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# IONIC MECHANISMS AND RECEPTOR PROPERTIES UNDERLYING THE RESPONSES OF MOLLUSCAN NEURONES TO 5-HYDROXYTRYPTAMINE

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SUMMARY

1. Molluscan neurones have been found to show six different types of response (three excitatory and three inhibitory) to the iontophoretic application of 5-hydroxytryptamine (5-HT). The pharmacological properties of the receptors and the ionic mechanisms associated with these responses have been analysed.

2. Four of the responses to 5-HT (named $A$, $A'$, $B$ and $C$) are consequent upon an *increase* in membrane conductance whereas the other two (named $\alpha$ and $\beta$) are caused by a *decrease* in membrane conductance.

3. The $A$-response to 5-HT consists of a 'fast' depolarization due to an increase mainly in $Na^+$-conductance; the $A'$-response is a 'slow' depolarization also associated with a $Na^+$-conductance increase. Receptors mediating the $A$- and $A'$-depolarizations have different pharmacological properties and may exist side by side on the same neurone.

4. Both the $B$- and $C$-responses are inhibitory. The $B$-response is a 'slow' hyperpolarization due to an increase in $K^+$-conductance, the $C$-response is a fast hyperpolarization associated with an increase in $Cl^-$-conductance.

5. The $\alpha$-response to 5-HT is a depolarization which becomes reduced in amplitude with cell hyperpolarization and reverses at $-75$ mV; it is caused by a decrease in $K^+$-conductance. The $\beta$-response is an hyperpolarization which increases in amplitude with cell hyperpolarization and reverses at $-20/-30$ mV. It results from a decrease in conductance to both $Na^+$ and $K^+$ ions.

6. The receptors involved in the 5-HT responses associated with a conductance increase may be recognized by the action of specific antagonists: 7-methyltryptamine blocks only the $A$-receptors, 5-methoxygramine only the $B$-receptors and neostigmine only the $C$-receptors. Curare blocks the $A$- and $C$-receptors and bufotenine, the $A$-, $A'$- and

*B*-receptors. No specific antagonists have yet been found for the 5-HT responses caused by a conductance decrease.

7. The significance of the multiplicity of receptors is discussed. Their functional significance at synapses is analysed in the following paper.

## INTRODUCTION

Recent work on the nervous system of gastropod molluscs has produced a fair amount of evidence in favour of the idea that 5-hydroxytryptamine (5-HT) plays a synaptic transmitter role at identified central and peripheral synapses (Cottrell, 1970; Paupardin-Tritsch & Gerschenfeld, 1973*a*; Cottrell & Macon, 1974; Mayeri, Koester, Kupfermann, Liebeswar & Kandel, 1974; Gerschenfeld & Paupardin-Tritsch, 1974).

In early papers it was shown that 5-HT applied iontophoretically depolarizes and excites some molluscan neurones (Gerschenfeld & Stefani, 1966) and hyperpolarizes and inhibits the firing of other neurones (Glaizner, 1967; Gerschenfeld, 1971). This paper deals with an analysis of both the ionic mechanisms and the characterization of the receptors involved in these excitatory and inhibitory actions. In the following paper the participation of some of these receptors and ionic mechanisms in the functioning of identified synapses will be described. The present work has demonstrated that the iontophoretic application of 5-HT on to molluscan neurones may in reality cause three different types of excitation: two of them, one slow and the other fast, involve an increase in $Na^+$-conductance, whereas the third is caused by a decrease in $K^+$-conductance. There are also three kinds of inhibition that can be evoked by 5-HT: two of these are due to an increase in the membrane conductance to either $Cl^-$ or $K^+$ ions; the third is generated by a decrease in both $Na^+$- and $K^+$-conductances. Moreover, it has been found possible to characterize the four distinct receptors involved in those actions of 5-HT causing an increase in the ionic membrane conductances.

Preliminary accounts of some of this work have already been given (Gerschenfeld & Paupardin, 1973; Paupardin-Tritsch & Gerschenfeld, 1973*b*).

## METHODS

The experiments were performed on specimens of the land snail *Helix aspersa* and the sea slug *Aplysia californica*. The snails were kept in the refrigerator at 4° C until 48–72 hr before the experiments, when they were transferred to room temperature (20–24° C), fed and hydrated. The *Aplysia* specimens were obtained from Dr R. Fay, Pacific Marine Co (Venice, California) and kept in oxygenated aquaria.

In the case of *Helix*, the shell was removed and the peri-oesophageal ganglionic ring and its associated nerves were dissected free and pinned to the layer of soft

plastic (Elastomere No. RTV 141 AEB Rhône-Poulenc) in the bottom of a Lucite chamber; the ganglia were bathed in a continuously flowing physiological saline (modified from Chiarandini, 1964) containing (mM): Cl 144, Na 120, K 5, Mg 3·5, Ca 6, D-glucose 5, and Tris-Cl (pH 7·4) 5.

In the case of *Aplysia*, the cerebral, the buccal or the abdominal ganglia were removed and mounted in the same way. The circulating fluid was artificial sea water (ASW) of the following composition (mM): Na 480, K 10, Cl 610, Ca 10, Mg 50 and Tris-Cl (pH 7·8) 10.

In both species the connective tissue enveloping the ganglia was removed by careful dissection to expose the soma of the unipolar neurones located in the periphery of the ganglia. The neurones were impaled with double-barrelled micropipettes (1 $\mu$m diameter) pulled with a de Fonbrune microforge and filled with 0·6 M-$K_2SO_4$. They generally showed resistances, measured in snail saline or ASW, of 10–25 MΩ; the pipettes were rejected if the coupling resistances between the barrels was greater than 200 kΩ. One of the barrels was used for recording via a unity gain amplifier connected both to a CRO and to a 280 Brush pen-recorder. The other was used to pass constant current pulses across the membrane (for measuring the input resistance of the neurone) and/or steady currents to set the membrane potential to desired levels. The bath was connected to earth via a saline agar AgCl bridge.

5-HT was applied iontophoretically on the neurones from single micropipettes filled with a saturated solution of 5-HT creatinine sulphate complex (pH 3·2). The spots sensitive to 5-HT were located under microscopic control; they were generally found in the region of the axon hillock (easy to identify in the snail neurones by a dark pigment accumulation) or beyond it (see Stefani & Gerschenfeld, 1969). Both in the snail and in *Aplysia*, the larger part of the soma was insensitive to 5-HT. The iontophoretic currents varied between 10 and 100 nA and were applied for 50–1000 msec. The intensity was monitored either on the oscilloscope or with a microammeter. No braking currents were used.

In many experiments, the ionic content of the extracellular medium was altered. Changes in the $K^+$ content of either the snail saline or the ASW were made by adding or removing KCl, without compensation for the changes in osmolarity. When $Cl^-$ ions were partially or totally removed from the snail saline, they were replaced by the impermeant sulphate or isethionate ions. When sulphate was used, sucrose was added to maintain a constant osmolarity. The $Na^+$ concentration of the extracellular medium was changed by replacing NaCl by Tris-Cl at a suitable pH. Alterations in the external concentration of $Ca^{2+}$ or $Mg^{2+}$ were obtained by either removing or adding $CaCl_2$ or $MgCl_2$, without compensation for the osmolarity changes.

Antagonists of 5-HT and other blocking agents tested were dissolved in the bathing medium and applied to preparation through the circulating system.

All the experiments were performed at room temperature (20–24° C).

## RESULTS

The main types of response to the iontophoretic application of 5-HT which will be described were observed equally in snail and *Aplysia* neurones; only the so-called *C*-response (see below) has not yet been observed in *Aplysia*. However, the illustrations in this paper have been taken mainly from experiments on snail ganglia.

*5-HT responses resulting from an increase in the neuronal membrane permeability*

*A- and A'-responses.* Previous work (Gerschenfeld & Stefani, 1966; Stefani & Gerschenfeld, 1969) demonstrated that the iontophoretic application of 5-HT on to certain neurones of the snail *Cryptomphallus aspersa* caused depolarization and excitation of these cells, probably as a result of an increase in the membrane permeability to $Na^+$ ions. These depolarizing responses could be blocked by $10^{-5}$–$10^{-4}$ M (+)-tubocurarine (TC).

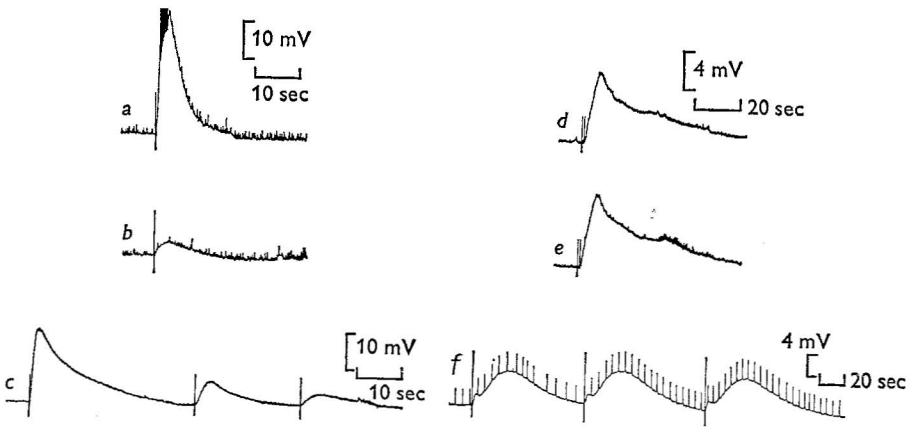


Fig. 1. Differences between the *A*- and the *A'*-responses to 5-HT. *a*, *A*-response to 5-HT in a neurone hyperpolarized by inward current to −80 mV, consisting of a large depolarization which brings the membrane potential to the firing level. The small rapid depolarizations correspond to 'spontaneous' synaptic potentials. *b*, the perfusion of a $10^{-4}$ M solution of TC to the preparation almost completely blocks the 5-HT response. *c*, in another neurone, also at a membrane potential of −80 mV, three successive iontophoretic applications of 5-HT result in *A*-responses which become progressively reduced in amplitude because of the receptor desensitization. *d*, an *A'*-response to 5-HT in a neurone whose membrane potential was previously driven to −80 mV. Notice the difference in time base with *a*, *b*, *c*. *e*, the bath application of a $10^{-4}$ M solution of TC does not affect the response. *f*, in another neurone, continuously bathed with a TC solution, the repetitive application of 5-HT does not cause desensitization of the responses as in *c*. The rapid depolarizations are spontaneous 'axonic' spikes generated far from the impaled soma.

