Actions of neuropeptides on activity of myenteric neurons

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1. Introduction

The enteric nervous system (ENS) is a complicated system containing as many as 100 millions of neurons, approximately equal to the number of neurons in the spinal cord. The ENS, having afferent neurons with sensory receptors, interneurons and efferent neurons with effectors such as smooth muscles and glands, is independent of CNS, though it is linked to CNS by the autonomic neurons. The ENS and CNS have common transmitters of peptides, called "brain-gut peptides". For example, pituitary adenylate cyclase activating peptide (PACAP), first found in the brain, and vasoactive intestinal peptide (VIP), first found in digestive tract, have the similar structure containing the same sequence of amino acid residue in 68%. We investigated the role of PACAP in ENS of guinea-pigs.

2. Materials and methods

(1) *Animal preparation*

Guinea-pigs (mature, male, body weight = 250-300 g) were killed by a blow to the head and subsequent severance of the cervical spinal cord followed by exsanguinations from the carotid artery. This method was approved by the Committee for Animal Experimentation of Tokyo Medical and Dental University. Segment of distal ileum, 1 cm long, was excised and into which a glass rod was passed. The outside layer, myenteric plexus-longitudinal muscle preparation, was removed and placed inside out on Sylgard in a 1.0 mL experimental chamber, then was fixed with 20-40 pins (10-20 μm in diameter). A ganglion (100 μm) was selected under microscope and immobilized by careful pinning closely around it with pins (10 μm in diameter) and cut the segment (500 μm × 1000 μm) out with a razor (Fig.1). Experimental chamber was then mounted on the stage of a microscope (Zeiss) with Normarski defferential interference contrast optics and continuously superfused (3 ml/min) with Krebs solution warmed to 36°C (composition in mM : NaCl 117; KCl 4.7; $CaCl_2$ 2.5; $MgCl_2$ 1.2; NaH_2PO_4 1.2; $NaHCO_3$ 2.5; Glc 11.5; equilibrated with 95% O_2 - 5% CO_2, pH = 7.4).

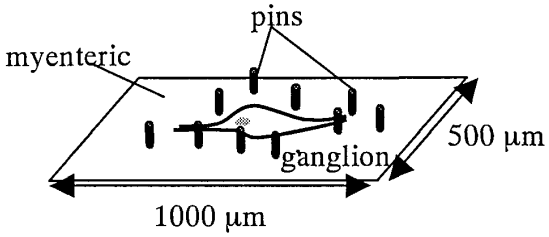

Fig.1 Schematic of the experimental chamber.

(2) *Drugs*

PACAP (His-Ser-Asp-Gly-Ile-Phe-Thr-Asp-Ser-Tyr-Ser-Arg-Tyr-Arg-Lys-Gln-Met-Ala-Val-Lys-Lys-Tyr-Leu-Ala-Ala-Val-Leu-NH_2) were purchased from Peptide Institute.

(3) *Electrophysiological measurements*

After inserting a microelectrode (tip resistance 50 ±10 MΩ, tip diameter 1 μm, filled with 2M KCl) into a nerve cell body on the experimental chamber, action potential was evoked by anodal current passed through the electrode by using a bridge circuit in an amplifier (CEZ-3100, Nihon kohden). Then the microelectrode potential φ vs. Ag/AgCl electrode immersed in Krebs solution were measured and dose-response data were obtained for concentration of PACAP c (0-1 μM) in Krebs solution. The membrane resistance R of the neuron was estimated from the amplitude of electrotonic potentials produced by cathodal current (0.1-0.3 nA for 50-100 ms at 0.2 Hz) (Fig. 2).

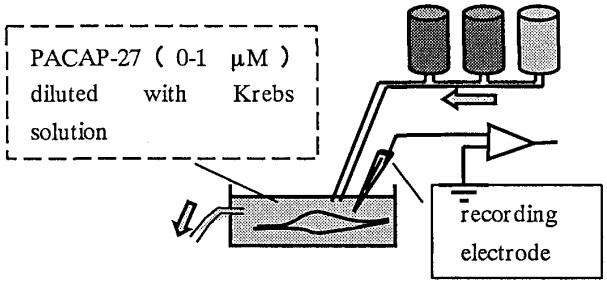

Fig. 2 Schematic of the experimental arrangement.

Myenteric neurons were classified into S neurons and AH neurons: S neurons respond to stimulation of incoming fibers with fast excitatory post synaptic potentials (fast EPSPs), whereas AH neurons were evoked hyperpolarizations that last up to 10-20 s (Fig. 3). In this experiment, the presynaptic excitation was

evoked by electrical stimulation (for 0.5 ms, 0.1Hz) using a glass capillary electrode (tip diameter 10 μm, filled with Krebs solution) close to the preganglionic neuron.

