

PRANDIAL INCREASES OF LEPTIN AND OREXIN IN THE BRAIN MODULATE SPATIAL LEARNING AND MEMORY

Yutaka Oomura¹⁾, Shuji Aou²⁾, Kouji Fukunaga³⁾, Sigeki Moriguchi³⁾ and Kazuo Sasaki⁴⁾

Abstract Leptin is well known to be involved in the inhibition of feeding, thermogenesis, reproduction and neuroendocrine functions through its actions on the rodent hypothalamic receptors. Leptin facilitated the presynaptic transmitter release and postsynaptic sensitivity to the transmitters in the hippocampal CA1 neurons. Thus long-term potentiation (LTP) and the phosphorylation of Ca²⁺/calmodulin protein kinase II (CaMK II) were facilitated in the CA1 neurons. Therefore behavioral performance related to spatial learning and memory was improved by leptin in vivo applications. Orexin-A produced by glucose-sensitive neurons in the lateral hypothalamic area (LHA) and released during food intake facilitates feeding. Orexin-A suppressed LTP and CaMK II phosphorylation without affecting the presynaptic transmitter release. Therefore behavioral performance on learning and memory was impaired. The present studies suggest that leptin and orexin signalings in the brain may have important implications for cognitive function.

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Key words: Leptin; Orexin; CaMK II phosphorylation; Facilitation and impairment of learning and memory in rodents

1. Introduction

Leptin, an adipocytokine encoded by an obesity gene and expressed in adipose tissue, is increased in rat plasma during eating, the rise being from approximately 2.3 to 3.8 ng / ml (i.e. to 2.4×10^{-10} M). Approximately 1/5000 of plasma leptin enters into the brain and binds to widely distributed long form leptin receptors (OBRb) in the neurons especially in the hypothalamus, modulates neuronal activity and affects feeding behavior, thermogenesis and neuroendocrine status. For example, leptin inhibits food intake through the inhibition of glucose-sensitive neurons in the LHA and medial part of arcuate nucleus, feeding center, and facilitates the glucoreceptor

neurons in the ventromedial nucleus and lateral part of the arcuate nucleus, satiety center¹⁾. Thus leptin is one of endogenous satiety substances. Leptin also activates the neuronal activity of the parvocellular neurons in the paraventricular nucleus and modulates the sympathetic nerve activity, thus the thermogenesis and neuroendocrine functions. OBRbs have been found to be expressed in the CA1 region of the hippocampus. These findings suggest that leptin may affect the synaptic plasticity and behavioral performance related to learning and memory. Indeed, it has been reported that the hippocampal synaptic plasticity is modulated by leptin in rats with intact leptin receptors^{2,3)}, whereas it is impaired in leptin receptor deficient rodents^{4,6)}. In the present

Department of Integrative Physiology, Graduate School of Medical Sciences, Kyushu University¹⁾, Department of Brain Science and Engineering, Graduate School of Life Science and Systems Engineering, Kitakyushu Institute of Technology²⁾, Department of Pharmacology, Faculty of Pharmacy, Tohoku University³⁾ and Division of Bio-Information Engineering, Faculty of Engineering, University of Toyama, Toyama⁴⁾

¹⁾ Maedashi 3-1-1, Higashi-ku, Fukuoka, 812-0054, Japan.

Tel. 092 642 6087, Fax. 092 642 6093,

y_oomura25@hotmail.com

²⁾ Hibikino 1-22-101, Wakamatu-ku, Kitakyushu, 808-0135, Japan. Tel. 093 695 6105, Fax. 093 695 6105, aou@brain.kyutech.ac.jp

³⁾ Aoba 6-3, Aoba-ku, Sendai, Japan. 980-8578, Japan. Tel. 022 795 6836, Fax. 022 795 6835, fukunaga@mail.pharm.tohoku,

⁴⁾ Gofuku 3150, Toyama, Japan 930-8555. Tel. 0764 45 6719, Fax. 0764 45 6723, sasaki@eng.u-toyama.ac.jp

report, first we mention the effects of leptin on behavioral performance in spatial learning and memory task, LTP at Schaffer collateral-CA1 synapses, presynaptic and postsynaptic activities in hippocampal CA1 neurons and CaMK II activity in CA1 region that exhibits LTP, and show that the low physiological dose of leptin enhances behavioral performance and LTP, whereas the high dose of leptin, namely plasma concentration, impairs them³.

Orexin-A produced in the glucose-sensitive neurons in the lateral hypothalamic area and released into the brain during food intake. Orexin-A facilitates the glucose-sensitive neurons and suppresses the glucoreceptor neurons, thus orexin-A is one of endogenous hunger substances^{1,7}. Orexin-A exhibits this function through orexin 1 receptor (OX1R) and orexin 2 receptor (OX2R)^{7,8} and has the opposite effects of leptin, namely impairments of learning and memory performance and LTP⁹.

