Activation of GABA_A Receptors in the Accessory Olfactory Bulb Does Not Prevent the Formation of an Olfactory Memory in Mice

Tomoko OTSUKA¹⁻³), Masatsugu HASHIDA²), Tatsuzo OKA¹) and Hideto KABA^{2,3})*

¹)Department of Veterinary Physiology, Faculty of Agriculture, Kagoshima University, Kagoshima 890-0065, ²)Department of Physiology, Kochi Medical School, Nankoku, Kochi 783-8505 and ³)CREST, Japan Science and Technology Corporation, Saitama 332-0012, Japan

(Received 27 October 2000/Accepted 22 March 2001)

ABSTRACT. When female mice are mated, they form a memory to the pheromonal signal of their male partner. The neural mechanisms underlying this memory involve changes at the reciprocal dendrodendritic synapses between glutamatergic mitral cells and γ -aminobutyric acid (GABA)-ergic granule cells in the accessory olfactory bulb (AOB). Blockade of GABA_A receptors in the AOB leads to the formation of an olfactory memory. In an attempt to disrupt memory formation at mating, we used local infusions of the GABA_A receptor agonist muscimol into the AOB during the critical period for memory formation. Muscimol across a wide range of doses (1–1000 pmol) did not prevent memory formation. The resistance of this memory to GABA_A receptor activation may reflect the complexity of synaptic microcircuits in the AOB.

KEY WORDS: accessory olfactory bulb, GABA_A receptor, olfactory memory.

J. Vet. Med. Sci. 63(7): 807–809, 2001

The reproductive physiology of mammals is strongly influenced by pheromones, with some of the most dramatic effects elicited in mice. Urinary male pheromones activate the vomeronasal system, by way of the vomeronasal receptors, and promote mating by inducing estrus in female mice [12]. However, if this pheromonal effect is induced within three days after mating, implantation of the embryos is prevented. This is referred to as the olfactory block to pregnancy and often occurs if a recently mated female is exposed to the pheromones of an unfamiliar male. To prevent the male partner from blocking pregnancy of his mate, the female forms a memory for the pheromones of her male partner. Several features which characterize the neural basis of this olfactory recognition memory have now been elucidated [1, 2, 8]. Memory formation depends on vaginocervical stimulation at mating [13], but requires a prolonged exposure of 4–6 hr to male pheromones immediately after mating [18]. The formation, but not recall, of the memory depends on mating-induced enhancement of α -adrenergic transmission in the accessory olfactory bulb (AOB), the first relay in the vomeronasal system [7, 13, 18]. This memory lasts for at least 30 days following mating unless pregnancy ensues [11]. The neural changes underlying the long-lasting memory occur in the AOB [11].

Microcircuits in the AOB include the prominent reciprocal dendrodendritic synapse between mitral and granule cells. Glutamate released from mitral cell dendrites activates the dendrites of granule cells, which in turn mediate γ -aminobutyric acid (GABA)-ergic dendrodendritic inhibition back onto mitral cell dendrites [6]. This feedback inhibition at the reciprocal synapses regulates mitral cell activity [7, 15]. The formation of the pheromonal memory involves

changes at the reciprocal synapses [3, 16]. The GABA-mediated feedback inhibition through the granule-to-mitral synapse is very powerful and is the main means for mediating control of output from the AOB, activating neuroendocrine mechanisms via the hypothalamus [7, 10, 15]. Blockade of GABA_A receptors by local infusions of bicuculline, a GABA_A receptor antagonist, into the AOB produces an olfactory memory without the occurrence of mating [11], suggesting that the relief of mitral cells from granule cell-mediated feedback inhibition is important for memory formation. We have therefore examined whether memory formation is disrupted by local infusions of muscimol, a GABA_A receptor agonist, into the AOB during the critical period for memory formation.

The mice used in this study were adult males and virgin females of the Balb/c strain (Japan SLC, Hamamatsu, Japan) and CBA males (Charles River Japan, Atsugi, Japan). The animals were housed singly at 23°C with a reversed 12:12 hr light cycle (lights on 18.00, lights off 6.00). Fifty-three female mice were used for the study. Procedures conducted in the dark phase were under red light. Food and water were available *ad libitum*.

The estrous cycles of the females were monitored daily by taking vaginal smears. Mating was carried out naturally by placing an intact male into the home cage of a single estrous female. The majority of matings were completed 15–40 min later. To demonstrate pregnancy block, females were killed by cervical dislocation six days after mating and uterine horns were examined for implantation sites. The absence of implantation sites confirmed a positive pregnancy block.

Female Balb/c mice had permanently indwelling 25-gauge stainless steel cannulas stereotaxically implanted under Avertin (tribromoethanol) anesthesia at least 5 days prior to mating. In order to avoid trauma to the AOB, these

* CORRESPONDENCE TO: KABA, H., Department of Physiology, Kochi Medical School, Nankoku, Kochi 783-8505, Japan.

cannulas were implanted just anterior to the AOB (co-ordinates relative to the intersection of the superficial sinuses and the surface of the olfactory bulb: longitudinal +0.5 mm, lateral ± 0.8 mm, vertical -0.3 mm).

For the purpose of investigating the local action of the GABA_A receptor agonist muscimol (Sigma, St. Louis, MO, U.S.A.) in the AOB on the olfactory block to pregnancy, females received bilateral infusions of the drug at 0 and 1.5 hr after mating. In one group, infusions were made at 0, 1 and 2 hr after mating. The drug was infused over 5 min in a volume of 0.5 µl through 33-gauge stainless steel cannulas connected to manually operated 10-µl microsyringes. The infusion cannulas were left in place for one additional minute after infusion. Muscimol was dissolved in saline.

