Electrophysiology of mammalian hypothalamic and interpeduncular neurons in vitro

N. Ogata

Department of Pharmacology, Faculty of Medicine, Kyushu University, Fukuoka 812 (Japan), 17 November 1978

Summary. Electrical activities of the hypothalamic and interpeduncular neurons were studied in vitro in brain slices prepared from the guinea-pig brain stem. Neurons preserved resting membrane potentials comparable to those of neurons in vivo, responded to stimulation of the afferent fibres, and retained stable spontaneous firings for more than several hours.

Microelectrophoresis is the most common technique for application of drugs to neurons in the mammalian brain. However, this technique has several well-known disadvantages. To avoid the disadvantages inevitably involved in in situ preparations, the isolated brain stem preparation of the guinea-pig including the hypothalamus and interpeduncular nucleus has been developed in the present study.

This in vitro preparation has enabled us to study the effect of drugs in precisely controlled concentrations on the electrical activities of the brain stem neurons in modified ionic environments.

Materials and methods. The whole brain of the adult guinea-pig of either sex was taken out of the skull, and placed on a filter paper soaked with Krebs solution with the ventral surface of the brain upward. The hemispheres were then divided at the midline with a slight tilt to 1 side to include the habenulo-interpeduncular tract in the slice (figure 1,A). A block of the brain stem including the thalamus, hypothalamus, mamillary body and interpeduncular nucleus was made by cutting away a large portion of the brain stem, hippocampus and cortices (the dotted area). The block was put on the cutting table with the midline sagittal surface upward, and a slice of about 400–600 μm thick parallel to the surface was made using a stainless steel slicer and a guide (the shaded area). In figure 1,B, an example of the brain stem slice is illustrated. Incubation, stimulation and recording procedures have been described in detail[2].

Results and discussion. The electrical responses in the interpeduncular nucleus by stimulation of the habenulo-interpeduncular tract were studied. In intracellular recordings, neurons responded to the tract stimulation with an excitatory postsynaptic potential which generated an action potential. Spontaneous firings of the neuron were observed in all the brain areas investigated, i.e., the hypothalamus, mamillary body and interpeduncular nucleus. The extracellular spontaneous spikes were usually positive-negative biphasic with relatively large amplitudes (about 5–20 mV) suggesting that they were recorded in close proximity to a neuron generating action potentials, and could be recorded for more than several hours stably. A variety of firing patterns were observed depending on the areas recorded. In short, in the interpeduncular nucleus, sporadic firings (0.5/min – 10/sec) with regular interspike intervals were the most prevailing firing pattern; in the mamillary body, intermittent burst discharges with interspike intervals of 6–15 msec which were observed only in this area in the present study were the dominant firing pattern in addition to the sporadic firings similar to those in the interpeduncular nucleus; and in the hypothalamus, sporadic firings with irregular interspike intervals or periodic grouped firings dominated. In intracellular recordings, spontaneous firings in the hypothalamus were frequently succeeded by a large hyperpolarization reminiscent of the inhibitory postsynaptic potential. The resting membrane potential of the brain stem neurons were 50.2 ± 8.4 mV without significant difference among the 3 areas. To obtain preliminary data on the pharmacological properties of the brain stem neurons in vitro, effects of several drugs were examined on the spontaneous firings of the neurons. L-glutamate (10^{-5} M) and pentylenetetrazol (7×10^{-3} M) increased the firing rate of neurons in all 3 portions of the brain stem. When acetylcholine (10^{-7} M) and substance P (10^{-7} M) were applied to the interpeduncular neurons, most neurons increased the firing rate. On the other hand, noradrenaline (10^{-7} M) showed generally a depressant action. Some of these data are illustrated in figure 2. Reduction of calcium from the medium increased the spontaneous firing rate, and was antagonized by increase of magnesium.