2. Results-1 Effects of leptin on learning and memory on rodents

2. 1. Facilitation of learning and memory and synaptic plasticity

The effects of leptin on the Morris water maze performance were carried out. Short swimming times to find a hidden platform inside the pool indicate more rapid learning. Thirty min before the task on day one various concentrations of leptin was intra-venously injected. On day one there was no difference between the leptin and control vehicle groups. On days 2-4, swimming time became shortened day by day in all groups including the vehicle control group. This showed the training effect. The only value significantly shorter than that for the control vehicle injected group was obtained on day 2 for the 50 $\mu\text{g}/\text{kg}$ leptin iv group ($p < 0.05$) as shown in Fig.1 A. Swimming time was significantly longer for the 500 $\mu\text{g}/\text{kg}$ group than the control group on day 2-4 ($p < 0.01$). In the probe test the swimming

time in the goal area (up to 60 s) was measured in the five groups ($n=5$ in each group). As shown in Fig.1 B, the 50 $\mu\text{g}/\text{kg}$ leptin group spent significantly more time swimming and the 500 $\mu\text{g}/\text{kg}$ group spent significantly shorter time in the goal area on probe test days 1 to 3 than the control group. These results indicate that 50 $\mu\text{g}/\text{kg}$ leptin group consolidates memory process so that it can last at least for 2 days after the Morris water maze test³.

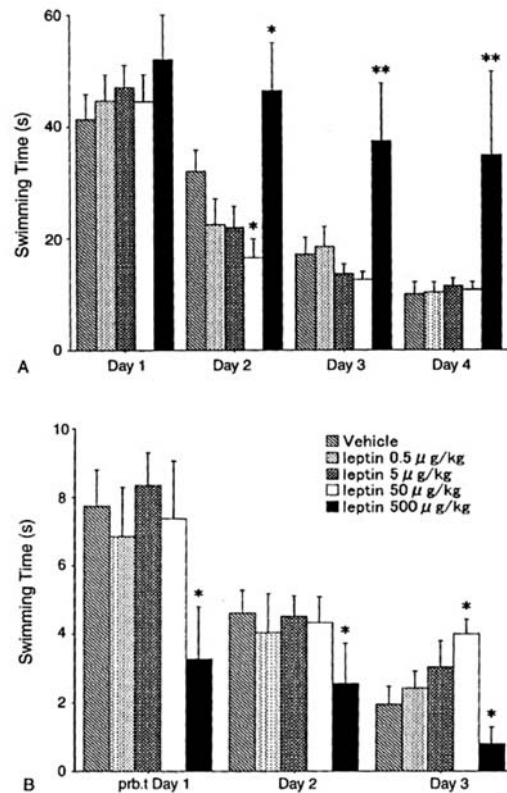


Figure 1 Enhancement of Morris water maze performance by the low concentration of leptin and impairment by the high concentration of leptin. Leptin 0.5 $\mu\text{g}/\text{kg}$ group, $n = 16$, other group, $n = 20$. The shortest swimming time (A) occurred on day 2 in the 50 $\mu\text{g}/\text{kg}$ leptin group (compared to vehicle group). Swimming time for finding the platform was significantly greater at 500 $\mu\text{g}/\text{kg}$ than in control on days 2 to 4. (B) Probe test after withdrawing the platform. The 50 $\mu\text{g}/\text{kg}$ leptin group spent a longer time in the goal-area than the vehicle group on probe test (prb. t) day 3 ($p < 0.05$), whereas the 500 $\mu\text{g}/\text{kg}$ leptin group spent less time than the vehicle group on probe test days 1-3 ($p < 0.05$). * $p < 0.05$. (Adapted from Oomura *et al.*, 2006)

Fig. 2 A and B provide the effects of various concentrations of leptin on LTP on CA1 neurons in hippocampal slice preparations. The percentage of the increase in field (f) EPSP amplitude in the 10^{-12} M leptin was approximately 250 %, whereas that in the normal Krebs-Ringer treated slices was approximately 150 %. At the 10^{-10} M leptin LTP was suppressed³.

