excitation. However, two tryptamine analogues tested showed an inhibitory effect. One compound, 5,6-dihydroxytryptamine, had a biphasic action, while the other, 6-hydroxytryptamine, always inhibited cell activity. The following compounds, in doses up to 1 μmol, failed to show any 5-HT-like activity: tryptamine, tyramine, N-methyltryptamine, 5-hydroxy-indole, 5-hydroxy-indolyl acetic acid, 5-methoxy-gramine, lysergic acid diethylamide, α-methyltryptamine, 5-methyltryptamine.

It is concluded that for potent 5-HT-like activity agonists should contain the following groups: an indole nucleus; either a hydroxyl or methoxy in the 5 position; a terminal unsubstituted nitrogen. Addition of methyl groups to the terminal nitrogen generally reduced potency.

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The amiloride receptor

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The diuretic drug amiloride (3, 5-diamino-6-chloropyrazinoylguanidine) is a potent inhibitor of sodium transport in epithelia, and is believed to prevent the passive entry of sodium ions to the transport mechanism (Crabbé & De Weer, 1969; Gatzy, 1971) by combining with receptors in the mucosal surface of the epithelia. Alterations in the calcium concentration affect the potency of amiloride in a way which suggests that calcium may be involved in the interaction of amiloride with its receptor. Evidence for this was obtained as follows.

The sodium-dependent oxygen consumption of pieces of toad bladder epithelium was measured. Amiloride (10^{-4} M), a concentration more than sufficient to completely inhibit sodium transport, abolished the sodium-dependent oxygen consumption in solutions containing 1 mM calcium. In the absence of calcium, amiloride (10^{-4} M) had no effect on the sodium dependent oxygen consumption. This result clearly indicates the requirement for calcium, but does not locate its site of action.

Sodium transport in frog skin and toad bladder can be measured as the short circuit current (SCC). Unfortunately, when the epithelia are placed in calcium-free solutions with EGTA the cells become separated and SCC disappears. To overcome this difficulty the epithelia were bathed on the serosal surface with solutions containing calcium, while the mucosal solutions were calcium-free and contained EGTA. Under these conditions the epithelia continued to transport sodium. Dose-response curves relating SCC to amiloride concentration were obtained in the presence and absence of calcium. The curves obtained in the absence of calcium were very flattened compared to those obtained in the presence of calcium.

The data are consistent with the assumption that amiloride can block the entry of sodium to the transport mechanism only when it forms part of a ternary complex with

calcium and the receptor. The fraction of receptors in the ternary form is given by p, where

$$p = \frac{AM\,K_2K_3}{1+AK_1+MK_2+AM\,K_2K_3}$$

and where A and M are the concentrations of amiloride and calcium respectively, and where K_1 and K_2 are the affinity constants for amiloride and calcium with the receptor and K_3 is the affinity constant for amiloride and the receptor-metal complex. Other ions (Ln^{3+}, Mn^{2+}, Mg^{2+}, Sr^{2+}) are able to substitute for calcium.

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Distribution of bound ^{3}H-benzilylcholine mustard in subcellular fractions of smooth muscle from guinea-pig ileum

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Studies on the uptake of the irreversible muscarinic blocking agent benzilylcholine mustard (BCM) (Gill & Rang, 1966) indicated that it would be a useful label for the muscarinic receptor (Rang, 1967). Preliminary experiments on the isolation of receptor material labelled with tritiated BCM are described.

Longitudinal muscle strips from guinea-pig ileum were exposed to 2×10^{-9} M ^{3}H-BCM for 30 min in Krebs solution at 37°C and then washed for 30 minutes. Pharmacological experiments show that this treatment causes about 95% receptor blockade.

The strips were then homogenized in 0·3 M sucrose and fractionated by differential centrifugation to yield three precipitates containing debris (P1; 3×600 *g* for 20 min); mitochondria (P2; $2\times10{,}000$ *g* for 20 min) and microsomes (P3; 100,000 *g* for 1 h) and a high-speed supernatant (SN3). Initially almost half of the protein and radioactivity was found in P1; however, all but about 8% of the activity could be removed from P1 by resuspending it and sonicating the suspension for 12 seconds. The microsomal fraction (P3) contained about 47% of the radioactivity and 11% of the protein. The distribution of acetylcholinesterase (Ellman, Courtney, Andres & Featherstone, 1961) and the membrane marker 5′-nucleotidase (Ipata, 1967; Song & Bodansky, 1967) closely paralleled that of the radioactivity in all fractions, suggesting that the radioactive label was attached mainly to cell membranes.

If 30 nM atropine is present during labelling very few receptors should be labelled by BCM, any uptake being due mainly to non-specific sites. Under these conditions it was found that uptake was reduced by 70%. The residual activity was distributed in the same way as the specific label which suggests that the non-specific sites were also on the membrane.

The P3 pellet was dissolved in 1% sodium dodecyl sulphate (SDS). Electrophoresis at pH7 on 5% polyacrylamide gels containing 1% SDS (Maizel, 1969) revealed three