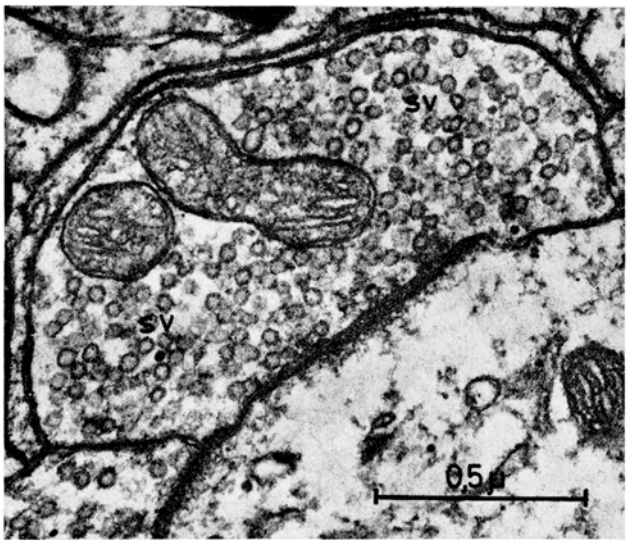

Fig. 2. Acrolein fixation. The figure shows an axo-dendritic synapses in the spinal cord 1 h after strychnine treatment. The synaptic vesicles (SV) in presynaptic ending are mainly round in shape.

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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Fig. 1. Control. Iris from α-MMT-treated cat incubated in Krebs-Henseleit alone. Vesicles of the nerve terminal are empty. × 55,000.

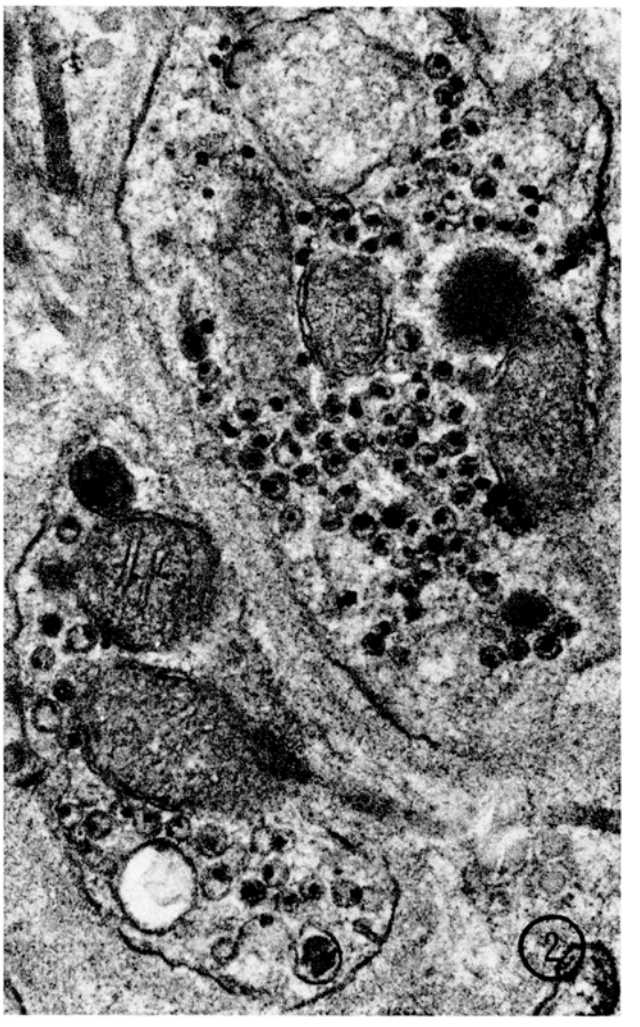

Fig. 2. Iris from α-MMT-treated cat incubated in 500 µg/ml 5-HT. Virtually all of the vesicles in the adrenergic nerve terminal contain a dense core. × 55,000.

Results in the vas deferens paralleled those seen in the iris. These results confirm and extend the previous radioautographic[3] and biochemical[4,5] findings.

It is interesting to note that, at least under these experimental conditions, 5-HT is capable of substituting for NE in the vesicles of adrenergic nerve terminals in spite of its different chemical structure. The results indicate that effective false transmitters in adrenergic nerve terminals are not restricted to phenethylamines but can be found also in the group of indolamines.

As in the case of 5-HO-DA[2] cholinergic nerve terminals do not accumulate the exogenously introduced 5-HT, indicating that the ability for uptake and storage of biogenic amines is restricted to adrenergic nerve terminals.

In addition our results reveal that not every vesicle is filled to capacity nor does every vesicle necessarily contain a dense core as is the case after treatment with 5-HO-DA[2]. However, this phenomenon may well be explained if one takes into account the importance of the fixation technique on the preservation of amines in the form of osmiophilic dense cores[8–10].

In summary, exogenous 5-HT, an indolamine, has been localized in the vesicles of adrenergic nerve terminals from the iris and vas deferens of cats as an osmiophilic dense core after the depletion of the normally occurring physiological transmitter NE.

Résumé. L'incubation d'iris et de canal déferent de chat dans des solutions contenant de la 5-hydroxytryptamine (5-HT) a permis de localiser spécifiquement la 5-HT exogène dans les vésicules des terminaisons nerveuses adrénergiques au microscope électronique. La recherche de «faux» transmetteurs adrénergiques ne se limite donc pas aux seules phényléthylamines mais s'étend également au groupe des indolamines.

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