A Slow Voltage-Dependent Na^+ -Current Induced by
5-Hydroxytryptamine and the G-Protein-Coupled
Activation Mechanism in the Ganglion
Cells of *Aplysia*

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Abstract Application of 5-hydroxytryptamine (5HT) induces a slowly depolarizing response in the neurons of *Aplysia* abdominal ganglion. In voltage-clamped cells, 5HT induced a slow inward current that increased steeply with membrane depolarization from -85 mV showing a negative slope conductance, but never reversed into outward when hyperpolarized beyond the equilibrium potential for K^+ . The 5HT-induced response was markedly augmented in Ca^{2+} -free media, but depressed in Na^+ -free media, and unaffected by a change in external potassium. Intracellular injection of guanosine 5'-O-(2-thiodiphosphate) (GDP β S) significantly depressed the 5HT response in a dose-dependent way. Injection of cholera toxin (CTX) selectively blocked the 5HT-induced response, the effect being irreversible. Neither 3'-deoxyadenosine, an inhibitor of adenylate cyclase, nor H-8, an inhibitor of protein kinase A, depressed the 5HT response. 3-Isobutyl-1-methylxanthine (IBMX) did not augment the 5HT response appreciably. The 5HT responses were not depressed at all during a saturated response to Br-cyclic AMP injected intracellularly. It was concluded that the 5HT response is produced by opening of the voltage-dependent Na^+ -channels with activation of CTX-sensitive G-protein but not necessarily with an increase in intracellular cyclic AMP.

Key words: serotonin receptor, GTP-binding protein, *Aplysia* neuron, cholera toxin, voltage-dependent slow inward current.

Serotonin (5-hydroxytryptamine: 5HT) induces a variety of responses: fast depolarizing or hyperpolarizing type and slowly depolarizing or hyperpolarizing type in both invertebrate and mammalian neurons (GERSCHENFELD and PAUPAR-

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DIN-TRITSCH, 1974; see also the review by BOBKER and WILLIAMS, 1990). Our present paper concerns only electrogenesis of the slowly depolarizing type of response. This type of response is often associated with an apparent decrease or appearance of negative conductance. As for ionic mechanism of this response, the following three different results have been reported independently: the response is produced mainly by 1) closing of K^+ -channels (DETERRE *et al.*, 1981, 1982; SIEGELBAUM *et al.*, 1982; WALSH and BYRNE, 1985), 2) opening of voltage-dependent Ca^{2+} -channels (PELLMAR and CARPENTER, 1980), 3) opening of voltage-dependent Na^+ -channels (KIRK *et al.*, 1988). Since the difference in results might be due to the difference in neurons they examined, our first attempt in this study is to examine which ionic mechanism is involved in the neurons we identified in the abdominal ganglion of *Aplysia*. Our experiment proved that the response was produced mainly by opening of voltage-dependent Na^+ -channels, the results being consistent with those of KIRK *et al.* (1988).

Our second attempt in this study is to elucidate what kind of G-protein and effector enzyme are involved in generation of this response. The investigators who emphasized the importance of K^+ -channel closing as the major ionic mechanism suggested that the receptor stimulation would activate adenylate cyclase and protein kinase A, and that closure of K^+ -channels could be due to phosphorylation by protein kinase A (DETERRE *et al.*, 1981, 1982; SIEGELBAUM *et al.*, 1982; WALSH and BYRNE, 1985). PELLMAR and CARPENTER (1980), who described the importance of Ca^{2+} -channel opening as the major ionic mechanism, also considered earlier that the receptor stimulation might activate adenylate cyclase, but they reported later that there was no detectable increase in intracellular cyclic AMP after receptor stimulation (PELLMAR, 1981). KIRK *et al.* (1988), who described that the response is produced mainly by opening of the voltage-dependent Na^+ -channels, also considered that the receptor stimulation would activate adenylate cyclase, and that cyclic AMP might be directly responsible for opening of Na^+ -channels (KIRK *et al.*, 1988; TAUSSIG *et al.*, 1989). In contrast, our present experiments demonstrate that CTX-sensitive G-protein is mediating this response but suggest that neither adenylate cyclase activation nor intracellular accumulation of cyclic AMP are involved in generation of this response.

