Time-Dependent Effect of Glutamate on Long-Term Potentiation in the Suprachiasmatic Nucleus of Rats

Yukiko Nisikawa¹, Takao Shimazoe¹,*, Shigenobu Shibata² and Shigenori Watanabe¹

¹Department of Pharmacology, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka 812-8582, Japan
²Department of Pharmacology, Faculty of Human Sciences, Waseda University, Tokozawa 359-1192, Japan

Received January 24, 2002 Accepted August 9, 2002

ABSTRACT—The effect of glutamate on optic nerve stimulation-evoked field potentials in rat suprachiasmatic nucleus (SCN) was examined in vitro. Glutamate application for 20 min induced long-term potentiation (LTP) of the field potentials in the SCN at nighttime, whereas that induced a weak LTP at daytime. On the other hand, application for 40 min induced LTP in the SCN during the daytime, whereas it induced a weak one at nighttime. These results indicate that the effect of glutamate is dependent on the application time and that the effect is influenced by the duration of glutamate exposure.

Keywords: Glutamate, Suprachiasmatic nucleus, Long-term potentiation

Long-term potentiation (LTP) of synaptic transmission is caused by tetanic stimulation of afferent fibers in the hippocampus, and it has been vigorously studied in the hippocampus in vitro and in vivo because of the involvement of memory function. Many reports showed that the N-methyl-D-aspartate (NMDA) receptor is involved in the formation of LTP (1). We demonstrated that tetanic stimulation of the optic nerve produces LTP in the suprachiasmatic nucleus (SCN) postsynaptic field potential (2). We also reported that the NMDA receptor is indispensable for the induction of LTP after tetanic stimulation (3).

The mammalian SCN is well known as a primary oscillator in circadian systems (4). Entrainment of circadian rhythms to the environmental light-dark cycle is mediated via the direct retino-hypothalamic tract (RHT) (5). With regard to chemical neurotransmission in the RHT, several lines of evidence suggest that excitatory amino acids (EAAs) are involved in the transduction of photic information to the SCN. Retinal terminals in the SCN have been found to be immunoreactive for EAA, and the immunoreactivity is significantly higher compared with their post synaptic dendrites and non-retinal terminals (6). EAAs are released when the optic nerves are stimulated (7-9). The photic entraining effect is blocked by EAA receptor antagonists (10, 11).

We reported that LTP in the SCN is induced not during subjective nighttime but during subjective daytime after tetanic stimulation (2). This result might depend on released glutamate from RHT terminal by tetanic stimulation. In this experiment, therefore, we first examined whether glutamate application induces LTP on optic nerve stimulation-induced field potential. We then studied whether LTP formation is influenced by exposure time of glutamate (20 min and 40 min) and the application time (daytime and nighttime) in rat SCN slice.

Adult Wistar rats (200 – 300 g) were housed under a normal 12:12 h light-dark cycle for at least 2 weeks before the experiments with food and water ad libitum. Each animal was decapitated under ether anesthesia and brains were quickly removed from the skull. The horizontal hypothalamic slice including the SCN (530 – 550-μm thickness) was prepared as described previously (2). One slice obtained from one animal and was preincubated with warmed artificial cerebrospinal fluid equilibrated with 95% O₂ / 5% CO₂ at 32°C. The composition of the control artificial cerebrospinal fluid was 129 mM NaCl, 1.3 mM MgSO₄, 22.4 mM NaHCO₃, 1.2 mM KH₂PO₄, 4.2 mM KCl, 10 mM D-glucose and 2.5 mM CaCl₂. This buffer was maintained at pH 7.3 – 7.4. After preincubation (more than 1 h), the slice was transferred to a recording chamber and kept in a constant flow medium (4 ml/min). Optic nerve stimulation-induced postsynaptic field potentials corresponding to EPSP were recorded from the SCN slice using a glass microelectrode. Insulated stainless wires (ø; 0.2 mm) were placed on the optic nerve approximately 1-mm rostral to the optic chiasm. A single pulse stimulation with duration of 0.08 ms at 0.5 – 1.5 mA for optic nerve stimulation at a rate...
of 0.1 Hz was used, because this has previously been shown to produce field potential in the ventrolateral SCN (2).