In a new survey of these responses, it has been found that both *Aplysia* and *Helix* neurones may actually show two different types of depolarizing responses to 5-HT involving an increase in $Na^+$ membrane conductance; these will be referred to as *A*- and *A'*-responses.

For the convenience of the reader, some results of the kind previously

reported are shown in Fig. 1*a–c*. Fig. 1*a* shows that the application of 5-HT on to a neurone, artificially hyperpolarized to −80 mV, depolarizes the cell by 35 mV. The neurone reached threshold in less than 2 sec and the potential returned to the initial level in about 15 sec. This response will be called *A*-response; it could be blocked by TC (Fig. 1*b*) and other various agents (see below) and could be intensely desensitized by repeated 5-HT application (Fig. 1*c*).

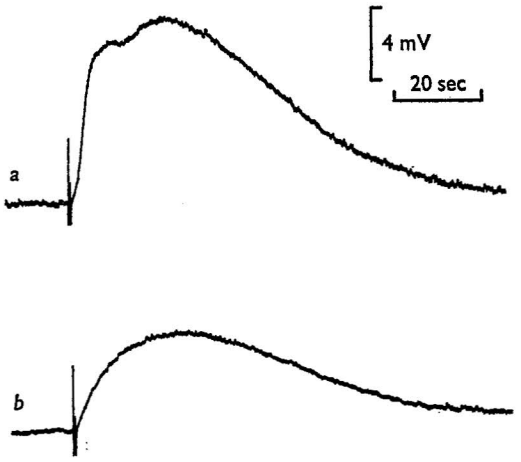


Fig. 2. Composite *A–A′* response to 5-HT. *a*, iontophoretic application of 5-HT on a neurone previously hyperpolarized by inward current to −80 mV results in a complex depolarizing response showing two components. The neurone was spontaneously active at its resting potential of −40 mV. *b*, bath application of a $10^{-4}$ M solution of TC blocks the faster component (*A*-response), without affecting the slower component (*A′*-response).

An example of the second type of depolarization (the *A′*-response) is shown in Fig. 1*d*. This response had a slower rising phase, reaching its peak in more than 6 sec and returning to the original level much more slowly, the falling phase lasting more than 50 sec. This type of response is not affected by TC (Fig. 1*e*) nor by other agents which block the *A*-responses (see below). The possibility that the difference in time courses of the *A*- and *A′*-responses is due to a different location of the receptors cannot be completely rejected. However, it may be noted that the time course of the *A′*-response was always 'slow' no matter where on the cell the 5-HT was applied. Another difference between the *A*- and *A′*-responses is that the desensitization shown by the *A′*-responses when 5-HT applications are repeated is very weak (Fig. 1*f*).

As Fig. 2*a* shows, some neurones responded by a depolarization which combined both *A*- and *A′*-responses. The composite nature of such

responses could be demonstrated by applying TC (Fig. 2*b*) or other blocking agents (see below) which suppressed the *A*-component but spared the *A'*-component.

As in the case of excitatory transmitter actions in the majority of invertebrate and vertebrate neurones studied (see Ginsborg, 1973) it was not possible to observe an inversion of the *A*- or *A'*-responses by depolarizing the neurones: when outward current was passed across the membrane of these neurones, it was unable to drive the somatic membrane potential beyond $-5$ mV because of the rectification which developed above

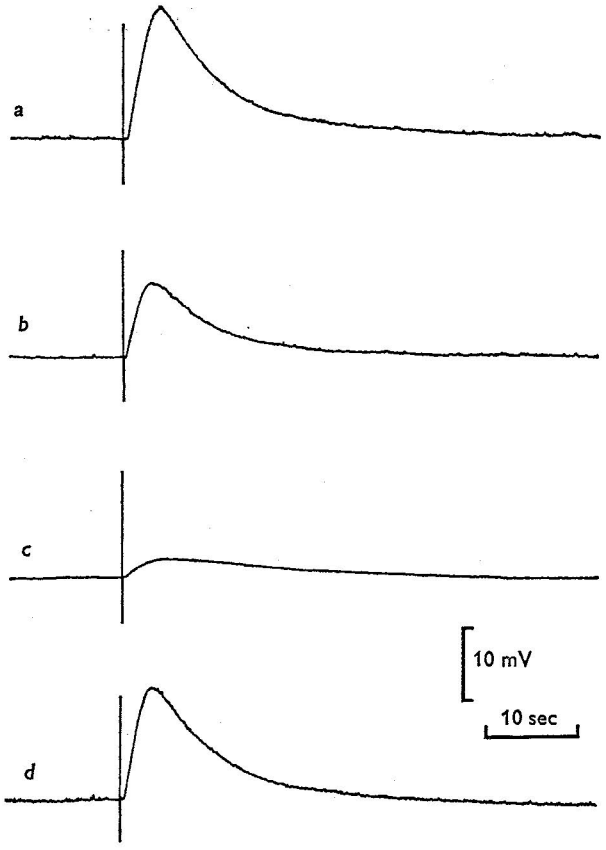


Fig. 3. Effects of altering $[Na]_o$ on the *A*-response to 5-HT. A series of 5-HT applications to a neurone previously hyperpolarized by inward current to $-80$ mV. *a*, control *A*-response in a normal external medium containing 120 mM-NaCl. *b*, the response becomes reduced in amplitude when half of the NaCl content of the medium (60 mM) is replaced by Tris-Cl. *c*, the *A*-response is almost abolished by total replacement of the external NaCl by Tris-Cl. *d*, the *A*-response recovers its original amplitude when replaced in a normal $Na^+$-containing medium.

$-30$ mV. However, for both the $A$- and $A'$-responses, values for the reversal potential ($E_A$, $E_{A'}$) could be approximately estimated by extrapolation and were found to be near the zero potential level.

It thus seems likely that an increase in $Na^+$-conductance was involved in these 5-HT depolarizing responses and an attempt to check this was

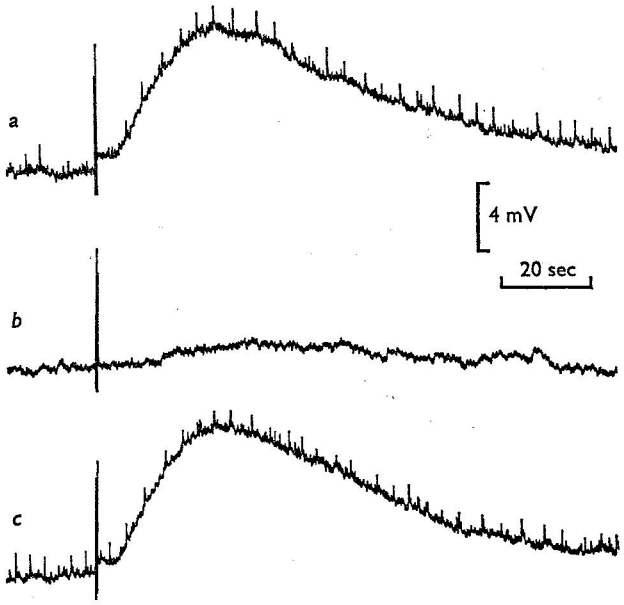


Fig. 4. Effect of changes in $[Na]_o$ on the $A'$-response to 5-HT. *a*, $A'$-response in a neurone whose membrane potential is set at $-80$ mV, bathed in a normal external medium. *b*, the response is almost abolished by removing the whole NaCl content of the environment and by replacing it with Tris-Cl. *c*, the response recovers its original amplitude after bathing the preparation in a normal medium.

made by investigating the effects of changes of $Na^+$ external concentration on the responses to 5-HT. Fig. 3*a* shows a control $A$-response in a snail neurone which clearly diminished when half of the NaCl content of the saline was replaced by Tris-Cl (Fig. 3*b*) and almost disappeared when the whole NaCl content of the medium was replaced by Tris-Cl (Fig. 3*c*). These effects of NaCl removal were reversible (Fig. 3*d*) and were not due to alterations in $(Cl)_o$, since removal of the $Cl^-$ ions from the medium did not significantly alter the $A$-responses.

A similar mechanism appears to underly the $A'$-responses. Thus, Fig. 4 shows that $A'$-responses were affected in the same way as the $A$-responses when the whole NaCl content of the environment was replaced by Tris-Cl

(Fig. 4*b*). Here again the alteration of $(Cl)_o$ alone did not affect the response. The effects of removing NaCl were easily reversible (Fig. 4*c*).

