Action of *p*-Chlorophenylalanine on the Synaptic Vesicles from Rat Pineal Nerves

In previous works we have shown that Champy-Maillet techniques reveal two components in the synaptic vesicles of rat pineal nerves[1,2]. These components are: the central core and the matrix which comprises the space between the vesicle membrane and the core. Both of them are differently stained, depending on the composition of the Champy-Maillet mixture employed. Those containing zinc iodide give a full reaction with the matrix leaving a paler core, while those containing potassium iodide specially react with the core.

DL-*p*-chlorophenylalanine (*p*-CPA) specifically depletes serotonin in pineal nerves, while noradrenaline and dopamine levels are not reduced[3,4]. We have studied the action of this drug on the synaptic vesicle components revealed by the Champy-Maillet techniques.

Rats were decapitated and pineal glands were fixed in mixtures containing 1 part of osmium tetroxide and 3 parts of a soluble iodide. The mixture containing zinc

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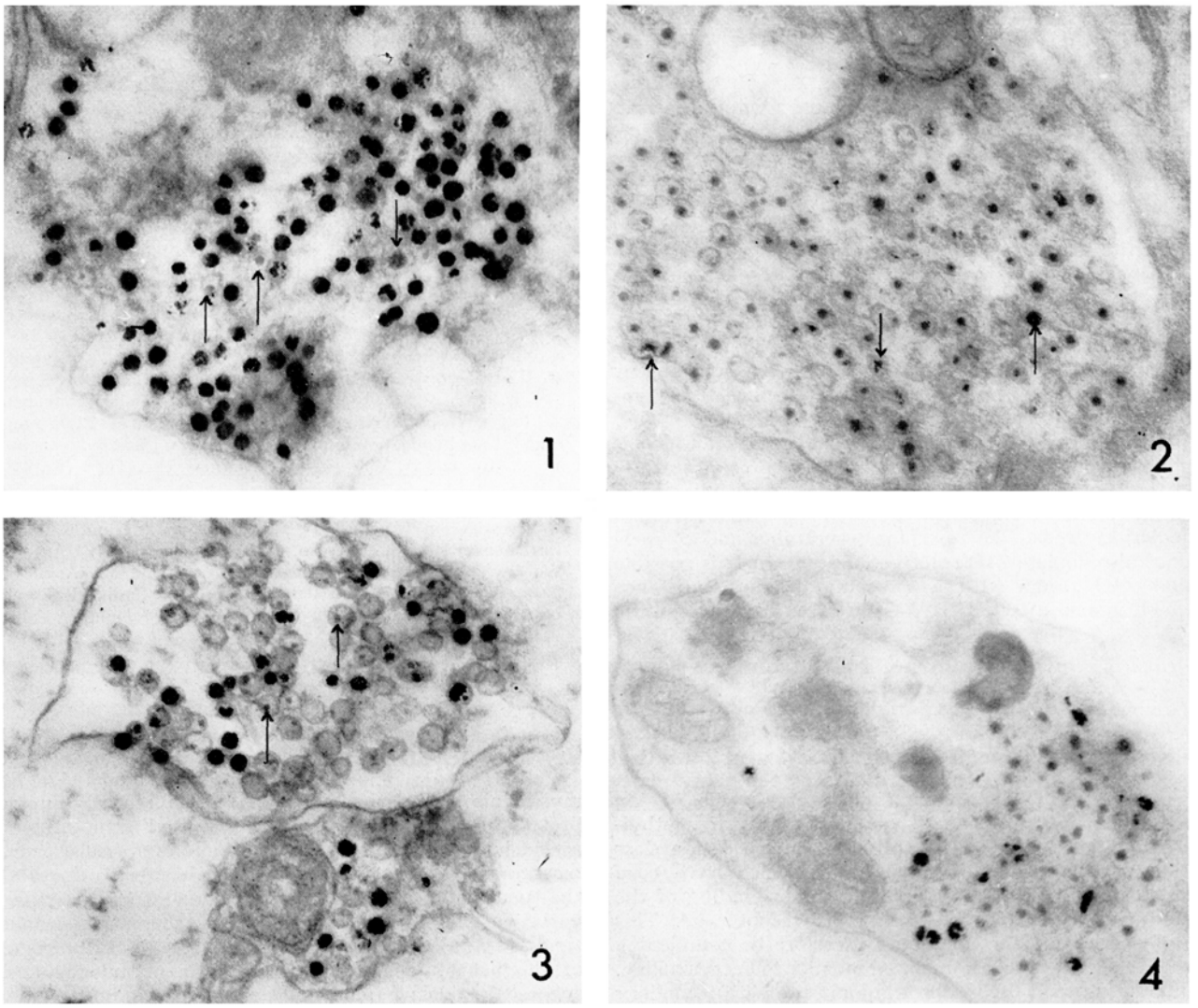

Figs. 1 and 2. Nerve endings from normal pineal glands.
Fig. 1. Gland fixed with osmium tetroxide-zinc iodide (ZIO). In many vesicles the matrix is deeply stained, while in some of them only a pale core (arrows) can be seen. The grids were stained with lead citrate. ×60,000.
Fig. 2. Gland fixed with osmium tetroxide-potassium iodide (KIO-7). An electron dense core can be observed in most of the vesicles. There is a partial reaction of the matrix in some vesicles (arrows). The grids were stained with lead citrate. ×60,000.

