J. exp. Biol. ***175****, 33–44 (1993)*
Printed in Great Britain © The Company of Biologists Limited 1993

AUGMENTATION OF BURSTING PACEMAKER ACTIVITY BY SEROTONIN IN AN IDENTIFIED *ACHATINA FULICA* NEURONE: AN INCREASE IN SODIUM- AND CALCIUM-ACTIVATED NEGATIVE SLOPE RESISTANCE *VIA* CYCLIC-AMP-DEPENDENT PROTEIN PHOSPHORYLATION

KOZO FUNASE

School of Allied Medical Sciences, Nagasaki University, Nagasaki 852, Japan

KAZUKO WATANABE

Department of Physiology, Gifu University School of Medicine, Gifu 500, Japan

and MINORU ONOZUKA

Department of Anatomy, Gifu University School of Medicine, Gifu 500, Japan

Accepted 28 September 1992

Summary

The mechanism of serotonin (5-HT) action on bursting activity was examined in a bursting pacemaker neurone of the snail *Achatina fulica*. 5-HT augmented both the depolarizing and post-burst-hyperpolarizing phases of the bursting cycle in a dose-dependent manner. This biogenic amine also enhanced the negative slope resistance (NSR), which was normally detectable at membrane potentials between −40 and −20mV, and produced another NSR at voltages between −20 and 0mV. The former NSR disappeared in Na^+-free saline and the latter was abolished by replacement with Co^{2+}-substituted Ca^{2+}-free saline. Both isobutylmethylxanthine, extracellularly applied, and intracellularly applied cyclic AMP simulated a 5-HT effect on the current–voltage relationships. In contrast, the 5-HT effect was suppressed in a dose-dependent manner by prior treatment with a cyclic-AMP-dependent protein kinase inhibitor, isoquinoline sulphonamide. Similar suppression was observed after intracellular injection of a cyclic-AMP-dependent protein kinase inhibitor isolated from bovine muscle. These results suggest that 5-HT may augment the bursting pacemaker activity by its stimulatory effect on both the slow Na^+ channels and the Ca^{2+} channels through cyclic-AMP-dependent protein phosphorylation.

Introduction

In molluscan neurones, such as those of *Aplysia californica* (Barker and Smith, 1976, 1978; Smith *et al.* 1975; Wilson and Wachtel, 1978), and *Euhadra peliomphala* neurones (Onozuka *et al.* 1986, 1988*a*, 1991*a*,*b*), it has been demonstrated that bursting activity is

Key words: serotonin, bursting activity, negative slope resistance, protein phosphorylation, *Achatina fulica*.

accompanied by a negative slope resistance (NSR) region in the steady-state current–voltage (*I–V*) curve of these neurones. It has been proposed that this NSR region plays an essential role in the maintenance of slow membrane oscillations during bursting activity (Wilson and Wachtel, 1978), although the reason for the involvement of an NSR in bursting activity is not understood (Epstein and Marder, 1990). Recently, we identified in the suboesophageal ganglion of *Achatina fulica* a spontaneously bursting neurone (PON) possessing a similar *I–V* relationship (Funase, 1990*a*). In this neurone, serotonin (5-HT) has been found to bring about an increase in the magnitude of this NSR and the induction of another NSR at more positive voltages on the *I–V* curve, as well as an enhancement of bursting pacemaker activity (for a brief report, see Funase, 1990*b*). However, the underlying intracellular mechanism(s) responsible for 5-HT augmentation of bursting activity with NSRs has yet to be elucidated.

It has been shown that 5-HT elevates the level of adenosine 3′,5′-cyclic monophosphate (cyclic AMP) in some molluscan neurones (Greenberg *et al.* 1987; Sweatt *et al.* 1989). An increase in the level of intracellular cyclic AMP ($[\text{cyclic AMP}]_i$), in turn, causes an activation of a protein kinase that has multiple effects on the membrane properties of these neurones (Baxter and Byrne, 1989, 1990; Levitan *et al.* 1987). Since one of the major effects of cyclic AMP is mediated by protein phosphorylation (for reviews, see Browning *et al.* 1985; Greengard, 1976; Kostyuk, 1986; Krebs and Beavo, 1979), it is conceivable that 5-HT may be involved in the augmentation of the bursting pacemaker activity through cyclic-AMP-dependent protein phosphorylation. In the present study, we tested this possibility by examining the effects of cyclic-AMP-dependent protein kinase inhibitors on 5-HT action on the membrane properties of the PON from *Achatina fulica*, using several pharmacological agents and the voltage-clamp technique in combination with pressure injection.

