Estrogen-Sensitive Neurons with Preoptic Projection in the Lower Brain Stem of the Female Rat

MASAZUMI KAWAKAMI AND SHIRO OHNO

Second Department of Physiology, Yokohama City University School of Medicine, 2-33 Urafunecho, Minamiku, Yokohama 232

Abstract

Fifty-one neurons in the ventrolateral part of the medulla oblongata were antidromically activated by electrical stimulation of the suprachiasmatic part of the preoptic area in urethane-anesthetized, ovariectomized and estrogen-primed female rats. Two types of antidromic responses were distinguished on the basis of their spike configurations and antidromic spike latencies. One type ("fast spikes") was characterized by a fast and smooth rising phase and a shorter duration of the initial positive deflection. The other type ("slow spikes") had a notch in the rising phase and took a longer time to complete the initial deflection. Mean antidromic spike latency for the fast spikes was 9.8 msec while the value for the slow spikes was 30.2 msec. Ionophoretic injection of estradiol was accomplished on 37 of the 51 antidromically identified cells, of which 21 showed slow responses and 16 responded with fast spikes. In cells with slow spikes, estradiol facilitated (n=9) or suppressed (n=3) their generation of action potentials. None of cells with fast responses changed their activity in response to estradiol. It is evident from the present experiment that neurons in the ventrolateral part of the medulla oblongata send their axons directly to the suprachiasmatic part of the preoptic area which plays an important role in the control of the ovulatory surge of LH and that some of these neurons themselves are the sensitive sites of estradiol.

The suprachiasmatic part of the preoptic area (POSC) has been pin-pointed as a nodal point of the neural mechanism which produces the preovulatory surge of LH (Kawakami and Kimura, 1976; Kawakami et al., 1978). Immunohistochemical studies demonstrated that the perikarya of the LH-RH neurons are mainly located in the POSC (Setáló et al., 1976; Ibata et al., 1979). Afferent connections from structures outside the preoptic-hypothalamic complex are important in the rat for the maintenance of normal cyclic ovarian function (Halász, 1969). We have demonstrated recently that lesions of the ventrolateral part of the medulla oblongata (VLMO), which disrupted direct hypothalamic projection of this area, abolished a preovulatory surge of LH (Kawakami and Ando unpublished observation). In the present experiment, the VLMO was explored for neurons with axonal projections to the POSC, and their response to microionophoresed estradiol was examined.

Materials and Methods

Wistar female rats weighing 200-300 g were housed under controlled conditions of light (lights on from 0500 to 1900) and temperature (23-25°C). Free access to water and laboratory chow was allowed at all times. The animals were ovariecto-
mized 2 to 3 weeks before the beginning of the experiments. Subcutaneous injection of estradiol benzoate (20 μg in sesame oil) was given 3 days prior to recording.

Surgical procedures were carried out under light urethane anesthesia (1.2 g/kg body weight, injected intraperitoneally at 0900). The animal was placed in a stereotaxic apparatus in a prone position, with the incisor bar placed at the same height as the center of the ear bars. Craniotomy was made in the parietal area and the dura was removed. The exposed cerebral cortex was covered with warm agar solution in saline. The rectal temperature was maintained between 35 and 38°C.

Coaxial bipolar electrodes were constructed from stainless-steel tubing (0.5 mm outer diameter) and stainless-steel wire (coated diameter: 0.15 mm). Except for the tip areas, they were insulated with Epoxy Resin. These electrodes were used to stimulate axons of the ascending medullary cells antidromically from the suprachiasmatic part of the preoptic area (POSC). Stereotaxic coordinates of the POSC in the system of Albe-Fessard et al. (1966) were: anterior 7.9-8.2 mm, lateral 0.1-0.2 mm and depth 3.0 mm. Stimulating electrode was inserted into the brain at right angles to the reference plane of the stereotaxic system through a small hole in the skull and the dura and fixed to the skull with dental cement.

The ventrolateral part of the medulla oblongata (VLMO) was systematically explored while the electrical stimulation to the POSC was delivered at 0.6 Hz, at an amplitude of 1.0-1.5 mA, with negative rectangular pulses of 0.1 msec duration. Microelectrode tracks were made in a series in the traverse plane approximately 6.5-7.0 mm posterior to the center of the ear bars. The penetrations were inclined 45 degrees posteriorly to the frontal vertical plane to get access to the posterior brain stem.