Cannulated females were allowed to mate with a Balb/c male. The females were left in the presence of the stud male's pheromones for 6 hr following mating before removal to a clean cage. On the afternoon of the day following mating, either the familiar (stud Balb/c) or strange (CBA) male was introduced to the female's cage and left with her for 48 hr. Four days later the females were killed and examined for implantation sites. Effectiveness of re-exposure (test exposure) to the familiar stud male in blocking pregnancy was used to evaluate whether memory formation had been prevented. When a low level of pregnancy block was obtained upon re-exposure to the familiar stud male, a comparison was made with the effectiveness of test exposure to strange male pheromones in blocking pregnancy. Data were analyzed with the Fisher exact probability test.

The results are summarized in Fig. 1. Two infusions of the GABA_A receptor agonist muscimol across a range of doses (1–100 pmol) during the critical period for memory formation resulted in a low level of pregnancy block to the

stud male (groups 2–4). For all three groups the level of pregnancy block was not significantly different from that for the saline-infused control (group 1) re-exposed to the stud male. The low level of pregnancy block could not be attributed to a disruptive effect of muscimol on the AOB structure because in females given 100 pmol muscimol a significant pregnancy block was induced by the strange male ($P<0.05$; group 5). Even the highest dose and frequency were still without effect on memory formation (group 6), despite the occurrence of drug-induced seizures. These results indicate that memory formation was not prevented by local infusions of muscimol into the AOB at any of the doses and frequencies used.

We had expected that infusions of the GABA_A receptor agonist muscimol into the AOB during the critical period for memory formation would interfere with the formation of the pheromonal memory, so the resistance of this memory to activation of GABA_A receptors was surprising. In relation to this, muscimol challenges to the AOB using the technique of *in vivo* microdialysis have been shown to increase the levels of noradrenaline without affecting the levels of excitatory neurotransmitters [3]. An increase in noradrenaline in response to muscimol challenges has also been reported in the sheep main olfactory bulb [14] and is probably due to the action of the GABA_A receptor agonist on the noradrenergic presynaptic terminals [4]. Therefore, such local control of noradrenaline release by activation of GABA_A receptors is probably important for enhancing the release of noradrenaline in the vicinity of the AOB mitral cells responding to male pheromones and thereby leading to the formation of the pheromone-specific memory as previously suggested [5]. This hypothesis is supported by the finding that bicuculline-induced memory lacks the pheromone specificity of the memory formed at mating and appears to generalize to

Fig. 1. Effects of local infusions of muscimol into the AOB on memory formation in the context of pregnancy block. Numbers in brackets refer to time (in hr) of drug infusions relative to mating. Numbers of animals tested are given in parentheses. * $P<0.05$ compared with the saline-infused group (Fisher exact probability test).

the pheromones of at least one other strain [11].

Pharmacological studies of pheromonal learning in the AOB have suggested that disrupting mating-induced memory formation is more difficult than creating an olfactory memory in the absence of mating [7, 9, 17]. This might reflect that multiple short-term exposures to male pheromones during the critical period after mating would be sufficient to produce a pheromonal memory. Accordingly, we cannot rule out the possibility that multiple short-term activation of mitral cells between muscimol infusions could have induced memory formation by circumventing the action of the GABA_A receptor agonist.

ACKNOWLEDGEMENTS. We would like to thank Dr. Peter Brennan for his advice during the preparation of this manuscript. This work was supported in part by research grants from the Ministry of Education, Science, Sports and Culture of Japan and The Mitsubishi Foundation.

REFERENCES

1. Brennan, P. A. and Keverne, E. B. 1997. *Prog. Neurobiol.* **51**: 457–481.
2. Brennan, P., Kaba, H. and Keverne, E. B. 1990. *Science* **250**: 1223–1226.
3. Brennan, P. A., Kendrick, K. M. and Keverne, E. B. 1995. *Neuroscience* **69**: 1075–1086.
4. Gervais, R. 1987. *Brain Res.* **400**: 151–154.
5. Gervais, R., Holley, A. and Keverne, B. 1988. *Chem. Senses* **13**: 3–12.
6. Jia, C., Chen, W. R. and Shepherd, G. M. 1999. *J. Neurophysiol.* **81**: 345–355.
7. Kaba, H. and Keverne, E. B. 1988. *Neuroscience* **25**: 1007–1011.
8. Kaba, H. and Nakanishi, S. 1995. *Rev. Neurosci.* **6**: 125–141.
9. Kaba, H., Hayashi, Y., Higuchi, T. and Nakanishi, S. 1994. *Science* **265**: 262–264.
10. Kaba, H., Li, C.-S., Keverne, E. B., Saito, H. and Seto, K. 1992. pp. 49–54. *In*: Chemical Signals in Vertebrates 6 (Doty, R. L. and Müller-Schwarze, D. eds.), Plenum Press, New York.
11. Kaba, H., Rosser, A. and Keverne, B. 1989. *Neuroscience* **32**: 657–662.
12. Keverne, E. B. 1983. *Trends Neurosci.* **6**: 381–384.
13. Keverne, E. B. and de la Riva, C. 1982. *Nature* **296**: 148–150.
14. Lévy, F., Guevara-Guzman, R., Hinton, M. R., Kendrick, K. M. and Keverne, E. B. 1993. *Behav. Neurosci.* **107**: 662–668.
15. Li, C.-S., Kaba, H., Saito, H. and Seto, K. 1990. *Neurosci. Lett.* **120**: 231–233.
16. Matsuoka, M., Kaba, H., Mori, Y. and Ichikawa, M. 1997. *Neuroreport* **8**: 2501–2504.
17. Okere, C. O., Kaba, H. and Higuchi, T. 1996. *Neuroscience* **71**: 349–354.
18. Rosser, A. E. and Keverne, E. B. 1985. *Neuroscience* **15**: 1141–1147.