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PHARMACOLOGICAL INDUCTION OF PLASTICIZATION IN THE ABDOMINAL CUTICLE OF *RHODNIUS*

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SUMMARY

Injections of 5-hydroxytryptamine (5-HT, serotonin) are found to cause plasticization of the abdominal cuticle of *Rhodnius* larvae. This plasticization is a direct action of 5-HT on some element in the body wall; the central nervous system is not required. It is probable that 5-HT acts directly at a receptor on the epidermal cells. The relationship between structure and plasticizing activity for a number of 5-HT analogues has been investigated. The receptor resembles other 'classical' 5-HT receptors in its requirements, but is unlike the 5-HT/diuretic hormone receptor of *Rhodnius* Malpighian tubules.

INTRODUCTION

In the course of work on the control of Malpighian tubule function in *Rhodnius*, it was observed that injections of 5-hydroxytryptamine into the insect's haemolymph led to a plasticization of the abdominal cuticle, apparently identical to that which occurs when the insect feeds and which has been described by Bennet-Clark (1962). In this paper the results of a detailed study of this pharmacological induction of plasticization are presented. It will be shown that the action of 5-HT is independent of the central nervous system, and that it is very likely that 5-HT acts by mimicking the action of the transmitter normally released from the axon terminals of the abdominal body wall's nerve supply.

METHODS

All insects used were 5th instar *Rhodnius* taken from a large laboratory culture 1–2 weeks since their last ecdysis. In the construction of the dose–response curves all insects tested were from the same feeding batch, and all were tested on the same day so that variation between insects was reduced to a minimum.

The effects of drugs were tested by injecting a solution of the test substance in Ringer solution into the insect's haemolymph. The composition of the standard *Rhodnius* Ringer solution is given by Maddrell (1969). The pH of the test solution was in all cases adjusted to pH 7·0 after the addition of the drug by the addition of either HCl or NaOH. Test solutions were injected into the haemocoel via a metathoracic leg from an 'Agla' micrometer syringe, an SWG 28 hypodermic needle making a snug fit with the cut leg. In almost all cases 10 μl of solution was injected. The volume of the haemolymph before injection was taken to be 17·5 μl, a mean value determined for

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5th instar *Rhodnius* from the same culture about 10 days after their last ecdysis by measuring the dilution of [^{14}C]inulin injected into the haemocoel by the same method (B. O. C. Gardiner, personal communication). The concentrations of the injected test substances in the haemolymph were calculated from this figure.

The extensibility of the abdominal cuticle of insects injected with 5-HT was found to attain a maximum value after 20–30 min (see Fig. 1). Accordingly, insects were tested for their response to the drugs about 30 min after injection.

The extensibility of the abdominal integument was tested by cutting a loop from the body wall and measuring the rate of extension (creep) 30 sec after the application of a 5 g load to the loop. Details of the preparation of such cuticle loops may be found in another paper (Reynolds, 1974*a*), where the testing apparatus is also described. The rate of creep after 30 sec has been taken here to represent a measure of the degree of plasticization of the abdominal cuticle. It is not possible to relate this measure in any simple way to more conventional measures of a material's mechanical properties (its elastic modulus, for example), but it was considered that a measure of the ***rate*** of extension was most relevant to the insect's requirements in this matter. The method also has the advantage of great rapidity, which was of considerable importance when testing large numbers of experimental insects in a short time.

The drugs used were: 5-Hydroxytryptamine creatinine sulphate complex (Sigma), tryptamine HCl (Sigma), 5-Methyltryptamine HCl (Sigma), 5-Methoxytryptamine (Sigma), 5-Fluorotryptamine HCl (Fluka AG), 5-Hydroxytryptophan (Sigma), Tryptophan (Sigma), N-Acetyl,5-hydroxytryptamine (Sigma), N,N-Dimethyl,5-hydroxytryptamine bioxalate (Bufotenine) (K and K Labs, California), *N*,*N*-Dimethyltryptamine (Ralph N. Emmanuel), 2-Bromolysergic acid diethylamide bitartrate (BOL) (Koch-Light and Sigma), Dopamine HCl (BDH), Tyramine HCl (Koch-Light).

The results are expressed as a percentage of the maximum response obtainable from the particular batch of insects used, which was determined by testing the cuticular extensibility of insects injected with 5-HT at 10^{-4} M in Ringer solution. Zero response is defined as that given by insects injected with normal Ringer alone. Zero and maximum responses were determined for each experiment.

RESULTS

The response to 5-hydroxytryptamine

Small quantities of 5-HT dissolved in Ringer solution were found to induce plasticization of the abdominal cuticle on injection into the haemolymph of *Rhodnius* 5ths. The response to injected 5-HT is immediate, but does not become maximal until 20–30 min (see Fig. 1). The level of this maximum response is such that the extensibility of the abdominal cuticle is about the same as that shown by feeding insects. Fig. 2 shows a dose–response curve for the plasticizing effect of injected 5-HT. The abscissa shows the estimated concentration of 5-HT in the haemolymph after injection (see Methods).

