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PHARMACOLOGY OF 5-HYDROXYTRYPTAMINE RECEPTORS IN FROG SKIN EPITHELIUM

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1 5-Hydroxytryptamine (5-HT) stimulates active sodium transport and decreases the passive mucosal to serosal chloride permeability across frog skin. The relative importance of the different regions of the 5-HT molecule in the mediation of these responses has been studied using a range of structurally related compounds.

2 Substitution in the ethyl amine side chain of 5-HT (5-hydroxytryptophan) results in decreased receptor affinity and intrinsic activity; removal of the side chain (5-hydroxyindole) abolishes activity. Methoxy substitution of the 5-OH moiety of 5-HT has no effect on intrinsic activity but reduces affinity; displacement of the hydroxyl group to position 6 diminishes intrinsic activity and affinity.

3 It is concluded that both of the 5-HT-induced physiological effects are mediated via a single receptor which is distinct from α- and β-adrenoceptors.

Introduction

In a recent study (Dalton, 1977a) it was shown that 5-hydroxytryptamine (5-HT) has two effects on ion transport across isolated skin of the frog: it stimulates active sodium transport and decreases the passive chloride permeability in the mucosal to serosal direction. The purpose of the present work was to study the relative importance of the various regions of the 5-HT molecule in determining its potency and intrinsic activity and to elucidate whether the dual action of 5-HT on ion transport is mediated via a single receptor, as the dual action of 5-HT on insect salivary glands appears to be (Berridge & Prince, 1972), or mediated via different receptors as are the multiple effects of 5-HT on postsynaptic membranes (Gerschenfeld & Paupardin-Tritsch, 1974).

Methods

Frogs, *Rana temporaria*, were rapidly pithed. The ventral skin was removed, stretched across a double Ussing-type chamber and incubated in Ringer solution (composition (mEq/l): Na^+ 113.5, K^+ 3.5, Cl^- 116.5, HCO_3^- 2.4, Ca^{2+} 0.89, pH 7.8) aerated with washed compressed air. After 45 min the Ringer solution was renewed and after a further 15 min a standard amount of the compound under test, dissolved in Ringer, was added to the serosal medium of one half chamber, the other half acting as control. All compounds tested (5-hydroxytryptamine creatinine sulphate complex, tryptamine hydrochloride, 5-methoxytryptamine hydrochloride, 6-hydroxytryptamine creatinine sulphate complex, 5-hydroxytryptophan, tryptophan, gramine, 5-hydroxyindole, adrenaline, yohimbine hydrochloride and dichloroisoprenaline hydrochloride) were obtained from Sigma Co., freshly prepared before use since it has been shown that 5-HT rapidly loses its activity in solution (Dalton, 1976) and titrated to pH 7.8. Short circuit current (s.c.c.) and membrane potential were measured by conventional techniques (Ussing & Zerahn, 1951); s.c.c. readings were corrected to unit area of skin ($\mu A/cm^2$).

Competitive inhibition by 5-hydroxyindole (5-OH indole) and ethyl amine was studied by adding 5-OH indole or ethyl amine to the serosal medium of preincubated frog skin followed by standard agonist (5-HT, tryptamine or 5-hydroxytryptophan (5-HTP)).

The effect of adrenoceptor stimulation on the 5-HT response was investigated by adding standard adrenaline (concentration range 10^{-7} M to 10^{-4} M) to the serosal medium of preincubated frog skin followed, at maximum response, by 5-HT 10^{-5} M.

To test the effect of adrenoceptor blockade, frog skin was preincubated in the normal way and then either yohimbine (10^{-3} M, α-blocking agent) or dichloroisoprenaline (10^{-3} M, β-blocking agent) added to the serosal medium of both half chambers; after 20 min 5-HT 10^{-5} M was added to the serosal medium of one half chamber and adrenaline 10^{-6} M to the other. Dichloroisoprenaline was used in preference to propranolol since while it does not exhibit agonistic properties on frog skin at the concentration used, it does antagonize (competitively) β-adrenoceptor