Materials and methods

Preparations

The suboesophageal ganglion of *Achatina fulica* was isolated and pinned to the Sylgard base of a 0.1ml perfusion chamber. After a 15-min incubation with 0.7% trypsin (type III, Sigma Chemical Co.) at room temperature (22±1°C), the connective tissue sheath overlying the dorsal surface of the ganglion was dissected with microforceps. All experiments were performed on the PON neurone (Funase, 1990*a*,*b*). In some experiments the neurone was axotomized by cutting the septum between the visceral and right parietal ganglion to exclude synaptic inputs. This axotomy was performed at the time the preparation was set up for recording.

Solutions

The neurone was superfused at a flow rate of $3\,\text{ml}\,\text{min}^{-1}$ with normal saline containing (in $\text{mmol}\,\text{l}^{-1}$): 80 NaCl, 4 KCl, 10 $CaCl_2$, 5 $MgCl_2$, 10 Tris–HCl (pH7.4). Na^+-free saline was made by substituting Tris for Na^+ and Ca^{2+}-free saline by replacing Ca^{2+} with an equimolar amount of Co^{2+}.

Electrophysiological recordings

The arrangements for recording, stimulating and voltage-clamp circuitry were conventional. A voltage detection electrode and current injection electrode using a Nihon-Kohden voltage-clamp amplifer (CEZ-1100) were employed as described elsewhere (Onozuka *et al.* 1986, 1988*a*). A neurone was kept at a holding voltage (V_h) of −50mV and stepped to various membrane potentials (5s in duration). The time between successive voltage steps was at least 30s to minimize possible interference from the preceding change of voltage. Steady-state current was measured at the end of the 5-s step, and leakage current was subtracted, assuming that the leak was linear over the voltage steps used.

Pressure injection

The third microelectrode was used for pressure injection of test materials into the neurone according to our previous method (Funase, 1990*a*,*b*; Onozuka *et al.* 1988*b*, 1991*b*). The electrode was filled with 0.1mol l^{-1} KCl (pH7.2) containing cyclic-AMP-dependent protein kinase inhibitor isolated from bovine muscle (PKI; type V) or cyclic AMP and 0.1% (w/v) Fast Green dye. A pressure of 2kgcm^{-2} of 1s duration was used, except when otherwise specified. To test the effect of pressure *per se*, a solution without test material was injected into the neurone. No significant change was noted in the *I–V* curve or the electrical activity.

Chemicals

Isobutylmethylxanthine (IBMX), cyclic AMP, tetraethylammonium chloride (TEA^+) and PKI were obtained from Sigma Chemical Co. and isoquinolinesulphonamide (H-8), a membrane-permeable inhibitor of cyclic-AMP-dependent protein kinase, from Seikagaku Kogyo Co. IBMX and TEA^+ were dissolved in normal saline.

Results

Effect of 5-HT

In agreement with a previous finding (Funase, 1990*a*), a PON neurone in normal saline usually exhibited bursting pacemaker activity characteristic of a slow potential oscillation (Fig. 1A). Under voltage-clamp, this neurone (*N*=15) showed an inward current over a wide range of membrane potentials, giving a dip in the *I–V* curve at membrane potentials between −40 and −20mV, characteristic of an NSR (Fig. 1B). This NSR will hereafter be referred to as the first NSR.

When the PON neurone (*N*=10) was superfused with 5-HT, the amplitude of both the depolarizing and the post-burst-hyperpolarizing phases of the bursting cycle was increased, although the duration of both phases was irregular (Fig. 2A). The *I–V* relationship shifted downwards with increasing concentrations of 5-HT, and the peak inward current, which gives the first NSR, became more pronounced (Fig. 2B). In addition, 5-HT produced another downward dip in the *I–V* relationship (hereafter referred to as the second NSR) at membrane voltages between −20 and 0mV. The magnitude of