2. 2. Effects of leptin on the presynaptic and postsynaptic activities

2. 2. 1. Presynaptic activity

Measurement of spontaneous mEPSCs in CA1 neurons in hippocampal slice preparations can be used to obtain additional information on how leptin affects presynaptic transmitter release in the process by which LTP is induced. As shown in Fig. 3, though no change in the mEPSCs

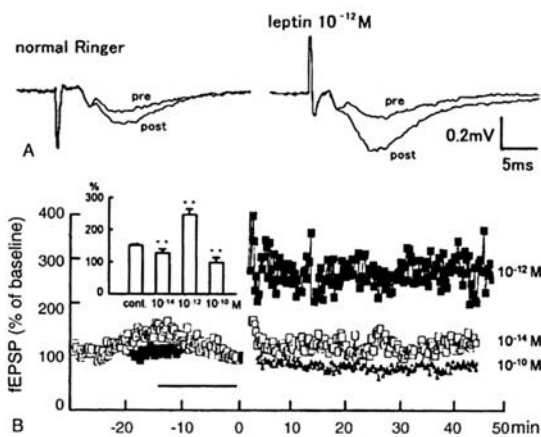


Figure 2 Leptin effects on LTP in CA1 neurons. (A) Example records of fEPSPs 20 min before (pre) and after (post) tetanization. Left, normal Krebs- Ringer, right, 10^{-12} M leptin. Leptin applied for 15 min just before tetanic stimulation. (B) Percent change of fEPSP amplitudes. Tetanic stimulation took place at zero time (see abscissa). Solid horizontal lines, leptin application for 15 min. Ordinate, amplitudes of fEPSPs as percentage of baseline (100%). Inset, mean amplitudes of fEPSPs (as percentage of baseline) following leptin administration; measurements were made over a 10 min period. (30-40 min after tetanic stimulation) 10^{-14} , 10^{-12} , 10^{-10} M leptin, $n = 3$; control, $n = 6$. Leptin groups are all significantly different from the controls. $** p < 0.01$. (Adapted from Oomura et al., 2006)

frequency the amplitudes were increased by approximately 130% in 10^{-12} M leptin, whereas no significant change was taken place in 10^{-10} M leptin³.

2. 2. 2. Postsynaptic activity

Postsynaptic responses evoked in CA1 neurons by various transmitter candidates can be used to obtain additional information on how leptin might be affecting postsynaptic receptors in the process in leading to LTP induction. The intracellularly recorded postsynaptic responses of CA1 neurons in hippocampal slice preparations evoked by quisqualic acid (Quis, 10 mM in 150 mM NaCl), γ -aminobutyric acid (GABA, 0.5 mM in 150 mM NaCl), and N-methyl-aspartic acid (NMDA, 10 mM in 150 mM NaCl) ejections

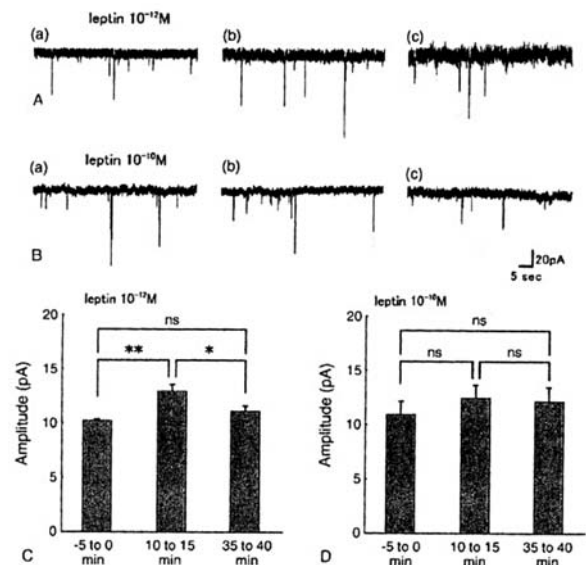


Figure 3 Effect of leptin on spontaneous mEPSCs in CA1 neurons. (A) and (B) Examples of high sweep records of mEPSC at 5 min (a) before, 10 min (b) and 35 min (c) after 10^{-12} M ($n = 3$) and 10^{-10} M leptin ($n = 4$) applications, respectively. Leptin application, 5 min. Membrane potential, clamped at -70 mV. (C) and (D) Effects of 10^{-12} and 10^{-10} M leptin on mEPSC amplitudes, respectively. Left, middle and right histograms in (C) and (D) are mean amplitudes for 5 min periods just before, from 10 min to 15 min and from 35 to 40 min after leptin application, respectively. Significant increase in mEPSC amplitude following 10^{-12} M leptin was seen. $*p < 0.05$, $**p < 0.01$. (Adapted from Oomura et al., 2006)

electrophoretically through the glass capillary multibarreled electrodes placed on the apical dendrites of the same recording neurons were obtained in normal Krebs-Ringer solution. The measurements were repeated in the same neuron over the next 15 min with 10^{-12} or 10^{-10} M leptin in the perfusate, and then repeated again in normal Krebs-Ringer solution. In four success neurons, as shown in Fig. 4A, the applications of 10^{-12} M leptin concentration did not show any changes