Recordings of extracellular potentials and ionophoretic application of estradiol hemisuccinate (17β-17-estradiol hemisuccinate, Teikoku Hormone Mfg. Co.) were accomplished with three-barreled glass micropipettes which were constructed from 3.0 mm outer diameter Pyrex tubes. Recordings were made by means of one of the barrels which was filled with 0.5 M sodium acetate solution. Pontamine sky blue 6B was added to the solution to make a final concentration of 2% to allow making of the recording loci by ionophoresis. The other two barrels of the electrodes were filled with 0.25 M estradiol hemisuccinate in distilled water, and 0.15 M NaCl (physiological saline), respectively. The DC resistance of the recording barrel was 10-30 MΩm, and those of the barrels containing estradiol solution and normal saline were approximately 100 MΩm or more. Extracellular potentials were amplified by the conventional method. A digital computer with A-D converter function (San-Ei Sokki, 7T07) was
used to visualize and store waveforms of antidromic responses. A constant current source designed expressly for microionophoresis (DIA medical Co., DPI-10) was used to generate a cathodal current of 5-40 nA. Current injection with this amplitude through the saline-containing barrel produced no appreciable change in the neuronal discharge.

At the end of each recording session, 10 μA cathodal current was passed through the recording barrel for 10 min and 10 μA anodal current for 30 sec through the stimulating electrode. The animal was then perfused with 10% formalin which was followed by a 3% potassium ferricyanide and ferrocyanide solution. The electrode positions were determined histologically in frozen slices.

Fig. 2. Antidromic activation of neurons induced by electrical stimulation of their axons. COLLISION: The first stimuli (open triangle) applied just after spontaneous spike discharges (open circle) failed to evoke unit firings because of autocancellation by collision of orthodromic and antidromic impulses on the axon, whereas the second stimuli (solid triangle) induced unit activity (solid circle) at a constant latency. CONSTANT LATENCY: The response was superimposed 10 times.

**Results**

**Antidromic Identification of VLMO Cells**

Fifty-one neurons with ascending projections were identified in 40 rats in the VLMO at the mid-olivary level, lying laterally or ventrally to the lateral reticular nucleus (Fig. 1). Identification was made by antidromic activation following electrical stimulation in the POSC. Antidromic responses were characterized by a constant latency of the spike potential from the stimulus and the ability to follow high frequency pulse stimuli. In the cells with spontaneous activity, collision of the anti-
Fig. 3. Representative samples of two types of antidromic spikes in VLMO cells elicited by POSC stimulation. Shown in A is a fast spike with short spike duration. Examples of slow spike which had a notch in the rising phase and a longer duration (B). These two types of cells differed in their distribution of antidromic spike latencies (C and D). Calibration is 2 msec and 0.5 mV.

dromic spike with the spontaneous spike was tested. Antidromic characteristics of typical responses are shown in Fig. 2.

The antidromically activated units had constant latencies which ranged from 6 msec to 39 msec. Two types of antidromic spike potentials were distinguished on the basis of their waveforms. One type ("fast spikes") was characterized by a sharp and smooth rising phase. The other ("slow spikes") had a notch in the rising phase. Mean antidromic spike latency for the fast spikes was 9.8 msec (range: 6–20 msec), while the value for the slow spikes was
The duration of the action potential was shorter in the fast spikes than in the slow spikes (Fig. 3). The cells which responded to the antidromic stimuli with fast spikes had discharge rates less than 1.0 Hz or none at all. The mean firing rate of cells with slow spikes was 2.4 Hz (range: 1.5–5.0 Hz).

**Effects of Microionophoresed Estradiol Hemisuccinate**

Thirty-seven antidromically identified neurons were successfully tested with the steroid ester, and 12 of them responded with either excitation or inhibition. Fig. 4 shows typical examples of the effect of estradiol hemisuccinate microionophoresed on single VLMO neurons with POSC projection. Cathodal (electrode negative) currents of 5–40 nA were used to eject the steroid ester. Within this range, the changes in the neuronal discharge rate in either direction, were roughly a function of the current intensity.