Figs. 3 and 4. Nerve endings from pineal glands treated with *p*-chlorophenylalanine.
Fig. 3. Gland fixed with ZIO. The matrix reaction has disappeared in most of the vesicles. The cores are reduced in size and electron density (arrows). Compare with Figure 1. The grids were stained with lead citrate. ×60,000.
Fig. 4. Gland fixed with KIO-7. The reaction of the cores and the matrix is similar to that of the control. Compare with Figure 2. ×60,000.

iodide at pH 5.5 will be called ZIO, and the one containing potassium iodide at pH 7.4 will be called KIO-7. (For detailed fixation schedules see ref. [2]). Treated rats were given 2×300 mg/kg i.p. of *p*-CPA, separated by a 48 h interval. They were killed 24 h after the last injection. Control rats received equivalent volumes of saline.

The ZIO reaction in pineal nerves (Figure 1) has already been described[1]. Most vesicles show a heavily stained matrix and a paler core. In *p*-CPA treated rats (Figure 3) the matrix reaction disappears in about 60% of the vesicles. Central cores can still be seen, however, they have a smaller diameter and less electron density than those in controls.

KIO-7 mixtures show a dense core in about 70–75% of the vesicles (Figure 2). The matrix does not react with KIO-7 as much as it does with ZIO. Only small dense spots can be seen in the matrix of some vesicles. After treatment with *p*-CPA (Figure 4), no alteration can be observed, either in the staining of the cores or in the reaction of the matrix.

It seems that *p*-CPA depletes ZIO reactive material both from the matrix and the core of synaptic vesicles from rat pineal nerves. With the Wood technique, serotonin and catecholamines can be localized histochemically in the core[4,5]. This can be related to the smaller ZIO reaction of the core in *p*-CPA treated rats. However, *p*-CPA also depletes ZIO reactive material from the matrix, where no indol or catecholamines can be localized with the Wood technique. Two hypothesis may be formulated: a) there is a serotonin store in the matrix which is not revealed by the Wood technique but which in some way reacts with ZIO. This possibility is enhanced by experiments made in vitro done in our laboratory. It has been found that serotonin, 5-hydroxytryptophan, tryptophan and melatonin reduce ZIO, giving a heavy precipitate. Catecholamines and their precursors, dopa and phenylalanine, also precipitate ZIO in the test tube. A relation of the matrix with catecholamine stores cannot be excluded as catecholamine-depleting drugs, like reserpine[1], tyramine and oxypertine[6] also deplete ZIO reactive material. b) It is possible that ZIO reacts with a serotonin-different substance which is also affected by *p*-CPA. KOE and WEISSMAN[7] observed that *p*-CPA reduces the normal increase of brain serotonin and 5-hydroxyindolacetic acid resulting from 5-hydroxytryptophan administration, and they postulated a *p*-CPA inhibition of 5-hydroxytryptophan uptake. Perhaps the matrix reactive material represents a binding site for 5-hydroxytryptophan.

After treatment with *p*-CPA, the cores revealed by KIO-7 remain observable. The same happens with those revealed by osmium tetroxide, but not with those revealed by glutaraldehyde-osmium tetroxide[4]. Perhaps KIO-7 reveals catecholamine stores as osmium tetroxide does, or it may be that KIO-7 reacts with some other core component of an unknown character. Inasmuch as KIO-7 fixation shows a greater number of cores than osmium tetroxide, it is possible that KIO-7 reveals a different kind of catecholamine binding.

Resumen. Se demuestra que la *p*-clorofenilalanina depleciona los componentes de las vesículas sinápticas de los nervios pineales de la rata revelables por la mezcla tetróxido de osmio-yoduro de zinc pero no afecta a los revelables por tetróxido de osmio-yoduro de potasio.

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[9] We are grateful to Miss MARGARITA LÓPEZ and Mr. ALBERTO SAENZ for their technical assistance, and to Pfizer Medical Research Laboratories for kindly supplying *p*-chlorophenylalanine.

Cholinergic Properties of 1-Methyl and 2-Methylpentyltrimethylammonium Salts

Although the cholinergic stimulant actions of the carbon analogue of acetylcholine, the pentyltrimethylammonium ion, are well established[1], this cation does not possess the ester oxygen atoms which have been implicated in hydrogen bonding or ionic binding of the neurotransmitter to the cholinergic receptors[2,3]. This aliphatic ammonium ion could therefore be complexing with the receptors in a modified manner to acetylcholine, interacting with the receptor anionic site with secondary binding factors accruing from some form of allosteric hydrophobic bonding[4].

In order to seek clarification of this consideration we have examined the agonistic properties of 1-methyl- and 2-methylpentyltrimethylammonium salts. Substitutions of this nature on the acetylcholine molecule do not grossly detract from the high potency of the parent substance although there is profound receptor differentiation. Thus, 1-methyl substitution is compatible with nicotinic stimulation while 2-methyl substitution favours muscarinic stimulation[5]. CHOTHIA[6] has recently interpreted these findings to develop 'essential' structures of acetylcholine acceptable to nicotinic and muscarinic receptors. We considered that if such substitutions on the pentyltrimethylammonium cation caused similar variations in agonistic specificities then this would signify a parallel between the mode of receptor interaction of the neurotransmitter and its carbon analogue and suggest the relative importance of oxygen binding. A lack of such differentiation would serve to indicate that lipophilic binding of the aliphatic chain is of great

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