The concentration required in the haemolymph to give a 50% response is about $3{\cdot}3 \times 10^{-7}$ M; the threshold for a response is about 5×10^{-8} M. The injection of 10 μl of Ringer solution alone caused no change in the mechanical properties of the cuticle a

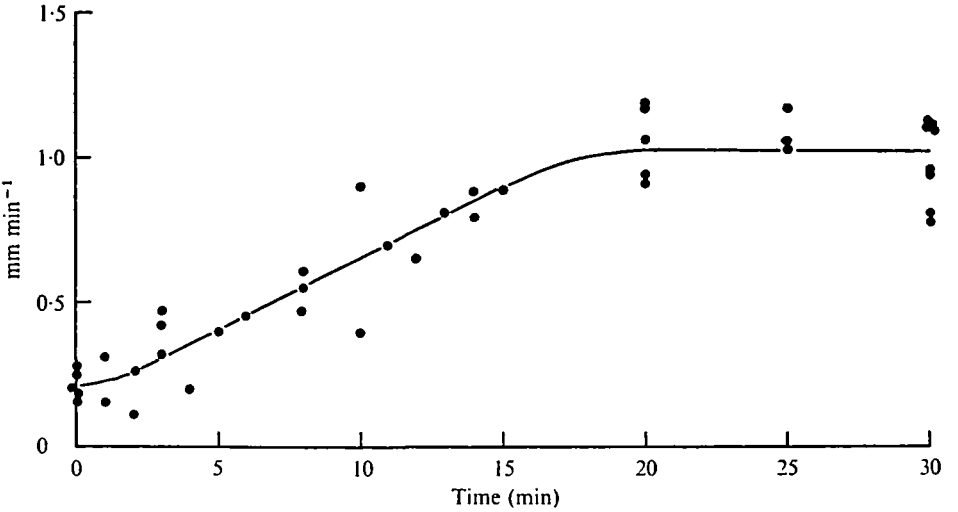


Fig. 1. The extensibility of the abdominal cuticle of insects injected with 10^{-4} M 5-HT in Ringer solution: time course. Injections were made at 0 min.

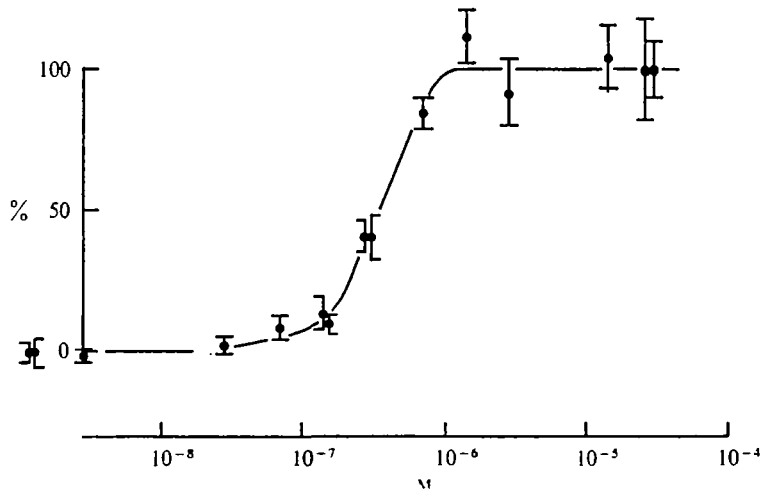


Fig. 2. The extensibility of the abdominal cuticle of insects injected with 5-HT dissolved in Ringer solution; dose–response curve. Cuticle samples were tested 30 min after injection. The points are mean values and are shown together with the extent of ± the standard error of the mean (S.E.).

compared with uninjected control insects. Injections of 10^{-4} M solutions of 5-HT in Ringer were routinely used to establish the maximal response that was available. Post-prandial plasticization of the cuticle is an active response of the epidermal cells (Bennet-Clark, 1962), being abolished by damage to the cells or by poisoning with inhibitors. Likewise, the plasticization response to 5-HT injections was completely inhibited by prior treatment with 10 mM sodium azide (see Table 1).

To exclude the possibility that 5-HT might act on the central nervous system to produce plasticization, a number of insects were taken and the nerve supply to the abdomen severed centrally. The operative incision was sealed and the insects were

Table 1. *Plasticization of* Rhodnius *abdominal cuticle: the effect of pre-treatment with sodium azide*

Azide (10^{-3} M) + 5-HT (10^{-4} M): creep rate = $0{\cdot}26 \pm 0{\cdot}07$ mm.min^{-1}.
Control 5-HT (10^{-4} M): creep rate = $1{\cdot}06 \pm 0{\cdot}18$ mm.min^{-1}.

Azide and 5-HT were injected in Ringer solution. Azide was injected about 5 min before 5-HT. The total volume injected was 10 μl. Means ± S.E.

Table 2. *Plasticization of* Rhodnius *abdominal cuticle: the effect of cutting the abdominal nerves*

Denervated: 5-HT (10^{-4} M): creep rate = $1{\cdot}14 \pm 0{\cdot}11$ mm.min^{-1}.
Control 5-HT (10^{-4} M): creep rate = $1{\cdot}19 \pm 0{\cdot}06$ mm.min^{-1}.

The denervation was performed about 1 h before injection of 5-HT, which was in Ringer solution. Control insects were sham operated. Means ± S.E.

allowed to recover. Subsequently, these insects were injected with 5-HT in Ringer solution (10^{-4} M), when their abdominal cuticles were found to become plasticized in the same way as those of unoperated insects. The success of the operation was checked on dissection (see Table 2). This experiment indicated that the action of 5-HT is directly on the abdominal body wall, not via the central nervous system. This does not, however, exclude the possibility that 5-HT might act on severed nerve endings still attached to the body wall – for instance, by causing the release of another factor stored in these nerve endings, which is in turn responsible for inducing plasticization of the cuticle.

