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Activation of Gonadotropin-Releasing Hormone Receptors Produces Neuronal Excitation in the Rat Hippocampus

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Abstract

Whole-cell patch-clamp recordings of evoked action potentials were made in CA1 and CA3 pyramidal neurons of rat hippocampal slices. Previously we have demonstrated that activation of gonadotropin-releasing hormone (GnRH) receptors induces a long-lasting enhancement of synaptic transmission mediated by ionotropic glutamate receptors in CA1 pyramidal neurons of rat hippocampal slices. Here, we further studied whether activation of GnRH receptors could modulate intrinsic neuronal excitability in CA1 and CA3 pyramidal neurons of rat hippocampal slices. The use of a specific GnRH analog, leuprolide (10^{-8} M), elicited a relatively long-term increase in evoked action potentials in CA1 and CA3 pyramidal neurons, respectively. The GnRH receptor-induced increase in evoked action potentials in both CA1 and CA3 pyramidal neurons could be abolished by a potent GnRH receptor antagonist, [acetyl-3,4-dehydro-Pro[1],D-p-F-Phe[2],D-Trp[3,6]]-LHRH (10^{-8} M). The present study suggests that activation of GnRH receptors can lead to an increase of intrinsic neuronal excitability of both CA1 and CA3 pyramidal neurons in the rat hippocampus, an important integrative region for reproductive process, both endocrinologically and behaviorally.

Key Words: gonadotropin-releasing hormone receptor, CA1 neurons, CA3 neurons, hippocampus, leuprolide, neuropeptide

Introduction

The gonadotropin-releasing hormone (GnRH) released from the hypothalamus can act onto the pituitary gland and the other extrapituitary sites within the brain (9, 20). Previous studies indicated that high densities of GnRH receptors were present in the regions of the brain, including CA1-CA3 regions of the hippocampus (8, 14, 16, 23). Furthermore, the GnRH receptor in the hippocampus was reported to be associated with the control of sex behavior since infusions of GnRH either centrally or peripherally produced specific effects on sex behavior in rats and mammals under appropriate conditions (4, 7, 13, 19, 24, 25). In addition, sex steroids could also modulated the concentration of GnRH receptors in the hippocampus, suggesting that hippocampal GnRH was involved in sexual behavior (1).

Understanding of the modulation of GnRH receptors in the hippocampus is a special interest in view of the importance of neuromodulatory actions of neural peptides on neurons in the central nervous system. Previous studies suggested that the hippocampus could serve as an important integrative region for reproductive process, both endocrinologically and behaviorally (11, 14, 26). For instance, electrical stimulation within the hippocampus inhibited ovulation in female rats (11, 26). Ablations of hippocampal structures also enhanced the expression of sexual behavior (11, 14, 26). However,

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it remains unclear about underlying mechanisms responsible for the actions of GnRH on hippocampal neurons, especially the CA3 pyramidal neurons. Recently, we reported that activation of GnRH receptors by a transient application of leuprolide, a specific GnRH analog, elicited a long-lasting enhancement of synaptic transmission mediated by ionotropic glutamate receptors in CA1 pyramidal neurons of the rat hippocampus (28). In the present study, we further investigated the possibility that activation of GnRH receptors could modulate intrinsic neuronal excitability in CA1 and CA3 pyramidal neurons of the rat hippocampal slices. Here, we provided evidence that activation of GnRH receptors led to a long-term increase of evoked action potentials in both CA1 and CA3 pyramidal neurons of the rat hippocampus.

Materials and Methods

Slice Preparation

Hippocampal slices were prepared from female Sprague-Dawley rats (200-250 g), as described previously [28, 29]. The slices (350-400 μm) were transversely cut with a vibroslicer (Campden Instruments, UK) and were immediately transferred to an incubating chamber provided with humidified 95% O_2/5% CO_2 gas at room temperature. After an incubation period of at least 1 hr, a slice was transferred to a submerged-type, constant flow recording chamber (volume approximately 1.0 ml) perfused about at a rate of 1.5-2.0 ml/min with oxygenated gas (95% O_2/5% CO_2) at 30.0±0.5°C. The slice was then held down and fixed with a U-shaped piece of platinum wire.