A preliminary account of this work has previously been reported (KUDO *et al.*, 1989).

METHODS

Preparation and perfusion media. The abdominal ganglion of *Aplysia kurodai* was dissected out and fixed in a perfusing chamber. The connective tissues covering the ganglion cells were carefully removed under the dissecting microscope to expose the cells to the perfusing medium. The cells were normally perfused with a physiological medium with an ionic composition similar to that of *Aplysia* blood (cf. SATO *et al.*, 1968): (in mM) Na^+ , 587; K^+ , 12; Cl^- , 671; Ca^{2+} , 14; Mg^{2+} , 52. The

pH was adjusted to 7.4 with Tris and HCl, and the cells were continuously perfused at a constant flow rate of $5 \text{ ml} \cdot \text{min}^{-1}$ and at the temperature $23 \pm 2^\circ\text{C}$. The effective perfusing volume of the chamber was 0.2 ml. Na^+ -free media were prepared by replacing NaCl with Tris and HCl. Modification of the K^+ -concentration was made simply by adjusting the concentration of KCl, neglecting a slight change in osmolarity. A Ca^{2+} -free medium was made by replacing CaCl_2 with equimolar MgCl_2 .

Evaluation of 5HT-induced response. Two microelectrodes filled with 1.8 mM potassium citrate with a resistance of 2–3 $\text{M}\Omega$ were introduced into a single cell of the RC cluster (FRAZIER *et al.*, 1967) under binocular microscopic control. One electrode was connected to the input of a preamplifier installed in a Dagan 8500 clamp unit in order to record the change in membrane potential. The other electrode was connected to the output of an operational amplifier in the same unit in order to pass the current across the cell membrane. The membrane current was measured at the virtual ground of another operational amplifier of the clamp unit.

Either current- or voltage-clamp method was used to evaluate the response of the RC cells to bath-applied or pressure-ejected 5HT, or to intracellularly applied cyclic AMP. In voltage-clamp methods, the responses to these agents were evaluated at various membrane potentials, thereby permitting the determination of the current-voltage relationship of the drug action. In addition, repeated 800 ms 5 nA or 5–10 mV pulses in current- or voltage-clamp modes, respectively, were applied across the cell membrane to permit the evaluation of the change in the membrane conductance during a given response.

Drugs. Drugs used were serotonin (5HT), adenosine cyclic 3',5'-monophosphate (cyclic AMP), 3-isobutyl-1-methylxanthine (IBMX), dopamine (DA), acetylcholine (ACh), cholera toxin (CTX), pertussis toxin (PTX), adenosine triphosphate (ATP), nicotinamide adenine dinucleotide (NAD), dithiothreitol (DTT), guanosine 5'-O-(2-thiodiphosphate) ($\text{GDP}\beta\text{S}$), N-[2-(methylamino)ethyl]-5-isoquinolinesulfonamide (H-8), 3'-deoxyadenosine, and ADP-ribosylating factor (ARF).

ACh, DA, 5HT, and IBMX were dissolved into the normal artificial *Aplysia* medium and applied to the neuron by a constant flow described above. The duration of transmitter application was adjusted so as to observe just a beginning of the maximal plateau response, for example, 15 s at 1 mM and 20 s at 0.1 mM in the case of 5HT. After each application of a drug, the cells were washed with the normal *Aplysia* medium for at least 10–20 min before testing the effects of other chemicals. In analyzing the effects of Na^+ -free, K^+ -free, or Ca^{2+} -free media on the 5HT-induced response, these media were applied 8 min before testing the 5HT response, unless otherwise specified.