An attempt was made to study the effects of 5-HT on an *in vitro* preparation of the abdominal integument. Loops of cuticle with epidermis attached were cut from the abdomen and were immersed in solutions of 5-HT in Ringer. The plasticizing effect produced was very much smaller than that which would have been produced by the same concentration of 5-HT *in vivo* (see Table 3). This may be understood by considering that the mechanism by which the cuticle is plasticized probably depends upon the modification of the composition of the aqueous phase inside the cuticle, in particular, upon the intracuticular pH. (Evidence for this statement is presented in another paper; Reynolds, 1974*b*.) The cut edges of the loops of cuticle used in these experiments represent two major diffusion pathways through which this controlled intracuticular environment may leak away, and by which the heavily buffered bathing medium may gain access to it. Hence, the epidermis is greatly hampered in its task of modifying this medium's composition in order to produce plasticization of the cuticle.

Nevertheless, despite the small magnitude of the response, a dose–response curve may be drawn up for the plasticizing effect of 5-HT on loops of abdominal cuticle *in vitro*. Its major features are similar to the curve for injected 5-HT, a 50% response being caused by about 6×10^{-7} M 5-HT. Cuticle loops stripped of the epidermis did not respond to 5-HT *in vitro* (see Table 3).

The effect of 2-Bromolysergic acid diethylamide

2-Bromolysergic acid diethylamide (BOL) has been found to be a potent inhibitory agent in most serotinergic systems in which it has been tested.

BOL in Ringer solution was injected about 25 min before the injection of 5-HT

Table 3. *Plasticization of* Rhodnius *abdominal cuticle: the response of cuticle loops to* 5-*HT* in vitro

(Loops were soaked for 30 min in the media shown, when they were tested for their extensibility. pH was 7·0 in all cases. Means ± S.E.)

Cuticle loops with epidermis intact:	
10^{-4} M 5-HT in Ringer:	creep rate = 0·28 ± 0·03 mm.min^{-1}
Ringer:	creep rate = 0·19 ± 0·01 mm.min^{-1}
Cuticle loops stripped of the epidermis:	
10^{-4} M 5-HT in Ringer:	creep rate = 0·10 ± 0·01 mm.min^{-1}
Ringer:	creep rate = 0·11 ± 0·01 mm.min^{-1}

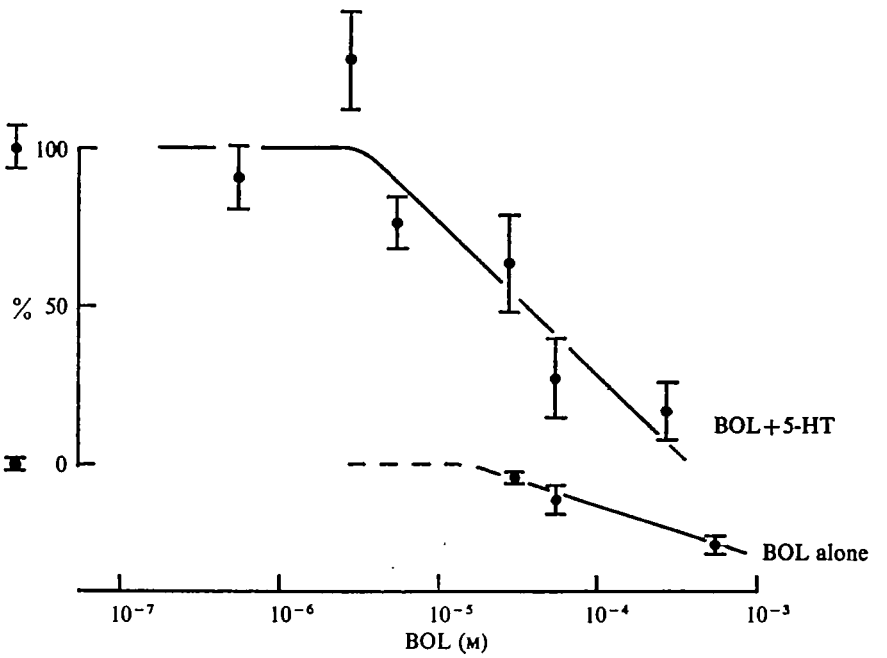


Fig. 3. The inhibition of the plasticization response to injected 5-HT by 2-bromolysergic acid diethylamide (BOL). BOL was injected in Ringer solution 25 min before 5-HT, also in Ringer solution. The abscissa shows the (calculated) final concentration of BOL in the haemocoel. The estimated final concentration of 5-HT was 5×10^{-5} M. The cuticle samples were tested 30 min after the injection of 5-HT. Means ± S.E.

(also in Ringer solution) into the haemocoel of *Rhodnius* 5th instar larvae. The final concentration of 5-HT in the haemolymph was estimated at 5×10^{-6} M. BOL was found to be antagonistic at relatively low levels to the plasticizing action of 5-HT. An estimated level of 5×10^{-5} M BOL in the haemolymph inhibited the response to 5-HT by more than 50 % (see Fig. 3). This action of BOL suggests that the action of 5-HT may be directly at a classical 5-HT receptor, rather than indirectly at a nerve terminal to produce the release of some other active substance.