Solutions and Drugs

The control artificial cerebrospinal fluid (aCSF) consisted of (in mM): NaCl (124), KCl (3.5), $CaCl_2$ (2), $MgCl_2$ (1), NaH_2PO_4 (1.25), $NaHCO_3$ (26), D-glucose (10), pH 7.4. The solution used in the patch clamp electrode contained (in mM): KCl (130), NaCl (10), EGTA (10), HEPES (10), $CaCl_2$ (1), $MgCl_2$ (3), MgATP (3), MgGTP (0.2), pH 7.2 (using KOH). The osmolarity of the two solutions was kept at 305±5 mOsm. Drugs were dissolved in sterile water for a stock solution and were stored at -20°C. All drugs in aCSF were prepared immediately before each experiment from the frozen stocks and were administrated via bath application under light protection. Methylene blue was used as an indicator which arrived the recording chamber about 20-25 sec after the start of the perfusion. This dye was washed out approximately within 2-2.5 min after it was switched off. Leuprolide, a specific GnRH analog (28), and a potent GnRH receptor antagonist [acetyl-3,4-dehydro-Pro^1,D-p-F-Phe^2,D-Trp^{3,6}]-LHRH (3, 28) were purchased from Sigma Chemical Company (St Louis, MO, USA).

Whole cell recordings A recording electrode (4-7 MΩ) made from a microelectrode puller (P-97, Sutter Instrument Co., USA) established a tight seal between the pipette and the cell membrane (seal resistance > 3 GΩ). Responses recorded were filtered at 1 KHz (low-pass filter, Axopatch 1D, Axon Inc., USA), digitized at a sampling rate of 15 KHz, and analyzed off-line using a IBM compatible computer and a commercial software (pClamp 6.04, Axon Inc., USA). In addition, previously described procedures (5, 12, 21, 28, 29), such as the series resistance (< 20 MΩ), initial resting membrane potential (at least -60 mV), and the input resistance (> 200 MΩ) were used to verify the adequacy of the clamping and the health of a cell. Unless otherwise stated, the cell membrane potential was held at -65 mV under the current-clamp mode throughout the experiments.

Statistics

For statistical analysis, the firing rate of evoked action potentials for each experiment was normalized relative to the control value prior to leuprolide application. Data are given as mean Ñb standard error of mean (SEM). Statistical differences were first determined by one-way ANOVA followed by Bonferroni t-test for multiple comparisons. A significant level of $\alpha = 0.05$ and $P < 0.05$ was applied to all tests.

Results

To determine whether intrinsic neuronal excitability in CA1 pyramidal neurons of rat hippocampal slices could be modulated by the activation of GnRH receptors, leuprolide, a specific agonist of GnRH receptors, was perfused to hippocampal slices for 10 min. In these experiments, the membrane potential was held at -65 mV (a simulation of neuronal resting membrane potential) under current-clamp mode. A single CA1 pyramidal neuron was injected with a 1-s depolarizing current (40 pA) to evoke action potentials. As demonstrated in Figure 1, evoked action potentials were obtained prior to and following leuprolide application (10^{-8} M). Employment of leuprolide elicited a long-term increase in the firing rate of evoked action potentials (5 slices from 5 animals). This long-term increase in the firing rate was characterized by a slow, gradual

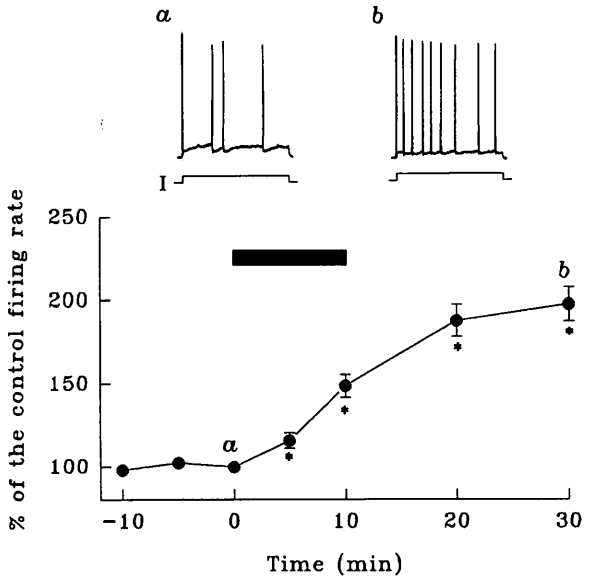

Fig. 1. Activation of GnRH receptors induced a long-term increase of neuronal excitability in CA1 pyramidal neurons of the hippocampus. The firing rate of evoked action potentials in response to a 1-s depolarizing current (I) prior to and 30 min following a 10-min application of 10^{-8} M leuprolide (solid bar). Insets *a* and *b* correspond to the plots of evoked action potentials labeled *a* and *b* from the experiments subjected to leuprolide, respectively. An asterisk indicates significantly different from the control level at time point = 0 min.

