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PHYSIOLOGICAL ANALYSIS OF GNRH RELEASE FROM THE BRAIN SLICE OF THE DWARF GOURAMI (*Colisa lalia*)

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GnRH has been known as a peptide hormone that regulates gonadotropin release from the pituitary. Recent studies have shown that there are multiple GnRH systems with different functions throughout the vertebrate brain. The terminal nerve (TN) GnRH cells are known to project their axons widely in the brain and are suggested to function as a neuromodulator by releasing GnRH peptide. However, GnRH release in the brain from TN-GnRH cells has not been investigated thus far. Here, we prepared brain slices of the dwarf gourami in order to analyze GnRH release from TN-GnRH system in comparison with that from the preoptic area (POA)-GnRH system. The brain slices were divided into two parts, POA-GnRH slices and TN-GnRH slices. The slices were incubated in the medium containing several kinds of agonists, and GnRH released into the medium was measured by radioimmunoassay (RIA). In TN-GnRH slices, high $[K^+]_o$ medium (depolarizing stimulus) stimulated GnRH release but an excitatory amino acid transmitter glutamate did not. The sexual difference of GnRH release in TN-GnRH slices was much smaller in comparison with that in the POA-GnRH slices. We also examined the electrical activity of TN-GnRH cells by current clamp recording to analyze the relation between the electrical activity and the GnRH release activity. By comparing the results of electrical recording of TN-GnRH cells and RIA in response to high $[K^+]_o$ stimulation and glutamate application, it is suggested that the GnRH release which was evoked by high $[K^+]_o$ stimulation of TN-GnRH slices results from the strong direct depolarization of TN-GnRH axon terminals and varicosities and not from the increased soma action potential frequencies.

INVOLVEMENT OF CA^{2+} CURRENTS IN THE MODULATION OF PACEMAKER ACTIVITY OF TERMINAL NERVE GNRH NEURONS BY GNRH PEPTIDE

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According to our working hypothesis, the terminal nerve (TN)-GnRH system functions as a neuromodulatory system that regulates many long-lasting changes in animal behaviors. We have already shown by using in vitro whole brain preparations of a small fish (dwarf gourami) that the pacemaker activities of TN-GnRH neurons are modulated biphasically by salmon GnRH, which is the same molecular species of GnRH produced by TN-GnRH neurons; the modulation consists of initial decrease and late increase of firing frequency. In the preset study, we investigated the possible involvement of Ca^{2+} currents in the modulation of pacemaker activities. Bath application of Ni^{2+} or La^{3+} slowed down the pacemaker frequency and attenuated the rate of the late increase of pacemaker frequency by GnRH. Furthermore, voltage-clamp experiments suggested that (1) low-voltage-activated and (2) high-voltage-activated (HVA) Ca^{2+} current were present in the TN-GnRH neurons, and the activation threshold of HVA Ca^{2+} current was shifted to more negative potentials by GnRH. Next, we examined the effects of specific Ca^{2+} channel blockers. Current- and voltage-clamp experiments suggested that nifedipine (a blocker of L-type Ca^{2+} channels) did not affect the pacemaker frequency and its modulation. However, ω-conotoxin GVIA blocked the modulation of pacemaker activities.

These results suggest that (1) some kinds of Ca^{2+} currents contribute to the generation and modulation of pacemaker activities (2) HVA Ca^{2+} current (possibly N-type Ca^{2+} current) is facilitated by GnRH so that it increases the frequency of pacemaker activities.

Dissociation of gonadotropin-releasing hormone neurons and their electrophysiology

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The terminal nerve (TN)-gonadotropin-releasing hormone (GnRH) neurons project their fibers widely throughout the brain, and it has been suggested that TN-GnRH system may function as a neuromodulator by releasing GnRH in such wide area of the brain. Moreover, it has been suggested by EM observations and electrochemical recordings that the exocytotic release of GnRH occurs not only from the axon terminals and varicosities but also from the cell bodies. Here, we tried to establish a protocol for dissociating TN-GnRH cells to use them for future studies such as simultaneous recordings of exocytotic GnRH release, electrophysiological activity, and Ca2+ imaging of GnRH neurons. The whole brain of the dwarf gourami, which is a favorable material for the study of GnRH neurons, was dissected out and cut into small blocks that contained a large cluster of TN-GnRH neurons on each side of the brain. They were mildly digested in a papain solution, and TN-GnRH neurons were dissociated by mild pipetting. These acutely dissociated TN-GnRH neurons were large and spherical cells with few processes. Therefore, they could be stably patch-clamped in a very good space clamp condition. The ion channel properties of these neurons were basically similar to those of the intact TN-GnRH neurons. In a preliminary study, we loaded the dissociated neurons with FM1-43, a membrane-impermeable styryl dye that become trapped within recycled vesicles. Destaining of FM1-43 was recorded after depolarizing stimulation of the cells with a perfusing medium containing high concentration of K^+, suggesting exocytotic release of GnRH from the cell body.