*B-responses.* In other molluscan neurones (located in both pallial ganglia

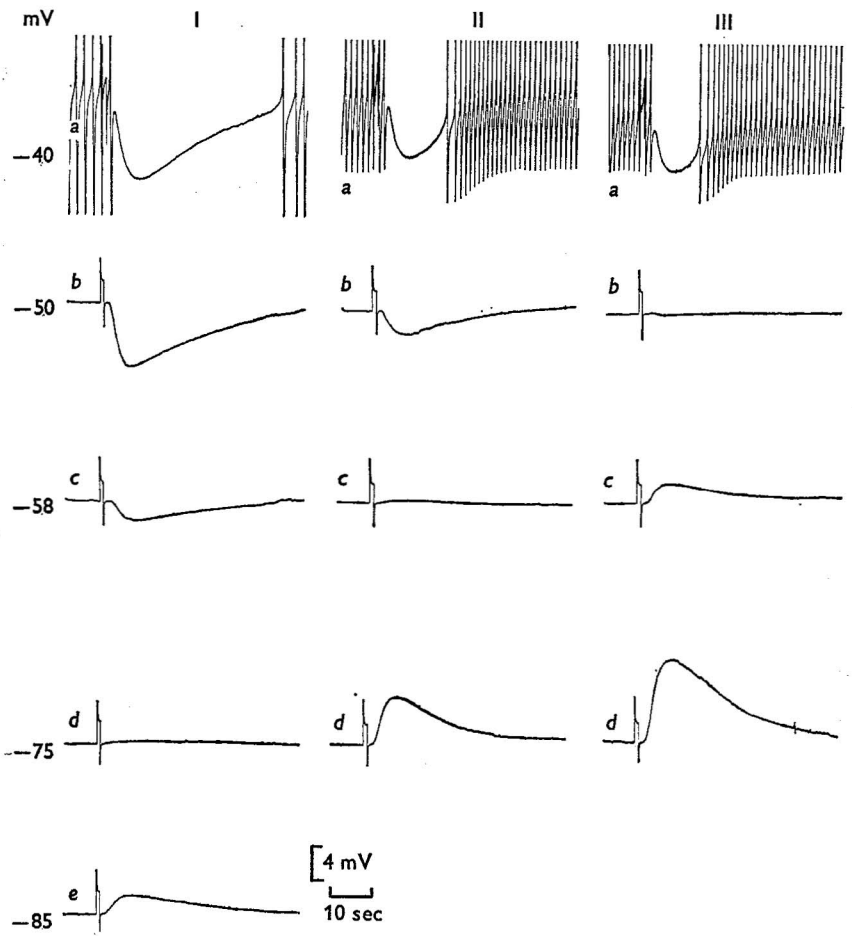


Fig. 5. Effect of changes in $[K]_o$ on the *B* responses to 5-HT. I. 5-HT applications on a neurone bathed in a normal medium containing 5 mM-K. *a*, at the resting potential (−40 mV), the application results in an hyperpolarizing *B*-response. When the neurone is hyperpolarized by inward current to −50 and −58 mV (*b*, *c*), the *B*-response decreases in amplitude. In *d*, at −75 mV, the effect of the 5-HT application is null (reversal potential, $E_B$). *e*, at −85 mV, the *B*-response shows a reversed polarity. II. Similar series of 5-HT applications to the same neurone after increasing the extracellular $K^+$ concentration from 5 to 10 mM. The reversal potential of the *B*-response is shifted to −58 mV (*c*). III. Another series of 5-HT applications to the same neurone in the presence of an external $K^+$ concentration of 15 mM. $E_B$ is deplaced now to −50 mV (*b*).

of *Helix* and in the buccal and abdominal ganglia of *Aplysia*), the iontophoretic application of 5-HT resulted (Fig. 5 I*a*) in a different kind of response, which will be called a *B*-response. It consisted in a hyperpolarization lasting for at least 40 sec and causing the inhibition of spike firing. When such neurones were hyperpolarized by passing inward current across the membrane (Fig. 5 I*b–d*) the amplitude of the 5-HT response decreased gradually with the increasing hyperpolarization, reaching a null value ($E_B$) near $-75$ mV. It is known that the value of $E_K$ in molluscan neurones is near $-75$ to 80 mV (Moreton, 1968; Kehoe, 1972; Russell & Brown, 1972) and thus it seemed possible that $E_B$ might be identified with $E_K$.

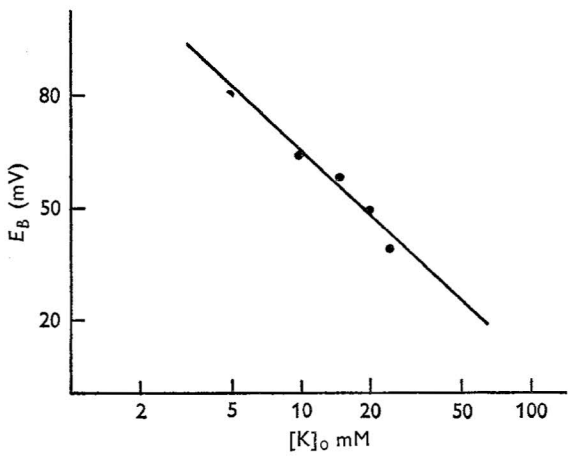


Fig. 6. Relation of the values in mV of the reversal potential of the *B*-responses to 5-HT ($E_B$) and the external $K^+$ concentrations in a semi-logarithmic scale. The experimental points are close to a straight line of slope 58 mV for a decade change in $[K]_o$, representing the theoretical values of $E_K$ according to the Nernst equation.

When the normal $K^+$ concentration in the extracellular medium (5 mM) was increased two (Fig. 5 II) or three times (Fig. 5 III) the value of $E_B$ shifted from $-76$ to $-58$ and $-51$ mV respectively. Fig. 6 shows that when the values of $E_B$ obtained in another neurone were plotted against the logarithm of $[K]_o$, the values of $E_B$ accord well with the theoretical values predicted by the Nernst equation. Moreover, changes in $[Cl]_o$ did not affect either the polarity of the *B*-responses or the value of $E_B$. It can be concluded that the *B*-responses to 5-HT are due to a selective increase in the membrane permeability to $K^+$ ions.

*C-responses.* In a few cells of the right pallial ganglion of the snail, another kind of hyperpolarizing response to the iontophoretic application of 5-HT was observed; it will be called *C*-response. As illustrated in Fig. 7 I, the

*C*-response reversed at about $-55$ mV. Another feature which evidently distinguishes it from the *B*-response is its greater speed. Moreover, it does not result from a rise in $K^+$-conductance, since increasing $[K]_o$ from 5 to 15 mM did not bring about any significant alteration in the values of $E_C$. By contrast, the replacement of the $Cl^-$ content of the extracellular environment by the impermeant $SO_4^{2-}$ ions caused a reversal of the *C*-responses (Fig. 7 II). In this condition, the value of $E_C$ shifted to near the zero potential level. Therefore, it can be concluded that the *C*-response is due to an increase in the membrane conductance to $Cl^-$ ions.

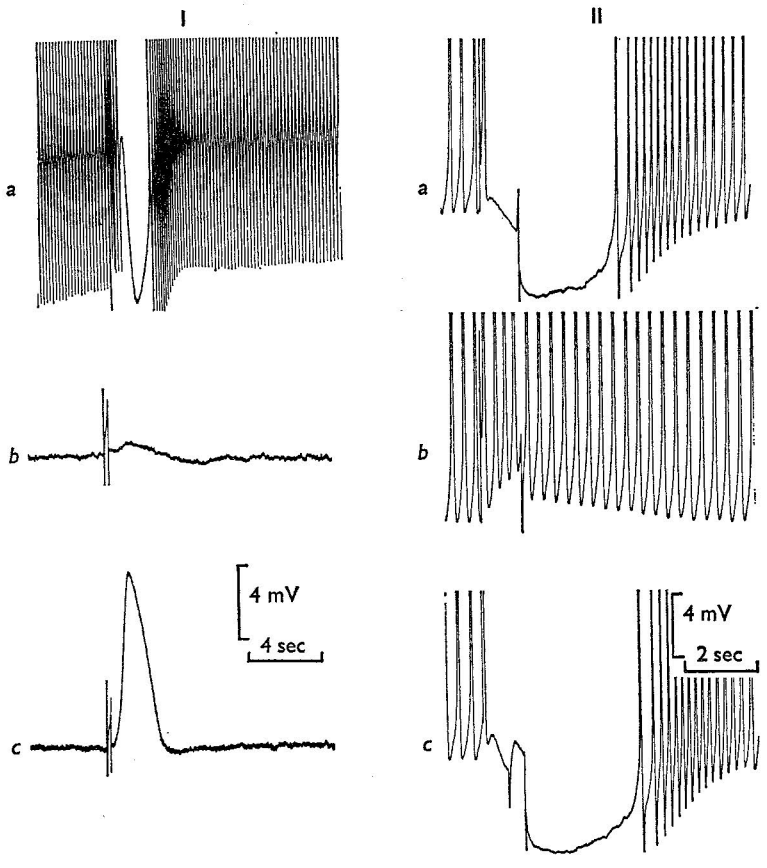


Fig. 7. I. *a*, *C*-response to 5-HT in a neurone at the resting potential of $-40$ mV. *b*, reversal potential level of the *C*-response at $-55$ mV. *c*, driving the membrane potential to $-80$ mV, the *C*-response reverses its polarity and appears as a fast depolarization. II. *a*, a *C*-response recorded in another experiment in a neurone bathed in a normal medium. *b*, replacement of the whole external $Cl^-$ by $SO_4^{2-}$ ions causes the reversal of the *C*-response. *c*, the response recovers its previous polarity after replacing in the bath containing a normal $Cl^-$ concentration.

*5-HT responses resulting from a decrease in the neuronal membrane permeability*

The four types of response to 5-HT described above, *A*, *A'*, *B* and *C*, all involving an increase in the membrane ionic conductance, can easily be interpreted according to the 'classical' theory of synaptic transmission (Fatt & Katz, 1951, 1953; see Ginsborg, 1973). However, in addition to these responses, it was possible to observe in other cells, both in *Helix* and in *Aplysia*, two kinds of 'atypical' responses to 5-HT which could not be so interpreted and which must be attributed to *decreases* in the membrane conductance to environmental ions. To distinguish them from the 'classical' 5-HT responses described above, they will be designated by the Greek letters $\alpha$ and $\beta$.

*$\alpha$-responses.* The responses shown in Fig. 8 were recorded from a neurone located in the right pallial ganglion of *Helix*. In *a*, it was hyperpolarized to $-39$ mV to prevent spike firing. The iontophoretic application of 5-HT then caused a depolarization which reached threshold and gave rise to two spikes. However, when the neurone was further hyperpolarized by passing inward current across the membrane, instead of the expected increase, a decrease in the amplitude of the 5-HT responses was observed (Fig. 8*b*, *c*). At $-75$ mV (Fig. 8*d*) no response was observed and beyond this value ($E_\alpha$) the response became a hyperpolarization (Fig. 8*e*). No increase in membrane conductance could explain the gradual decrease of the response and its inversion. As an alternative, an attempt was made to interpret the responses in terms of the model proposed and analysed by Weight & Votava (1970) for the slow excitatory synaptic potentials recorded in vertebrate sympathetic ganglion neurones. In this model, the transmitter induces a decrease in membrane conductance.