Thus, the neurons in vitro preserved resting membrane potentials comparable to those of neurons in vivo, responded to the afferent stimulation with an ordinary type of excitatory postsynaptic potentials, retained stable spontaneous firings for more than several hours, and responded to the drugs in a predictable manner. All these observations indicate that the in vitro neurons are quite 'healthy'. The present results, however, might not be directly related to the in situ situation, because there are several discrepancies in electrical activity between preparations in vitro and in vivo[3,4], e.g., low rate of spontaneous firings in the in vitro preparation. Some of these discrepancies may be due to the absence of connections with adjacent brain areas in in vitro therefore resulting in smaller amounts of afferent volleys to

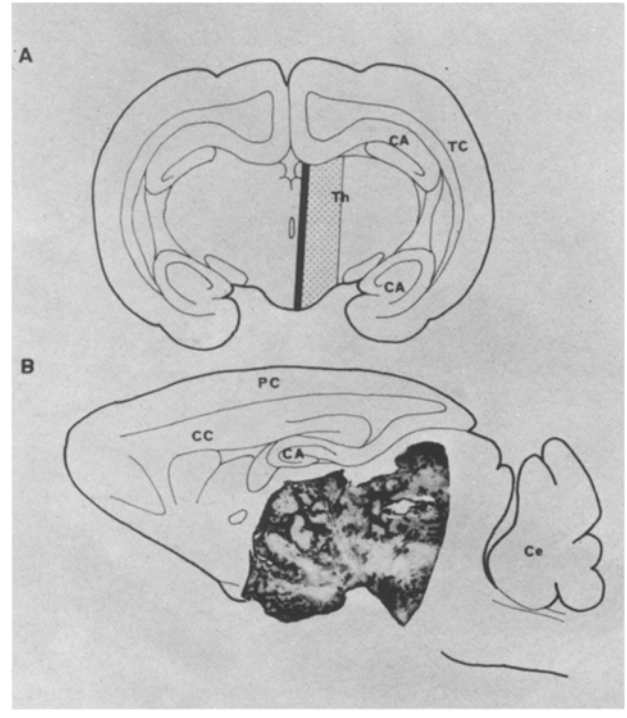

Fig. 1. Schematic drawings of the brain to illustrate spatial orientation of the brain stem slice. *A* Coronal section; *B* Sagittal section. Details, in the text. TC, temporal cortex; CA, cornu Ammonis; Th, thalamus; PC, parietal cortex; CC, corpus callosum; Ce, cerebellum.

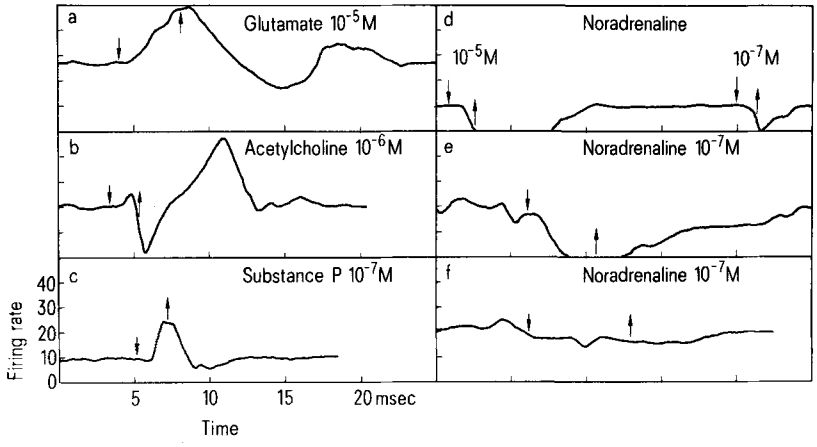

Fig. 2. Effects of various drugs on the spontaneous firing rate of the neurons in the brain stem slice. Extracellular single cell discharges recorded in magnetic tapes were digitized by an A-D converter, and processed by a general-purpose computer Nihon-Kohden ATAC 1200. Firing rate is indicated in the ordinate as the number of spikes per 10 sec. Upward and downward arrows represent the commencement of the drug application and washing by the standard medium, respectively. *A-C* Interpeduncular nucleus; *D* and *E* mamillary body; *F* Hypothalamus. Noradrenaline (10^{-7} M) usually depressed the firing rate of the hypothalamic neurons. However, in some neurons, it exerted no effect as shown in *F*.

neurons in vitro. Therefore, it might be considered alternatively that the in vitro neuron is relevant to the examination of drug effect on the discrete central neuron without interference from the surrounding neuronal activities as compared with the in vivo neuron. In view of the recent findings that acetylcholine[5] and substance P[6] play an important role in the habenulo-interpeduncular system, and that several putative peptide transmitters such as vasoactive intestinal polypeptide (VIP)[7] or endorphins[8] are rich in the hypothalamus, the brain stem slice will be a useful experimental model for pharmacology of the hypothalamic and interpeduncular neurons.