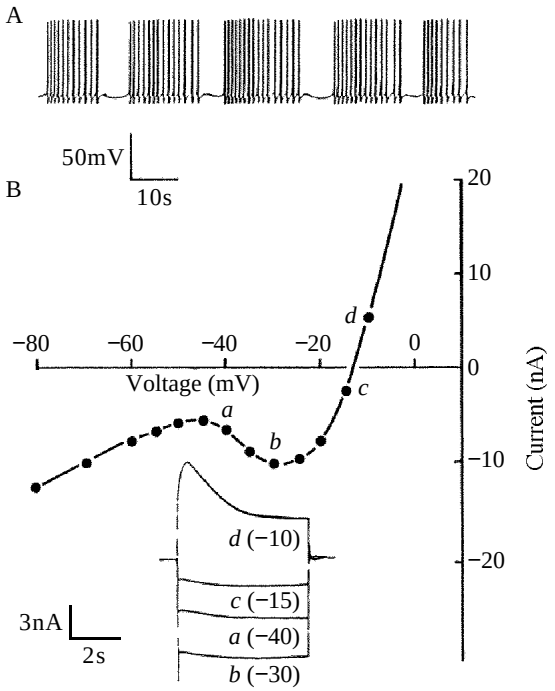

Fig. 1. Characteristic membrane properties of the PON neurone. (A) Bursting pacemaker activity. (B) The steady-state *I–V* curve obtained by plotting the currents measured by shifting a membrane potential from a V_h of −50mV to various voltage levels (5s in duration). The current was measured at the end of each 5-s step. Examples of actual records were taken from the points marked by corresponding letters on the curve. The resting membrane potential of the neurone was about −50mV.

the second NSR was also dose-dependent. These effects of 5-HT were virtually reversible upon rinsing with 5-HT-free normal saline (*d* in Fig. 2A,B). Essentially similar results were obtained with axotomized cells (*N*=5) (data not shown), indicating that the action of 5-HT upon the neurone is direct and not mediated synaptically. Levels of 5-HT below $0.5\,\mu mol\,l^{-1}$ did not affect either the membrane potential or the *I–V* curve. In subsequent experiments, $10\,\mu mol\,l^{-1}$ 5-HT was used, unless otherwise specified, since this concentration always brought about both first and second NSRs.

Effect of the action of 5-HT in Na^+-free or Ca^{2+}-free saline

The ionic mechanism of the action of 5-HT on bursting activity was examined by removing extracellular Na^+ or Ca^{2+}, since both Na^+ and Ca^{2+} influx contribute to the rising phase of the action potential in molluscan neurones (Adams *et al.* 1980*a*,*b*). In addition, the *I–V* relationship of the net 5-HT current ($I_{5\text{-HT}}$) was obtained from the difference between the currents in the presence and absence of this bioamine in the perfusate. Filled circles in Fig. 3 show the *I–V* relationships in which the second NSR was larger than the first NSR. When the neurone (*N*=7) was superfused with Na^+-free saline containing 5-HT, the first NSR completely disappeared, whereas the second NSR was