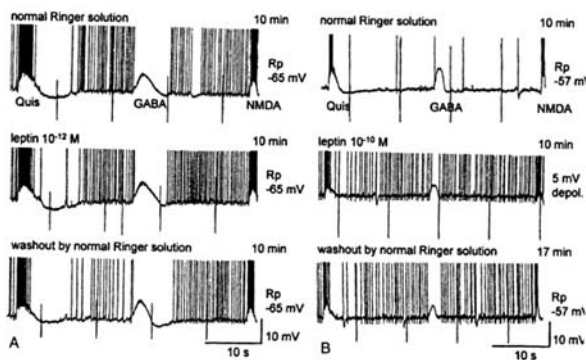


Figure 4 Effect of leptin on intracellularly recorded postsynaptic responses induced in a CA1 neuron by electrophoretic application (25 nA for 1 s) of Quis, GABA and NMDA. (A) and (B) Effect of 10^{-12} and 10^{-10} M leptin application, respectively. Upper traces in (A) and (B), 10 min after recording had stabilized in normal Krebs-Ringer solution. Middle traces in (A) and (B) 10 min after 10^{-12} and 10^{-10} M leptin application, respectively. No observable changes in postsynaptic responses were seen following 10^{-12} M leptin application, whereas suppression of postsynaptic responses to all three transmitters was seen following 10^{-10} M leptin application. Membrane potential (Rp: -65 mV) was not changed by application of 10^{-12} M leptin, but a 5 mV depolarization (depol) occurred following 10^{-10} M leptin application. Lower traces in (A) and (B), 10 and 17 min after washout respectively. (Adapted from Oomura et al., 2006)

in the postsynaptic responses to the Quis, NMDA and GABA. However in 10^{-10} M leptin the postsynaptic responses to these transmitter candidates were all attenuated, as shown in Fig. 4 B. At that time the membrane potential of the CA1 neurons were about 5 mV depolarized³⁾.

2. 3. Effect of leptin on intracellular Ca^{2+} in single isolated CA1 neurons

As mentioned above, the different effects caused by the different leptin concentrations could be related to the $[Ca^{2+}]_i$ in the postsynaptic CA1 neurons. We measured the $[Ca^{2+}]_i$ concentration in single CA1 neurons at different leptin concentrations. The basal level of $[Ca^{2+}]_i$ was 115 ± 44 nM ($n = 6$). Application of leptin of 10^{-12} and 10^{-10} M induced marked dose dependent increase in $[Ca^{2+}]_i$ was 329 ± 97 nM ($n = 6$) and 739 ± 250 nM ($n = 6$) respectively³⁾.

2. 4. Effect of leptin on CaMK II activity

The activation and autophosphorylation of CaMK II is one of the molecular signal transduction mechanisms known to LTP and to learning and memory performance¹⁰⁾. We measured the effect of leptin on the activity of CaMK II in the rat CA 1 regions of slices in which LTP had been induced as described above. Control slices were stimulated and potentiated, but not exposed to leptin. As shown in the Table, 10^{-12} M leptin induced a significant increase in Ca^{2+} -independent CaMK II activity ($p < 0.05$) without a significant increase in total CaMK II activity, but 10^{-10} M leptin showed the significant

Table 1 Effect of leptin on CaMK II activity. (Adapted from Oomura et al., 2006)

Table 1 - Effects of leptin on CaMK II activity			
	(A) Ca^{2+} -independent activity (nmol/mg/min)	(B) Total activity (nmol/mg/min)	" Ca^{2+} -independence" (A/B \times 100%)
Control ($n = 8$)	25.3 ± 6.87	232.5 ± 15.46	15.3 ± 3.38
10^{-12} M leptin ($n = 10$)	$42.3 \pm 5.15^*$	239.1 ± 18.65	18.0 ± 3.25
10^{-10} M leptin ($n = 8$)	$31.3 \pm 3.74^{\#}$	221.6 ± 21.02	$14.2 \pm 2.55^{\#}$

* $p < 0.05$ in comparison with control.

[#] $p < 0.05$ in comparison with 10^{-12} M leptin.

lowering of Ca^{2+} -independent CaMK II activity. These results indicate that a specific dose-dependent increase in CaMK II activity at 10^{-12} M leptin is associated with the induction of LTP facilitation³⁾.

