concentration of AChM, [A], by the expression (Kitz & Wilson, 1962):

$$1/k_{app} = 1/k_2 + \frac{1}{k_2 K_a} \cdot \frac{1}{[A]}$$

where $K_a = k_1/k_{-1}$ the apparent binding affinity of AChM.

Experiments were carried out using strips of the longitudinal muscle from guinea-pig small intestine. The equipotent molar ratio for AChM compared to ACh was 4. To determine the free receptor fraction before and after treatment with AChM we have measured the extent of the binding of [^{3}H]-propylbenzilycholine mustard ([^{3}H]-PrBCM), an irreversible muscarinic antagonist (Burgen, Hiley & Young, 1973). The strips were exposed to various concentrations (10^{-6} M to 5×10^{-5} M) of the aziridinium ion form of AChM in Krebs-Henseleit solution at 30°C for a given time in the presence of eserine (10^{-6} M), washed for 30-40 min and then exposed to 2.4 nM [^{3}H]-PrBCM for 10 minutes. The extent of inhibition of [^{3}H]-PrBCM uptake was not significantly altered on varying the washing period after AChM between 1 and 120 minutes. Approximately 30% of the uptake of [^{3}H]-PrBCM could not be blocked by AChM. This has been taken to represent the non-specific portion of the [^{3}H]-PrBCM binding. A plot of $1/k_{app}$ against $1/[A]$ yielded a reasonable straight line, from which values of 1×10^5 M^{-1} for K_a and 2×10^5 s^{-1} for k_2 were derived. The value for K_a is comparable with the value of 1.1×10^5 M^{-1} for ACh deduced from its action as a competitive inhibitor of [^{3}H]-PrBCM uptake (Burgen, Hiley & Young, 1973).

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Factors contributing to the binding of [^{3}H]-5-hydroxytryptamine to butanol extracts of rat brain

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Fiszer & De Robertis (1969) reported the binding of 5-hydroxytryptamine (5-HT) to butanol extracts of mammalian brain and in view of the potential importance of this finding to the isolation and characterization of drug receptors we have investigated some of the parameters which control this binding.

Water washed rat mid-brain was suspended in 50% w/v sucrose and extracted into butanol at room temperature. After washing the extract with deionized water the butanol extracts were dehydrated *in vacuo* at 35°C. Aliquots (6 ml) were incubated with [^{3}H]-5-HT (5×10^{-7} M) at room temperature for 10 min and loaded onto a column of LH_{20} Sephadex equilibrated in chloroform. Radioactivity was eluted with a discontinuous gradient of chloroform: methanol of increasing polarity (Fiszer & De Robertis, 1969). Fractions (5 ml) were collected and sampled for radioactivity, protein and phosphorus. A typical elution pattern for [^{3}H]-5-HT is shown in Figure 1. The

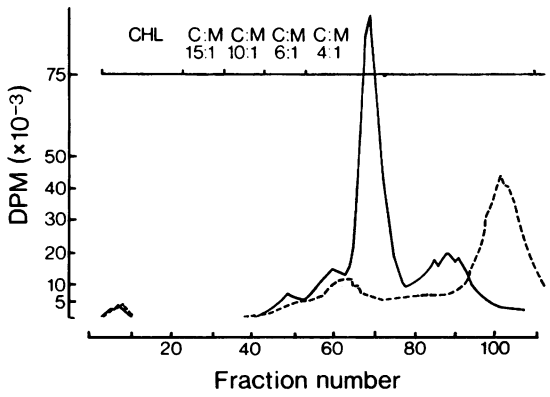

Fig. 1 Hydrophobic chromatography on LH_{20} Sephadex of a butanol extract of rat brain labelled with [^{3}H]-5-HT. Dotted line, butanol; solid line, butanol extract of brain. [^{3}H]-5-HT concentration 5×10^{-7} M. Fractions 5 ml. Discontinuous gradient of chloroform : methanol (C : M).

recovery of radioactivity from the column varied from 75-85% of that added to the butanol. The degree of hydration of the butanol extract has a marked effect on both the 'binding' of 5-HT to the butanol extract and its behaviour during column chromatography. In the presence of excess water no selective binding of 5-HT could be seen, whereas, after dehydration of the butanol extract a discrete peak of radioactivity could be eluted from the column. However, the best results were obtained following the addition of 6% water to the butanol, which resulted in a sharp peak of radioactivity clearly distinguished from that for 'free' [^{3}H]-5-HT (Figure 1). The peak of radioactivity is reduced in a concentration-dependent manner by the addition of lysergic acid diethylamide ($3.2 \times 10^{-7} - 2.6 \times 10^{-6}$ M) to the butanol extract before the binding of [^{3}H]-5-HT. Further investigations have involved biochemical studies to characterize the component(s) which are essential for the observed binding of 5-HT to butanol extracts of rat brain.

S.G. is an M.R.C. Scholar.

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A two-state (allosteric) model for sodium channels in transporting epithelia

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Recent results with activators and inhibitors of sodium transport in epithelia have exposed some problems in drug mechanisms. Using a labelling technique (Cuthbert, 1973) the number of functional sodium channels in the mucosal surface of frog skin was measured before and after treatment with vasopressin, a hormone known to increase mucosal sodium permeability. No apparent increase in channel density was observed (Cuthbert & Shum, 1974) yet the nominal current passing through the channels was increased.

Some indication that the channels were modified by hormone was obtained using amiloride, an agent known to block sodium channels in frog skin and to interact competitively with sodium for the channel. After hormone treatment the concentration-inhibition curve for amiloride was moved to the right in a parallel manner.

Consideration of the experimental findings showed that they could not be reconciled with the Michaelis-Menten approach to receptor interactions. Thus the possibility was explored that the channels could exist in 'open' and 'closed' forms and that activators and inhibitors of transport at the mucosal surface might operate by altering the proportions of 'open' to 'closed' forms at any instant.

The allosteric model outlined below has properties which are consistent with the experimental findings. Suppose channels exist in closed (T) and open (R) forms, and let amiloride have a higher microscopic affinity constant for the T form, and let the agent generated by the hormone have a higher microscopic affinity constant for the R form. Sodium ions are assumed to bind equally to both R and T forms, and to the same sites as amiloride. Translocation of sodium will only occur with the R (open) form of the channel. Thus amiloride is a competitive inhibitor, and the hormonal agency an allosteric activator of sodium ion translocation. The model can be described by the equation (Monod, Wyman & Changeux, 1965).

$$\bar{R} = \frac{1}{1 + \frac{T_0}{R_0}\left[\frac{1 + c\alpha + d\beta}{1 + \alpha + \beta}\right]^m \left[\frac{1 + e\gamma}{1 + \gamma}\right]^n}$$

where $\bar{R}$ is the proportion of channels in the open form. The constants c, d and e are the ratios of the dissociation constants for the R and T forms of the channel (K_R/K_T) with amiloride, sodium and the hormonal agency respectively, and where α, β and γ are the ratios of concentrations to the dissociation constants (K_R) for the same three substances respectively. T_0 and R_0 are the proportions of the two forms of the channel in the absence of ligands, and m and n refer to the number of binding sites.

It should be emphasized that although the theory used in adequate for the data, it is not unique.

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