Brief Notes

Submicroscopic Changes of the Nerve Endings in the Adrenal Medulla after Stimulation of the Splanchnic Nerve*.

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Electron microscope studies of synaptic junctions have demonstrated the presence of a characteristic submicroscopic component designated by De Robertis and Bennett (1) the *synaptic vesicles.* This component was observed in the neuropile of the nerve cord of the earthworm, and in frog sympathetic ganglia (1) and reported simultaneously in synapsis of the central nervous system (2, 3) and the neuromuscular junction (3). Furthermore, the widespread occurrence of similar vesicles in synapses of the arthropods and in the central nervous system and retina of mammals was emphasized (4) and their presence in many types of synaptic junctions and nerve endings has been widely recognized (5, 6).

Synaptic vesicles were found to be preferentially distributed in the presynaptic side of the synapse and to have a close relationship to the synaptic membrane. These and other morphological characteristics led to speculations that acetylcholine and other chemical compounds active in synaptic transmission might be bound to the synaptic vesicles. Moreover, it was presumed that the movement of vesicles, to and possibly across the synaptic membrane, was an indication of the flow and discharge of these chemical mediators into the space immediately adjacent to the postsynaptic membrane (1).

Further indirect support of this hypothesis was found in the fact that the synaptic vesicles of the retinal rods and cones underwent considerable changes in size under prolonged darkness (7). Furthermore, in the acoustic ganglion they became agglutinated and lysed very early after destruction of the organ of Corti (8). The latter alternative changes should be correlated with the early physiological deterioration of synaptic transmission in nerve degeneration. However, so far there has not been a direct proof of the physiological significance of synaptic vesicles in the transmission of the nerve impulse.

Ever since the discovery of the synaptic vesicles it has been realized that a more direct approach to this problem might be found by inducing changes in the synaptic vesicles by physiological or electrical stimulation. Several attempts made in collaboration with Prof. Amassian of Washington University, Prof. Luco of the University of Santiago (5), and Prof. Berry of Cornell University in synapses of the sympathetic ganglia, failed or gave inconclusive results, mainly

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because of technical difficulties and the lack of time to solve them.

The investigation of this problem had to be postponed, but was reopened when it was realized that the adrenal medulla might be a better experimental material than the sympathetic ganglia. Suitable material for this experiment must fulfill the following conditions: (*a*) it must be easily accessible to electrical stimulation; (*b*) all or a great proportion of the nerve fibers must make a terminal contact within the tissue; (*c*) the concentration of nerve endings should be large enough for them to be found easily in the small fields revealed by the electron microscope; (*d*) a postsynaptic action potential or another type of signal should be available for recording.

The adrenal medulla fills most of these conditions since it receives almost all of its nerve supply by way of the homolateral splanchnic nerve which can easily be stimulated. These nerve fibers innervate the chromaffin cells directly rather than through intercalated neurones (see literature in 9). Furthermore the nerve endings, like other preganglionic autonomic endings, are of the cholinergic type (10). The nerve fibers and the endings synapsing with the chromaffin cells are very numerous (9) and are not difficult to find in sections with the electron microscope. Although this type of nerve ending has many characteristics resembling other cholinergic neuroeffectors and peripheral synapses, it seems to have some special physiological and pharmacological properties (see literature in 11). It is not known, moreover, whether there is an electrical action potential that accompanies the secretion of adrenaline, nor is it definitely determined whether the adrenal neuroeffector junction is intercalated between the two conducting elements (11). In spite of these uncertainties, this type of junction is generally considered to be of a synaptic nature, and this viewpoint will be followed here.

The signal which can be recorded in this system, is the amount of adrenaline, noradrenaline, or total catechols secreted into the adrenal vein under electrical stimulation (11, 12) or the analysis of the histochemical and submicroscopic changes of the stimulated adrenal cell with the electron microscope. Some observations on these changes made on the same material are being reported elsewhere (13).

A detailed account of our experiments, techniques, and findings will be published in the future. This preliminary paper is only intended to report the changes that occur in the synaptic vesicles after prolonged stimulation with electrical pulses of different frequencies.