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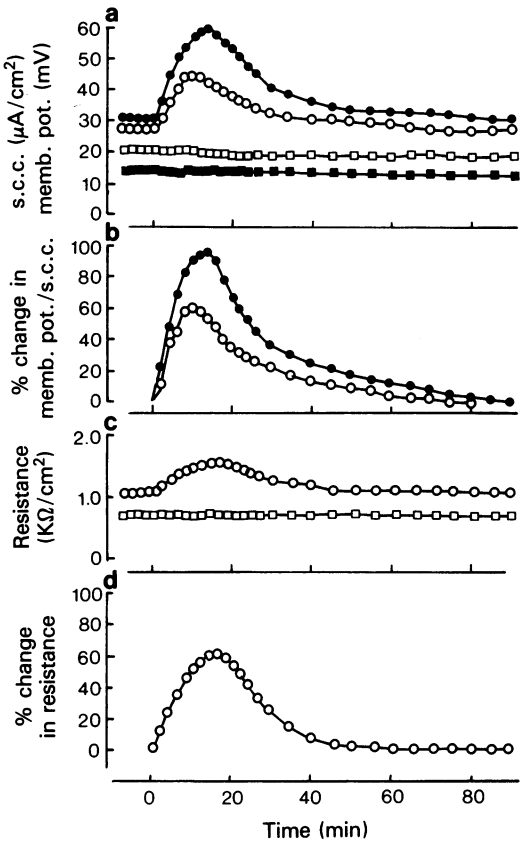

Figure 1 The response of frog skin to the serosal addition of 5-hydroxytryptamine (5-HT) 10^{-5} M. (a) Shows the change in membrane potential and s.c.c. in control tissue: memb. pot. (■); s.c.c. (□); and in response to the addition, at time zero, of 5-HT 10^{-5} M: memb. pot. (●); s.c.c. (○). (b) Shows the response to 5-HT plotted as the % change in membrane potential (●) and s.c.c. (○). (c) Shows the change in transepithelial resistance, calculated from the membrane potential and s.c.c. by Ohm's Law, in control tissue (□) and in response to the addition of 5-HT 10^{-5} M (○). (d) Shows the resistance response to 5-HT 10^{-5} M plotted as the % change. Each point represents the mean of 8 experiments.

responses; incubation of frog skin with propranolol leads to an irreversible decline in s.c.c. and increase in transepithelial resistance (unpublished observations).

Results

The s.c.c. and transepithelial resistance across frog skin increase on the application of 5-HT to the serosal medium (Figure 1). It has been shown (Dalton, 1977a) that in the absence of chloride ions from the incubation medium 5-HT increases both s.c.c. and membrane potential but there is no change in transepithelial resistance; thus it was shown that the increase in s.c.c. is equal to the increase in active sodium transport and the total increase in transepithelial resistance is equal to the decrease in mucosal to serosal chloride permeability. Responses were calculated as the percentage change in s.c.c. and resistance relative to the control tissue; concentration-response curves were obtained by plotting the maximum change in s.c.c. and resistance vs. log concentration of the compound inducing the observed change. Figure 2 indicates that compounds structurally related to 5-HT induced similar responses but varied in their concentration-response characteristics. The potency (pD_2 values) and relative activities (expressed as equi-potent molar ratios) of the compounds tested are summarized in Table 1.

As indicated in Figure 2 the concentration-response characteristics of the s.c.c. (sodium transport) and resistance (mucosal-serosal permeability) changes induced by the addition of agonist were very similar: removal of the 5-OH group of 5-HT (tryptamine) or its substitution (5-methoxytryptamine) resulted in a decreased affinity for the receptor but had little effect on the intrinsic activity. However, displacement of the −OH group to position 6 resulted in decreased affinity and intrinsic activity. Removal of the ethyl amine side chain (5-OH indole) resulted in a total loss of activity whilst alteration of the side chain (5-HTP) resulted in a diminished affinity and intrinsic activity. Removal of the 5-OH group and alteration of the side chain (tryptophan and gramine) resulted in a very low affinity and intrinsic activity.

