The Anterior Preoptic Neurons of the Frog, *Rana catesbeiana* and Toad, *Bufo bufo japonicus*: Control of Mating Behavior

Akihisa Urano
Department of Regulation Biology, Saitama University, Urawa, Japan

The anterior part of the preoptic nucleus (APON), especially its ventral half, is thought to be an androgen sensitive center for male mate-calling behavior in many anuran species. An electrophysiological study of this locus in *Rana pipiens* has revealed the presence of neurons responsive to auditory stimulation by playback of con-specific mating calls, and injections of pituitary homogenates significantly increase the percentage of these units excited by the calls. Responsiveness of APON neurons can thus be modulated by hormonal input signals.[1] On the other hand, a neuroanatomical retrograde horseradish peroxidase study has indicated that neuronal activity of APON may be controlled by many neural inputs from several discrete brain areas such as the limbic cortex, the posterior part of the preoptic nucleus, the thalamus, and the reticular formation. These results strongly suggest that APON neurons are controlled by both neural and hormonal input signals. However, it is not known whether individual APON neurons have anatomical features indicative of these two functions. In the present study, cytoarchitecture of the APON was examined by electron microscopic techniques to obtain information that would permit further understanding of neural and hormonal control mechanisms of male mating behavior.

Materials and methods

Adult frogs and toads of both sexes were used as experimental animals. They were anesthetized with tricaine methanesulfonate. The brains were fixed with transcardial perfusion of a fixative, containing 2% glutaraldehyde and 2% paraformaldehyde, following brief perfusion with frog saline. Whole brains were taken out and further fixed in the fixative of the same composition. The fixed brains were examined by scanning electron microscopy (SEM) for observation of the surface structure of the ventricular wall of the preoptic recess, and observation of Golgi-stained and gold-toned APON neurons after removal of surrounding tissues. Semi-thin sections of Golgi-stained neurons were examined by scanning transmission electron microscopy (STEM). For Golgi-staining and gold-toning, the rapid-Golgi method of Peters[2] was adopted with slight modification. The Golgi-stained brains were cut at 200 μm by use of a vibratome. Thick vibratome sections were digested with 8N HCl (60°C, 1h) followed by collagenase (1 mg/ml, 37°C, 4h). This treatment removed tissues surrounding the silver-impregnated and gold-toned neurons, but stained neurons still kept their forms almost intact.

The ventricular wall of the preoptic recess

The dorsal half of the ventricular wall of APON is heavily ciliated, but ciliated ependymal cells are sparse in the ventral half. The most prominent surface structures are bulbous protrusions, which might be intraventricular dendritic end bulbs of APON neurons and cytoplasmic extensions of ependymal cells.[3] Debris from these bulbous protrusions may indicate that some liquor contacting cells secrete their inclusion into the cerebro-spinal fluid by a holocrine activity.

At the light microscopic level, liquor contacting cells in the APON can be classified mainly into three types; ependymal cells, tanycytes and liquor contacting neurons. Surfaces of the liquor contacting neurons whose somata lie adjacent to the ventricular wall are rather smooth, whereas neurons whose somata lie at some distance from the ventricular wall are covered with a considerable number of deposits as well as ordinary neurons. The size of these deposits are compatible with that of synaptic nerve endings. It would appear,

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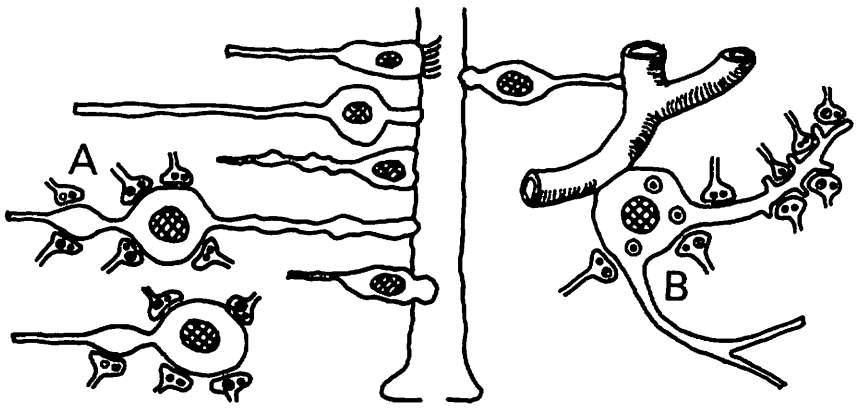

Fig. 1. APON neurons showing features which suggest an integration of both neural and hormonal information. *A,* liquor contacting neurons at some distance from the ventricular wall of the preoptic recess, *B,* blood capillary contacting a neuron which has a spined dendritic process.

therefore, that nerve terminals are impinging on some liquor contacting neurons. Since the liquor contacting neurons are considered to be receptor cells relaying hormonal information from the cerebro-spinal fluid, these neurons at some distance from the ventricular wall may integrate both neural and hormonal glands.

The thick vibratome sections were embedded in an Epon-Araldite mixture in flat trays so that the sections could be studied under the light microscope. The stained neurons were first drawn with a camera lucida, and were then sectioned at 0.1–0.2 μm for electron microscopic observations. Among several of the Golgi-stained and gold-toned neurons examined, the histological features indicate that they are receiving both neural and hormonal signals. A dendritic process from the neuron runs to the white matter lateral to the neuronal mass of APON, while another, thicker, dendritic process, and a part of the soma, makes contact with the wall of the blood capillary. Thus, neural information from other parts of the brain may be transmitted via the dendritic spines localized in the white matter, while contact with sex-steroid hormones is also possible from the blood capillary (Fig. 1). The neuron contains dense-cored elementary granules and seems to be neurosecretory or peptidergic. The APON neurons thus display features which suggest they may integrate both neural and hormonal signals, and elicit peptidergic output signals to other neurons.

References

[1] Urano, A. & Gorbman, A. (1981). *J. Comp. Physiol.* **141**, 163–71.
[2] Peters, A. (1978). In *Neuroanatomical Techniques*, pp. 187–94. Society for Neuroscience.
[3] De Waele, G. & Dierickx, K. (1979). *Cell Tiss. Res.* **203**, 53–64.