BOL injected alone was found to cause the abdominal cuticle to become less extensible than normal, so that with an estimated 5×10^{-3} M BOL present in the haemolymph, the rate of creep after 30 sec under test was reduced by 25 % of the normal value (see Fig. 3). This suggests that there may be a tonic control of cuticular

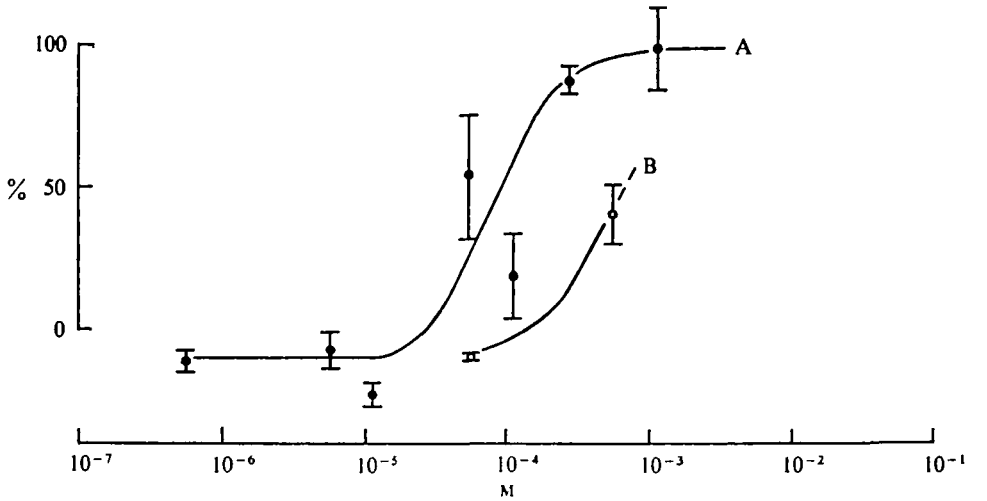


Fig. 4. Dose–response curve: (A) tryptamine, (B) 5-Methyltryptamine.

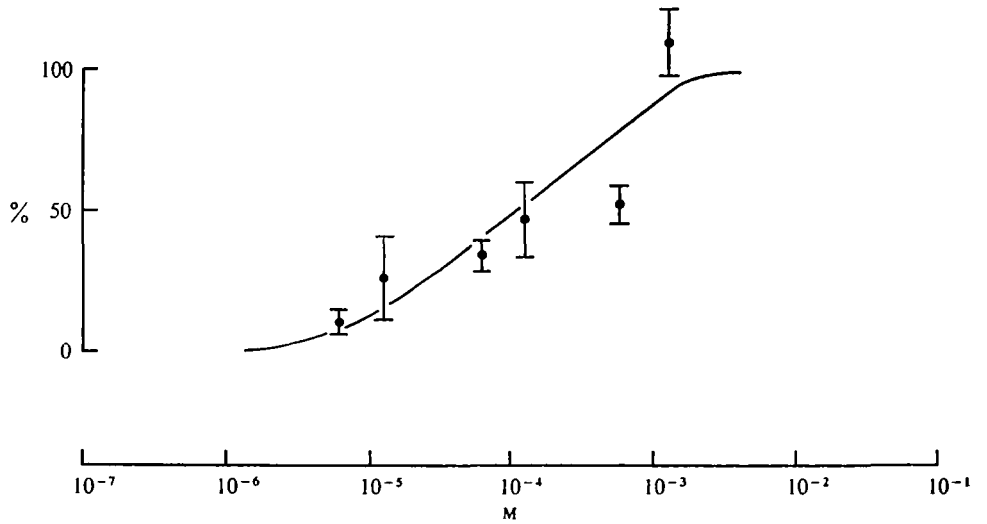


Fig. 5. Dose–response curve: 5-methoxytryptamine.

extensibility in the *Rhodnius* abdomen, and that this tonic control is antagonized by BOL. This, in turn, suggests that it is likely that 5-HT, BOL and the normal transmitter responsible for plasticization all act at the same site.

In order to investigate this possibility, a number of insects were pre-treated by injection with 10^{-3} M BOL in Ringer solution (10 μl). The insects were then given the opportunity to feed. They appeared to behave normally until presented to the living rabbit upon which they would normally have fed. However, although the treated insects showed initial excitement and extended their probosces, they seemed unable to probe normally and did not feed. Evidently the BOL treatment had deranged their feeding behaviour.

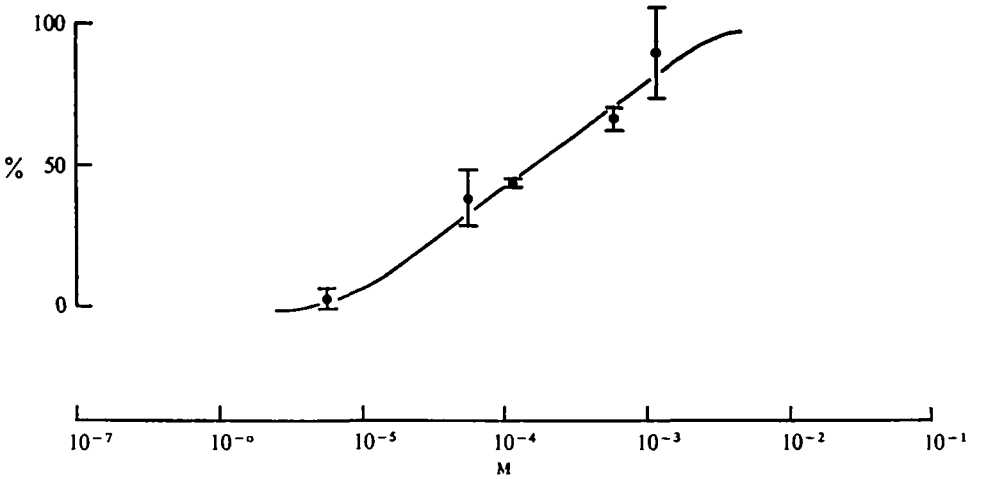


Fig. 6. Dose–response curve: 5-Fluorotryptamine.

