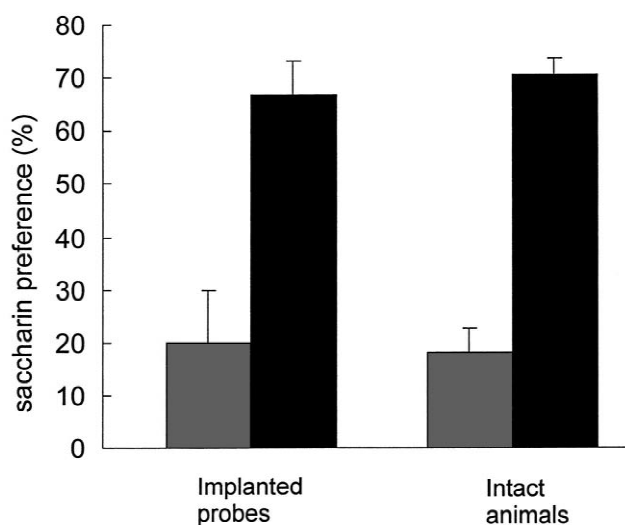


Fig. 2. Conditioned taste aversion (CTA) following bilateral implantation of the dialysate probes into the parabrachial nucleus (PBN) to test for the possible interference with CTA formation. CTA is evaluated in terms of the saccharin preference (SP) expressed by a percentage portion of saccharin consumption (*S*) related to the total fluid intake ($S + H_2O$), i.e. $(S / (S + H_2O) \times 100)$. The smaller is the value of SP, the more pronounced is CTA. The intact animals were submitted to the same procedure but no surgery was performed. The gray columns, SP after pairing of the fluid intake with i.p. administration of LiCl. The black columns, saline was used instead of LiCl (controls). Each point represents mean \pm S.E.M. of at least five measurements.

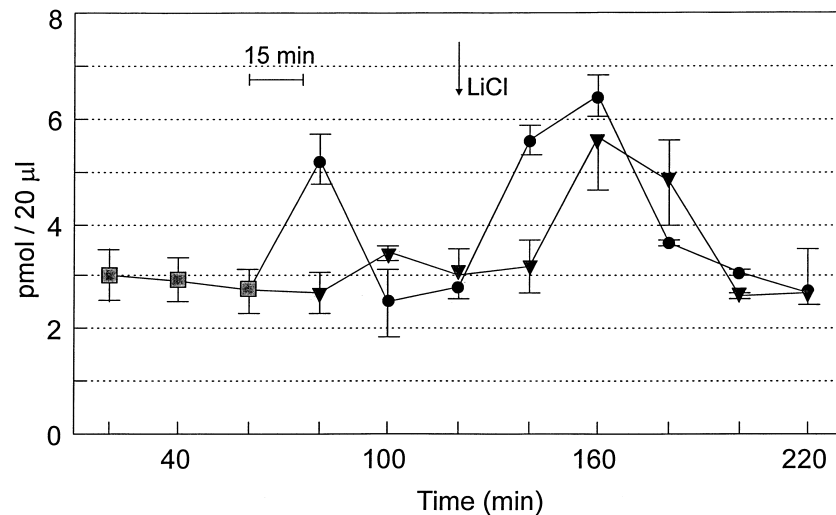


Fig. 3. Glutamate concentration in single 20- μ l microdialysis fractions after drinking of water (triangles) or saccharin (filled circles) before and after LiCl administration (fractions: 60–120 min and 120–220 min, respectively). The squares designate baseline samples. The 15-min bar represents a period of drinking. Each point represents the mean \pm S.E.M. of at least five measurements.

already in the first post-lithium fraction (5.59 ± 0.44 vs. 2.87 ± 0.27 ; $t_{(9)} = 3.72$; $N = 11$; $P < 0.01$) and persisted in the second fraction (6.44 ± 0.41 vs. 2.87 ± 0.27 ; $t_{(9)} = 8.20$; $N = 11$; $P < 0.003$). It appears that saccharin consumption somehow facilitates the lithium-induced release of glutamate during CTA formation, the difference between glutamate in the first post-lithium fraction following saccharin intake (5.59 ± 0.44) and that after drinking of water (3.19 ± 0.53) is statistically significant ($t_{(8)} = 3.93$; $N = 10$; $P < 0.01$; Fig. 2).

The present experiments demonstrate the possibility of monitoring extracellular glutamate in the PBN, a proposed site of the formation. The high concentration of glutamate, found after 20 min of perfusion, does not necessarily reflect such a long persistence of increased glutamate concentration in the extracellular space. It may last for seconds but can be detected in diluted form in the eluate collected over 20 min.

By monitoring the extracellular glutamate in the PBN, we have found that during acquisition of CTA, there is an enhancement of this excitatory neurotransmitter. To our knowledge this is the first direct evidence for the participation of glutamatergic neurotransmission in PBN during acquisition of CTA. As mentioned in the introductory part of this paper, there is only one report in which brain microdialysis was used to monitor glutamate during CTA (not necessarily during the acquisition phase). Increased glutamate was found in amygdala but not in the lateral hypothalamus [25]. These data together with our present results are in accordance with the previous findings based on indirect methods, i.e. on the use of antagonists of glutamate receptors. They strongly suggest the essential role of glutamate neurotransmission in the CTA phenomena. Applying these drugs, into the nuclei of the gustatory-visceral pathways (nucleus of the solitary tract, amygdala,

insular cortex) resulted in impairment of CTA formation [7,10,11,18,21]. Brain microdialysis has an advantage in the possibility to quantifying the relative changes in the extracellular concentrations of the transmitters. However, this technique does not allow the assessment of the type of receptors which are activated by glutamate. Both NMDA and non-NMDA receptors have been suggested to be engaged [21]. The metabotropic glutamate receptor may be a candidate of the non-NMDA type [18]. In our preliminary experiments microdialysis application into PBN of DL-2-amino-3-phosphonopropionic acid (AP-3), a strong antagonist of the metabotropic glutamate receptor, disrupted acquisition of CTA. Under the same experimental conditions the NMDA receptor antagonists, AP-5 and MK-801, have been only partially effective even at relatively high concentrations.

Although CTA possesses some features that differ from those of more common paradigms of learning (“The Memory of a Special Kind” [2]), it has all the attributes of an associative form of learning. The role of glutamate neurotransmission may be a common denominator for various kinds of memory formation. Thus, for instance, the role of excitatory amino acids has been suggested for long term potentiation [17], spatial learning [5,11], contextual pavlovian fear conditioning [8], inhibitory avoidance [15], and several models of synaptic plasticity [3]. However, the detailed mechanism by which glutamate transmission may be activated during various kind of memory formation is only speculative. The molecular mechanism by which CS contributes to the effective association with US is also not known.

The present data suggest that saccharin consumption (CS) may form a kind of a persisting trace that apparently facilitates the subsequent action of lithium (US). This facilitation might be interpreted as a potentiation of the

glutamate neurotransmission and might serve as a basis for the CTA acquisition. Whether only the metabotropic glutamate receptor is the target of the released glutamate and to what extent, if at all, NMDA and some other glutamate receptors might also be involved in this mechanism remains to be demonstrated.

Acknowledgements

The authors thank Dr. J. Bures for his interest and encouragement and Mrs. M. Fialova for skillful technical assistance. This study was supported by Grant A7011706 from the Academy of Sciences of the Czech Republic.

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