5-OH indole, whilst having no agonistic effects on frog skin acted as an inhibitor of 5-HT, tryptamine and 5-HTP. Concentration-response curves for these agonists were obtained in the presence of 3×10^{-5} M, 10^{-4} M, 3×10^{-4} M, 10^{-3} M and 3×10^{-3} M concentrations of 5-OH indole; Figure 3 shows that in the presence of increasing concentrations of 5-OH indole the concentration-response curves for 5-HT were shifted to the right along the concentration axis and there was a decrease in the maximal response exhibited by the tissue. However, even in the absence of 5-OH indole the magnitude of the 5-HT responses diminished at concentrations greater than 10^{-5} M. At concentrations above 10^{-4} M 5-HT, s.c.c. is further inhibited but resistance increases.

Ethyl amine, tested at the same concentrations as 5-OH indole, had no effect on the responses of frog skin to 5-HT, tryptamine or 5-HTP. Figure 4 shows Schild plots (Schild, 1947; 1957) for the action of 5-OH indole on the 5-HT responses. Similar plots were obtained for the action of 5-OH indole on the tryptamine and 5-HTP responses; Table 2 shows that

5-OH indole was an equipotent inhibitor of the 5-HT, tryptamine and 5-HTP responses.

The effect of adrenaline on the magnitude of the 5-HT responses is shown in Figure 5. Fassina, Carpendo & Fiandini (1968) have shown that the effective adrenaline concentration range in stimulating s.c.c. is between 10^{-8} M and 2×10^{-6} M, higher concentrations lead to auto-inhibition in a concentration-dependent fashion; thus two concentrations of adrenaline were chosen in the lower dose range (10^{-7} M and 10^{-6} M) and two concentrations in the auto-inhibitory range (10^{-5} M and 10^{-4} M). At all concentrations tested, adrenaline induced an increase in s.c.c. across the tissue and a transient increase in transepithelial resistance but no change in the magnitude of either the s.c.c. or resistance response to 5-HT could be detected.

Yohimbine (Figure 6) inhibited the resistance response to adrenaline and reduced the s.c.c. response to adrenaline. Dichloroisoprenaline inhibited the s.c.c. response to adrenaline but had little effect on the resistance response to applied adrenaline; neither compound affected the 5-HT responses.

Discussion

In these experiments it was not possible to separate the active sodium transport response of 5-HT from its chloride permeability response: either both responses occurred or neither. The binding characteristics of the receptor inducing the sodium transport response are identical with the binding characteristics of the receptor inducing the chloride permeability response and the ability of the receptor-hormone complex(es) to catalyse the two responses is the same so that it seems likely that the mediation of both responses is via a single receptor. However, the two responses do not share a common mode of action (Dalton, 1977a): increased sodium transport is associated with an increase in the mucosal permeability to sodium ions whilst the reduction in chloride permeability is associated with an influx of calcium ions across the mucosal membrane which then appear to act as a secondary messenger in mediating the response. Binding of an active compound to the 5-HT receptor thus induces, on the one hand, a decrease in the mucosal permeability barrrier to sodium ions

Table 1 Potency (pD_2 values) and relative activities (expressed as equi-potent molar ratios relative to 5-hydroxytryptamine (5-HT)) of 5-HT analogues in stimulating s.c.c. (active sodium transport) and increasing transepithelial resistance (decrease in passive chloride permeability) across frog skin

	Short-circuit current		*Transepithelial resistance*	
Reference agonist	*Potency of agonist: pD_2*	*E.p.m.r.*	*Potency of agonist: pD_2*	*E.p.m.r.*
5-HT	5.30	1.0	5.25	1.0
Tryptamine	4.85	2.8	4.77	3.0
5-CH_3O-tryptamine	4.45	7.0	4.48	6.0
6-HT	4.30	10.0	4.40	7.0
5-HTP	4.30	10.0	4.35	8.0
Tryptophan	3.85	28.0	3.90	23.0
Gramine	3.85	28.0	3.90	23.0
5-OH indole*	0	0	0	0
Ethyl amine*	0	0	0	0

* Either alone or in combination.