3. Results-2 Impairment effects of orexin on long-term potentiation and spatial learning and memory in rodents

3. 1. Introduction

Hypothalamic peptide, orexin-A produced in the glucose-sensitive neurons in the LHA and dorsomedial hypothalamic nucleus stimulates food intake^{1,7)}. Orexin-A containing terminals were found in the hippocampus, amygdala, central gray, locus coeruleus, dorsal raphe, dorsal motor nucleus of the vagus, perirhinal sensory and motor cortex, and the cerebellum^{7,8)}. Two types of orexin receptors, OX1R and OX2R distribute widely in the CNS: high levels of OX1R mRNA are found in the hippocampal formation, and OX2R mRNA is found in the cerebral cortex. Consequently, the activation of functional orexin circuits can influence mainly learning and memory and arousal^{8,9)}. The purpose of the present study was to determine the effects of different doses of orexin-A on 1) performance in the Morris water maze test and 2) on LTP and paired-pulse facilitation in the Schaffer collateral synapses.

3. 2. Orexin-A impairs learning and memory in the water maze in rodents

The doses of orexin-A were selected based on their influences on the food intake^{1,7,8)}. Control, 5 μl ACSF (n = 10), 1 and 10 nmol orexin-A in 5 μl (n = 6, 8 respectively) were i.c.v. injected. In Fig.5A, the mean swimming time shows significant differences between the two orexin-A groups, namely orexin-A group was performing less efficiency than the control, particularly on block 2 and 3. The results of the probe test in Fig.5 B was more pronounced regarding the

different doses of orexin. In the goal area, there was a significant dose related impaired retention, in term of where the platform had been located⁹⁾.

3. 3. Orexin-A depresses LTP in Schaffer collateral –CA1 synapses in hippocampal slices

As shown in Fig.6 A and B, after the tetanic stimulation, 10 nM orexin-A reduced the potentiation of fEPSPs amplitudes. Thus LTP was suppressed. There was no effect of orexin-A, even 30 nM concentration, applied after the tetanus (C). The effects of 10 nM orexin-A was the approximately the same as those of 30 nM orexin.

In normal Krebs-Ringer solution with a low frequency stimulation, fEPSP mainly results from

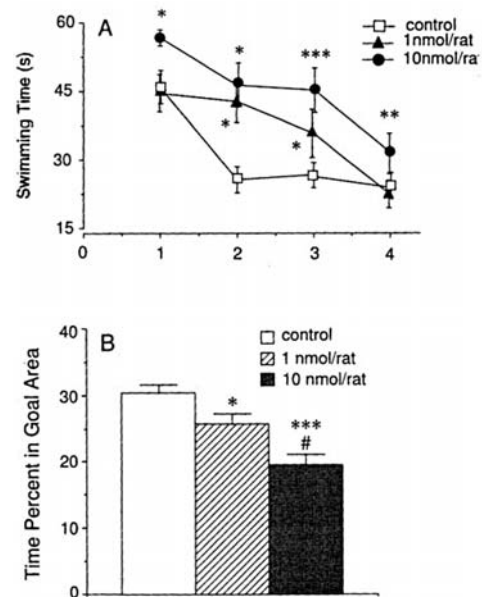


Figure 5 Effect of i.c.v. injection of orexin-A (1, 10 nmol) on water maze performance in an invisible platform condition. (A) Escape latency. Impairment in swimming time occurred on blocks 2 and 3 in the 1 nmol group and on blocks 1, 2, and 3 in the 10 nmol group. Swimming time was significantly longer at 1 and 10 nmol than the control. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (vs. control). (B) Probe test on day 2 after trials with the platform (block 4), a free-swim that without the platform. Goal area one quadrant of the pool where the platform was formerly located, 70 cm in diameter. The swimming time % in the goal area. Significantly shorter times are seen in the 1 and 10 nmol groups than in the control group. (Adapted from Aou et al., 2003)

2-amino-3-hydroxy-5-methyl-isoxazole (AMPA) response. After a tetanic stimulation, N-methyl-D-aspartate (NMDA) response is activated and lead to LTP. Because orexin-A had no effect on the base line fEPSP in the normal Krebs-Ringer solution, orexin-A had no effect on AMPA response. In order to record the NMDA component of EPSP, we used 10 μ M 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) to block the AMPA response and changed to a solution with low 0.1 mM Mg^{2+} and 50 μ M picrotoxin

