EXCITATORY AND INHIBITORY SYNAPSES
IN THE CAT SPINAL CORD

Koji Uchizono

Department of Physiology, Faculty of Medicine, University of Tokyo

Synaptic vesicles were independently recognized by many electron microscopists. Conclusive evidence has been presented that the synaptic vesicles contain preformed packets of the transmitter substance. Further investigations have indicated that there are at least three types of dense patches at the synaptic sites, of which only two are functional synapses. Four criteria were given for distinguishing type 1 from type 2 synapses. In the type 1 synapse the synaptic cleft is wider (300 Å) as against 200 Å for type 2; the postsynaptic membrane is thicker and denser; the dense patches are much more extensive. The third type of membrane thickening is not associated with any accumulation of vesicles. The distribution of the two types of synapses on the surface of neurons has different patterns according to the types of neurons. In pyramidal cells of the cerebral cortex a very important synaptic contact occurs on the spine. The synapses on these spines are exclusively type 1. On the other hand the synapses on the somas of pyramidal cells of the neocortex are always of type 2. Type 1 synapses also occur exclusively on the somas of the hippocampal pyramids and of the cerebellar Purkinje cells of the cat. The synaptic endings on somas of the Purkinje cells of the cerebellum are from the basket cells and likewise the synapses on the somas of hippocampal pyramidal cells are from the hippocampal basket cells. It has been conclusively demonstrated that these latter cells exert a powerful postsynaptic inhibition on the pyramidal cells. The same situation exists between cerebellar basket cells and Purkinje cells. Type 2 synapses are therefore postulated to be inhibitory in function. The synapses on apical dendrites of pyramidal cells presumably must be excitatory. Therefore it seems probable that the type 1 synapses are excitatory. It has been suggested that the distinction between excitatory and inhibitory synapses on morphological grounds may be possible, but so far it has been unsuccessful to identify two different types of synapses electron microscopically. However, investigations carried out electron microscopically in our laboratory on the cerebellar Purkinje cells of the cat have revealed that two different types of
synapses occur, the one with spherical vesicles on the dendrites and the other with ovoid or ellipsoidal vesicles on the soma of that neuron. Preliminary report was made elsewhere\(^\text{13}\). The present paper concerns mainly with the excitatory and inhibitory synapses in the anterior horn of the cat spinal cord.

**METHODS**

Cats were anaesthetized with nembutal. Perfusion with Locke's solution was conducted through a canula inserted into the left ventricle of the heart. When the exsanguination was confirmed macroscopically on the surface of laminectomized cat spinal cord \((L_4-S_1)\), the perfusate was changed to the fixative containing formalin until the whole animal body was fully fixed. Contracture of the whole muscular systems and the stiffness of joints of the body suggested the completeness of fixation. Some parts of the spinal cord were cut into small pieces which were re-immersed in the ice-chilled Palade fixative. Doublefixed materials were dehydrated in a series of ethyl alcohol and embedded in the epoxy resin. Ultrathin sections were made with LKB Ultrotome and electron microscopic investigations were carried out with Akashi TRS 80.

**RESULTS**

As shown in Fig. 1 two different types of synapses were recognized on the dendrite of neurons in the anterior horn of the cat spinal cord, the one contains vesicles of almost the same shape of spherical profile, while the other is filled with vesicles of irregular shape and size of ellipsoidal profile. Distinction between two types of synapses was sometimes difficult, especially when viewed at low magnifications (Fig. 1, A), but no difficulty was experienced when viewed at relatively higher magnifications as shown in Fig. 1, B and C.

The schematic diagram of two types of synapses is presented in Fig. 2, which characterizes the difference between two synapses. The synapses which contain spherical vesicles are tentatively named E-type (indicative of excitatory), while the other which has ellipsoidal vesicles is called I-type (indicative of inhibitory) synapse.

As already demonstrated in the synapse on the cerebellar Purkinje cells of the cat, the synapses which contain spherical vesicles may be excitatory in function, while the synapse filled with ellipsoidal vesicles is assumed to be inhibitory in nature. In the spinal cord of the cat it also may be possible to assume that the spherical vesicles are excitatory and the ellipsoidal vesicles are inhibitory just as in the case of cerebellar Purkinje cells\(^\text{13}\), although there is no definite evidence to lead us to that conclusion. In the anterior horn of the cat spinal cord two types of synapses surround the dendrites and somas of various types of neurons, moto- and interneurons, very densely. Distribution of excitatory and inhibitory (tentative as it may be) synapses is
Fig. 1. Synapses on the dendrite of neuron in the anterior horn of the cat spinal cord.
A: Three synapses make contact with the dendrite.
   E: Excitatory synapse, I: Inhibitory synapse D: Dendrite × 60,000
B: Excitatory synapse which contains spherical vesicles. × 160,000
C: Inhibitory synapse which contains ellipsoidal vesicles. × 160,000
different from neuron to neuron, but in motoneurons the number of excitatory synapses is less than that of inhibitory ones as shown in FIG. 3, which was made from the montage of several electron micrographs taken from a motoneuron in the spinal cord.

Motoneurons are easily distinguished from other interneurons by their size, abundance in Nissl bodies and some other kinds of characteristics. In the motoneuron shown in FIG. 3 in the ratio of inhibitory synapses to excita-
tory ones is about 3:2. It has been established that the synaptic transmission of motoneuron collaterals on Renshaw cells of the spinal cord and neuromuscular junctions of the skeletal muscle in the cat is cholinergic and excitatory in function. It has been proved that the shape of synaptic vesicles of neuromuscular junction of vertebrate is also spherical when viewed with the new fixation methods using formalin. It is interesting to note that in some neurons in the anterior horn of the cat spinal cord the surface of neuron-soma is surrounded almost exclusively with inhibitory synapses. It is suggested that this type of neurons is different from motoneurons, but exact nature of this cell is not yet established.

SUMMARY

New fixation methods in electron microscopy using formalin have revealed that there are two different types of synapses in the anterior horn of the cat spinal cord. The one type of synapse contains vesicles of spherical shape of about 500 Å diameter, while the other synapse contains ellipsoidal vesicles of the smaller size than the former. In comparison with Purkinje cells in the cat's cerebellum which have been identified as having excitatory (spherical) and inhibitory (ellipsoidal) synapses on the dendrite and soma of neuron respectively, it may be concluded that the motoneuron of the cat's anterior horn has two types of synapses, of which one contains excitatory synaptic vesicles (spherical) and the other contains inhibitory synaptic vesicles (ellipsoidal).

REFERENCES