To test this hypothesis, the effect of 5-HT on the input resistance of a neurone showing an $\alpha$-response was first analysed. Fig. 9*a* shows two of a series of electrotonic potentials obtained by passing constant inward current across the cell membrane. In Fig. 9*b*, the same current pulses were applied to the membrane during the application of a $5 \times 10^{-5}$ M solution of 5-HT (the solution depolarized the cell, but the membrane potential was reset to the initial value by means of a steady additional current). It is evident that the 5-HT caused a marked increase in input resistance, the effect being reversed by the removal of the antagonist (Fig. 9*c*).

To investigate the ionic species involved in the membrane conductance decrease observed, a series of experiments as in Fig. 9*d–k* was performed. After recording an $\alpha$-response reversing at $-78$ mV (Fig. 9*d–g*), the $K^+$ content of the medium was doubled. This resulted in a shift in the value of $E_\alpha$ to $-60$ mV (Fig. 9*h–k*) which fitted well with the expected

displacement of $E_K$ predicted by the Nernst relation, of about 17 mV at 20° C. It thus seems that the $\alpha$-responses to 5-HT result from a *selective decrease* of the membrane conductance to $K^+$ ions.

*$\beta$-responses.* In other identified neurones (two in the right pallial

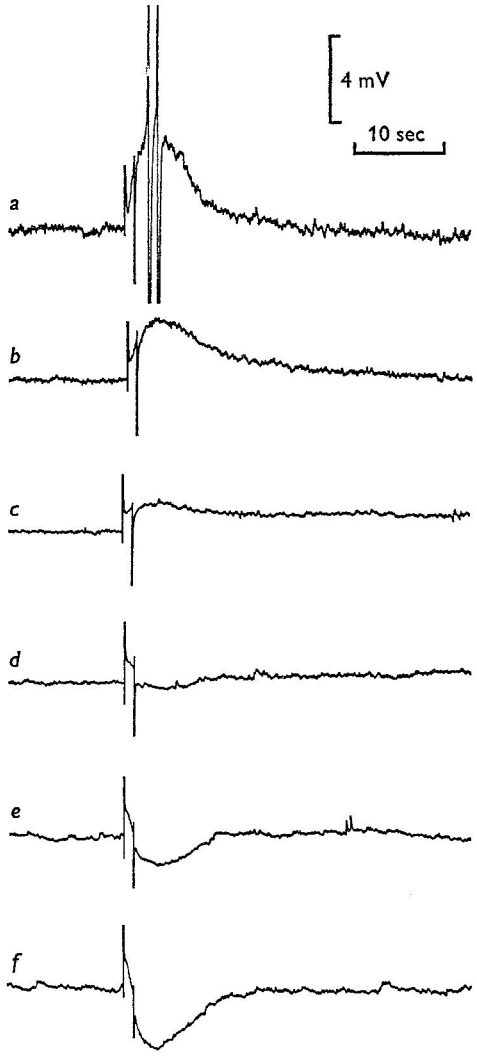


Fig. 8. $\alpha$-response to 5-HT. *a*, 5-HT application to a neurone slightly hyperpolarized to $-39$ mV results in a depolarization reaching threshold and in spike firing. *b*, *c*, when the membrane potential is successively driven to $-45$ and $-60$ mV, the $\alpha$-response decreases in amplitude. *d*, no effect of 5-HT is observed at $-75$ mV (reversal potential, $E_\alpha$). *e*, *f*, beyond $E_\alpha$, at $-85$ and $-95$ mV, the polarity of the response is reversed (see text).

ganglion of *Helix* and one in each buccal ganglion of *Aplysia*), another variety of 'atypical' response was observed which consisted in an hyperpolarization (Figs. 10 and 11) and which will be called $\beta$-responses.

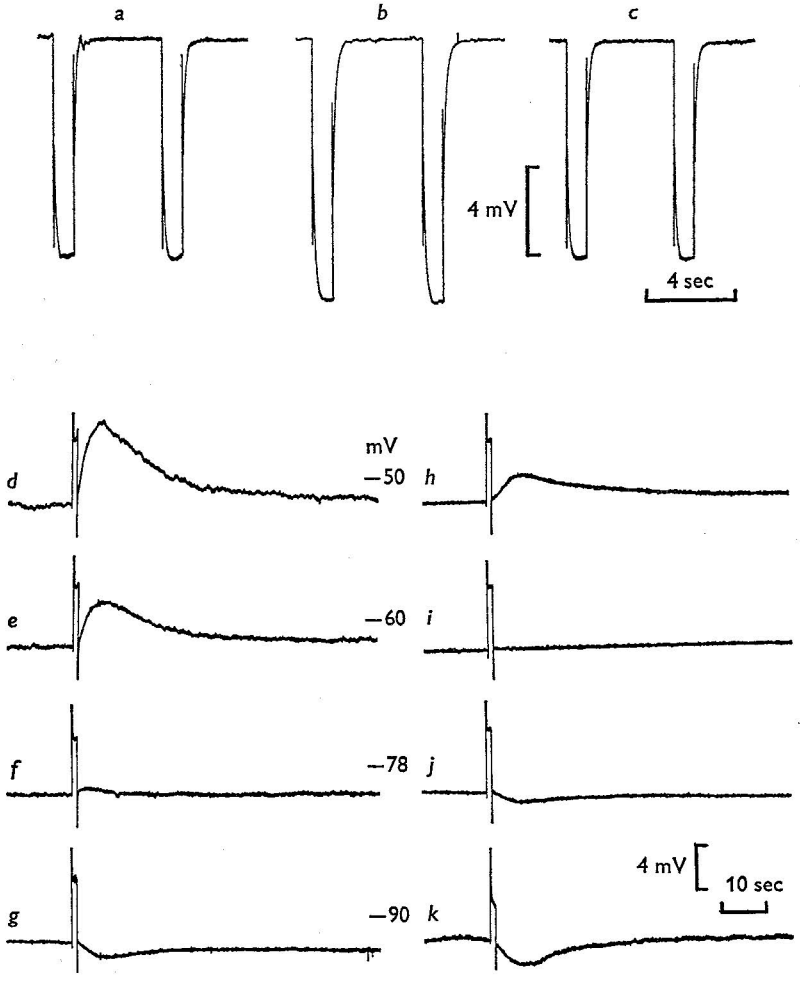


Fig. 9. Effect of changes in $[K]_o$ on the $\alpha$-response. *a*, in a neurone hyperpolarized by inward current to $-50$ mV, square current pulses are passed across the membrane to measure the input resistance. *b*, bath application of a $5 \times 10^{-5}$ M solution of 5-HT causes an evident increase in the amplitude of the electrotonic potentials. *c*, the effects of 5-HT disappear when it is washed out from the preparation. *d–g*, 5-HT iontophoretic applications to the same neurone bathed in a normal external medium result in $\alpha$-response which reverses at $-78$ mV (*f*). *h–k*, when the external $K^+$ concentration is increased from 5 to 10 mM, the reversal potential of the $\alpha$-response shifts to $-60$ mV (*i*) (see text).

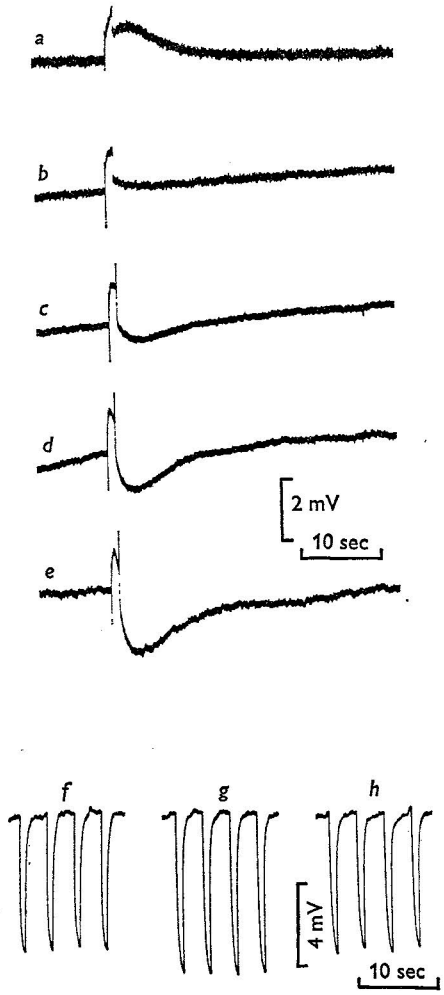


Fig. 10. $\beta$-responses. In a neurone which does not discharge action potentials at any level, probably due to traction during the ganglion dissection, $\beta$-responses are recorded in response to the iontophoretic applications of 5-HT. At the resting level, $-40$ mV (*c*), the 5-HT application produces an hyperpolarization which increases in amplitude with increasing polarization (*d*, *e*). When the cell is depolarized, at $-30$ mV (*b*), an equilibrium level is reached and, at $-20$ mV (*a*), the $\beta$-response becomes reversed.

In *f*–*h*, the input resistance of a neurone responding by a $\beta$-response to 5-HT was measured by passing square current pulses. *f*, control electrotonic potentials; *g*, the electrotonic potentials increase when the preparation is bathed in a $5 \times 10^{-5}$ M solution of 5-HT. *h*, removal of 5-HT from the bath (see text).

Records of Fig. 10 were obtained from a silent neurone, whose spike mechanism was probably inactivated because excessive traction was exerted on the soma during the connective tissue removal. In spite of this soma inexcitability, the neurone showed a resting potential of $-40$ mV and a $\beta$-response could be recorded when 5-HT was applied iontophoretically (Fig. 10*c*). When the neurone was hyperpolarized by passing inward current across the membrane, the amplitude of the $\beta$-response increased (Fig. 10*d*, *e*) and when the cell was depolarized, the $\beta$-response diminished in amplitude and finally reversed beyond $-30$ mV ($E_\beta$) (Fig. 10*a*, *b*). In the majority of the cases, the neuronal firing did not allow a direct recording of the reversal potential, and in these cases $E_\beta$ was estimated by extrapolation from the amplitudes of the responses observed at subthreshold potentials. The values of $E_\beta$ calculated from the data of eight experiments varied in a range between $-15$ and $-38$ mV. In spite of this dispersion, the $\beta$-responses could be only explained by a mechanism involving a reduction in membrane ionic conductance either to both $Na^+$ and $K^+$ or to both $Na^+$ and $Cl^-$.