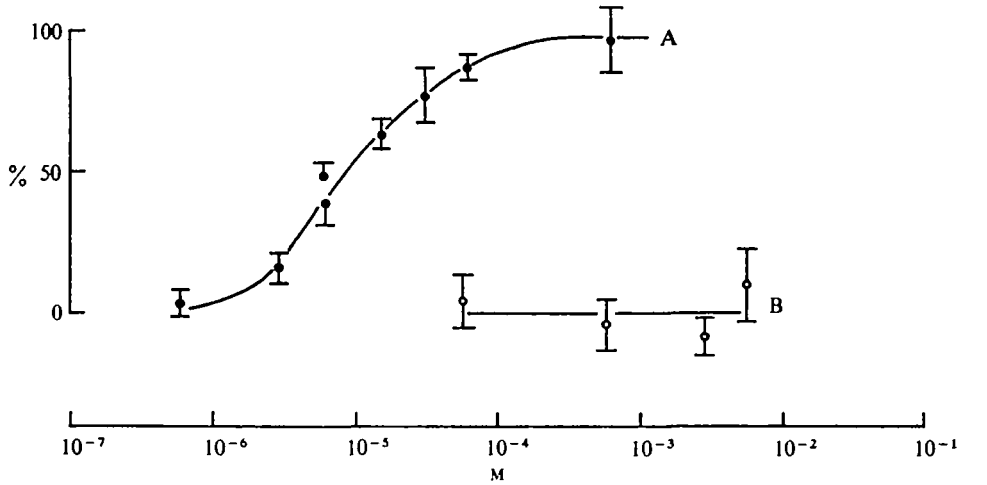


Fig. 7. Dose–response curve: (A) bufotenine, (B) *N*,*N*-dimethyltryptamine.

The structural requirements of the 5-HT receptor

A number of structural analogues of 5-HT were found to cause plasticization of the abdominal cuticle on injection in Ringer solution. The structural requirements for activity at the 5-HT receptor site were investigated by drawing up dose–response curves for these substances (Figs. 4–10).

(i) *Substitution at the 5-position* (see Table 4; Figs. 4–6)

Substitution of the hydroxy group at the 5-position resulted in a loss of activity in all cases, though all of the compounds tested here were active. Polar substituents at the 5-position (5-fluoro and 5-methoxytryptamine) did not seem to give markedly more activity than did non-polar ones (tryptamine and 5-methyltryptamine). Hydroxylation at the 5-position also increased the activity shown by ligands substituted at other sites; e.g. both tryptophan (Fig. 7) and *N*,*N*-dimethyltryptamine (Fig. 8) were inactive

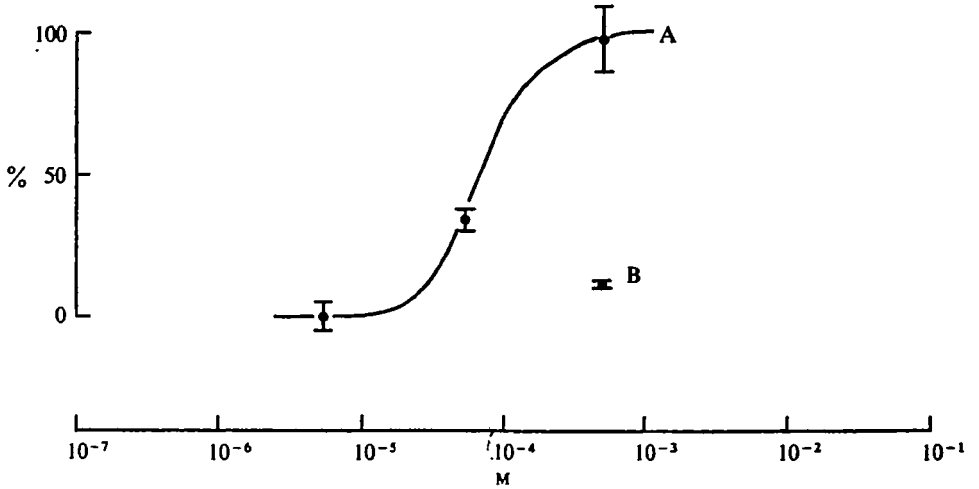


Fig. 8. Dose–response curve: (A) 5-hydroxytryptophan, (B) tryptophan.