After observation of a stable response from the SCN over 30 min, drugs were added to the artificial cerebrospinal fluid and was perfused for up to 20 min or 40 min through the experimental chamber. The amplitude of postsynaptic field potentials before glutamate application was set as 100%. LTP was defined as when a significant increase was continued for more than 60 min. The drugs used in this study were L-glutamate (Yoneyama Yakuhin Kogyo Co., Ltd., Tokyo). The amplitude of postsynaptic field potential before glutamate application was set as 100%. Data were expressed as means \pm S.E.M. Statistical significance was determined by ANOVA and differences between means were tested for significance \((P<0.05)\) using ANOVA followed by Scheffe’s S-test.

The study was approved by the Committee of Animal Care of Graduate School of Pharmaceutical Sciences at Kyushu University.

A field potential was caused by stimulation of the optic nerve in the ventrolateral SCN. Bath application of glutamate (10 \(\mu\)M) for 20 min at zeitgeber time (ZT) 13 enhanced the negative wave of the SCN field potential \((n=4, \text{Fig. 1A})\). The application of glutamate (10 \(\mu\)M) for 20 min at ZT 20 enhanced the negative wave of the SCN field potential as well as application at ZT 13 \((n=3, \text{Fig. 1A})\). Amplitude of the field potential was rapidly increased until 60 min and then slowly increased until 120 min after bath application. Figure 1B shows the traces of the postsynaptic field potentials recorded before and 120 min after glutamate application at ZT 13. In contrast, the application of 10 \(\mu\)M glutamate for 20 min at ZT 4 showed no observable effect on the response; i.e., failed to cause LTP formation \((n=3, \text{Fig. 1A})\). The changes in the LTP formation was significantly different between application at ZT 4 or at ZT 13 and at ZT 20 \((P<0.01, P<0.01, \text{respectively}; \text{ANOVA followed by Scheffe’s S-test})\).

On the other hand, bath application of glutamate (10 \(\mu\)M) for 40 min at ZT 4 enhanced the negative wave of the SCN field potential \((n=4, \text{Fig. 2})\). Amplitude of field potential was rapidly increased until 60 min and then slowly increased until 120 min after glutamate application,
whereas application of 10 μM glutamate for 40 min at ZT 13 or at ZT 20 showed no observable effect on the response; i.e., failed to cause LTP formation (ZT 13, n = 3; ZT 20, n = 4; Fig. 2). The changes in the LTP formation was also significantly different between application at ZT 4 and at ZT 13 or at ZT 20 (P<0.01, P<0.05, respectively; ANOVA followed by Scheffe’s S-test).

The RHT is the principal entrainment pathway in the mammalian circadian systems (5, 12). Glutamate has been proposed as a neurotransmitter released by retinal ganglion cells whose axons comprise the RHT.

We examined whether the effect of glutamate for 20 min application has time-dependency on optic nerve stimulation-evoked field potentials in the SCN. We demonstrated that 10 μM glutamate application for 20 min at ZT 13 or at ZT 20 enhanced the amplitude of the optic nerve stimulation-evoked field potentials, whereas the application at ZT 4 failed to cause LTP formation. This time-dependency is similar to that obtained from administration of light pulses to intact animals maintained in constant darkness.

We also examined whether the effect of glutamate is dependent on the duration of application time on optic nerve stimulation-evoked field potentials in the SCN. We showed that 10 μM glutamate application for 40 min at ZT 4 enhanced the amplitude of the optic nerve stimulation-evoked field potentials, whereas the application at ZT 13 or at ZT 20 failed to cause LTP formation. We previously reported that LTP is induced only by daytime tetanic stimulation (2). Therefore, the effect of 40 min application of glutamate seems to be equivalent to that of tetanic stimulation.

The reason for this opposite effect is unclear at present. Similar data is reported in in vivo experiments. The phase-response curve (PRC) of wheel running activity rhythm obtained by microinjection of glutamate or aspartate into the SCN mimicked not that of the light pulse type but that of dark pulses (13). One possibility for these opposite effects is that glutamate administration facilitated the release of serotonin, GABA and/or neuropeptide Y from afferent fiber terminals, and then, the action of the dark pulse type might be caused. Then, Wagner et al. (14) reported that the opposite action of GABAergic neurons between daytime and nighttime plays an important role in circadian systems. Therefore, the different effect of glutamate between daytime and nighttime may be caused by these GABAergic mechanisms.

