Ultrastructure of Spinal Cord Synapses During Strychnine Intoxication

Excitatory and inhibitory potential recorded in the central nervous system (CNS)[1] have hinted at the possibility of differentiating also morphologically 2 types of synapses according to their function[2-4].

It has been suggested that those synapses might differ from the fine structure of synaptic junction[5], or from the different shape of the presynaptic vesicles. Some authors claim that the inhibitory synapses have a population of ellipsoidal vesicles, while the excitatory have round-shaped ones[1-4,6-8]. Because this last difference so far described was not clear cut, and depended largely on the fixatives used[8], another approach was tried. We thought that it might be possible to modify the ultrastructure of synapses, using some well-known special-acting drugs on the synaptic transmission. For this purpose we chose strychnine which is a typical anti-inhibitory drug, acting on the synapses by selective blocking of the post-synaptic inhibition[1]. The observations here reported concern the synaptic terminals in the anterior horn of the spinal cord.

Material and methods. Four days before the experiment, teflon tubing was placed permanently in the right common carotid of 300 g Sprague-Dawley male rats and fixed to the skin of the cervical region of the rat. In this manner it was possible to perfuse the animals with fixatives without using anaesthetic drugs which, on their own, could modify the ultrastructure of the CNS.

Six animals were kept as controls. 18 rats were treated with 5 mg/kg i.p. of strychnine chlorhydrate and placed under artificial respiration. 6 animals were sacrificed at the appearance of convulsion, six 1 h and six 2 h later. At the prescribed time the animals were perfused through the teflon tubing for 3 min with Ringer lactate at 6 °C and afterwards with a fixative solution at 15 °C for 15–20 min. The fixatives used were the following: 2.2% acrolein (Eastman O.C. filtered through active coal at 10 °C) in 0.1 *M*, phosphate buffer pH 7.4, final osmolarity 610 ± 10 milliosmol[6], 2.5% glutaraldehyde (Fisher C. distilled according to FAHIMI and DROCHMANS[7] in 0.1 *M* phosphate buffer pH 7.4, final osmolarity 450 ± 10 milliosmol. After aldehyde fixation small pieces of the spinal cord were postfixed with 1% OsO_4 in 0.1 *M* Sörensen buffer pH 7.4 for 2 h at 4 °C, and embedded in Epon 812 or Vestopal W. Sections obtained with a LKB Ultrotome microtome and stained with the uranyl acetate-Pb citrate double stain, were examined with Philips EM 200 electron microscope.

Results and discussion. In all animals regardless of the treatment given, no difference could be found in the morphology of the synaptic endings, either axosomatic or axodentritic. The number of synaptic vesicles present in nerve endings was very variable; they were evenly distributed in the synaptic terminals with an increase of concentration near the synaptic junction.

The vesicles (300–600 Å diameter) were generally round in shape, but in a few presynaptic endings they had an ellipsoidal or elongated shape. However in many cases both types of vesicles were present in the same nerve endings. Vesicles population showed no difference in presynaptic endings with the perikaryon, or cone of origin, or with small, medium, or large dendrites. Because many authors supported the difference between excitatory and inhibitory synapses by the different shape of their vesicles, we have taken this particular point into account, and calculated in 250 presynaptic endings the percentage of ellipsoidal vesicles in each group of animals. The results are given in the Table.

Strychnine treatment did not change the percentage of ellipsoidal vesicles in the population of the presynaptic vesicles. Instead this percentage differed strongly according to the fixative used, glutaraldehyde (Figure 1) or acrolein (Figure 2), as shown in the Table.

After strychnine intoxication the synaptic junctions never changed.

A morphological change could be expected in the synaptic fine structure affecting either the number, distribution and shape of vesicles or the morphology of the synaptic junction after so great an alteration of the spinal cord function, provoked by the anti-inhibitory activity of strychnine. However, in our investigation, we could not find any great difference in the morphology of the synaptic junctions (membranes, pre- and post-synaptic apparatus) nor in the distribution and percentage of the different

No. of ellipsoidal vesicles/1000 synaptic vesicles in spinal cord nerve endings after strychnine treatment and after different fixation

Fixatives	Controls	Time after strychnine treatment		
		5 min	1 h	2 h
Glutaraldehyde	40 ± 5	45 ± 5	39 ± 5	44 ± 5
Acrolein	22 ± 2	19 ± 2	23 ± 3	21 ± 2

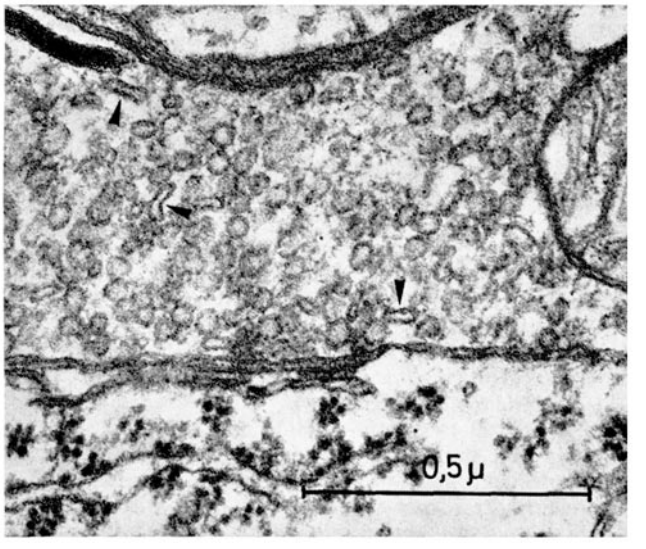

Fig. 1. Glutaraldehyde fixation. The figure shows an axo-somatic synapses in the spinal cord 1 h after strychnine treatment. Many ellipsoidal and elongated vesicles can be clearly seen among the round-shaped ones (arrows).