Table 2 Competitive inhibition by 5-hydroxyindole of the 5-hydroxytryptamine (5-HT), tryptamine and 5-hydroxytryptophan (5-HTP)-induced s.c.c. and transepithelial resistance responses in frog skin

	Potency of 5-OH indole antagonism			
Reference agonist	*Short-circuit current*		*Transepithelial resistance*	
	pA_2	*Slope of Schild plot*	pA_2	*Slope of Schild plot*
5-HT	3.62	1.10	3.66	1.07
Tryptamine	3.76	1.14	3.69	1.10
5-HTP	3.84	1.05	3.51	1.08

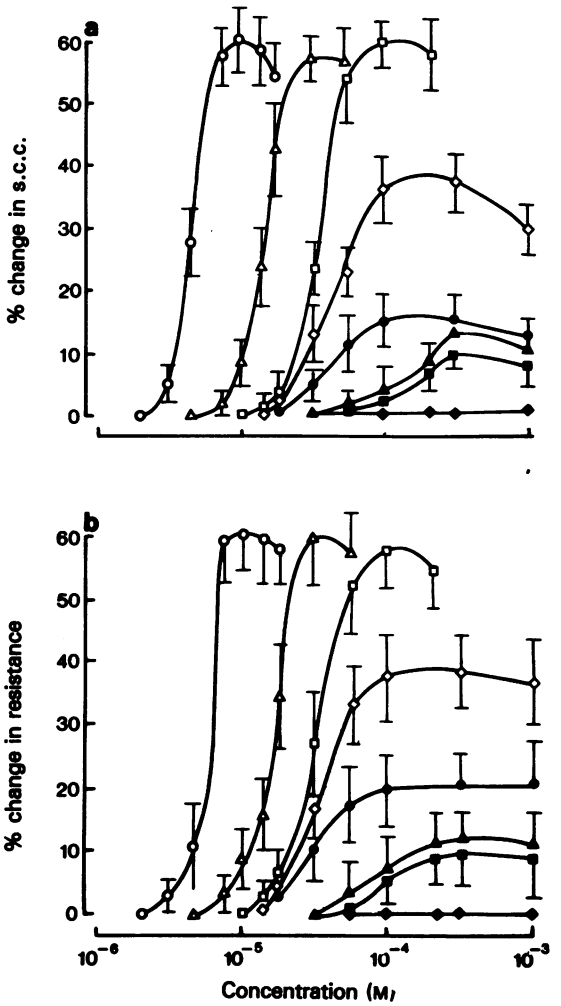

Figure 2 The concentration-response characteristics of different compounds in increasing s.c.c. (a) and transepithelial resistance (b) measured across frog skin. Responses are expressed as the maximal % increase in s.c.c. and resistance induced by a standard concentration of test compound: 5-hydroxytryptamine (○); tryptamine (△); 5-methoxytryptamine (□); 6-hydroxytryptamine (◇); 5-hydroxytryptophan (●); tryptophan (▲); gramine (■); 5-hydroxyindole/ethyl amine—either singly or in combination (◆). Each point represents the mean of 8 experiments; vertical lines show s.e.mean.

