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SHORT COMMUNICATIONS

The identification of acetylcholine in presynaptic terminals isolated from brain

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ALTHOUGH it is known that acetylcholine (ACh) is released from the surface of the intact mammalian cerebral cortex,[1] definite evidence is lacking that this substance originates from presynaptic terminals. For instance it could be a by-product of metabolic processes in brain.[2] In view of the current interest in the identity of cholinomimetic substances in brain,[3] it was thought desirable to give a preliminary report of some recent findings which indicate that the cholinomimetic activity of presynaptic terminals is chiefly due to ACh.

The fractionation of sucrose homogenates of rat brain[4] was carried out at 0–4°, the low temperature minimising the possible synthesis[5] both of ACh and of other cholinomimetic substances not originally present in significant concentrations in the intact tissue. This is particularly important since brain extracts can synthesise both ACh and other cholinomimetic substances *in vitro*.[6] The active cholinomimetic substance was extracted from the "crude mitochondrial" fraction from brain by heating the particles at 100° for 10 min at pH 4. It is known that on subfractionation the cholinomimetic activity of this fraction is recovered from a layer of the density gradient which corresponds to presynaptic terminals.[4, 7] Substance P was inactivated by incubating the extract with chymotrypsin, after neutralising to pH 7·5. The extract was then heated at 80° for 10 min to precipitate the chymotrypsin and to inactivate catechol amines. This procedure did not destroy ACh in control experiments (see also ref. 4). After centrifuging, the supernatant fluid was assayed against standard solutions of acetylcholine bromide on several biological test preparations.

On the superfused guinea pig ileum, the blood pressure of the rat treated with neostigmine, the superfused rectus abdominis muscle of *Bufo marinus* in which the cholinesterase was inactivated with tetraethylpyrophosphate, and on eserinised rabbit auricles the results of the parallel assays agreed within 8 per cent. The ACh content of the extract was 0·9 μg/g of fresh brain weight. This figure is of the same order as that obtained by other workers.[4, 7] However, on isolated toad atria, either in the presence or absence of eserine, the estimated ACh content was about 25 per cent less than in the other tests. This low figure on toad atria is attributed to the presence of an interfering substance since when administered to the perfused toad heart, the extract had a stimulant effect at low doses, but in higher doses it produced a mixed stimulant and ACh-like depressant effect. The depressant, but not the stimulant effect, was abolished by treatment with alkali.

The effects produced by the extract were similar to those produced by ACh with regard to blocking by atropine, potentiation by eserine and destruction by alkali, and also with respect to rate of onset and duration of action. On the toad rectus muscle the different rates of onset and duration of effect of different cholinomimetic substances were particularly marked. ACh, propionylcholine, n-butyrylcholine and acetyl-β-methylcholine produced fairly rapid contractures with slow recovery, in contrast to a number of other substances, including γ-aminobutyrylcholine and the methyl esters of β-propiobetaine and γ-butyrobetaine, which caused slowly developing contractures with slow recovery. It is therefore significant that on this preparation the effect of the extract was similar to that of ACh.

Since parallel biological assays have so far distinguished between all choline esters examined, including pyruvylcholine,[8] and those which have been reported to be present in brain extracts, such as ACh, propionylcholine, n-butyrylcholine, γ-aminobutyrylcholine and betaine esters, it may reasonably be concluded on the present evidence that the cholinomimetic activity of an extract of presynaptic terminals is due to ACh and not to a related ester.

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Phthalanilide interaction with nucleic acids*

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THE substituted phthalanilides are a series of basic compounds which have been shown to have exceptional therapeutic activity against certain experimental neoplasms, notably leukemias in rodents.[1, 2]

In preliminary experiments we have observed that a significant portion of the phthalanilide present in a mouse ascitic leukemia cell (P-388 line), after treatment *in vivo*, is localized in the nuclear fraction after centrifugal fractionation of the cell components. These findings, and reports of interactions of DNA with polybasic compounds such as spermidine[3] and with the actinomycins[4] which inhibit DNA-dependent RNA synthesis,[5, 6] have led us to investigate the phthalanilides for their capacity to interact with DNA and RNA.

MATERIALS AND METHODS

The phthalanilides used in this study were supplied by the Cancer Chemotherapy National Service Center and are as follows: NSC 35843: 4′,4″-di(2-imidazolin-2-yl)terephthalanilide dihydrochloride; NSC 53212: 4′,4″-di(2-imidazolin-2-yl)isophthalanilide dihydrochloride; NSC 60339: 2-chloro-4′,4″-di(2-imidazolin-2-yl)terephthalanilide.

Highly polymerized salmon-sperm DNA, yeast RNA, and polyadenylic acid were obtained from commercial sources. The DNA preparations from ascitic leukemia cells, which were either sensitive or resistant to NSC 60339, were prepared by the method described by Marmur.[7]

Spectral observations were made at room temperature with a Zeiss spectrophotometer, model PM QII.

RESULTS AND DISCUSSION

Mixtures of NSC 60339 (2 to 20 μg/ml) and salmon-sperm DNA (5 to 125 μg/ml) in dilute aqueous solutions result in the formation of a complex, as is evidenced by a 13-mμ bathochromic shift of the absorption maximum of the phthalanilide from 292 to 305 mμ (Fig. 1, a). The hyperchromic maximum of the difference spectrum at 325 mμ is taken to be an index of complex formation and is proportional to phthalanilide concentration at saturating levels of DNA (Fig. 1, b). It was determined that increasing the DNA concentration above 1·2 μg DNA/μg NSC 60339 did not increase the amount of complex formed (Fig. 2).

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