Neurons tested with the microionophoresis of estradiol ester included 21 cells which showed slow spikes when invaded antidromically, and 16 cells which were associated with fast spikes. It was found that all the 12 neurons which responded to estradiol belonged to the cells which generate slow spikes. Of these, 9 were facilitated and 3 were inhibited. None of 16 cells with fast spikes responded to the steroid.

**Discussion**

*Technical considerations.* The present results indicate that the VLMO contains neurons which are driven from the POSC, a forebrain structure. The action potentials in individual cells evoked by electrical stimulation occurred at constant latencies and followed repetitive stimuli at high frequencies. The response could be blocked at appropriate intervals by spontaneous
spikes. These characteristics indicate that the responses were obtained by direct activation of the axons of VLMO cells and that the action potentials were conducted antidromically along the axon from the POSC to the VLMO. It is likely, therefore, that the activated neurons could be part of the medullary cell population which was visualized by retrograde uptake of markers (Sakumoto et al., 1978; Loewy et al., 1981) or by tritiated amino acid autoradiography (Loewy et al., 1981) to have direct connection with the forebrain.

The existence of subsets of neurons with different configurations of antidromically evoked potentials is known for several neuronal pools (Mountcastle et al., 1969; Humphrey and Orrie, 1978; Sakuma and Pfaff, 1980). They are attributed to differences in either geometry, size or membrane characteristics between each cell group (Kernell, 1966). Therefore, slow and fast spikes might be recorded from different subsets of medullary neurons which are similarly projected to the POSC.

Technical problems concerning ionophoretic application of estradiol hemisuccinate have been documented in detail (Kelly et al., 1977a; 1977b). Present observation of the gradual onset and decay of
the response, as well as the ineffective current injection through the saline-containing barrel, are evidence that the responses were caused by ionophoretically ejected estradiol ester and not by current effects.

**Physiological considerations.** It could not be a mere coincidence that the VLMO cells with POSC projections were located in the area which was known to be the origin of the Al noradrenergic fiber bundle (Dahlström and Fuxe, 1964; Ungerstedt, 1971; Jacobowitz and Palkovits, 1974). Sakumoto et al., (1978) have successfully demonstrated by a combined horseradish peroxidase and monoamine oxidase staining method that hypothalamic projection of this area is truly noradrenergic. Our observation that bilateral VLMO implantation of a minute amount of dietyldithiocarbamate, a potent dopamine-β-hydroxylase inhibitor, was as effective as an electrolytic VLMO lesion in blocking spontaneous ovulation (Kawakami and Ando unpublished observation) indicates the involvement of a catecholaminergic mechanism in this area in the control of gonadotropin secretion. Surgical interruption of the ascending Al fiber bundle at the midbrain abolished the proestrous surge of LH (Clifton and Sawyer, 1979). Selective chemical destruction of ascending noradrenergic fibers has a comparable effect (Martinovic and McCann, 1977; Langlier and McCann, 1977).

A number of peripheral stimuli such as coitus or cervical prove, which are known to affect gonadotropin secretion (Taleisnik et al., 1966) must be transmitted by ascending pathways traversing the lower brain stem. The cells identified in the present study could be a link in such a system.

The time-course of the estrogen-induced changes in neuronal discharges implies that these changes are elicited by direct action of the steroid hormone on the neuronal membrane, rather than mediated by its genomic action (McEwen et al., 1979). Therefore, it is not known at present if neuronal responses seen in the present experiment are related to the autoradiographic demonstration of estradiol uptake by catecholaminergic neurons in this area (Madhabananda and Stumpf, 1981).

Since a local implant crystalline estradiol in the VLMO was ineffective in promoting changes in serum LH level in ovariectomized estrogen-treated rats (Kawakami and Ando, unpublished report), VLMO neurons are unlikely to be a site of estrogen action in the feedback control of gonadotropins. Rather, they probably participate in the regulation of the anterior pituitary by mediating peripheral effects and providing a hormonese sensitive bias in the major neuroendocrine mechanism in the preoptic-hypothalamic region.

**Acknowledgement**

The authors wish to express their thanks to Dr. Y. Sakuma, Niigata University School of Medicine, for help in preparation of this manuscript.

This work was supported in part by Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

**References**