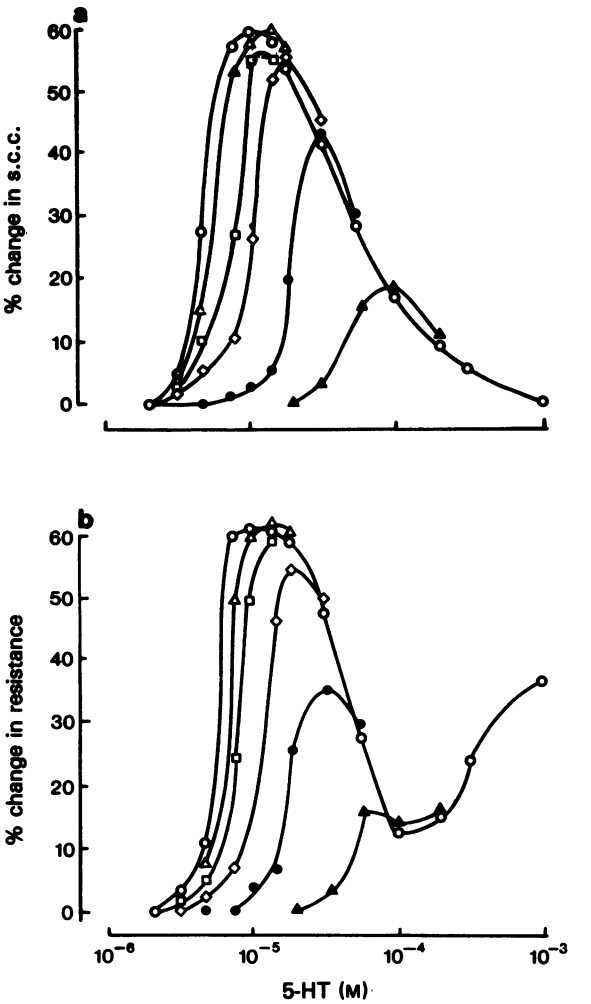

Figure 3 Inhibition of the 5-hydroxytryptamine (5-HT) responses on s.c.c. (a) and resistance (b) across frog skin by 5-hydroxyindole. The graphs are the concentration-response curves for 5-HT in the absence (○) and in the presence of 3×10^{-5} M (△); 10^{-4} M (□); 3×10^{-4} M (◇); 10^{-3} M (●) and 3×10^{-3} M (▲) 5-hydroxyindole. Each point represents the mean of 8 experiments.

and, on the other hand, an influx of calcium ions across the mucosal border.

The inability of ethyl amine and 5-OH indole to stimulate either physiological response could be due to the failure of the compounds to bind to the receptor(s) or to the inability of the receptor-hormone complex to catalyse the responses. In the latter case the compound should act as a competitive inhibitor of the response(s) to active compounds. It was found that ethyl amine did not inhibit either the sodium transport or the chloride permeability response of frog skin to the agonists tested but that 5-OH indole was an inhibitor of both responses. The concentration-response curves of the agonists 5-HT, tryptamine and (partial agonist) 5-HTP were shifted to the right along the concentration axis and the maximal response was diminished in the presence of

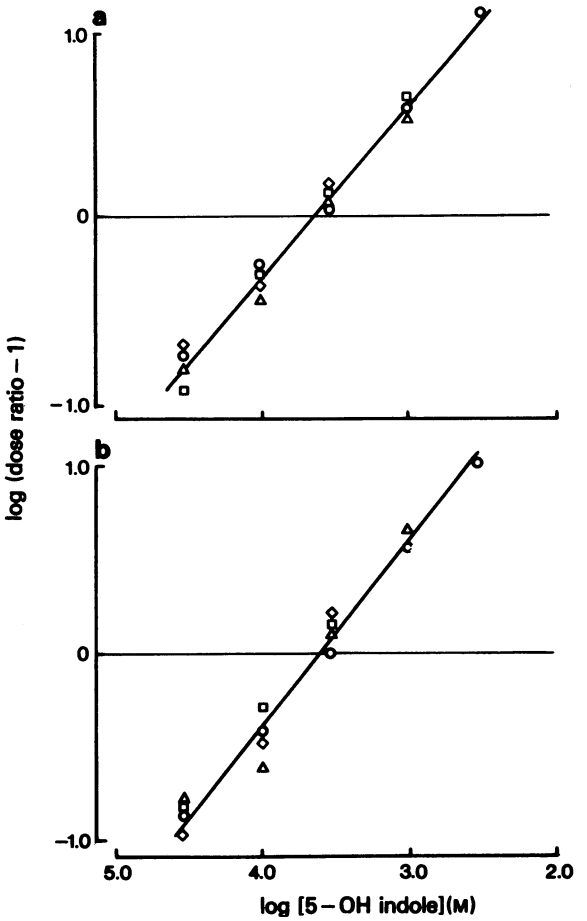

Figure 4 Schild plots for the inhibition of the 5-hydroxytryptamine (5-HT) responses on s.c.c. (a) and resistance (b) across frog skin. Dose-ratio is the concentration of 5-HT producing a given response in the presence of 5-hydroxyindole (5-OH indole) relative to the concentration of 5-HT alone which produces the same response. Values correspond to 12.5% (○); 25% (△); 37.5% (□) and 50% increases in s.c.c. and resistance. The lines drawn are calculated by regression.