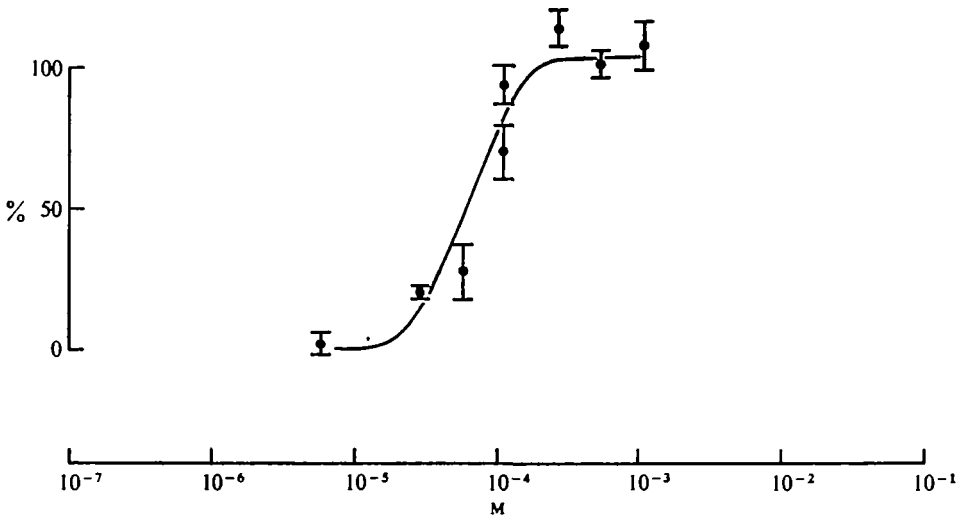
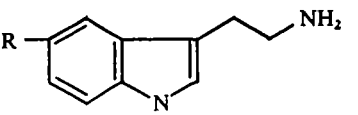


Fig. 9. Dose-response curve: *N*-acetyl,5-hydroxytryptamine.

Table 4. *Activities of 5-HT analogues substituted at the 5-hydroxy position*



		50% response at:
R=OH	5-Hydroxytryptamine	$3{\cdot}3 \times 10^{-7}$ M
R=H	Tryptamine	5×10^{-5} M
R=OMe	5-Methoxytryptamine	5×10^{-5} M
R=F	5-Fluorotryptamine	7×10^{-5} M
R=Me	5-Methyltryptamine	3×10^{-4} M

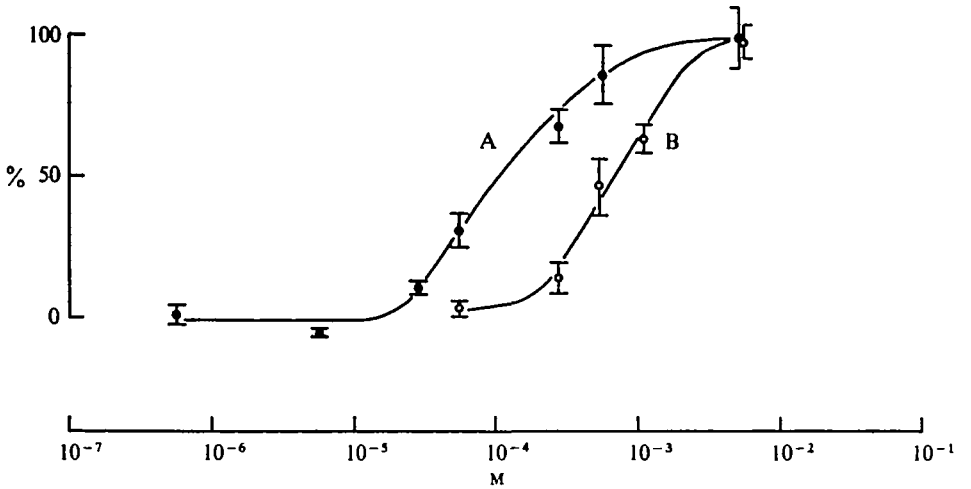
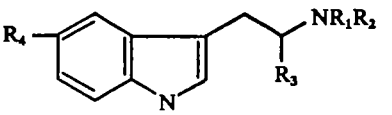


Fig. 10. Dose–response curve: (A) dopamine, (B) tyramine.

Table 5. *Activities of 5-HT analogues substituted at or near the terminal amino group*



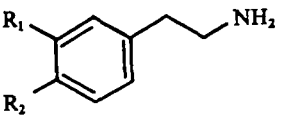
R_1	R_2	R_3	R_4		50% response at:
H	H	H	OH	5-Hydroxytryptamine	$3{\cdot}3 \times 10^{-7}$ M
Me	Me	H	OH	Bufotenine	$3{\cdot}7 \times 10^{-6}$ M
Me	Me	H	H	*N,N*-Dimethyltryptamine	—
$CO.CH_3$	H	H	OH	*N*-Acetyl, 5-hydroxytryptamine	$3{\cdot}0 \times 10^{-5}$ M
H	H	COOH	OH	5-Hydroxytryptophan	$3{\cdot}5 \times 10^{-5}$ M
H	H	COOH	H	Tryptophan	—

in the range of concentrations tested, whereas their 5-hydroxylated counterparts were active, giving a 50% response at $3{\cdot}5 \times 10^{-5}$ M (5-hydroxytryptophan), and at $3{\cdot}7 \times 10^{-6}$ M (bufotenine, *N,N*-dimethyl,5-hydroxytryptamine) (see section (ii) below).

(ii) *Substitution at or near the terminal amino group* (see Table 5; Figs. 7–9)

Substitution here impaired activity in all cases. The effect of methylation at the terminal amino group (bufotenine) appears less marked than does acetylation (*N*-acetyl,5-hydroxytryptamine) or the introduction of a carboxyl group into the aliphatic side chain near to the terminal amino group (5-hydroxytryptophan). In the former case there would be steric hindrance of the terminal charged group's access to a binding site for that group, whereas with the introduction of a polar group, as in the second two cases, there would be an additional effect on the charge distribution within the molecule. It is known that intramolecular hydrogen bonding occurs in tryptophan, which would also severely restrict the free movement of the aliphatic side chain of the molecule.