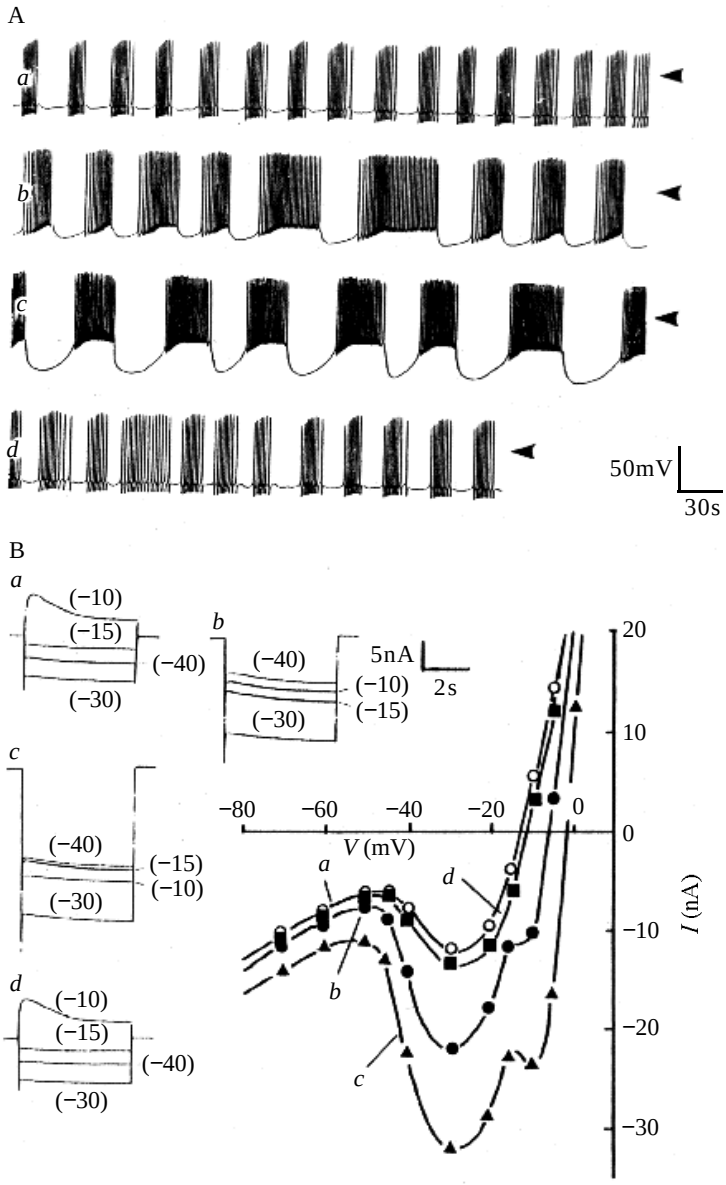

Fig. 2. Effects of 5-HT on the bursting activity (A) and the steady-state *I–V* curve (B) of the neurone. Electrical activities and currents were measured before (*a*, open circles) and 5min after introduction of 1 (*b*, filled circles) or 10 μmol l^{-1} 5-HT (*c*, filled triangles). The arrowheads in A indicate the reference potential level. Resting membrane potential appeared to be slightly shifted in a depolarizing direction dependent on the concentration of 5-HT. Traces in B show examples of actual records in response to a membrane potential shift lasting for 5s from a V_h of −50 to −40, −30, −15 and −10mV. Note that the effects of 5-HT were reversed approximately 30min after rinsing with normal saline (*d*, filled squares).

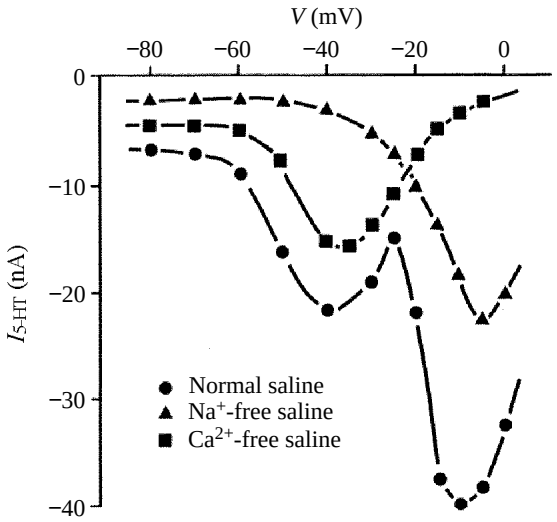

Fig. 3. Effect of removal of Na^+ or Ca^{2+} from the saline on the 5-HT-induced steady-state *I–V* relationship. Currents were measured 5min after superfusion with normal (filled circles), Na^+-free (filled triangles) or Ca^{2+}-free saline (filled squares) containing 10 μmol l^{-1} 5-HT. Na^+-free saline was made by substituting $Tris^+$ for Na^+, while Ca^{2+}-free saline was made by replacing Ca^{2+} with Co^{2+}, as described in Materials and methods. Data are expressed as the *I–V* relationship of the net 5-HT current ($I_{5\text{-HT}}$) obtained by plotting the difference in the current obtained in the presence and absence of this bioamine in the perfusate.

reduced by about 50% (filled triangles in Fig. 3). In contrast, the second NSR disappeared in response to perfusion with Co^{2+}-substituted Ca^{2+}-free saline, while the first NSR was reduced (filled squares in Fig. 3).

Effect of TEA^+ on I–V *relationships*

Since it has been suggested that augmentation of the post-burst hyperpolarization by 5-HT in the bursting pacemaker neurone of *Aplysia californica* might be due to an increase in the outward K^+ conductance (DePeyer *et al.* 1982), we also examined the effect of TEA^+ on the 5-HT-induced *I–V* relationship. As shown in Fig. 4, TEA^+ (*N*=5) slightly increased the magnitude of the first NSR and enhanced the size of the second NSR in a dose-dependent manner. In addition, the membrane potential at the peak of the second NSR was progressively shifted with increasing concentrations of TEA^+ towards the voltage at which the largest Ca^{2+} current has been shown to be generated in snail neurones (Onozuka *et al.* 1990).