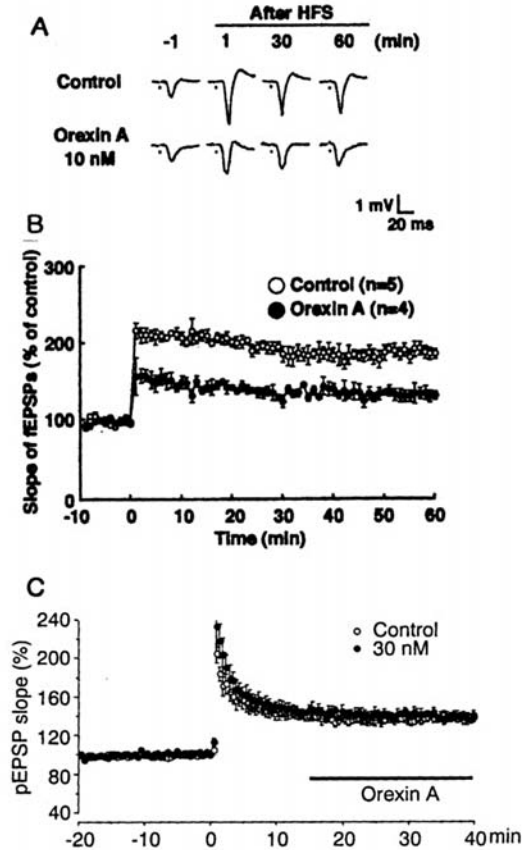


Figure 6 Effects of bath application of orexin-A on LTP. The mean \pm S.E.M. percent changes of slopes of fEPSP before and following a tetanic stimulation (time 0 on abscissa). Orexin-A was applied for 15 min just before tetanic stimulation. (A), fEPSPs amplitudes before and after tetanic stimulation. Upper, before orexin-A application, lower, after 10 nM orexin-A application. (B) LTP. 10 nM orexin-A was applied for 15 min just before tetanic stimulation. Orexin-A suppressed LTP. (C) No effect of orexin-A on the maintenance of LTP when orexin-A was applied after tetanus. (Adapted from Aou *et al.*, 2003)

to enhance the NMDA response. Under this condition, the amplitudes of fEPSPs decreased only after the application of orexin-A. This finding indicated that orexin-A only affected NMDA receptors.

3. 4. Effect of orexin-A on paired pulse facilitation

This experiment was carried out to determine the possible effects of 10 and 30 nM orexin-A on presynaptic release of transmitter. The results are summarized in Fig.7 where the percent change in the first pulse p1 and the ratio p2/p1 for each min is presented before and after the administration of the two doses of orexin-A

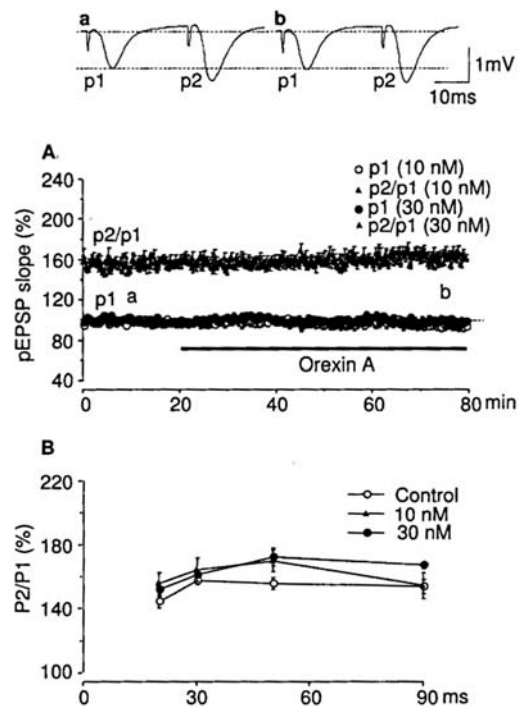


Figure 7 The presynaptic effects of orexin-A were investigated by mean of paired pulse stimulation. Upper recordings, first and second fEPSP at 30 ms stimulus interval. a and b, selected where indicated in lower part by corresponding letters. (A) amplitudes of first fEPSP and amplitude ratio p2/p1, interval 30 ms. Orexin-A had no effect on the p1 amplitude and ratio. Solid horizontal lines, orexin-A (10, 30 nM) application. (B) the amplitude ratio p2/p1 following orexin-A application as a function of the paired-pulse stimulus interval. No significant difference in the ratio occurred. Test stimuli, every 30 s (n=5 - 7, for each group). (Adapted from Aou *et al.*, 2003)

for 60 min ($n = 5$, each). Because there was no significant change in the fEPSP amplitudes and the p2/p1 ratios with an interpulse interval of 30 ms, another experiment was carried out utilizing intervals of 20, 50, 90 ms at the same doses of orexin-A, 10 and 30 nM ($n = 5$, each, in comparison with the control ($n = 7$). The results, as shown in Fig.7 B, were similar to those of the previous experiment. The mean \pm S.E.D. of p2/p1 ratio in percent for control, 10 and 30 nM orexin-A applications were not significant between the different intervals. As a result, there was no evidence of any orexin-A effect on the presynaptic transmitter release.