In a number of rabbits under nembutal anesthesia the left splanchnic nerve was dissected and stimulated with a Grass stimulator (model S4A) using supramaximal (8 volts) rectangular pulses of 1 msec. duration for 10 or 15 minutes. The frequencies varied between 8, 40, 100, and 400 pulses per second, but the main number of experiments were made with 100 and 400 per second during 10 minutes. Those frequencies were selected because Rapela and Covian (12) have reported in the dog a maximum secretion of catechols at frequencies of 40 to 100 per second and a considerable diminution in output with higher frequencies.

In thin sections of the adrenal medulla observed under the electron microscope the unmyelinated nerve fibers and the enlarged terminal nerve endings can be easily recognized (Fig. 1). In the nerve endings a typical vesicular component consisting of the synaptic vesicles (1) is observed. In addition mitochondria, some larger vesicles and, less often, neuroprotofibrils are found. The vesicles show in many instances a close relationship to the synaptic membrane. This phenomenon is more obvious in the specimens stimulated at 100 pulses per second, in which one can observe many vesicles attached to the membrane and a considerable increase of electron density on both sides of the synaptic membrane. With this kind of stimulation there is also a definite increase in the number of synaptic vesicles within the nerve endings.

The number of synaptic vesicles per square micron was determined in 8 sections of nerve endings for both normal and stimulated (100 pulses per second) rabbits. In the nerve endings of the control a mean of 82.6 vesicles per square micron was found, whereas in the stimulated animal the mean rose to 132.7.

Even more striking are the results observed in glands stimulated at 400 pulses per second. In this case a considerable depletion of synaptic vesicles takes place, together with other changes of the matrix and mitochondria. These will be described in detail elsewhere (see Fig. 2). Countings in 7 endings of different specimens gave a mean number of vesicles of 29.2 per square micron.

Although these results are not as yet preliminary a tentative interpretation may be advanced. The number of vesicles observed at a definite moment of the activity of the nerve ending can be interpreted as the result of a balance between two processes: one of formation of synaptic vesicles, and the other of release or destruction of the vesicles. It seems possible that under a stimulation of 100 pulses per second both the formation and release of synaptic vesicles are increased but the first process predominates, giving rise to a positive balance and an augmentation in the number of vesicles. In contrast, with a stimulation of 400 pulses per second a negative balance is engendered and there is a depletion of the vesicular component.

This assumed diminution of the synaptic vesicles may be due to the fact that the rate of formation cannot keep pace with the increased destruction and release. However an inhibition of the formation of vesicles, under the influence of the high frequency stimulus, might also take place. The results so far obtained do not permit a definite interpretation of the mechanisms involved in this depletion of synaptic vesicles. However, the facts that there is a smaller output of catechols from the gland (12) and a lesser release of catechol containing droplets from the adrenal cells (13), are suggestive of an impairment in the activity of the nerve endings and may indicate an actual fatigue of the synaptic junction.

The increase in number of synaptic vesicles with a stimulus of 100 pulses per second is probably related to an increment of the activity of the nerve endings. This results in a larger output of catechols from the adrenal gland (12) and in a considerable depletion of catechol containing droplets from the adrenal cells (13).

The finding of changes in the synaptic vesicles under electrical stimulation with different frequencies seems to confirm the presumption (1, 7, 8) that the synaptic vesicles may play a physiological role in the transmission of the nerve impulse across the synaptic junction.

SUMMARY

The nerve endings of the adrenal medulla of the rabbit were studied under the electron microscope in the normal condition and after prolonged electrical stimulation of the splanchnic nerve. With

a stimulus of 100 pulses per second for 10 minutes, there is an increase in the number of synaptic vesicles in the nerve ending. The mean number is of 82.6 vesicles per square micron in the normal and of 132.7 per square micron in the stimulated glands. With a stimulus of 400 pulses per second for 10 minutes, there is a considerable depletion of synaptic vesicles and other changes occur in the nerve endings. The mean number of vesicles is of 29.2 per square micron.

These results are interpreted as indicative of an increased activity of the ending in one case, and as a diminished activity and fatigue of the synaptic junction in the other.

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Explanation of Plate 201

Fig. 1. Electron micrograph of a thin section of the adrenal medulla of a normal rabbit showing a nerve ending interposed between several adrenal cells. The ending is surrounded by a synaptic membrane (*sm*) and contains a few mitochondria (*mi*) and many synaptic vesicles (*sv*). A total of 95.9 vesicles per square micron were counted in this specimen. In the adrenal cells mitochondria (*mi*) and catechol containing droplets (*cd*) are observed. ×49,000.