Effect of cyclic AMP or cyclic-AMP-dependent protein inhibitors

Because several actions of 5-HT in molluscan neurones are mediated through cyclic AMP which, in turn, activates a protein kinase (Deterre *et al.* 1981; Drummond *et al.* 1980; Levitan *et al.* 1987; Levitan and Levitan, 1988; Swandulla and Lux, 1984; Siegelbaum *et al.* 1982; Taussig *et al.* 1989), we hypothesized that cyclic AMP may mimic the action of 5-HT on the *I–V* curve. To test this hypothesis, we measured the

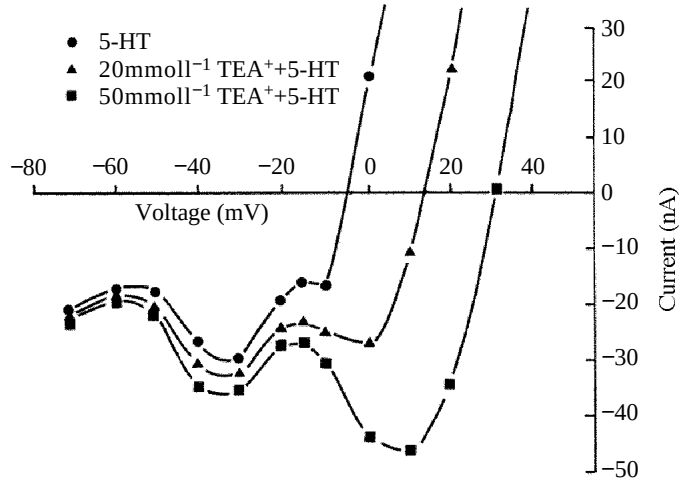

Fig. 4. Effect of TEA^+ on 5-HT-induced steady-state *I–V* relationships. Currents were measured 5min after introduction of 0 (filled circles), 20 (filled triangles) or 50mmol l^{-1} (filled squares) TEA^+ containing 10 μmol l^{-1} 5-HT. Note that the membrane potential of the peak of the second NSR was shifted to the positive side in a dose-dependent manner, implying that this NSR may be carried by extracellular Ca^{2+}.

currents when $[\text{cyclic AMP}]_i$ was elevated by either extracellular application of 100 μmol l^{-1} IBMX (*N*=10) or pressure injection of 1mmol l^{-1} cyclic AMP into the neurone (*N*=10). Fig. 5A shows the *I–V* curve of the net IBMX current (I_{IBMX}) obtained by substracting the currents measured before from those measured 20min after IBMX exposure. The pattern of this *I–V* curve was comparable to that of $I_{5\text{-HT}}$ (see filled circles in Fig. 3). A similar result was obtained in the neurones when cyclic AMP was applied through the intracellular microelectrode (Fig. 5B). However, when neurones (*N*=4) had been superfused with Na^+- and Ca^{2+}-free saline, 5-HT and cyclic AMP had no effect (data not shown).

We have previously found that the cyclic-AMP-dependent protein kinase inhibitors H-8 and PKI both depress cyclic-AMP-dependent protein phosphorylation in extracts from snail ganglia (Onozuka *et al.* 1988*a*; Watanabe and Funase, 1991). Accordingly, we examined the involvement of the phosphorylation in the effect of 5-HT on the *I–V* relationship of the neurone using these two inhibitors. As seen in Fig. 6A, $I_{5\text{-HT}}$ was inhibited in a time-dependent manner by injecting PKI into the neurone (*N*=10). The *I–V* relationships were also obtained in another experiment (*N*=4), first in normal saline after injection with PKI, then during perfusion with saline containing 5-HT, and finally during recovery in normal saline. PKI did not cause any shift of the resting *I–V* relationship; however, $I_{5\text{-HT}}$ was completely abolished by PKI (data not shown). Similarly, another inhibitor, H-8, also suppressed $I_{5\text{-HT}}$ in a dose-dependent manner, when added to the perfusate (Fig. 6B).