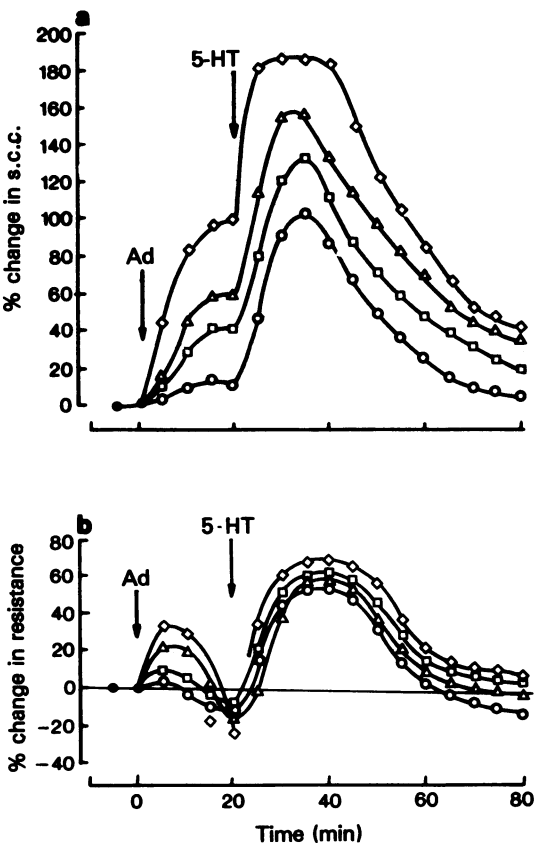

Figure 5 The effect of adrenaline (Ad) on the magnitude of the increase in s.c.c. (a) and resistance (b) induced by 5-hydroxytryptamine (5-HT) across frog skin. Adrenaline at the concentrations 10^{-7} M (□); 10^{-6} M (◇); 10^{-5} M (△) and 10^{-4} M (○) was added to the serosal medium at time zero, followed by 5-HT 10^{-5} M at maximal stimulation. Each point represents the mean of 5 experiments.

increasing concentrations of 5-OH indole. This diminished maximal response in the presence of 5-OH indole suggests that the inhibition may be non-competitive, however, as already noted the maximal response diminished in the absence of 5-OH indole. These observed actions of 5-HT creatinine sulphate complex have been shown to be due to the separate components of the complex (Dalton, 1977a): in the concentration range 10^{-5} M to 10^{-4} M, auto-inhibition of the 5-HT response occurs whilst at concentrations greater than 10^{-4} M, creatinine sulphate inhibits sodium transport and decreases the chloride permeability. These observations together with the observed unity slope of the Schild plots are taken to indicate that inhibition by 5-OH indole is competitive; furthermore it is an equipotent competitive inhibitor i.e. exhibits the same receptor binding affinity, for both responses of all the agonists tested as evidenced by the minimal variation in pA_2 value. The failure of ethyl amine to antagonize the 5-HT responses probably reflects a very low affinity for the compound and consequently its inability to bind to the receptor. Since ethyl amine was inactive as an agonist even when tested in combination with 5-OH indole the activity of 5-HT and analogues must depend upon these moieties being chemically combined; furthermore, the receptor appears to have