3. 5. Effect of orexin-A on CaMK II activity

As mentioned on the leptin effects on the facilitations of learning and memory and LTP, the two factors have the intimately close relationship. Orexin-A has the impairments in both learning and memory and LTP, thus we measured the CaMK II activation on hippocampal CA1 neurons under the orexin-A applications. Orexin-A at 10nM and 30 nM concentrations suppressed the Ca^{2+} -independent CaMK II activity. The activity levels were 25.5 ± 5.37 in the control, 20.2 ± 4.37 in 10 nM orexin-A and 18.6 ± 5.68 nmol/mg/min in 30 nM orexin-A ($p < 0.05$, at control to 10 nM orexin).

4. Physiological significance of leptin and orexin-A

The present results clearly indicate that the physiological concentration of leptin in the brain, 10^{-12} M facilitates LTP and phosphorylation of CaMK II, and enhances spatial learning and memory, but the plasma concentration of leptin 10^{-10} M impaired these. The close association between enhanced hippocampal LTP and facilitated learning and memory has been reported³. When we assume that a 200-250 g rat has a plasma volume of approximately 10 ml, 50 μ g / kg leptin intravenously applied would be equivalent

in plasma to about 10^{-8} M and 500 μ g / kg dose to 10^{-7} M. As mentioned in the introduction, less than 1/5000 of ^{125}I -leptin administered intravenously enters into the brain^{3, 11}, since 50 μ g / kg leptin is the physiological concentration in the brain, whereas 500 μ g / kg leptin is the pharmacological concentration³.

It is known that a moderate increase of $[Ca^{2+}]_i$, say 329 ± 97 nM in CA 1 neurons activates the Ca^{2+} -independent activity of CaMK II, whereas a large and prolonged increase of $[Ca^{2+}]_i$, say more than 600 nM at the 10^{-10} M leptin concentration inhibits it, possibly through the activation of calcineurin^{6,12,13}.

Calcineurin is a protein phosphatase that brings the CaMK II dephosphorylation. Thus high $[Ca^{2+}]_i$ at 10^{-10} M leptin, instead of activation of CaMK II may activate the calcineurin.

The present experiments also demonstrated that orexin-A inhibited the performance of rats in the invisible platform condition in the Morris water maze test, suggesting that orexin-A impaired the spatial performance but did not induce the abnormality in swimming ability, thigmotaxis, and vision in vivo condition. The present results also indicate that orexin-A inhibits the LTP in the hippocampus under in vitro conditions.

The paired pulse facilitation experiment indicated that the orexin-A induced inhibition of LTP is not mediated by the decrease in amount of transmitter released from presynaptic terminals but solely by the postsynaptic NMDA receptors while the AMPA receptors are not involved. The LTD, did not mentioned in the present results, but actually did not change, thus indicating no involvement of the calcineurin-inhibitor-1 phosphatase cascade¹⁴.

The concentration of orexin-A in the rat CSF is not yet known. We gave 1-10 nmol/rat orexin-A and the concentration can be estimated as 3.3 to 33 μ M when the volume of CSF is approximately 300 μ l¹⁵). Since the diffusion

between the ventricle and the hippocampus dose not exceed 2 %¹⁶⁾, the concentration of 3.3 to 33 μM orexin in CSF becomes 6.6×10^{-8} M to 6.6×10^{-7} M in the hippocampus. If we assume that the production of CSF is 0.5 % of the total volume per min then the concentration of orexin-A would be diluted to 40 % of the initial concentration. As a results, the concentration in the hippocampus becomes 2.6×10^{-8} M to 2.6×10^{-7} M.

5. Conclusion

Figure 8 shows the conclusive variegated physiological significance of leptin. During food intake, leptin released into plasma from the adipocytes reaches into the brain. The concentration of leptin in the brain is approximately 1/5000 of its plama concentration. Leptin diffuses into the brain parenchyma and first reaches the hypothalamus and suppresses food intake through the inhibition of the

activity of the glucose-sensitive neurons in the LHA and the medial arcuate nucleus, feeding related areas and through the activation of the glucoreceptor neurons in the ventromedial nucleus and the lateral part of arcuate nucleus, satiety related areas. Secondly, leptin reaches the hippocampus and facilitates synaptic plasticity and improves spatial learning and memory performance through the facilitations of LTP and CaMKII phosphorylation in the CA1 neurons. Thirdly, leptin reaches to the neurons in the parvocellular part of the paraventricular nucleus and facilitates the release of the corticosterone releasing hormone (CRH) and also activates not only hypothalamic-pituitary-adrenal axis but also efferent sympathetic outflow.