Discussion

In molluscan neurones, the NSR has been shown to be evoked after application of a

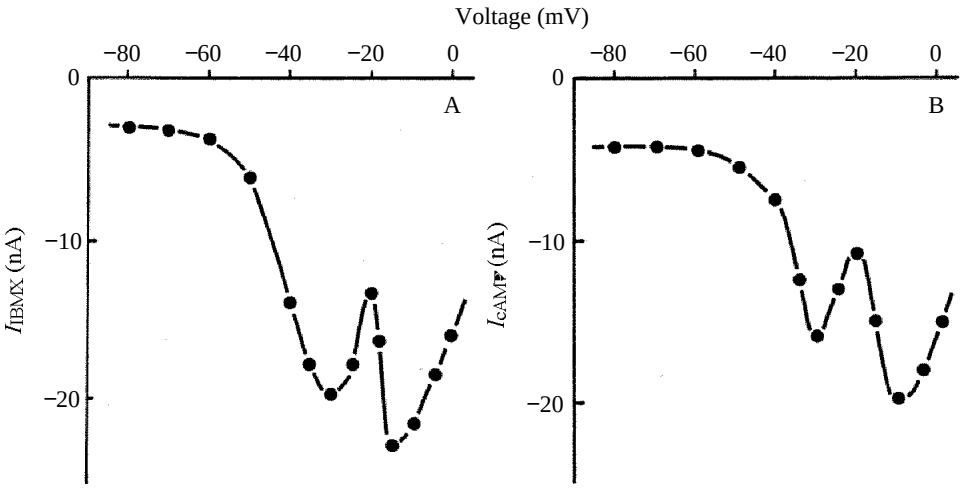

Fig. 5. Effect of an increase in [cyclic AMP]$_i$ on the steady-state *I–V* relationships. (A) Net IBMX current (I_{IBMX}) measured 20min after treatment with 100 μmol l^{-1} IBMX. Data are expressed as the steady-state *I–V* relationship of I_{IBMX} obtained by plotting the difference between the current in the presence and that in the absence of this phosphodiesterase inhibitor in the perfusate. (B) Net cyclic-AMP current (I_{cAMP}) measured 5min after pressure injection of 1mmol l^{-1} cyclic AMP into the neurone. Data are expressed as the steady-state *I–V* relationship of I_{cAMP} obtained by plotting the difference in the current before and that after the injection.

convulsant drug, pentylenetetrazole (Onozuka *et al.* 1991*a*) and several peptides or neurotransmitters, such as oxytocin (Funase, 1990*a*), vasopressin (Onozuka *et al.* 1986), FMRFamide (Ichinose and McAdo, 1988, 1989), egg-laying hormone (Kirk and Scheller, 1986; Kirk *et al.* 1988; Levitan *et al.* 1987) and dopamine (Matsumoto *et al.* 1988). An essentially similar response is seen in the *Aplysia californica* R15 neurone, which has a spontaneous bursting pacemaker potential (Wilson and Wachtel, 1978). It is widely accepted that the NSR is caused by an increase in Na^+ conductance (Barker and Smith, 1978; Carnivale and Wachtel, 1980; Smith *et al.* 1975; Wilson and Wachtel, 1978), Ca^{2+} conductance (Eckert and Lux, 1975; Gorman *et al.* 1982) or both (Johnston, 1976).

In the present experiments, the PON neurone always exhibited spontaneous bursts of firing. Moreover, the steady-state *I–V* relationship of this neurone showed an NSR with a pattern similar to that of the *Aplysia californica* R15 neurone (Levitan *et al.* 1987; Levitan and Levitan, 1988). It was also similar to the NSR present during bursting activity induced by pentylenetetrazole (Onozuka *et al.* 1991*a*) and by vasopressin (Onozuka *et al.* 1986) in *Euhadra peliomphala* neurones. The PON neurone also showed 5-HT augmentation of the amplitude of both the depolarizing and hyperpolarizing phases of the burst cycle. Furthermore, the first NSR disappeared on removal of Na^+ from the saline, while the second NSR disappeared on removal of Ca^{2+}, suggesting that 5-HT augmentation of the depolarizing phase may be due to its stimulatory effect on the opening of both Na^+ and Ca^{2+} channels associated with the NSR. In contrast, an augmentation of the post-burst hyperpolarization during exposure to 5-HT is attributed to