This hypothesis was first tested, as in the case of the $\alpha$-responses, by checking the effect of 5-HT on the membrane input resistance of a neurone showing a $\beta$-response. Fig. 10*f* shows the control electrotonic potentials. The perfusion of a $5 \times 10^{-5}$ M solution of 5-HT hyperpolarized the cell, and after resetting the membrane potential to the initial level, the amplitude of the electrotonic potential appeared remarkably increased (Fig. 10*g*). These potentials recovered their previous amplitude after the removal of the amine (Fig. 10*h*).

The involvement of $Cl^-$ ions in the $\beta$-responses is made unlikely by the fact that the amplitude of the $\beta$-responses or their reversal potentials were unaltered when half of the $Cl^-$ content of the extracellular medium was replaced by isethionate or sulphate ions. On the contrary, changes in both $[Na]_o$ and $[K]_o$ strongly influenced the values of $E_\beta$. Fig. 11*a–e* shows the behaviour of a $\beta$-response when the membrane potential was varied between the resting level of $-40$ and $-80$ mV. When the neurone was depolarized, the intensity of the firing hindered the direct measure of $E_\beta$, which was estimated to be about $-23$ mV by extrapolation (Fig. 11, graph). When half of the NaCl content of the extracellular environment was replaced by Tris-Cl, the reversal potential became directly observable at $-40$ mV (Fig. 11, *f–j* and graph). The participation of a change in $K^+$ conductance in the generation of the $\beta$-responses was also suggested by experiments of Fig. 12, which were performed on a neurone in which the response reversed in a normal saline environment at an estimated $E_\beta$ level of $-27$ mV (Fig. 12, *a–d* and graph). When $[K]_o$ was reduced from 5 to 2·5 mM, the calculated value of $E_\beta$ shifted to $-34$ mV (Fig. 12, *e–h* and graph).

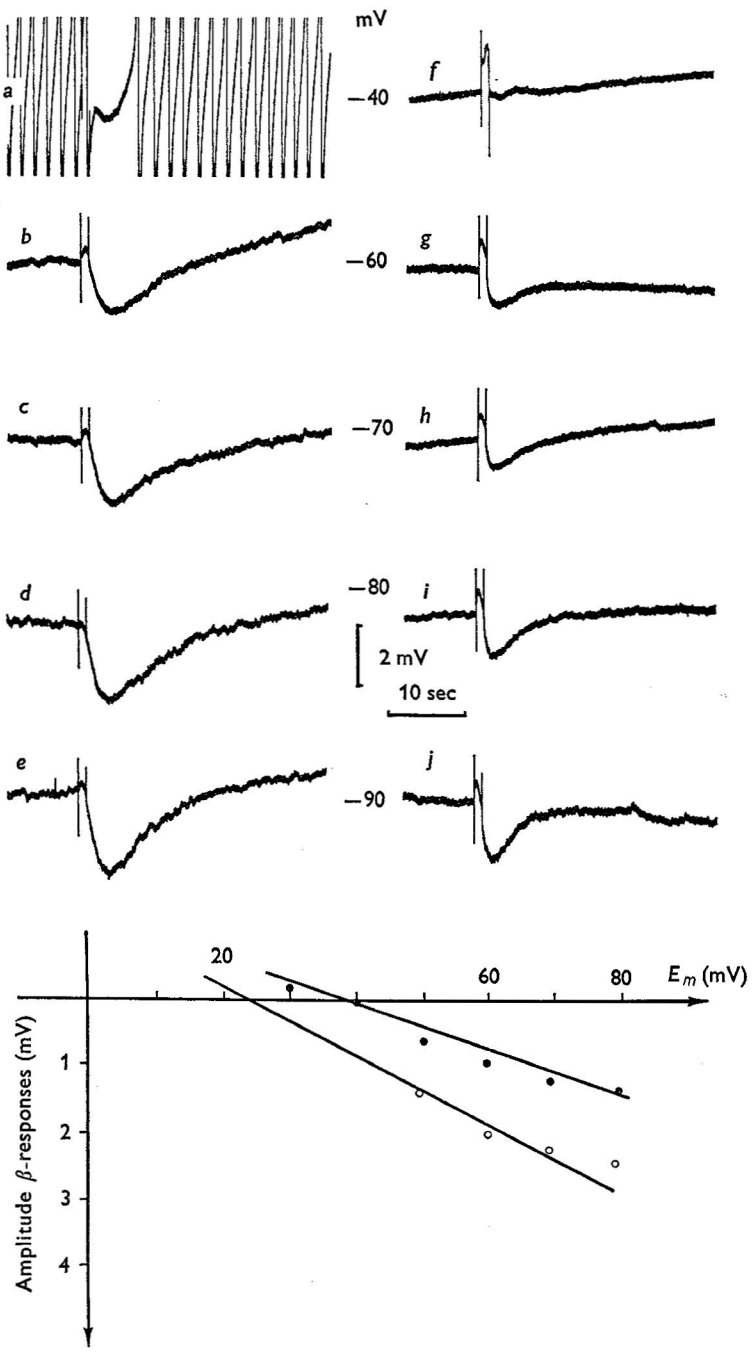


Fig. 11. For legend see facing page.

It can be thus concluded that the inhibitory $\beta$-response to 5-HT results from a *decrease* in the membrane conductance to both $Na^+$ and $K^+$.

The relative contributions of the simultaneous changes in $Na^+$- and $K^+$-conductance ($\Delta g_{Na}$ and $\Delta g_K$ respectively) may be treated in analogy with the vertebrate neuromuscular junction where ACh produces the exactly opposite effects, namely, an increase in $Na^+$- and $K^+$-conductances. If such an analogy is accepted, the ratio can be calculated as follows (Takeuchi & Takeuchi, 1960): since at the level of $E_\beta$, the sum of the ionic currents generated by 5-HT application ($I_{Na}+I_K$) is equal to zero, and

$$I_{Na} = \Delta g_{Na}\,(E_{Na}-E_\beta) \quad \text{and} \quad I_K = \Delta g_K\,(E_K-E_\beta)$$

we can write $$\Delta g_{Na}\,(E_{Na}-E_\beta)+\Delta g_K\,(E_K-E_\beta) = 0$$

and then $$\Delta g_{Na}/\Delta g_K = (E_K-E_\beta)/(E_\beta-E_{Na}).$$

In normal saline, the value of $E_K$ is about $-75$ mV according to the data already mentioned above and it would shift to $-92$ mV in a medium containing half of the normal $[K]_o$. From the data of Thomas (1972), $E_{Na}$ may be calculated to be 78 mV and would be expected to shift to 61 mV when $[Na]_o$ would be reduced to half of its normal value.

By using the data from both Figs. 11 and 12, it is possible to evaluate the respective contributions of the decrements in the $Na^+$- and $K^+$-conductances to the $\beta$-responses in both normal and modified ionic media. In the case of Fig. 11, the value of $\Delta g_{Na}/\Delta g_K$ should be equal to 0·51 for the neurone bathed in a normal medium, decreasing to 0·35 when the NaCl of the medium was replaced by Tris-Cl. In the case of Fig. 12, $\Delta g_{Na}/\Delta g_K$ would have a value of 0·46 in the normal environment containing 5 mM-$K^+$, but would increase to 0·52 when the preparation was immersed in a saline containing only 2·5 mM-$K^+$ (see Discussion).

*Influence of variations in* $[Ca]_o$ *and* $[Mg]_o$ *on the* $\beta$*-responses.* A possible interpretation of the mechanism involved in the turning-off of an ionic membrane conductance by 5-HT is that the amine would interfere in

Fig. 11. Effect of changes of $[Na]_o$ on the $\beta$-responses. *a*, $\beta$-response to 5-HT which inhibits the firing of a neurone, at $-40$ mV. *b–e*, this hyperpolarizing $\beta$-response gradually grows in amplitude when the neurone is hyperpolarized to $-50$, $-60$, $-70$ and $-80$ mV respectively. In *f–j*, half of the NaCl content of the external medium is replaced by Tris-Cl, causing a shift of the inversion potential of the $\beta$-response, ($E_\beta$), which becomes observable at $-40$ mV (*k*). The graph at the bottom relates the amplitude of the $\beta$-responses with the membrane potential values at which they were recorded. The open circles correspond to the experiment in the normal medium and the filled circles to the values recorded in a low $Na^+$ medium. Notice the shift of $E_\beta$ from the calculated value of $-23$ mV to the observed value of $-40$ mV (see text).