An after effect may be thought of as another explanation. Brief trains of high-frequency stimulation to mono synaptic excitatory pathways in the hippocampus cause an abrupt and sustained increase in the efficiency of synaptic transmission. This effect, first described in detail in 1973 (15), is called LTP. LTP has since been found in all excitatory pathways in the hippocampus, as well as in several other regions in the brain, and there is growing evidence that it underlies at least certain forms of memory. In the past 10 years, LTP in the hippocampus has become the dominant model of activity-dependent synaptic plasticity in the mammalian brain, and much progress has been made in elucidating the mechanisms underlying its induction and expression. If the after effect is regarded as the learning and memorizing phase of the circadian systems, LTP in the SCN may be the primary experimental model for investigating the synaptic basis of learning and memory. In any case, glutamate also has an effect on circadian systems during daytime. On the other hand, tetanic stimulation to Schaffer collaterals elicited LTP formation in hippocampal CA1 of the rat from both subjective day and subjective night (2). Thus, the induction of LTP in the SCN is time-dependent mainly because the SCN is a circadian oscillator.

In summary, the time-dependent effect of glutamate is shown on the formation of LTP in the SCN, and the effect is also dependent on the duration of glutamate application.

REFERENCES
1 Bashir ZI, Alford S, Davies SN, Randall AD and Collingridge GL: Long-term potentiation of NMDA receptor-mediated synaptic transmission in the hippocampus. Nature 349, 156 – 158 (1991)
2 Nishikawa Y, Shibata S and Watanabe S: Circadian changes in long-term potentiation of rat suprachiasmatic field potentials elicited by optic nerve stimulation in vitro. Brain Res 695, 158 – 162 (1995)
3 Nisikawa Y, Shimazoe T, Shibata S and Watanabe S: N-Methyl-D-aspartate receptors are indispensable for the formation of long-term potentiation in the rat suprachiasmatic nucleus in vitro. Brain Res 782, 303 – 305 (1998)
4 Inouye ST and Kawamura H: Persistence of circadian rhythmicity in a mammalian hypothalamic “island” containing the suprachiasmatic nucleus. Proc Natl Acad Sci USA 76, 5962 – 5966 (1979)
5 Moore NY and Card JP: Visual pathways and the entrainment of circadian rhythms. Ann NY Acad Sci 453, 123 – 133 (1985)
6 de Vries MJ, Nunes CB, van der Want J, de Wolf A and Meijer JH: Glutamate immunoreactivity in terminals of the retinohypothalamic tract of the brown Norwegian rat. Brain Res 572, 231 – 237 (1993)
7 Liou SY, Shibata S, Iwasaki K and Ueki S: Optic nerve stimulation-induced increase of release of 1H-glutamate and 1H-aspartate but not 1H-GABA from the suprachiasmatic nucleus in slices of rat hypothalamus. Brain Res Bull 16, 527 – 531 (1986)
8 Shibata S, Watanabe A, Hamada T and Watanabe S: Protein synthesis inhibitor blocks (R,S)-alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)-or substance P-induced phase shift of the circadian rhythm of neuronal activity in the rat suprachiasmatic nucleus in vitro. Neurosci Lett 168, 159 – 162 (1994)
9 Shirakawa T and Moore NY: Glutamate shifts the phase of the circadian neuronal firing rhythm in the rat suprachiasmatic nucleus in vitro. Neurosci Lett 178, 47 – 50 (1994)
10 Abe H and Rusak B: Physiological mechanisms regulating photic induction of Fos-like protein in hamster suprachiasmatic nucleus. Neurosci Biobehav Rev 18, 531 – 536 (1994)

11 Rusak B, Abe H, Mason R, Piggins HD and Ying SW: Neurophysiological analysis of circadian rhythm entrainment. J Biol Rhythms 8, S39 – S45 (1993)

12 Treep JA, Abe H, Rusak B and Goguen DM: Two distinct retinal projections to the hamster suprachiasmatic nucleus. J Biol Rhythms 10, 299 – 307 (1995)

13 de Vries MJ and Meijer JH: Aspartate injections into the suprachiasmatic region of the Syrian hamster do not mimic the effects of light on the circadian activity rhythm. Neurosci Lett 127, 215 – 218 (1991)

14 Wagner S, Castel M, Gainer H and Yarom Y: GABA in the mammalian suprachiasmatic nucleus and its role in diurnal rhythmicity [see comments]. Nature 387, 598 – 603 (1997)

15 Bliss TVP and Lømo TJ: Long-lasting potentiation of synaptic transmission in the dentate area of the anesthetized rabbit following stimulation of the perforant path. J Physiol (Lond) 232, 331 – 356 (1973)