Fig. 2. Electron micrograph of a similar field as in Fig. 1 but belonging to a gland whose splanchnic nerve had been stimulated at 400 pulses per second for 10 minutes. The most significative change is the great depletion of synaptic vesicles (*sv*). In this case only 28.7 vesicles per square micron were counted. *mi*, mitochondria, *sm*, synaptic membrane, *cd*, catechol containing droplets. × 42,000.

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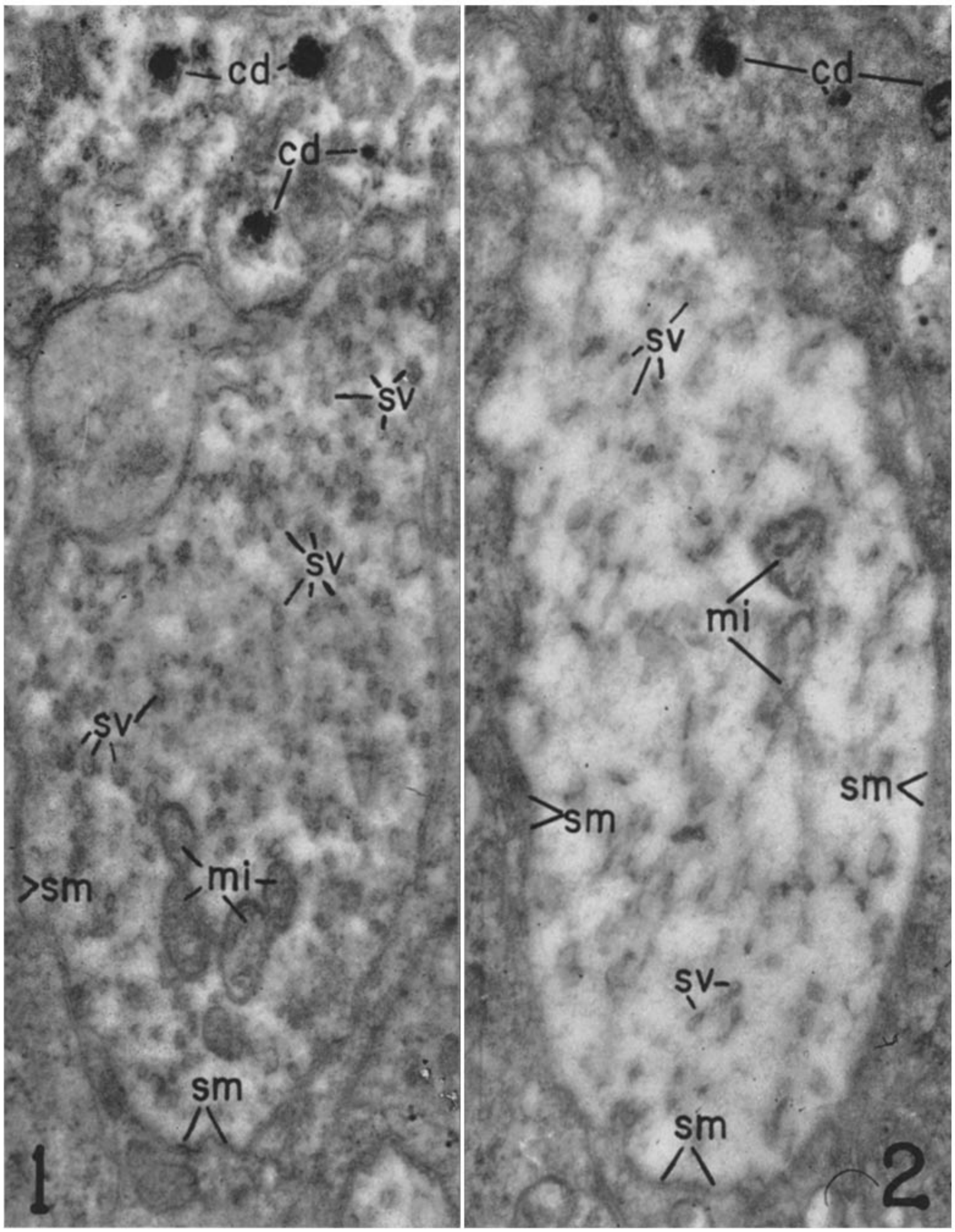

(De Robertis and Ferreira: Submicroscopic changes of nerve endings)