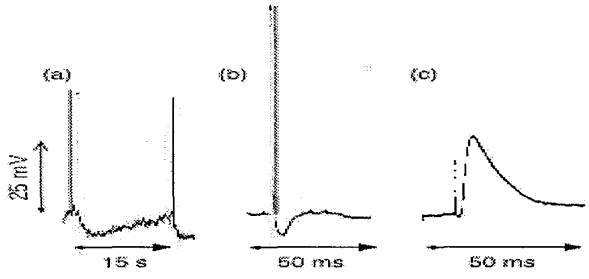

Fig. 3 Action potentials recorded from AH neuron (a) and S neuron (b) and Fast EPSPs from S neuron (c).

3. Results and discussion

A total of 13 neurons showed action potentials by injecting anodal current, where 2 neurons were found to have S electrophysiological behavior, 11 neurons AH behavior.

Application of PACAP in superfusion solution caused membrane depolarization in 2 of 2 S neurons (Fig. 4a), and in 8 of 11 AH neurons (Fig. 4b). On the other hand, it caused membrane hyperpolarization in 2 of 11 AH neurons.

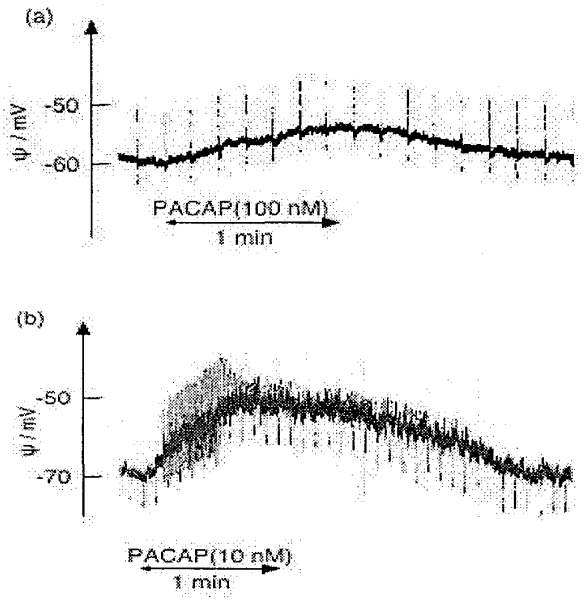

Fig. 4 Excitatory effect of PACAP on S(a) and AH (b) neuron.

The slope of the current-voltage characteristic of the cell membrane indicates a membrane resistance of 92 MΩ, as shown in Fig. 5.

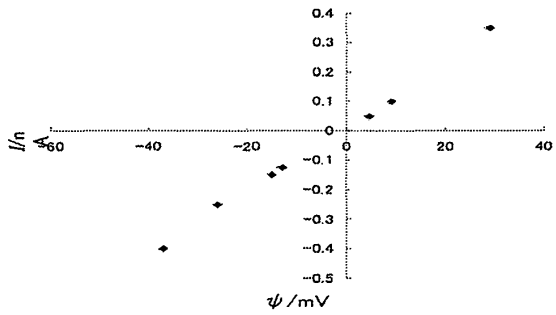

Fig. 5 Current-voltage charasteristic on AH neuron.

The variation of membrane potential change $\Delta\varphi$ with the concentration c of PACAP is shown in Fig. 6 as a Lineweaver-Burk plot using Eq. (1),

$$\frac{1}{\theta} \equiv \frac{\Delta\varphi_{\infty}}{\Delta\varphi} = \frac{K}{c} + 1 \qquad (1)$$

where $\Delta\varphi_{\infty}$ is saturated potential change. The plot showed good linearity with an intercept of 1, and the receptor's affinity to PACAP was obtained as $pK = 8.2$.

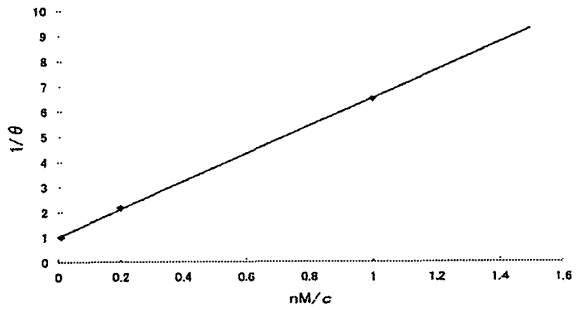

Fig. 6 Linweaver• Burk plot of PACAP

4. Conclusion

S and AH neurons in myenteric plexus were excited by superfusion of PACAP. The receptor's affinity to PACAP was found to be $pK = 8.2$.

[Acknowledgement]

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[References]

F. L. Christofi, J. D. Wood, "Effects of PACAP on morphologically identified myenteric neurons in guinea pig small bowel", *Am. J. Physiol.*, **264**, G412-421. (1993).