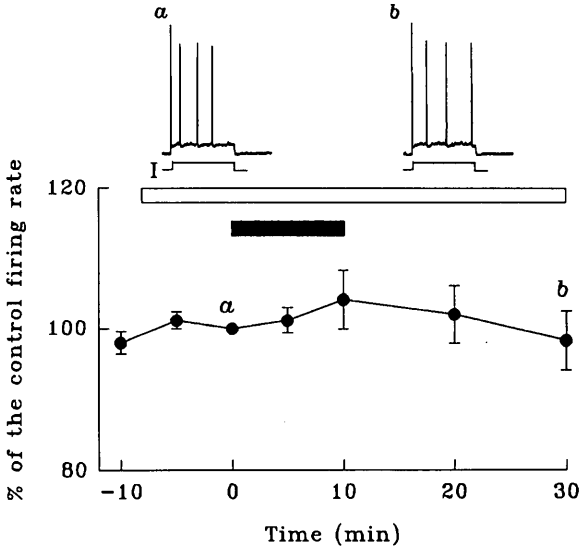

Fig. 2. The use of a potent GnRH receptor antagonist blocked the development of GnRH-induced long-term increase of neuronal excitability in CA1 pyramidal neurons of the hippocampus. A specific antagonist of GnRH receptors (opened bar), 10^{-8} M acetyl-3,4-dehydro-Pro^1,D-p-F-Phe^2,D-Trp^{3,6}]-LHRH, was administrated 7 min before, during, and 30 min following 10^{-8} M leuprolide application (solid bar). Insets *a* and *b* correspond to the plots of EPSCs labeled *a* and *b* from the experiments subjected to leuprolide, respectively.

increase in the number of firing rate, which persisted for at least 30 min. In two slices, this long-term increase could even last for 90 min (data not shown). The average of the firing rate of evoked action potentials obtained 30 min following leuprolide application (10.5 ± 0.8/sec, n = 5) was statistically different from that obtained under the control condition (4.1 ± 0.5/sec, n = 5).

To test whether the action of leuprolide on intrinsic neuronal excitability of CA1 pyramidal neurons (Fig. 1) was specific for GnRH receptors, leuprolide was administrated to the slices in the presence of acetyl-3,4-dehydro-Pro^1,D-p-F-Phe^2,D-Trp^{3,6}]-LHRH (a potent antagonist of GnRH receptors). As demonstrated in Figure 2, the use of acetyl-3,4-dehydro-Pro^1,D-p-F-Phe^2,D-Trp^{3,6}]-LHRH (10^{-8} M) caused no discernible effects on the baseline firing rate of evoked action potentials. However, this antagonist significantly blocked the development of leuprolide-induced increase in the firing rate of evoked action potentials. The average of the firing rate of evoked action potentials obtained 30 min following leuprolide application (4.6 ± 0.6/sec, n = 4) was not statistically different from that obtained under the control condition (4.1 ± 0.4/sec, n = 4).

To determine whether the intrinsic neuronal excitability in CA3 pyramidal neurons of the rat hippocampal slices could also be modulated by the activation of GnRH receptors, leuprolide was perfused to hippocampal slices for 10 min. The experimental protocols used in Figure 1 were repeated throughout the entire experiments. As illustrated in Figure 3 (5 slices from 4 animals), thirty minutes following leuprolide application (10^{-8} M), a long-term increase in the firing rate of evoked potentials was observed (9.3 ± 0.7/sec, n = 5) when compared to that under the control condition (4.6 ± 0.6/sec, n = 5). In some experiments, the long-term increase of neuronal excitability could even last for 60 min (data not shown).

To test whether the action of leuprolide on neuronal excitability of CA3 pyramidal neurons (Fig. 3) was specific for GnRH receptors, leuprolide was administrated to the slices in the presence of 10^{-8} M acetyl-3,4-dehydro-Pro^1,D-p-F-Phe^2,D-Trp^{3,6}]-LHRH. The administration of acetyl-3,4-dehydro-Pro^1,D-p-F-Phe^2,D-Trp^{3,6}]-LHRH did not affect the baseline firing rate of evoked action potentials (Fig. 4). Subsequently, this antagonist significantly abolished the generation of leuprolide-induced increase in the firing rate of evoked action potentials in CA3 pyramidal neurons. The average of the firing rate of evoked action potentials obtained 30 min following leuprolide application (4.4 ± 0.6/sec, n = 4) was not statistically different from that obtained under the control condition (4.2 ± 0.9/sec, n = 4).