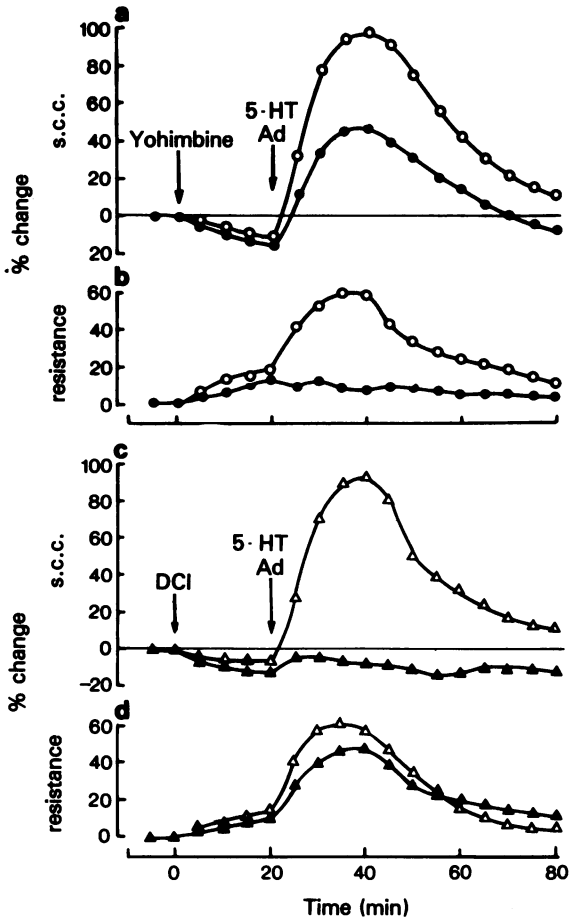

Figure 6 The effect of yohimbine (α-adrenoceptor blocking agent; a and b) and dichloroisoprenaline (β-adrenoceptor blocking agent; c and d) on the s.c.c. and resistance responses induced by 5-hydroxytryptamine (5-HT) and adrenaline (Ad) across frog skin. Yohimbine (10^{-3} M) or dichloroisoprenaline (DCI, 10^{-3} M) was added to the serosal medium at time zero followed, after 20 min, by either 5-HT (10^{-5} M; open symbols) or adrenaline (10^{-6} M; closed symbols). Each point represents the mean of 4 experiments.

rather exacting spatial requirements for activity at the 5- position of the indole nucleus, removal of the 5-OH group (tryptamine) or its substitution (5-methoxytryptamine) led to a decrease in affinity but little change in intrinsic activity; however, displacement of the -OH group to position 6 resulted in a much decreased affinity and intrinsic activity. Such a result has been observed in the Malpighian tubules and cuticle of the insect *Rhodnius* (Maddrell Pilcher & Gardiner, 1971; Reynolds, 1974) but this is not the case in most tissues studied e.g. rat stomach strip (Vane, 1959; Offermeier & Ariens, 1966), rat uterus (Barlow & Khan, 1959), molluscan heart (Greenberg, 1960), salivary glands of the blowfly, *Calliphora* (Berridge, 1972; Dalton, 1977b), pigeon erythrocytes (Campbell & Siddle, 1977)—substitution at position 5 of the indole nucleus or displacement of the 5-OH group in these tissues leads to decreased receptor affinity but the compounds still effect a maximal response.

Some 5-HT receptors, notably the *Calliphora* salivary gland receptor, integumental receptors in *Rhodnius* and pigeon erythrocyte receptors show affinity for other biogenic amines particularly the catecholamines which have been shown to compete for the receptor so that the possibility of 5-HT acting via adrenaline receptors in frog skin was investigated. Adrenaline and 5-HT induce similar effects in frog skin in that both induce an increase in s.c.c. and transepithelial resistance (Figure 5; Watlington, 1968); frog skin contains both α- and β-adrenoceptors: α-site stimulation results in an increase in skin resistance whilst β-site stimulation results in an increase in s.c.c. Thus adrenaline, which binds to both sites (Watlington, 1968) induces a dual effect. As seen in Figures 5 and 6, adrenaline and 5-HT did not compete for either of the α- or β-sites and the α- and β-blockers whilst interfering with the adrenaline response, had no effect on the 5-HT responses indicating that the 5-HT receptor(s) is distinct from the α- and β-adrenoceptors. Such a result is not altogether surprising since the increase in transepithelial resistance on α-stimulation is associated with a decrease in mucosal-serosal sodium transport whereas the 5-HT-induced change results from an effect on chloride permeability. The increase in s.c.c. on β-site stimulation is associated with increased active chloride transport in the serosal-mucosal direction whereas the 5-HT-induced change is associated with increased mucosal-serosal sodium transport.

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