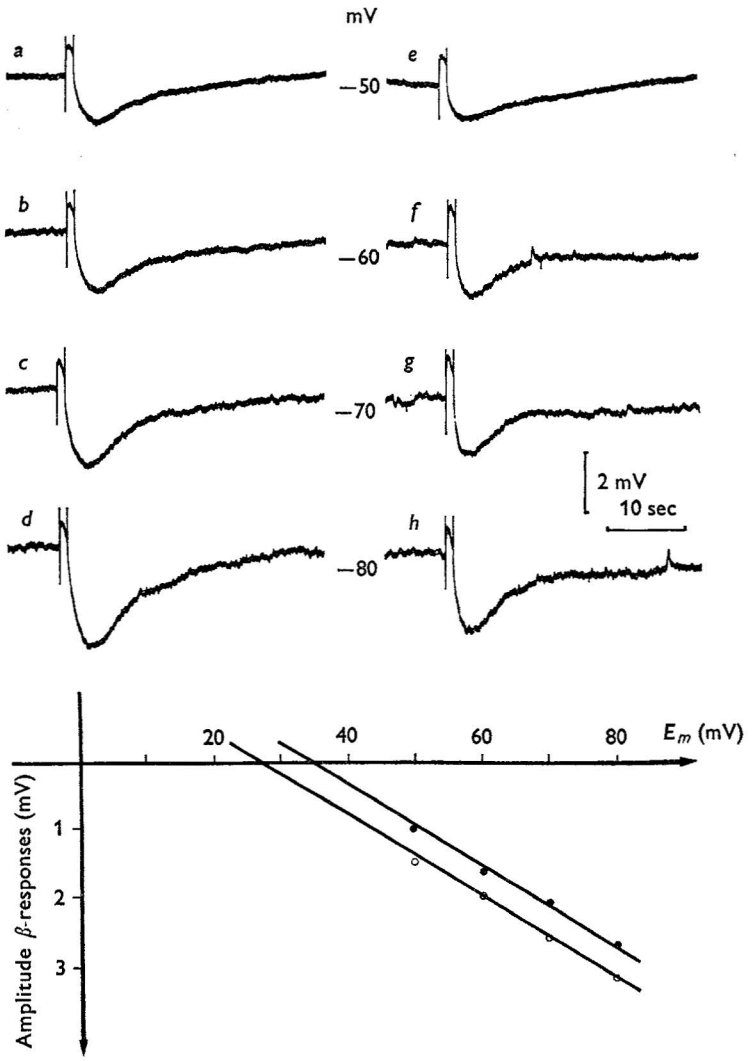


Fig. 12. Effects of changes of $[K]_o$ on the $\beta$-responses. *a*, $\beta$-response to 5-HT recorded in a neurone bathed in a normal medium containing 5 mM-$K^+$, at $-50$ mV. *b–d*, the $\beta$-responses increase with increasing polarization of the cell. *e–h*, a similar series of 5-HT applications to the same neurone, which is now bathed in a medium containing only 2·5 mM-$K^+$.

The graph at the bottom relates the amplitude of the $\beta$-responses recorded in a normal $[K]_o$ medium (open circles) or in a low $K^+$ medium (filled circles) with the membrane potential values at which the recordings were made. Notice the shift in the calculated values of $E_\beta$ from $-27$ to $-34$ mV when $[K]_o$ is reduced by half (see text).

some way with the release of a transmitter from another neurone. To test this hypothesis, the effects of reducing $[Ca]_o$, increasing $[Mg]_o$ and adding chelating agents to the medium to impair transmitter release were tested on $\beta$-responses. Fig. 13 I*a–c* corresponds to one of these experiments and shows that when the whole $Ca^{2+}$ content of the saline was removed, the $[Mg]_o$ increased four times and 5 mM-EGTA were added to the medium, although the $\beta$-responses became decreased in amplitude (Fig. 13 I*b*) they were still clearly observable. It is evident that in such ionic conditions, the

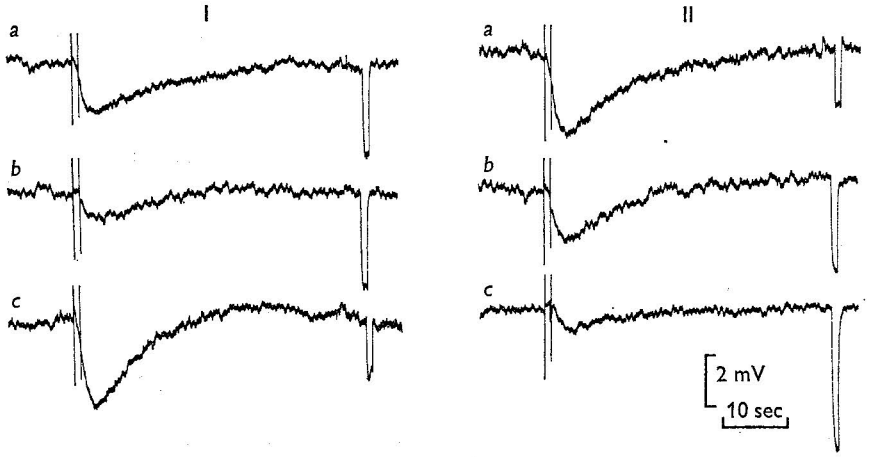


Fig. 13. Effect of changes in the divalent ions' external concentration on the $\beta$-responses. $\beta$-responses in a neurone hyperpolarized by inward current to $-60$ mV. At the end of each recording, a square current pulse was passed to measure the input resistance.

I. *a*, control $\beta$-responses in a normal medium containing 6 mM-$Ca^{2+}$ and 3·5 mM-$Mg^{2+}$. *b*, $\beta$-response in a medium with no $Ca^{2+}$ ions, 14 mM-$Mg^{2+}$ and also containing 5 mM-EGTA. *c*, the same 5-HT application is performed now in a medium without $Ca^{2+}$ and containing 3·5 mM-Mg and 5 mM-EGTA. II. $\beta$-responses recorded in the same cell at different $[Ca]_o$, keeping a constant $[Mg]_o = 3{\cdot}5$ mM. In *a*, $[Ca]_o$ is 2 mM, in *b*, 6 mM and in *c*, 20 mM (see text).

transmitter release due to the firing of neurones would probably be abolished. Moreover, when all the $Ca^{2+}$ ions were removed from the environment, without increasing the concentration of $Mg^{2+}$ ions and the same concentration of EGTA was present, the $\beta$-responses became markedly increased (Fig. 13 I*c*). A possible explanation of these variations in amplitude of the $\beta$-responses is suggested by the results shown in Fig. 13 II. In this neurone, a $\beta$-response was recorded in media containing a $[Ca]_o$ varying between 2 and 20 mM. The gradual increase in $[Ca]_o$ resulted in a gradual decrease in the amplitude of the $\beta$-response (Fig. 13 II *a–c*)

suggesting a probable non-specific impairment of the responses by divalent cations. It is also interesting to point out that the increase in the external concentration of either of the divalent cations was also associated with a parallel increase in the membrane resistance (Fig. 13).

From these experiments it may be concluded that the $\beta$-responses do not appear to result from the suppression by 5-HT of a transmitter release resulting from the firing of an interneurone.

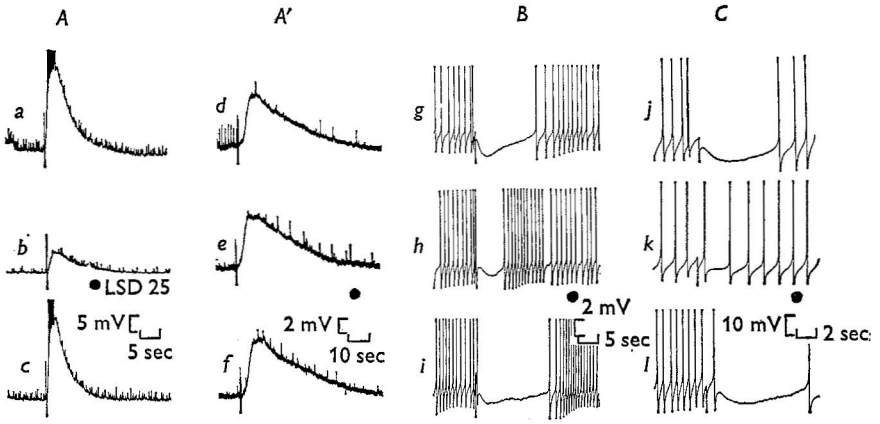


Fig. 14. Action of LSD 25 on 5-HT receptors. In this and following Figures, each vertical column of recordings ($A$, $A'$, $B$, $C$) corresponds to a series of homonymous responses to 5-HT. $a$–$c$, $A$-responses recorded in a neurone hyperpolarized by inward current to $-80$ mV. The depolarization reaches the firing level. $a$, control. $b$, bath application of $10^{-5}$ M LSD 25 blocks the response. $c$, the response recovers its original amplitude after removing the antagonist. $d$–$f$, $A'$-responses recorded in a neurone whose membrane potential was driven to $-80$ mV. $d$, control. $e$, response unaffected by a perfusion of $10^{-5}$ M solution of LSD 25. $f$, after removing LSD 25 from the bath. $g$–$i$, $B$-responses to 5-HT recorded in a neurone spontaneously firing at $-38$ mV. $g$, control. $h$, bath application of LSD 25 $10^{-5}$ M blocks the response. $i$, recovery of the response after removal of the blocking agent. $j$–$l$, $C$-responses to 5-HT in a neurone discharging action potentials at $-40$ mV. $j$, control. $k$, blockage of the $C$-response by LSD 25 $10^{-5}$ M. $l$, wash out of the antagonist.

### *Identification of 5-HT receptors*

A series of experiments were made to investigate the individuality and specificity of each of the receptors involved in the described 5-HT responses. This was attempted by searching for antagonist drugs which allow a specific blockade of each type of response. The blocking agents whose actions will be described in this paper were selected because they did not behave as agonists at the concentration used. This study was only partially

successful. Although a series of antagonists were found which permitted a rather good discrimination between the different receptors involved in the 5-HT responses associated with membrane conductance increases, no blocking agents were found for the $\alpha$- and $\beta$-responses. Therefore, the following data deal only with the $A$-, $A'$-, $B$- and $C$-responses.

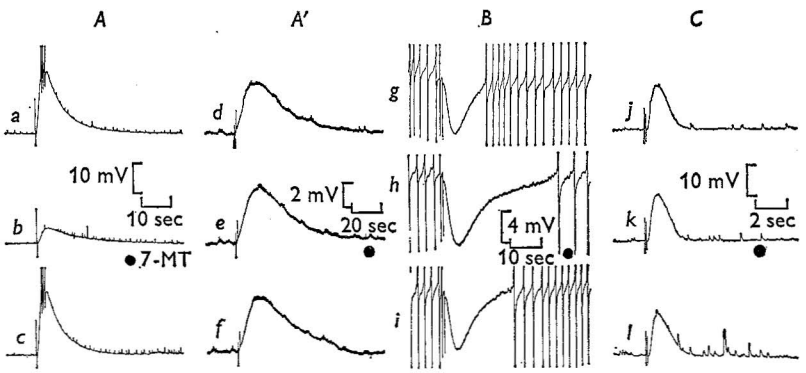


Fig. 15. Specific blockage of $A$-responses by 7-methyltryptamine (7-MT).