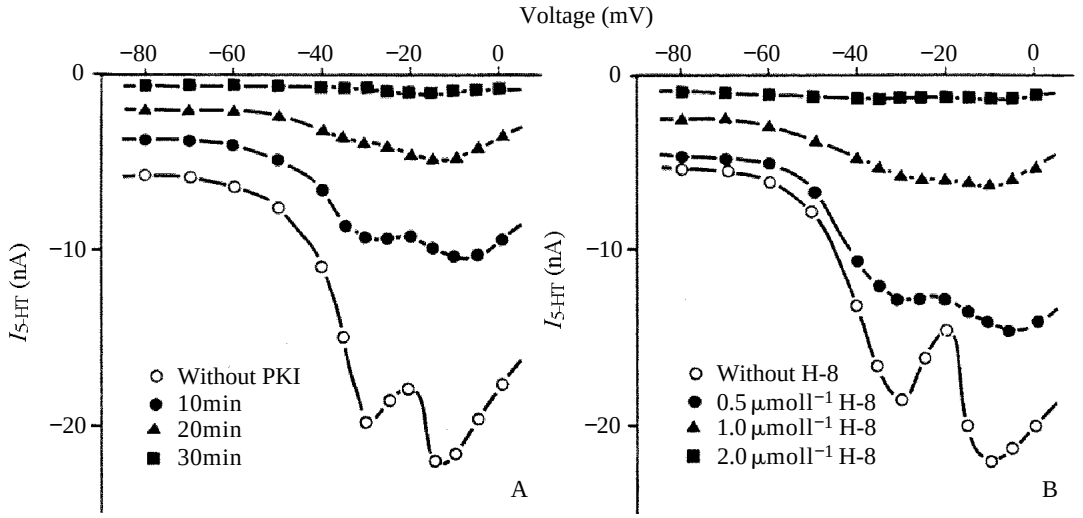

Fig. 6. Effect of cyclic-AMP-dependent protein kinase inhibitors on $I_{5\text{-HT}}$. (A) The effect of PKI. PKI (1mgml^{-1}) was pressure-injected into the neurone which had been superfused with 10 μmol l^{-1} 5-HT for 5min. Steady-state currents were measured before (open circles) and 10min (filled circles), 20min (filled triangles) or 30min (filled squares) after PKI injection. (B) The effect of H-8. Steady-state currents were measured in the 5-HT (10 μmol l^{-1}) saline without H-8 (open circles) and in the 5-HT saline containing 0.5 (filled circles), 1.0 (filled triangles) or 2.0 μmol l^{-1} H-8 (filled squares). All data are expressed as the steady state *I–V* relationship of $I_{5\text{-HT}}$ obtained by plotting the difference in the current in the presence and that in the absence of 5-HT in the saline.

an increase in the Ca^{2+}-dependent K^+ conductance coupled to the elevation of Ca^{2+} levels during the membrane depolarization.

Our data also show that both extracellular application of IBMX and intracellular injection of cyclic AMP simulate the 5-HT-induced effect on the *I–V* relationship in normal saline but that the effects are not detectable in Na^+- and Ca^{2+}-free saline. These results imply that 5-HT may augment the bursting activity by elevating $[\text{cyclic AMP}]_i$, which, in turn, affects Na^+- and Ca^{2+}-dependent NSRs. In *Euhadra peliomphala* neurones, Onozuka *et al.* (1986, 1988*a*) found that the NSR at membrane potentials between −40 and −20mV, which is completely dependent on extracellular Na^+, could be elicited by cyclic AMP. In addition, in the present experiments, the effect of 5-HT on the *I–V* relationship was abolished after intracellular injection of PKI or extracellular application of H-8, suggesting that both 5-HT augmentation of the Na^+-activated NSR and 5-HT development of the Ca^{2+}-activated NSR may be mediated by cyclic-AMP-dependent protein phosphorylation.

The mechanisms of the 5-HT response have been extensively studied in different *Aplysia californica* neurones. In the *Aplysia californica* sensory neurones, 5-HT produces a slow excitatory postsynaptic potential mediated by cyclic AMP, which contributes to presynaptic facilitation and behavioural sensitization of the animal's gill withdrawal reflex (Kandel and Schwartz, 1982). Work by Kandel and his co-workers (Klein *et al.*

1982) led to the identification of a K^+ current whose magnitude is reduced by 5-HT. This was named the S current. They also found that, in the cell-free membrane patch, this channel is modulated by cyclic-AMP-dependent protein phosphorylation (Shuster *et al.* 1986). In contrast, recent experiments carried out using the *Aplysia californica* R15 neurone, which is an endogenous burster, have shown that 5-HT augments the depolarizing and post-hyperpolarizing phases of the bursting cycle in this neurone and they suggest that this augmentation is due to an increase in the Ca^{2+} and K^+ currents *via* cyclic-AMP-dependent protein phosphorylation (Lemons *et al.* 1986; Levitan and Levitan, 1988). The results from the present study, therefore, may demonstrate a mechanism of 5-HT action that has not yet been reported.