Cyclic AMP, $\text{GDP}\beta\text{S}$, 3'-deoxyadenosine, and H-8 were dissolved in a solution of 200 mM KCl. CTX and PTX were dissolved into the solution containing 200 mM KCl, 10 mM ATP (for PTX), or 10 mM GTP (for CTX), 10 mM DTT, and 10 mM NAD. DTT was used to promote dissociation of the A-protomer from the

B-subunit of these toxins (KATADA *et al.*, 1983).

Intracellular application of cyclic AMP, GDP β S, 3'-deoxyadenosine, H-8, CTX, and PTX. These reagents were intracellularly applied into the cells by pressure injection method. Each reagent was injected intracellularly by pressure pulse, a pulse duration of 200 ms with a pressure of 1–2 kg $\cdot$ cm $^{-2}$. Intracellular concentrations of these reagents were estimated by measuring the size of a droplet ejected from the electrode before impalement, and by calculating the volume ratio of the droplet to the cell examined.

Fig. 1. 5HT-induced responses of a neuron in the RC cluster of *Aplysia* abdominal ganglion. A: Slowly depolarizing response with spike firings recorded under current clamp. Periodic downward deflections in the voltage record (*V*) show the electrotonic potentials induced by constant 800 ms inward current pulses (*I*) delivered every 5 s, indicating the changes in effective membrane resistance. B: Slow inward current response (*I*) of the same cell recorded under voltage clamp at -55 mV. The mode of voltage clamp is shown above. Note the appearance of negative conductance during the current response.

RESULTS

I. Characteristics of the 5HT-induced slowly depolarizing response

In current-clamp experiments, an application of 0.01 to 0.1 mM 5HT to the cell of RC cluster induced a depolarizing response of approximately 10–15 mV, leading to generation of action potentials (Fig. 1A). The response measured at resting potential was always associated with an apparent increase in membrane resistance, as shown in Fig. 1A. The current response of the same cell to 5HT was recorded under voltage clamp and is shown in Fig. 1B. This response is often associated with the reverse polarity of the conductance measuring pulses, indicating the appearance of a negative slope conductance. This type of response was designated as “slowly depolarizing response” because the time course of this response was much slower

Fig. 2. Voltage dependence of the slow inward current induced by 0.1 mM 5HT (inward down). The peak amplitude of the 5HT-induced current (I_{5HT}) recorded under the voltage clamp was plotted against the membrane potential (V). Resting potential: -57 mV. Note that I_{5HT} steeply increased when the membrane was depolarized from -85 mV but never reversed to the outward current when the membrane was hyperpolarized beyond the E_K (-95 mV).

than that of other depolarizing responses which are associated with a distinct increase in membrane conductance.

a. Voltage dependence of this response. The amplitude of the current response to a given concentration of 5HT varied when measured at different holding potentials under voltage clamp. The peak amplitude of the 5HT-induced current (I_{5HT}) at each membrane potential was plotted against membrane potential (V) as shown in Fig. 2. The I_{5HT} - V curve thus obtained showed the minimum inward current response near -85 mV, and no reversal potential of this response. The response markedly increased as the membrane was depolarized from -85 mV, showing the characteristic voltage dependence with a steep negative slope. Similar I_{5HT} - V curves were obtained with 25 cells in RC cluster.

b. Effects of the changes in extracellular ionic concentrations. In an attempt to determine the ionic mechanisms underlying the 5HT-induced response, experiments were performed in which the ionic gradients for K^+ , Ca^{2+} , and Na^+ were altered.

When the external K^+ concentration ($[K^+]_o$) was reduced to zero (K^+ -free medium), the amplitude of the 5HT-induced inward current did not change

Fig. 3. Testing the effects of the change in external K^+ concentration ($[K^+]_o$) on the response to 0.1 mM 5HT. A: Middle trace is the response recorded in $[K^+]_o$ -free medium. Top and bottom are responses recorded in normal K^+ concentration taken before and after the test. The membrane potential was clamped at -20 mV in order to increase further the driving force for K^+ (see the text). B: Similar records except the middle trace which was recorded in 4 times normal $[K^+]_o$ -medium ($4 \times K^+$). The cell was different from that shown in A.