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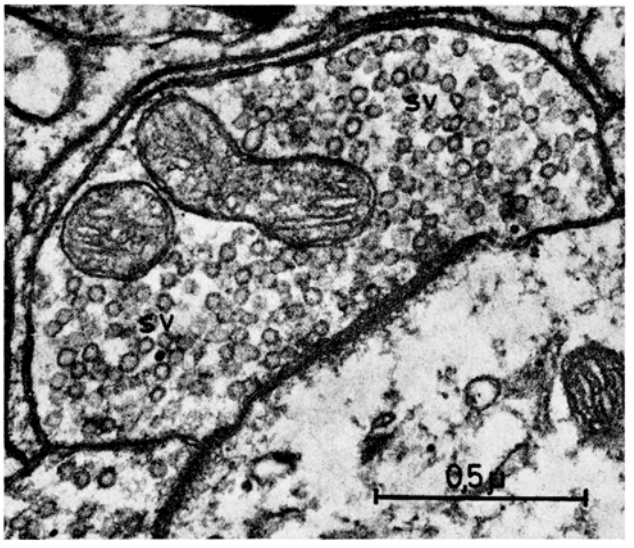

Fig. 2. Acrolein fixation. The figure shows an axo-dendritic synapses in the spinal cord 1 h after strychnine treatment. The synaptic vesicles (SV) in presynaptic ending are mainly round in shape.

shaped vesicles (round, ellipsoidal or elongated). If we can infer from our morphological data, it seems that the block of inhibition at the level of the post-synaptic membrane did not considerably affect the presynaptic terminals.

Although some authors distinguish the inhibitory synapses from the excitatory ones according to the shape of synaptic vesicles, our results show that these parameters are very variable according to the different fixatives used. It is possible to suppose that some differences detected in synapses could be due more to the action of the fixative than to a real morphological difference depending on their function.

Riassunto. Gli autori studiando la ultrastruttura delle sinapsi del midollo spinale durante intossicazione stricnina non trovano nessuna differenza nei componenti sinaptici attribuibile al trattamento stricnico. Rilevano invece variazioni nella morfologia delle vescicole presinaptiche in relazione a differenti fissativi utilizzati.

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Fine Structural Localization of Exogenous 5-HT in Vesicles of Adrenergic Nerve Terminals

It is now well established that adrenergic nerve terminals possess a population of vesicles 300–600 Å in diameter containing an osmiophilic dense core which represents the physiological neuro-transmitter norepinephrine (NE). It has been demonstrated by electron microscopy that these same terminals are capable of acquiescing structurally similar phenethylamines such as α-methylnorepinephrine and 5-hydroxydopamine (5-HO-DA), so-called 'false' transmitters[1,2]. As well, it has recently been shown by radioautography in rat vas deferens[3] and by biochemistry in guinea-pig vas deferens[4,5] that an indolamine, 5-hydroxytryptamine (5-HT), is capable of being accumulated and stored in sympathetic nerve terminals and liberated as a transmitter by sympathetic nerve stimulation[4]. Since it is possible to demonstrate 5-HT electron microscopically in blood platelets[6], it was thought that this might provide a favourable prerequisite to test electron microscopically if an indolamine is capable of being accumulated selectively in the adrenergic nerve terminals, as is the case for phenethylamines and more specifically to yield information on its discrete subcellular localization.

Cats were pretreated with α-methylmetatyrosine (α-MMT) 4 × 200 mg/kg over a period of 2 days before the commencement of the experiments. Iris and vas deferens were quickly removed and incubated in Krebs-Henseleit solution containing a concentration of 5-HT (10 μg/ml, 50 μg/ml or 500 μg/ml) at 37 °C for 30 min. Incubation of a portion of the tissue in Krebs-Henseleit without 5-HT served as control. After incubation tissues were fixed in glutaraldehyde, post-fixed in osmium tetroxide and embedded for electron microscopy. The electron microscopical examination of the iris was concentrated on the medium zone of the dilator pupillae, as this zone contains a large number of sympathetic nerves.

That α-MMT treatment successfully depleted the NE in the vesicles of the adrenergic nerve terminals was shown ultrastructurally. Those tissues incubated in Krebs-Henseleit alone revealed no or very few dense core vesicles within nerve terminals (Figure 1). Thus, any obvious accumulation of amine in the form of an osmiophilic dense core in the vesicles of nerve terminals from incubated tissue over and above this control level must be due to the selective and specific uptake and storage of exogenous 5-HT. As well, depletion of NE was confirmed by the biochemical determination of NE employing the method of BERTLER et al.[7] in residual parts of the organs not incubated in 5-HT. The NE content was reduced to less than 5% of control levels.

Figure 2 illustrates the appearance of iris after incubation for 30 min in 500 μg/ml 5-HT. From the large number of dense core vesicles present it is clear that 5-HT is capable of being taken up by the vesicles of adrenergic nerve terminals. As in the normal state there still exists a dual population of terminals, those with dense core vesicles and those with only empty vesicles. The regularly occurring empty vesicle-terminals have been shown to be of cholinergic origin and those with dense core vesicles of adrenergic derivation. Concentrations of 10 and 50 μg/ml 5-HT also result in dense cores in the adrenergic terminals but to a lesser extent than with the higher dose of 500 μg/ml.

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