1 Acknowledgment. I thank Prof. H. Kuriyama for discussions and encouragement.
2 N. Ogata, Experientia *34*, 1035 (1978).
3 Y. Oomura, T. Ono, H. Ooyama and M.J. Wayner, Nature *222*, 282 (1969).
4 N. Lake, Exp. Neurol. *41*, 113 (1973).
5 K. Kataoka, Y. Nakamura and R. Hassler, Brain Res. *62*, 264 (1973).
6 T. Hökfelt, J.O. Kellerth, G. Nilsson and B. Pernow, Science *190*, 889 (1975).
7 S.I. Said and V. Mutt, Eur. J. Biochem. *28*, 199 (1972).
8 J.S. Hong, H.-Y.T. Yang, W. Fratta and E. Costa, Brain Res. *134*, 383 (1977).

Receptor potential of rat taste cell to potassium benzoate

T. Sato and L.M. Beidler

Department of Physiology, Faculty of Dentistry, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo 113 (Japan) and Department of Biological Science, Florida State University, Tallahassee (Florida 32306, USA), 27 November 1978

Summary. Rat taste cells responded to relatively low concentrations of K-benzoate with a hyperpolarization and to the high concentrations with a depolarization. During both responses the membrane resistance of a taste cell decreased. Depolarization elicited by application of a combination of 0.25 M NaCl and 0.05 M K-benzoate was smaller than that by the NaCl alone, indicating a depressant action of K-benzoate.

The effect of low concentration of K-benzoate applied to rat taste receptors is quite unusual in that the spontaneous activity in the taste nerve is depressed and a transient excitation occurs when a water rinse is applied. At higher concentrations, the stimulus behaves as most other stimuli in that it excites the taste receptors, although a large response still occurs with a water rinse (figure 1). Miller[2] studied the single taste fibre response to low concentrations of K-benzoate. Several fungiform papillae on rat tongue, each of which has only 1 taste bud, are innervated by peripheral branches of a single chorda tympani nerve fibre[2]. During stimulation of 1 fungiform papilla with NaCl, stimulation of the other neurally connected fungiform papillae with a relatively low concentration of K-benzoate caused a depression of gustatory neural impulses being elicited by the NaCl stimulus[2]. To understand this mechanism we studied the electrical properties of receptor potentials in rat taste cells elicited by K-benzoate applied to the tongue surface.

Materials and methods. Female adult Sprague-Dawley rats, anesthetized with urethane, were used. The intracellular responses of single taste cells in the fungiform papillae were recorded with a 3 M KCl-filled micropipette. The details of method for recordings have been given elsewhere[3]. Distilled water was flowed continuously on the tongue surface and, when a taste stimulus solution was delivered, the water flow was switched to the stimulus flow. The method of stimulus application was the same as that described previously[3]. The experiments were carried out at room temperature of 25–28 °C.

Results and discussion. Figure 2 illustrates an example of the concentration-receptor potential curve for K-benzoate of a taste cell. The receptor potentials in response to 0.003 M, 0.03 M and 0.1 M K-benzoate solutions are shown in the inset. As seen in the 3 records, the receptor potentials were composed of an initial transient hyperpolarization and a subsequent hyperpolarization or depolarization of a relatively steady magnitude. An off-depolarization was initiated after K-benzoate was rinsed with water (arrows in 2 right records). Since the tongue was adapted to distilled water, of course, the application of the water did not produce any change in the membrane potential. In this cell, only hyperpolarizations occurred below 0.05 M of K-benzoate but depolarizations preceded by initial transient hyperpolarizations appeared at concentrations above 0.05 M. The amplitude of initial phasic hyperpolarizations