significantly in cells clamped at their resting potentials (-50 to -60 mV). Unaffected response in K^+ -free media was confirmed by an experiment in which the membrane was clamped at -20 mV (Fig. 3A). The inward current response recorded at this potential should show a greater increase in the K^+ -free media if the 5HT response should include a component due to a closure of K^+ -channels. When the $[K^+]_o$ was increased to 4 times normal ($4 \times K^+$), the 5HT-induced current was not altered significantly (Fig. 3B). Similar results were obtained from 12 cells in RC cluster. These results, together with the absence of reversal potential as mentioned above, suggested that the decrease in K^+ -permeability was not the major ionic mechanism involved in this slowly depolarizing response.

In the Na^+ -free media, the 5HT-induced inward current response were markedly depressed (Fig. 4A). The I_{5HT} - V curve shifted upward in low Na^+ (0.5 times normal Na^+) media with a little change in voltage dependence (data not shown). However, they did not disappear completely no matter how long Na^+ -free perfusion was continued. On the contrary, 5HT-induced inward current was markedly augmented in Ca^{2+} -free with normal Na^+ -media (Fig. 4B). The I_{5HT} - V curve simply shifted downward in Ca^{2+} -free media with little change in voltage dependence (data not shown). However, this response completely disappeared in

Fig. 4. A: Top is a control response to 0.1 mM 5HT. Middle is the same response but recorded in Na^+ -free media. Bottom shows the recovery of the response in normal Na^+ -media. B: Top is a control recorded from a different cell. Middle is the same response but recorded in Ca^{2+} -free media. Bottom shows the recovery of the response in normal Ca^{2+} -media. C: Top is a control recorded from a different cell. Middle shows a complete disappearance of the response in Na^+ - and Ca^{2+} -free media. Bottom shows the recovery of the response in normal Na^+ - and Ca^{2+} -media.

Fig. 5. Dose-response curves for 5HT obtained in the normal perfusing medium (open circle) and for 0.3 times normal Na^+ -medium (filled circle). 5HT-induced currents were normalized by the response to 0.3 mM 5HT obtained in the normal perfusing medium. Each point represents the mean value obtained from 5 cells voltage-clamped at -55 mV.

Ca^{2+} - and Na^+ -free media (Fig. 4C). Similar results were obtained in 20 cells of the same type. The results shown in Fig. 4A and C suggest that the 5HT-induced inward current consisted mainly of Na^+ -influx. Refer to DISCUSSION as for interpretation of Fig. 4B.

In order to examine if the depressing effects of Na^+ -free media on the 5HT-induced current were due to the decrease in the affinity of the receptor for 5HT, dose-response curves were obtained both in normal perfusing media and 0.3 times normal Na^+ -perfusing media. As seen in Fig. 5, the maximum value of the curve markedly decreased in the 0.3 times normal Na^+ media. On the other hand, the values of ED_{50} estimated from the curves showed no significant difference statistically because they were 0.026 ± 0.02 mM (mean $\pm$ S.D., $n=5$) for control and 0.04 ± 0.02 mM (mean $\pm$ S.D., $n=5$) for the 0.3 times normal Na^+ media. This result suggests that the depression was not due to the decrease in the receptor affinity.

II. Intracellular mechanisms involved in generation of this response

Many investigators have suggested that the 5HT-induced slow inward current is produced by an activation of adenylate cyclase and increase in intracellular cyclic AMP (SIEGELBAUM *et al.*, 1982; TAUSSIG *et al.*, 1989; WALSH and BYRNE, 1985). If the 5HT-receptor of this type could ultimately stimulate the adenylate cyclase, the receptor should activate a specific G-protein (G_s) since G_s is known to be a