INTERACTIONS BETWEEN THE NEURAL NETWORKS IN THE CRUSTACEAN STOMATOGASTRIC NERVOUS SYSTEM.

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The stomatogastric nervous system of decapod crustaceans is an important center for coordinated movements of the foregut. The stomatogastric ganglion contains pattern generating networks for movements of three distinct regions: the cardiac sac, gastric mill and pylorus. Motor patterns of the stomatogastric ganglion neurons in different networks were studied in the shrimp *Penaeus japonicus*. Most of these neurons participate in either the gastric or pyloric motor patterns. One of the gastric network neurons participates in strong pyloric activity, and can switch from the pyloric to the cardiac sac pattern. This neuron fires in antiphase with the cardiac sac dilator neurons. They construct a cardiac sac network. The cardiac sac pattern becomes vigorous when the pyloric rhythm is less active. All the gastric neurons can be active in time with the pyloric rhythm when the gastric rhythm is inactive. Some of the gastric neurons have the tendency to fire with the gastric rhythm in the absence of the strong pyloric rhythm. Once the pyloric rhythm is inactivated under some conditions or by hyperpolarization of the pyloric pacemaker neuron, they are released from the influence of the pyloric rhythm and fire with the gastric rhythm. Extensive interactions between the gastric and pyloric networks are seen in *Penaeus*, and several modes of interactions between the networks found in reptantians can be observed. Such interactions are general features of the stomatogastric nervous system in decapods. Neurons of the stomatogastric ganglion are part of a single neural network from which they can be assembled for configuration of the pattern generators under the appropriate modulatory conditions.

NITRIC OXIDE EXERTS INHIBITORY EFFECT ON FEEDING RESPONSE IN THE POND SNAIL, *Lymnaea stagnalis*

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In the present study, we used a NO specific electrode to measure an increase in NO concentration around the buccal ganglia in the central nervous system of *L. stagnalis* when the lips were stimulated by sucrose stimulus. The fictive feeding rhythm in the buccal ganglia was also monitored in the semi-intact preparations. When the B2 cells, putative NO-generative neurons, in the buccal ganglia were injured, NO response to sucrose was not induced around the buccal ganglia. At that time, the fictive feeding rhythm increased. Application of NO scavenger also increased the fictive feeding rhythm, irrespective of sucrose stimulation. The present results suggest that first; even the low concentration of constitutive NO precisely regulates the feeding rhythm by suppressing high frequency feeding responses; second, that the high concentration of NO released after activation of the feeding central pattern generator following appetitive stimulation of the lips suppresses the feeding rate, resulting in recurrent inhibition. This is the first direct evidence that NO can function to suppress rhythmic activity in the brain.

LOCALIZATION OF GHRELIN-IMMUNOPOSITIVE CELLS IN THE RAT HYPOTHALAMUS

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Ghrelin, which was isolated from the stomach as an endogenous ligand for the growth-hormone secretagogue receptor, is a new polypeptide consisting of 28 amino acid residues. In this study, we determined the localization of ghrelin-producing cells and neural projections in the rat brain by immunohistochemistry. Wistar male rats were perfused with a fixative containing 4% paraformaldehyde, and 60-?m-thick brain tissue sections were immunostained by the free-floating method. Ghrelin-immunopositive cells were observed in the paraventricular nucleus, supraoptic nucleus, supraoptic retrochiasmatic nucleus and ventral tuberomammillary nucleus. Interestingly, no ghrelin neuronal projections were found in the external layer of the median eminence, but projections to the posterior pituitary gland though internal layer of the median eminence were observed.

These results suggest that hypothalamic ghrelin does not directory stimulate GH secretion from the anterior pituitary gland via the median eminence, and that ghrelin has distinct physiological functions in the rat brain.

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