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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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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importance in receptor complexing of the pentyltrimethylammonium ion.

The muscarinic potencies of pentyltrimethylammonium bromide, (±)-1-methyltrimethylammonium iodide and (±)-2-methyltrimethylammonium iodide were determined in vitro on the guinea-pig ileum, using Tyrode solution containing $1 \times 10^{-4} M$ hexamethonium dichloride to dispel ganglion stimulant contributions to the measured responses. Drug responses were completely abolished when the compounds were administered in the presence of $1 \times 10^{-7} M$ atropine sulphate[7].

Nicotine-like stimulant actions of the compounds were conducted on the rectus abdominis muscle of the frog *Rana pipiens*[8]. Carbachol was used as the standard drug in all experiments and equipotent molar ratios were determined by complete four-point assays.

Although the pentyltrimethylammonium salts are less active than the standard, carbachol, the results clearly demonstrate that the nicotinic and muscarinic receptors can differentiate between the substituted aliphatic agonists (Table). The pentyltrimethylammonium and 1-methylpentylammonium salts are virtually equipotent nicotinic stimulants whereas 2-methylpentyltrimethylammonium iodide is considerably less active on a molar basis. The reverse situation holds true at the muscarinic receptor where 2-methylpentyltrimethylammonium iodide and pentyltrimethylammonium bromide are more potent than the 1-methylpentyltrimethylammonium salt.

The consistancy between the above results and those previously determined for the analogous acetylcholines[5,9] implies that the pentyltrimethylammonium ion fits the cholinergic receptors in a similar way to acetylcholine. Moreover, the quantitative variations noted (Table) indicates that the muscarinic receptor is more sensitive to exchange of the ester group for methylenes than the nicotinic receptor[10].

Equipotent molar ratios relative to carbachol

	Frog rectus abdominis (Nicotinic)	Guinea-pig ileum (Muscarinic)
Carbachol	1.00	1.00
Pentyltrimethylammonium bromide	2.14 ± 0.080	15.94 ± 2.58
(±)-1-Methylpentyltrimethyl ammonium iodide	2.89 ± 0.423	300.2 ± 20.60
(±)-2-Methylpentyltrimethyl ammonium iodide	140.1 ± 16.20	10.68 ± 0.372

Each experiment was repeated 3 times. The results show the equipotent molar ratios and the standard errors of the means.

Résumé. Étude des propriétés cholinergiques des iodures du méthyl-1 et du méthyl-2-pentyltriméthylammonium. Dans leur comportement agonistique, ces sels offrent des variations parallèles à celles de dérivés méthyles de l'acétylcholine. Le cation pentyltriméthylammonium est donc capable de s'associer aux récepteurs cholinergiques tels que l'acétylcholine.

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Removal of Acetylcholine During Perfusion of Liquor-Spaces and its Influence on Outflow Volume

Acetylcholine (Ach) appears in the effluent during perfusion of the cerebroventricular system[1] and intermeningeal spaces of the spinal cord with an anticholinesterase[2]. There is, however, not much information concerning the fate of Ach in these spaces. Bhawe[3] recovered 56% from cat cisterna magna after a single intraventricular injection of Ach. In the present work[4], Ach was perfused continuously through different compartments of CSF-containing spaces and its disappearance studied.

Methods. 26 cats of either sex were anaesthetized by an i.v. injection of 25 mg/kg sodium pentobarbital. The liquor-containing spaces were perfused as follows: a) the cerebral subarachnoid space, from the parietal cortex to cisterna magna[5]; b) the spinal subarachnoid space, from the cisterna magna to lumbosacral foramen[2]; c) the cerebroventricular system[1], from the left lateral ventricle either to cisterna magna or d) to aqueduct of Sylvius. Acetylcholine chloride (100 ng/ml) was added to the perfusion fluid[6] which was introduced at a rate of 0.1 ml/min. The content of Ach was checked by biological assay at the beginning and end of the experiment to exclude loss due to spontaneous hydrolysis. 20 min samples of perfusate were collected in graduated tubes and Ach content determined on guinea-pig isolated ileum[7]. Disappearance of Ach was calculated by comparing inflow-outflow amounts. In a separate series of experiments, pieces of dura mater and arachnoid were dissected from different regions of brain and spinal cord and cholinesterase activity was determined histochemically[8], as well as colorimetrically[9] using homogenates.

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