Fig. 6. Effects of intracellular application of GDP β S on 5HT-induced response (A) and ACh-induced response (B) of the same cell. Top traces show control responses both for 5HT and ACh. Lower three traces in A were recorded 1, 10, and 25 min after the first injection of GDP β S. Note that the second injection of GDP β S completely depressed the 5HT-induced inward current, while it has no effect on ACh-induced inward current. Resting membrane was clamped at -55 mV. Estimated intracellular concentration of GDP β S was $125\text{--}250\text{ }\mu\text{M}$.

regulatory (stimulatory) protein of this effector enzyme (GILMAN, 1987). In order to examine whether or not these sequential biochemical reactions are actually occurring during the 5HT-induced response of this type, we analyzed the effects of the following reagents which are known to affect the specific steps in these biochemical reactions.

a. Effect of GDP β S. Intracellular injection of GDP β S, a competitive inhibitor of GTP for the binding site at G-protein (ECKSTEIN *et al.*, 1979), did not cause any change in either slope conductance or potential level of the resting membrane. In contrast, GDP β S significantly depressed the 5HT-induced inward current (Fig. 6A). The depressing effect of GDP β S was dose dependent since 5HT-induced responses were further depressed by the second injection of GDP β S. The depressing effect was sustained as long as intracellular concentration of GDP β S was maintained and it was very specific for the 5HT-induced slow inward current because fast inward current response of the same cell to acetylcholine was not affected at all (Fig. 6B). Similar selective and irreversible depressing effect of GDP β S was confirmed with 12 other cells in three RC clusters used for identical experiments.

b. Effect of CTX. Intracellular injection of CTX did not cause any ap-

Fig. 7. Effects of CTX on the 5HT-induced inward current (A) and DA-induced K^+ -current (B) recorded from the same cell. Control responses are on the top traces and lower traces are 15, 30, and 40 min after an injection of CTX. Note the time-dependent gradual depressing effect on the 5HT-induced inward current but no effect on DA-induced outward current. The resting membrane was clamped at -55 mV. Estimated CTX concentration inside the cell, $2-5 \mu g \cdot l^{-1}$.

preciable change in the resting state of the membrane. It is well known that ADP-ribosylation of G_s is markedly facilitated by co-existence of ADP-ribosylating factor (ARF) (SCHLEIFER *et al.*, 1982). However, we confirmed that there was no effect on the resting membrane even when CTX was injected together with ARF (data not shown).

Nevertheless, injection of CTX gradually depressed the 5HT-induced inward current and finally blocked it completely within 35 min (Fig. 7A). The blocking effect of CTX on the 5HT-induced response was considered to be irreversible because the 5HT response never recovered even after continuous washing more than 5 h. The effect of CTX was specific for 5HT-induced inward current because DA-induced outward current recorded from the same cell was not affected at all by CTX (Fig. 7B). Previously we confirmed that this DA response was produced by activation of a different G-protein (G_i or G_o) following the receptor stimulation (SASAKI and SATO, 1987). Similar selective and irreversible blocking effects of CTX were confirmed with 15 cells with the same type of receptors.

c. *Effect of PTX.* PTX is known to ADP-ribosylate G_i and G_o type of G-protein and causes uncoupling between the receptor and G-protein, resulting in the block of certain transmitter-induced responses (COTE *et al.*, 1984). Intracellular injection of PTX completely blocked K^+ -dependent outward current induced by DA within 30 min after injection of PTX (Fig. 8A) whereas it did not block the

Fig. 8. Effects of PTX on the DA-induced outward current (A) and 5HT-induced inward current (B) recorded from the same cell. Control responses are on the top traces, and lower traces are recorded 15, 30, and 40 min after an intracellular injection of PTX. Note the lack of any effect on the 5HT-induced inward current while PTX completely blocked the DA-induced outward current response. The resting membrane was clamped at -55 mV. Estimated PTX concentration inside the cell, $2-5 \mu\text{g} \cdot \text{l}^{-1}$.