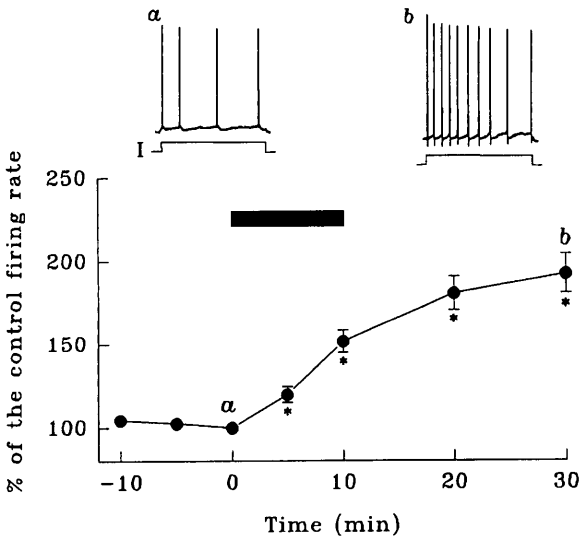

Fig. 3. Activation of GnRH receptors elicited a long-term increase of neuronal excitability in CA3 pyramidal neurons of the hippocampus. The experimental protocols used as in Fig. 1 were repeated throughout whole experiments. Insets *a* and *b* correspond to the plots of EPSCs labeled a and b from the experiments subjected to leuprolide, respectively. An asterisk indicates significantly different from the control level at time point = 0 min.

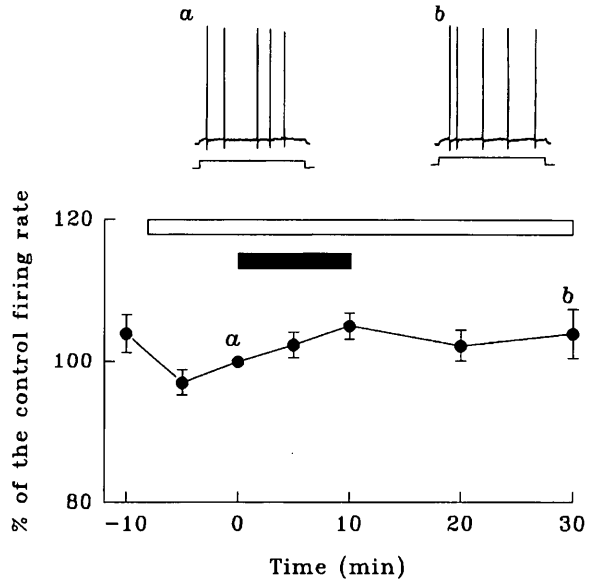

Fig. 4. The GnRH receptor antagonist blocked the generation of GnRH-induced long-term increase of neuronal excitability in CA3 pyramidal neurons of the hippocampus. The experimental protocols used as in Fig. 2 were repeated throughout whole experiments. Insets *a* and *b* correspond to the plots of EPSCs labeled *a* and *b* from the experiments subjected to leuprolide, respectively.

Discussion

The major finding of the present study was that activation of GnRH receptors produced a long-term increase of intrinsic neuronal excitability in both CA1 and CA3 pyramidal neurons of the rat hippocampus that could serve as an important integrative region for reproductive process, both endocrinologically and behaviorally (1, 4, 11, 19, 26).

The similar feature of the central effect of GnRH on intrinsic neuronal excitability in both CA1 and CA3 pyramidal neurons suggested that similar mechanisms activated by GnRH receptors may exist in both CA1 and CA3 pyramidal neurons. Recently, it became increasingly apparent that GnRH can activate a G-protein whose α subunit could correspond to and activate phospholipase C (PLC) (6, 10, 20, 22). The increased phosphoinositde turnover generated inositol-1,4,5-triphosphate (IP_3) releasing intracellular Ca^{2+} and early diacylglycerol (DAG), which were both required for activation of PKC. Furthermore, PKC was shown to participate in several important functions in the CNS, such as GnRH-induced sustained enhancement of synaptic transmission mediated by ionotropic glutamate receptors in the hippocampus (28), gonadotropin release as well as gene expression of gonadotropin subunits and PKCβ mRNA itself in pituitary gonadotrophs (2, 18, 20, 31). Nevertheless, it is still unclear whether PKC stimulated by GnRH receptor activation could also play a role in the modulation of intrinsic neuron excitability in both CA1 and CA3 pyramidal neurons of the hippocampus. The present study provided evidence that activation of GnRH receptors might initiate transient signals, such as the activation of inositol phosphates, and sustained signals, such as PKC-mediated phosphorylation of ion channels. The ion channels including calcium-dependent potassium channels responsible for the slow afterhyperpolarization of cell membrane potential, calcium channels and/or sodium channels were considered (15, 17, 27, 30).

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