*a–c*, $A$-responses to 5-HT recorded in a neurone hyperpolarized by inward current to $-80$ mV. The 5-HT application depolarizes the cell to the firing level. *a*, control $A$-response. *b*, the bath application of a $10^{-5}$ M solution of 7-MT blocks almost completely the $A$-response, which recovers after removal of the blocking agent (*c*).

*d–f*, $A'$-responses to 5-HT of a neurone hyperpolarized by inward current to $-70$ mV. The control response in *d* is not affected in *e* by the perfusion of a $10^{-5}$ M solution of 7-MT. *f*, after washing out the antagonist.

*g–i*, $B$-responses to 5-HT of a neurone discharging 'spontaneous' spikes at $-40$ mV. *g*, control response. *h*, the perfusion of 7-MT prolongs the inhibitory effect of 5-HT. This could be due to the blocking of an $A$-receptor located at the same membrane, whose responses were masked by the inhibition. *i*, the recovery of the response in normal medium.

*j–l*, $C$-responses recorded as depolarizing potentials in a neurone hyperpolarized by inward current to $-80$ mV. Perfusion of 7-MT to the preparation at *k* does not affect the response. *l*, after washing.

Previous studies (Gerschenfeld & Stefani, 1966; Stefani & Gerschenfeld, 1969) had already shown that among other drugs TC, the dyethylamide of lysergic acid (LSD 25) and tryptamine were able to block the $A$-responses. Curare was also shown to block the $C$-responses (Gerschenfeld, 1971) and to be inactive on both $A'$- (Fig. 1) and $B$-responses (Gerschenfeld, 1971). The effects of LSD 25 and tryptamine are similar when assayed on the different 5-HT responses. Fig. 14 shows that the perfusion of a $10^{-5}$ M solution of LSD 25 to four neurones, each showing a different 5-HT response, caused a block of the $A$-, $B$- and $C$-responses without affecting

the amplitude of the *A'*-depolarization (Fig. 14*d–f*), even when LSD 25 was applied in much higher concentrations.

From a series of more than fifteen structural analogues of 5-HT assayed in these responses, three had interesting actions: 7-methyltryptamine (7-MT), bufotenine and 5-methoxygramine (5-MG). Fig. 15 records the specific blocking effects of 7-MT on the *A*-responses (Fig. 15*a–c*). No other response was affected at all by this antagonist (Fig. 15*d–l*) even at higher

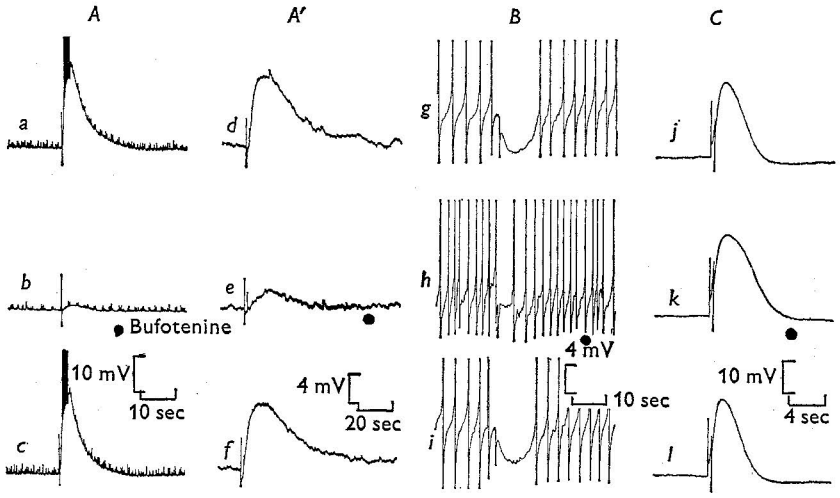


Fig. 16. Blocking effects of bufotenine $10^{-5}$ M solution on the *A*-, *A'*- and *B*-responses to 5-HT.

*a–c*, *A*-response in a neurone excited by 5-HT application at −80 mV. *a*, a control 5-HT depolarization which reaches the firing level. *b*, bath application of the antagonist causes a marked attenuation of the response. *c*, the response returns to the control amplitude after washing.

*d–f*, *A'*-responses recorded at −80 mV. *d*, control *A'*-response. *e*, response greatly reduced by bufotenine application. *f*, the response recovers its initial amplitude after removal of the bufotenine.

*g–i*, *B*-responses of a neurone recorded at −50 mV. The control response in *g* disappears under the action of bufotenine in *h*, and recovers after washing in *i*.

*j–l*, *C*-responses recorded as depolarizing potentials in a neurone at −80 mV. Bufotenine bath application, in *k*, only produces a slight prolongation of the response.

concentrations. Bufotenine (5-hydroxy-N-dimethyltryptamine) was reported to have agonist effects on snail neurones at concentrations of $10^{-9}$ M (Walker & Woodruff, 1972). However, at the concentrations used in the present work ($10^{-6}$–$10^{-5}$ M) such actions were not observed, neither on the membrane potential nor on the resting conductance of the neurones. Fig. 16 shows that a $10^{-5}$ M concentration of bufotenine blocked

the *A*-, *A'*-and *B*-responses (Fig. 16*a–i*) whereas it did not alter the *C*-responses (Fig. 16*j–l*).

5-methoxygramine has an action completely different from those of the other antagonists. Recordings of the four types of responses are shown again in Fig. 17, where the perfusion of a $5 \times 10^{-6}$ M solution of 5-MG was demonstrated to block specifically the *B*-response (Fig. 17*g–i*) without affecting any of the other three. Further increase in the concentration of 5-MG did not alter this selective behaviour.

Finally, it is of interest that neostigmine, an anticholinesterase agent, can specifically block the *C*-response without affecting any of the others.

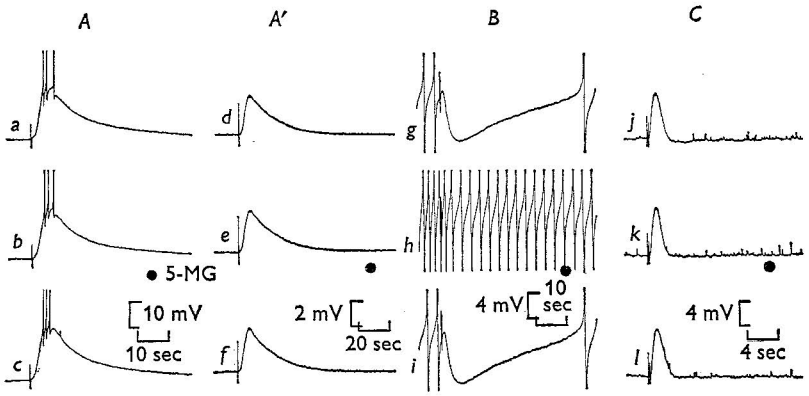


Fig. 17. Specific blockage of the *B*-response to 5-HT by 5-methoxygramine (5-MG).

*a–c*, *A*-responses in a neurone hyperpolarized by inward current to −80 mV. The depolarization reaches the firing level. *a*, control response. *b*, perfusion of a $5 \times 10^{-6}$ M solution of 5-MG has no effect on the response. *c*, removal of the 5-MG.

*d–f*, *A'*-responses also recorded in a cell at −80 mV. The response is not affected by 5-MG applied at *e*. *f*, removal of the 5-MG.

*g–i*, *B*-responses to 5-HT recorded in a spontaneously active neurone at −50 mV. *g*, control. *h*, bath application of a $5 \times 10^{-6}$ M solution of 5-MG blocks the response. *i*, the *B*-response recovers its characteristics after removing the antagonist.

*j–l*, *C*-responses recorded as depolarizing potentials in a neurone whose membrane potential is driven to −80 mV. *j*, control. *k*, response unaffected by bath application of $10^{-5}$ M-5-MG. *l*, the *C*-response after washing out 5-MG.

### *Multireceptor responses*

As in the case of ACh (Kehoe, 1967, 1972; Wachtel & Kandel, 1971) and dopamine (Ascher, 1972) several types of 5-HT receptors may be seen on the same cell. The activation of such receptors results in biphasic or multiphasic responses to the iontophoretic application of 5-HT, combining

excitatory and/or inhibitory effects. Some of them have been already illustrated (see Fig. 2 and Gerschenfeld & Paupardin, 1973). With the aid of specific antagonists it is generally possible to dissociate the components of these complex responses and to show that they result from the activation of two or more of the four receptors described (*A*, *A'*, *B*, *C*). A detailed analysis of these multireceptor responses is outside the scope of this paper.

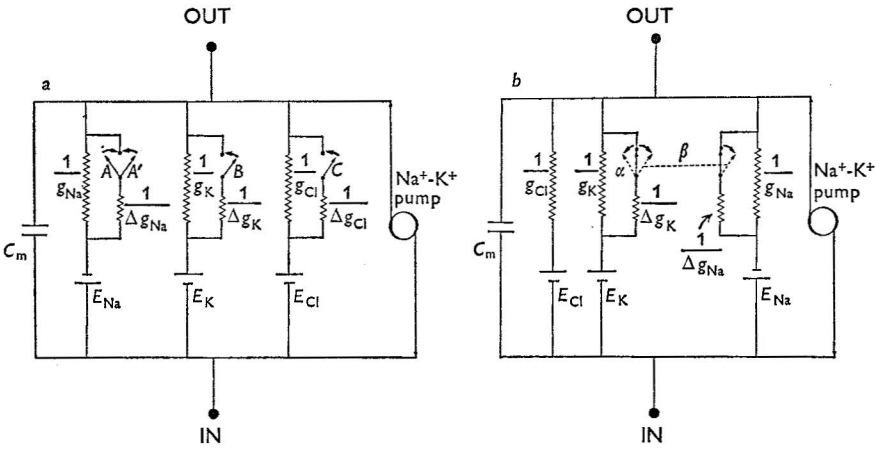


Fig. 18. Simplified membrane circuits representing the conductance changes induced by the activation of different 5-HT receptors in molluscan neurones.