Table 6. *Activities of other aromatic amines*



R_1	R_2		50% response at:
OH	OH	Dopamine	6×10^{-5} M
H	OH	Tyramine	4×10^{-4} M

(iii) *Other aromatic amines* (see Table 6; Fig. 10)

The ability of these drugs to cause plasticization implies that the receptor at which they act is not very specific in its requirements for the indole nucleus of 5-HT. Here, the nucleus is aromatic and flat, as it is in the indole case, but a nuclear nitrogen is lacking and the shape of the nucleus is quite different. The two other requirements of the receptor, as explored above, i.e. an aliphatic amino group and a nuclear hydroxyl group, are both present, however, allowing these molecules to be active at the receptor site.

(iv) *Antagonists*

Some substances which showed no agonistic activity were tested for their ability to inhibit the response of the abdominal body wall to exogenous 5-HT.

2-Bromolysergic acid diethylamide was found to be an effective inhibitor of the response to 5-HT at low concentrations (see above and Fig. 2).

N,*N*-dimethyltryptamine was neither an agonist nor an antagonist at concentrations up to 5×10^{-3} M (Fig. 7).

DISCUSSION

The action of 5-hydroxytryptamine

Bennet-Clark (1962) showed that cuticle plasticization in *Rhodnius* is an active response of the cells of the epidermis, and Nuñez (1963) and Maddrell (1966) showed that this response is under direct, local, nervous control. The evidence presented here shows that the response of the epidermis to exogenous 5-HT is not mediated by the central nervous system, but the possibility remains that the response may be due to the liberation by 5-HT of another agent from abdominal nerve endings, which is in turn responsible for the induction of plasticization. This would be analogous to the action of 5-HT at the 'M' receptor of the guinea pig ileum (Gaddum & Picarelli, 1957) and at the adrenergic receptors of the rat vas deferens (Nishino, Irikura & Takayanagi, 1970), where 5-HT acts indirectly via the nerve supply. This interpretation is difficult to refute, as it is not possible to remove the severed nerve endings from the abdominal body wall. However, two lines of evidence indicate that 5-HT probably does not act in this way:

(*a*) A wide range of structural analogues of 5-HT is effective in causing the epidermis to plasticize the cuticle. This wide range is similar to that effective at other known serotinergic receptors (see below for further discussion of this point). It is not similar to the rather restricted range of compounds which is effective where 5-HT

known to act indirectly by the release of another transmitter substance (Gaddum & Picarelli, 1957; Nishino *et al.* 1970).

(*b*) 2-Bromolysergic acid diethylamide (BOL) administered at relatively low levels blocks the plasticizing action of injected 5-HT. BOL administered alone appears to reduce a tonically maintained level of cuticle plasticity. BOL is a well-known inhibitor of classical 5-HT receptors; it is not known to inhibit the response to 5-HT of those tissues which respond via the liberation of another factor. The response of the integument to BOL alone is considered here to be evidence that the normal transmitter, BOL and 5-HT all act at the same site.

This is of course far from showing that 5-HT is identical with the normal transmitter active at the body wall. Although the activity of exogenous 5-HT has been demonstrated, the presence of 5-HT in the nerve supply to the epidermis has not been shown, nor has its release on stimulation of those nerves. A peptide neurosecretory factor, whose action is mimicked by 5-HT, might be responsible for the induction of plasticization *in vivo*. 5-HT is able to mimic the action of known peptide neurosecretory factors in the control of secretory function of the Malpighian tubules of *Rhodnius* and of *Carausius* (Maddrell, Pilcher & Gardiner, 1971), and in the control of Malpighian tubule muscle activity in *Carausius* (Pilcher, 1971). However, it should be pointed out that the wide range of 5-HT analogues which are effective in causing plasticization is quite unlike the rather restricted range of ligands which mimic the neurohormones mentioned above.

An outstanding puzzle has been Bennet-Clark's (1962) observation that injections of mammalian blood into the haemocoel of *Rhodnius* are able to cause the plasticization of the abdominal cuticle. The work of Nuñez (1963) and Maddrell (1966) has shown quite clearly that the plasticization response of the epidermis is under local nervous control. However, Maddrell (1966) was able to confirm Bennet-Clark's observation that there is indeed a factor in mammalian blood which induces plasticization, although it is unable to cross the gut wall under normal circumstances. The nature of this factor has remained a mystery. The suggestion is made here that this factor may be 5-HT, which occurs in mammalian blood in substantial quantities. Erspamer (1966) gives figures for a variety of species: oxblood, as used by both Bennet-Clark and Maddrell, contains 1·48 μg ml^{-1} in the plasma. All of the 5-HT is associated with the platelets, which are extremely fragile and liberate most of their 5-HT into the plasma on collection of the blood. Erspamer comments that analyses of blood serum usually only show about 50% of the whole blood's 5-HT, the rest being lost on separation. The data available are sufficient to show that injections of either whole blood or plasma could cause plasticization. The threshold for the response to injected 5-HT is about 5×10^{-8} M (see above). The concentration in oxblood plasma according to Erspamer's figure is $8{\cdot}4 \times 10^{-6}$ M, with the likelihood of about twice as much in whole blood. This would be more than enough to cause a maximal response. Maddrell comments that rabbit blood will also cause plasticization in *Rhodnius* when it is allowed to escape from the burst gut into the haemocoel. Erspamer's values for rabbit blood are in the ranges, 0·31–1·04 μg ml^{-1} (whole blood) and 0·3–1·72 μg ml^{-1} (plasma). These correspond to the range $1{\cdot}7$–$9{\cdot}8 \times 10^{-6}$ M. Again this would be sufficient to cause maximal plasticization of the cuticle on injection.