5HT-induced inward current response of the same cell (Fig. 8B), again indicating selective action of each toxin.

d. Effects of inhibitors of adenylate cyclase and protein kinase A. In order to examine whether the 5HT-induced response is the result of activations of adenylate cyclase and protein kinase A, we analyzed the effects of relatively selective inhibitors for each enzyme on the 5HT-induced response: 3'-deoxyadenosine, an adenylate cyclase inhibitor (HAZEKI and UI, 1981) and H-8, a protein kinase A inhibitor (HIDAKA *et al.*, 1984). Intracellular injection of 3'-deoxyadenosine or H-8 affected neither the resting state of the membrane nor the 5HT-induced inward current (Fig. 9A, B). Intracellular concentrations of both 3'-deoxyadenosine and H-8 were estimated to be $10-20 \mu\text{M}$. Biochemically, this concentration is known to be sufficient to block adenylate cyclase and protein kinase A, respectively.

e. Effects of IBMX on 5HT-induced response and Br-cyclic AMP-induced response. The response of a neuron to Br-cyclic AMP (a nonhydrolyzable analog of cyclic AMP) injected intracellularly was markedly augmented and prolonged by perfusion with 0.1 mM IBMX, a phosphodiesterase inhibitor (Fig. 10A). This observation indicates that the concentration of IBMX was sufficient to inhibit the phosphodiesterase activity. Nevertheless, the 5HT-induced inward current recorded from the same neuron was not augmented at all (Fig. 10B).

Fig. 9. Testing the effects of either 3'-deoxyadenosine (A) or H-8 (B) on the 5HT-induced responses of different cells. The 5HT-induced responses were recorded 2, 15, and 32 min after intracellular injection of 3'-deoxyadenosine or H-8. Top traces are controls. Membrane potential was clamped at -55 mV in all recordings. Thin arrows on the beginning of each trace denote the original level of the holding current before injection of each drug.

f. 5HT-induced response during the saturated response to Br-cyclic AMP. If 5HT-induced response is caused by an increase in concentration of intracellular cyclic AMP following receptor stimulation, this response would be occluded if examined during the saturated response to Br-cyclic AMP injected intracellularly. On the contrary, the 5HT-induced response was not depressed at all while the response to the second application of Br-cyclic AMP was appreciably occluded by the first response (Fig. 11).

DISCUSSION

I. Ionic mechanisms in the 5HT-induced inward current. The present study showed that 5HT induces a slow inward current in RC cluster neurons in *Aplysia* abdominal ganglia. This response often exhibited an apparent decrease in slope conductance or appearance of negative slope conductance.

Many investigators have also observed similar 5HT responses associated with a decrease in slope conductance in various neurons of different animals (in mollusc, DETERRE *et al.*, 1981, 1982; SIEGELBAUM *et al.*, 1982; WALSH and BYRNE 1985; in

Fig. 10. Effects of IBMX on Br-cyclic AMP-induced (A) and 5HT-induced (B) slow inward current responses of the same cell. Br-cyclic AMP was intracellularly applied to the cell in A. Note that 0.1 mM IBMX did not augment the 5HT-induced response, while it markedly increased the amplitude and duration of the Br-cyclic AMP-induced response. Membrane potential was clamped at -55 mV. Intracellular concentration of Br-cyclic AMP was estimated to be $10 \mu\text{M}$.

rat facial motoneurons, VANDERMAELEN and AGHAJANIAN, 1982; in rat nucleus accumbens, NORTH and UCHIMURA, 1989; in guinea-pig myenteric neurons, JOHNSON *et al.*, 1980). Most of these investigators concluded that 5HT-induced responses of this type are produced mainly by a decrease in membrane conductance toward K^+ , because the reversal potentials of these responses corresponded to the equilibrium potential of K^+ .