*a*, 5-HT responses involving an *increase* in membrane conductance. The closing of either the *A*- or the *A'*-switch or of both at the same time will introduce a shunt ($\Delta g_{Na}$) in the $Na^+$-conductance ($g_{Na}$), which becomes increased. In the same way, the closing of the *B*-switch and the *C*-switch by the activation of the homonymous receptors will cause an increase in the $K^+$ and the $Cl^-$ conductances ($g_K$ and $g_{Cl}$) respectively.

*b*, 5-HT responses caused by a *decrease* in the membrane conductance. The resting conductances to both $Na^+$ and $K^+$ are represented by two conductances in parallel ($g_{Na}$ and $\Delta g_{Na}$; $g_K$ and $\Delta g_K$). 5-HT produces an $\alpha$-response by opening the switch which will remove the shunt $\Delta g_K$, and thus decreases $K^+$ conductance. The $\beta$-response will result from the opening of the coupled switches controlled by $\beta$, thus decreasing both $Na^+$ and $K^+$ conductances.

Table 1 summarizes the ionic mechanism associated with the six different responses to 5-HT described here and Fig. 18 schematizes in two different equivalent membrane circuits the mechanism of these different 5-HT actions. In Fig. 18*a* are represented the responses involving the activation of a membrane conductance: the closing of one of the switches *A*, *A'*, *B* or *C* would produce the homonymous response. By contrast, in Fig. 18*b*, it is the opening of the switches $\alpha$ or $\beta$ which would turn-off

a component of the membrane conductance and cause the homonymous responses to 5-HT.

Table 2 summarizes the pharmacological properties of the receptors involved in the responses to 5-HT associated with a conductance increase.

TABLE 1. 5-HT responses of molluscan neurones

| Response | Effect | Reversal potential value (mV) | Ionic mechanism |
|---|---|---|---|
| *A* | 'Fast' depolarization | ~0* | Increase $g_{Na}$ |
| *A'* | 'Slow' depolarization | ~0* | Increase $g_{Na}$ |
| *B* | 'Slow' hyperpolarization | −75 | Increase $g_K$ |
| *C* | 'Fast' hyperpolarization | −56 | Increase $g_{Cl}$ |
| $\alpha$ | Depolarization | −75 | Decrease $g_K$ |
| $\beta$ | Hyperpolarization | ~ −30 | Decrease $g_{Na}$ and $g_K$ |

* Estimated by extrapolation.

TABLE 2. Effect of antagonists on the 5-HT responses of molluscan neurones associated with an increase in membrane conductance

| Antagonist | *A* | *A'* | *B* | *C* |
|---|---|---|---|---|
| (+)-TC | Blocks | None | None | Blocks |
| LSD 25 | Blocks | None | Blocks | Blocks |
| Tryptamine | Blocks | None | Blocks | Blocks |
| 7-MT | Blocks | None | None | None |
| Bufotenine | Blocks | Blocks | Blocks | None |
| 5-MG | None | None | Blocks | None |
| Neostigmine | None | None | None | Blocks |

## DISCUSSION

A survey of the responses to 5-HT in two molluscan nervous systems shows that only part of the neurones are sensitive to the iontophoretic application of this amine, responding to it in six different ways: three excitations and three inhibitions, involving five different ionic mechanisms.

Such a variety of responses for 5-HT is not such a surprising finding since a complex pattern of receptor and ionic mechanisms has already been demonstrated in the molluscan nervous system, in a study of the action of other transmitters such as acetylcholine (Tauc & Gerschenfeld, 1961; Kehoe, 1967, 1972; Wachtel & Kandel, 1971; Levitan & Tauc, 1972; see review Gerschenfeld, 1973) and dopamine (Ascher, 1972). Moreover, indication of such complexities have also been observed in the case of other transmitter candidates (Gerschenfeld & Lasansky, 1964). However, the multiplicity of mechanisms associated with the 5-HT responses is the most complex observed for any transmitter candidate in any nervous system.

What seems particularly interesting in this multiplicity is the redundancy of the 5-HT actions. Some experimental data on the possible physiological significance of this redundancy will be presented in the next paper. It is not hard to suppose that such redundancy probably offers a way of achieving different actions with different temporal properties, when comparing, for instance, the slow $A'$-excitation with the faster $A$-depolarization or the slow $K^+$-dependent $B$ inhibition with the faster $Cl^-$-dependent $C$ hyperpolarization. Three main factors could possibly account for these temporal differences: the antagonist-receptor binding-relaxation properties, the temporal properties of the ionic channels and/or some local difference in the inactivation mechanism of 5-HT. There are no grounds for supposing that the mechanism of inactivation would be different when 5-HT is applied on to the soma of different neurones. On the other hand, when comparing the $A$- and $A'$-responses, since both are associated with an increase in $Na^+$-conductance, it is not unreasonable to suppose that they involve the same ionic channels, and if this is so, the difference in the response must reflect differences between the $A$- and $A'$-receptors.

It is evident that the $B$- and $C$-responses must involve both different receptors and different ionic pathways. However, when the time course of these 5-HT responses is compared with those to ACh also involving conductance increases to $K^+$ or $Cl^-$ (Kehoe, 1972), it becomes clear that the $K^+$-dependent responses to both agonists are much more prolonged than the $Cl^-$-dependent ones. A similar difference in time course has been also observed between $Cl^-$- and $K^+$-dependent responses to glutamate in molluscan neurones (C. Lowagie & H. M. Gerschenfeld, unpublished). All these data suggest that the difference in time course between the $B$- and the $C$-responses may depend on some difference in the ionic channels. Nevertheless, more evidence is necessary to reject completely the possibility that the difference stems from the properties or the localization of the receptors. A way to investigate this question might be to study the 5-HT 'noise' in the same way as the ACh 'noise' was studied on the neuromuscular end-plate by Katz & Miledi (1972).

The experimental evidence demonstrating that each one of the four different actions of 5-HT resulting from an increase in ionic conductance is linked to the activation of a distinct receptor seems fairly conclusive: each receptor can be discriminated from the others by the use of specific antagonists (Table 4). As it was previously reported (Gerschenfeld & Paupardin, 1973) by manipulating the environmental divalent cations and adding chelating agents, it was possible in snail ganglia to suppress all synaptic activity and yet still to record the $A$-, $A'$-, $B$- and $C$-responses to 5-HT. It thus seems highly unlikely that these responses result from an indirect action of 5-HT on the firing of an interneurone. However this

does not rule out a possible effect of 5-HT on the *spontaneous* transmitter release from another neurone.

Responses such as the $\alpha$- and $\beta$-responses to 5-HT do not seem to be unique to molluscan neurones. The idea that turning-off a membrane permeability could produce a membrane potential change was postulated more than ten years ago by Kuffler (1960). A year later, Grundfest (1961) reported that 5-HT applied to lobster exoskeletal muscle fibres caused an increase of their resistance and the conversion of a graded response into a full spike. The operation of such a mechanisms by a synaptic transmitter was suggested by Krnjević & Schwartz (1967). The work by Weight and his collaborators (Weight & Votava, 1970; Weight & Padjen, 1973*a*) has recently brought experimental evidence supporting the idea that both the slow EPSPs and IPSPs recorded in frog sympathetic ganglion neurones are generated by decreases in either $K^+$- and $Na^+$-conductances respectively, and a similar conductance decrease mimicking the slow i.p.s.p. could be evoked in the same neurones by ACh (Weight & Padjen, 1973*b*). Other pharmacological studies have demonstrated that the inhibitory effects of iontophoretically applied norepinephrine, either to spinal motoneurones (Engberg & Marshall, 1971) or to cerebellar Purkinje neurones (Siggins, Oliver, Hoffer & Bloom, 1971) are also associated with an increase in membrane resistance.

It is possible that in the cases of the $\alpha$- and $\beta$-responses, 5-HT also acts on specific receptors. However, it is known only that these receptors are different from those involved in the other 5-HT responses. The observation that 5-HT does not produce resistance increases in every neurone of the ganglion and that it elicits the $\alpha$- and $\beta$-responses only when applied to restricted areas of identified neurones constitute arguments in favour of the existence of distinct receptor sites. The same arguments also indicate that the ionic channels involved in these responses are different from the channels regulating the general conductance of the neurone.

Little more can be concluded about the channels involved in the $\alpha$- and $\beta$-responses to 5-HT. The conductance ratio ($\Delta g_{Na}/\Delta g_K = 0{\cdot}5$) seems to differ from that at the frog muscle end-plate (1·3; Takeuchi & Takeuchi, 1960). In addition, at the end-plate the conductance ratio is independent from the external $Na^+$ concentration (Takeuchi, 1966), whereas in the case of the $\beta$-responses, $\Delta g_{Na}/\Delta g_K$ varies when the external concentration of $Na^+$ (and $K^+$) were altered.

Since the ionic channels turned off in the $\alpha$- and $\beta$-responses to 5-HT are permanently open and thus must contribute to the membrane potential of the neurones, it would be important to know why they are continuously turned on. Two possibilities, at least, can be envisaged: (*a*) they are a special type of channel, built-in in an 'open' conformation in the

structure of the membrane, or (*b*) they remain open as a result of the continuous action of a transmitter. The second possibility has been recently considered in connexion with the hyperpolarization, associated to a conductance decrease, observed in the horizontal cells of vertebrate retina (Tomita, 1965; Nelson, 1973). To explain such observation, it was postulated (Trifonov, 1968) that photostimulation would suppress the continuous release of the photoreceptors' transmitter. Recent experiments by Dowling & Ripps (1973) and by Cervetto & Piccolino (1974) would support such hypothesis since manipulations presumed to suppress transmitter release (an increase in external $Mg^{2+}$ ions, accompanied or not by a reduction in $Ca^{2+}$ ions) were shown to mimic the effect of photostimulation. As was already discussed for the case of the *A*-, *A'*-, *B*- and *C*-responses, it is not possible to rule out a continuous 'spontaneous' release of transmitter at the origin of the responses of the molluscan neurones to 5-HT.

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