Bennet-Clark found that dried human plasma contained an agent which could cause

cuticle plasticization. The agent was extractable with water but not with acetone, ethanol, chloroform or ether. Human plasma contains 5-HT, though at a lower level than many other mammals. The failure to extract the active agent in acetone, a good solvent for 5-HT, seems puzzling. However, it is worth noting that Weissbach, Waalks & Udenfriend (1958) found that solvent extraction of blood from several mammalian species (with butanol in this case, also a good solvent for 5-HT) yielded very poor recovery of 5-HT, so that solvent extraction could not be used to estimate levels of 5-HT in these tissues. Direct estimation of the blood showed 5-HT to be present in quantity, however.

The pharmacology of the response to 5-HT

Despite uncertainty as to the nature of the normal transmitter acting at the site, the results of the pharmacological characterization of the 5-HT receptor in the *Rhodnius* abdominal body wall are still of considerable interest.

It should be remembered that the assay procedure used in these experiments utilizes the response of the whole insect to injected substances; this is a complicating factor in that some of these substances have other pharmacological effects in *Rhodnius* (Maddrell *et al.* 1971), and may undergo metabolism to a greater or lesser extent. Nevertheless, a consistent relation between structure and activity can be seen in the results.

With some slight modifications the model of the *Calliphora* salivary gland 5-HT receptor constructed by Berridge (1972) accounts quite well for the behaviour shown here by the receptor of the *Rhodnius* abdominal body wall. The terminal amino group of the side chain of 5-HT and analogues is an essential feature for activity, and interference here, either steric (*N*,*N*-dimethylation in bufotenine) or affecting charge distribution (*N*-acetylation in *N*-acetyl, 5-hydroxytryptamine) or introducing an adjacent carboxyl group (as in 5-hydroxytryptophan), causes reduced affinity at the receptor. Although interference with charge distribution does cause a greater loss of activity than simple steric interference in the *Rhodnius* case, there is still appreciable activity in the case of 5-hydroxytryptophan.This is in contrast to the situation at the *Calliphora* receptor, where 5-hydroxytryptophan shows very much attenuated activity; the concentration required to give 50 % activity is 5×10^6 times greater than for 5-HT and a maximal response is not possible. It appears that the requirements of the *Rhodnius* receptor are less strict in this respect than those of the *Calliphora* receptor.

Substitution at the 5-position of the indole nucleus gives results which are similar to the *Calliphora* case but which are slightly different in detail. In *Rhodnius*, the abdominal body wall receptor shows a higher affinity for tryptamine (R=H) than for other compounds substituted here, except for 5-hydroxytryptamine itself (R=OH). The reverse is true in *Calliphora* and also in a number of other well investigated 5-HT receptors (e.g. the rat fundus strip, Vane, 1959; the rat uterus, Barlow & Khan, 1959). It may be that the *Rhodnius* receptor has more exacting *steric* requirements at the 5-position than these other receptors, which appear to have a principal requirement for a hydrogen bonding site here (Berridge, 1972).

It may be noted that the dose–response curves for 5-methoxytryptamine ($R=OCH_3$) and for 5-fluorotryptamine (R=F) both show considerable flattening. This is reminiscent of dose–response curves shown by lysergic acid diethylamide (LSD) and, to a lesser extent, by N-substituted tryptamine derivatives in the *Venus* heart (Greenberg

1960; Wright, Moorhead & Welsh, 1962) and in *Calliphora* salivary gland (Berridge & Prince, 1973). In these cases, the flattening of the curve is associated with drug-receptor interactions which are irreversible or only slowly reversible. However, as pointed out by Ariens, Simonis & van Rossum (1964), the extent of variance in the data may influence the slope of the dose–response curve. For this reason, it is probably unwise to infer too much from the slopes of the curves presented here, which are not obtained from single preparations, but which are built up statistically.

As in the case of the *Calliphora* salivary gland receptor there is some affinity shown for other biogenic amines which are not indole derivatives. Both dopamine and tyramine shown appreciable activity at the *Rhodnius* receptor. (The catecholamines, adrenaline and noradrenaline were not tested.) Evidently the indole nucleus is not essential for activity, provided that other requirements are met. Both the phenylamines active here have hydroxyl groups attached to an aromatic nucleus and an alkyl side chain of two carbon atoms terminated by an unimpeded charged amino group, as does 5-HT. The aromatic nucleus of the phenylamines is flat, as is the indole nucleus of 5-HT. The *Calliphora* receptor appears to be rather more selective than the *Rhodnius* receptor with respect to this point; the concentration of dopamine required to give a 50% response in *Calliphora* salivary glands is 10^6 times greater than for 5-HT, whereas in *Rhodnius* the concentration required is only 5×10^2 times greater than for 5-HT.

In general, the range of structural analogues of 5-HT which are active at the *Rhodnius* integumental 5-HT receptor is similar to those which have been described for other well-studied 5-HT receptors, with only minor deviations from the classical pattern. It is interesting that this is quite unlike the very much more restricted range of ligands which is effective at the receptors of the *Rhodnius* Malpighian tubules (Maddrell *et al.* 1971), which are known to be regulated in life by a hormone, probably peptide in nature, which is distinct from 5-HT.

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