On the other hand, the 5HT-induced responses of the neurons examined in this experiment showed neither reversal at E_{K} nor dependence on the external K^+ concentration, suggesting that the decrease in K^+ -conductance was not the major ionic mechanism. Instead, the responses we observed were markedly depressed when Na^+ was removed from the perfusing media. Accordingly, we concluded that the responses we examined in this experiment were produced mainly by opening of the voltage-dependent Na^+ -channels, which is partially inhibited by the presence of external Ca^{2+} . According to the recent work by KIRK *et al.* (1988), the 5HT-induced slow inward current recorded from the buccal ganglion cells of *Aplysia* is also due to opening of the voltage-dependent Na^+ -channels and not closing of K^+ -channels. The slow inward current responses associated with an appearance of negative slope conductance are not a feature specific to only this type of 5HT

Fig. 11. Top and bottom traces are control responses to 5HT recorded before injection of Br-cyclic AMP and after disappearance of Br-cyclic AMP response, respectively. Middle trace shows the Br-cyclic AMP-induced responses and 5HT-induced response superimposed. Note that the second response to Br-cyclic AMP indicated nearly saturated state of the response, while 5HT-induced response was not saturated but remained the same as the control. Membrane potential was clamped at -55 mV in all records.

response, but are also observed in responses of other receptors, namely, certain type of dopamine receptor (MATSUMOTO *et al.*, 1988), Phe-Met-Arg-Phe-NH₂ (FMRF-amide) receptor (ICHINOSE and MCADOO, 1988), and egg-laying hormone (ELH) receptor (KIRK *et al.*, 1988). All of these responses have been reported to be produced by opening of the voltage-dependent Na⁺-channels, but not by closing of K⁺-channels.

II. Involvement of G-protein. Recently, an involvement of G-protein in the receptor-operated response has been proved in various types of receptor activities. In our results, intracellular injection of GDP β S selectively depressed the 5HT-induced inward current without affecting the nicotinic response of the same cell which does not involve G-protein activation (SASAKI and SATO, 1987). The depression by GDP β S suggests the involvement of a certain type of G-protein in this 5HT-induced response. CTX is known to ADP-ribosylate α -subunit of G_s and activate G_i irreversibly (GILL and MEREN, 1978). However, intracellular injection of CTX did not cause any appreciable change in the holding current. The ineffectiveness of CTX on the resting membrane was confirmed by injection of CTX together with ADP-ribosylating factor (ARF), which is known to facilitate the

effect of CTX (SCHLEIFER *et al.*, 1982). Nevertheless, the 5HT-induced response was completely depressed 30–40 min after injection of CTX. The blocking effect of CTX was selective and irreversible. These results strongly suggest that 5HT-induced response of this type is mediated by the activation of the G-protein that is sensitive to CTX but probably not activated by CTX.

III. Does the 5HT-induced response we observed require an intracellular increase in cyclic AMP and subsequent activation of A-kinase? In the present experiments, injection of CTX during the resting state of the membrane did not cause any sustained inward current. This observation raised a question whether G_s type of G-protein really mediates the 5HT-induced response activating adenylate cyclase and A-kinase. However, injection of neither the adenylate cyclase inhibitor, 3'-deoxyadenosine, nor the A-kinase inhibitor, H-8, depressed the 5HT-induced response. Furthermore, the 5HT-induced response was not significantly augmented by the presence of phosphodiesterase inhibitor, IBMX, while the cyclic AMP-induced response was markedly augmented. In addition to these results, the 5HT-induced response was not occluded during the saturated response to cyclic AMP. These results are all against the premise that the 5HT-induced response we observed was the result of activation of adenylate cyclase, subsequent accumulation of cyclic AMP, and the activation of A-kinase.

Recently, YATANI *et al.* (1987, 1988) demonstrated that activated α_s -subunit of G_s can directly open the Ca^{2+} -channels coupled with the β -receptor in a reconstituted lipid membrane. The 5HT-induced slow inward current in our experiment seemed mainly due to the opening of voltage-dependent Na^+ -channel. This channel exhibited similar ionic permeability change and voltage dependence of the channel to those operated by cyclic AMP. Nevertheless, the opening of these channels does not seem to be regulated by cyclic AMP but by CTX-sensitive G-protein which is supposedly different from the classical G_s . Further experiments are needed to identify the exact nature of this G-protein.

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