Corticosterone released from the adrenal gland can also modulate LTP and promote the learning and cognitive processes¹⁷⁾. In fact, the clinical data suggest that CRH deficiencies

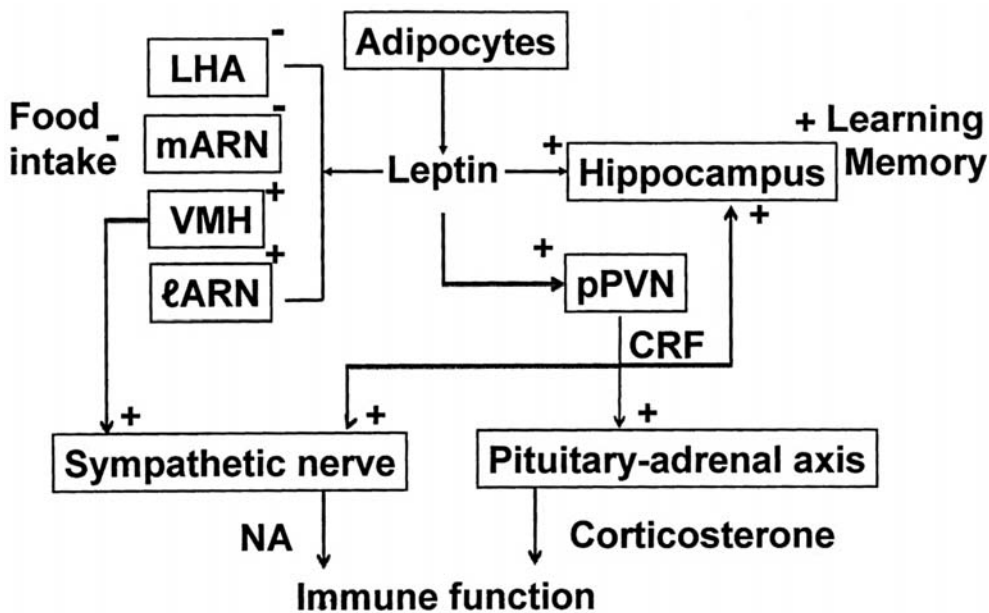


Figure 8 Deduced physiological action of endogenous satiety substance, leptin. Leptin released into plasma during food intake from the adipocytes enters into the brain only by 1/5000 of the plasma concentration. Leptin reaches the glucose-sensitive neurons in the LHA and the medial part of the arcuate nucleus (feeding related centers) and the glucoreceptor neurons in the ventromedial nucleus and lateral part of the arcuate nucleus (satiety related centers) and inhibits feeding. Then leptin reaches the hippocampus and facilitates spatial learning and memory. Leptin reaches the neurons in the parvocellular part of the paraventricular nucleus (pPVN) and activates these neurons causing CRH release. These neurons and CRH activate efferent sympathetic outflow and pituitary-adrenal axis. Splenic sympathetic activation and released corticosterone modulate immune function.

can be detected in the brain in patients with neurodegenerative dementia. The parallel activation of the sympathetic outflow to the adrenal gland and brown adipose tissue may show that leptin has a multifunctional roles in the control of neuronal and/or endocrinological activities. The iv administration of leptin induces hyperthermia corresponding to the postprandial hyperthermia, in awake and freely moving rats. Corticosterone and splenic sympathetic activation attenuate the immune system. Since noradrenalin released into the spleen, attenuates the natural killer activity and corticosterone reduces the antibody-antigen reaction. In other words, the autoimmune reaction is reduced. That is a benefit to the living machinery.

The endogenous satiety substance, leptin plays physiological role in the presence of alimentation. This evidence indicates that food intake maintains not only the body energy homeostasis but also primes the brain for important functions.

Zucker fatty rats and db/db mice who have the abnormal leptin receptors, therefore showed impairments of LTP and spatial learning and memory performance. These strains also showed lower basal levels of CaMK II activity in the CA1 region than the respective controls, and the levels did not respond to a brief tetanic stimulation⁶⁾.

Compared with one of the endogenous satiety substances leptin released from the adipocytes during feeding, orexin-A is one of the endogenous hunger substances released from the glucose-sensitive neurons in the LHA and other hypothalamic feeding related centers and promotes feeding. The activation of the LHA glucose-sensitive neurons also promotes the autonomic nervous system function, especially the sympathetic outflow. Thus orexin-A has an important role for keeping the healthy living body machinery. In spite of carrying such an important role, orexin-A has the other site of

function, namely plastic impairment. During food intake we should concentrate this feeding behavior.

The present studies, anyway indicate the importance of leptin and orexin-A signaling for cognitive function.

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