One question arises: how does 5-HT develop the Ca^{2+}-dependent second NSR? Based both on the results obtained by application of IBMX or intracellular injection of cyclic AMP and on the observation that 5-HT increases [cyclic AMP] in *Aplysia californica* neurones (Greenberg *et al.* 1987; Sweatt *et al.* 1989), two explanations are possible. One is that 5-HT may activate a cyclic-AMP-dependent protein kinase that, in turn, is linked to the opening of Ca^{2+} channels. The other explanation is that 5-HT-activated protein kinase may be involved in the development of a Na^+-dependent NSR, followed by voltage-dependent Ca^{2+} channel activation. The experiment carried out in Ca^{2+}-free saline resulted in the disappearance of the second NSR, which was reduced by removal of Na^+. Taken together, the results suggest that 5-HT causes the second NSR by phosphorylating either a Ca^{2+} channel directly or a channel-associated protein. This assumption may be indirectly supported by the observation that, when the outward K^+ current was suppressed by TEA^+, the voltage at the peak of the second NSR was shifted closer to the membrane potential at which the largest Ca^{2+} current is recorded in *Aplysia californica* (Adams *et al.* 1980*a*,*b*) and *Euhadra peliomphala* neurones (Onozuka *et al.* 1990), and that this phenomenon did not occur in the presence of a general protein kinase inhibitor 1-(5-isoquinolinylsulphonyl)-2-methylpiperazine (H-7) in the bathing medium (K. Funase, K. Watanabe and M. Onozuka, unpublished observation).

From the present results, we conclude that 5-HT augments the depolarizing phase of the burst cycle by its stimulatory effect on both the slow Na^+ channels and the Ca^{2+} channels through cyclic-AMP-dependent protein phosphorylation. In contrast, the post-burst hyperpolarization may be augmented by an increase in the Ca^{2+}-activated K^+ conductance. Our recent evidence for the activation of K^+ channels during bursting activity in snail neurones shows that intracellular injection of a Ca^{2+}/calmodulin-dependent protein kinase II causes both a prolonged hyperpolarization of the membrane and an enhancement of the outward K^+ current during pentylenetetrazole-induced bursting activity (Onozuka and Tsujitani, 1991).

This work was supported by grants from the Ichiro Kanehara Foundation, the Japan Brain Foundation, the Nukada Foundation, the Tokai Foundation of Encouragement of Academy, the Naito Foundation, the Narishige Neuroscience Trust and the Mochida Memorial Foundation for Medical and Pharmacological Research.

References

ADAMS, D. J., SMITH, S. J. AND THOMPSON, S. H. (1980*a*). Ionic currents in molluscan soma. *A. Rev. Neurosci.* **3**, 141–167.

ADAMS, W. B., PARNAS, I. AND LEVITAN, I. W.(1980*b*). Mechanism of long-lasting synaptic inhibition in *Aplysia* neurone R15. *J. Neurophysiol.* **44**, 1148–1160.

BARKER, J. L. AND SMITH, T. G.(1976). Peptide regulation of neuronal membrane properties. *Brain Res.* **103**, 167–170.

BARKER, J. L. AND SMITH, T. G. (1978). Electrophysiological studies of molluscan neurones generating bursting pacemaker potential activity. In *Abnormal Neuroneal Discharges* (ed. N. Charazonitis and M. Boisson), pp. 359–387. New York: Raven Press.

BAXTER, D. AND BYRNE, J. H. (1989). Serotonergic modulation of two potassium currents in the pleural sensory neurones of *Aplysia*. *J. Neurophysiol.* **62**, 665–679.

BAXTER, D. AND BYRNE, J. H. (1990). Differential effects of cyclic AMP and serotonin on membrane current, action-potential duration and excitability in somata of pleural sensory neurones of *Aplysia*. *J. Neurophysiol.* **64**, 978–990.

BROWNING, M. D., HUGANIR, R. AND GREENGARD, P. (1985). Protein phosphorylation and neuronal function. *J. Neurochem.* **45**, 11–45.

CARNIVALE, N. T. AND WACHTEL, H. (1980). Two reciprocating current components underlying slow oscillations in *Aplysia* bursting neurones. *Brain Res. Rev.* **2**, 45–68.

DEPEYER, J. E., CACHELIN, A. B., LEVITAN, I. B. AND REUTER, H.(1982). Ca^{2+}-activated K^+ conductance in internally perfused snail neurones is enhanced by protein phosphorylation. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4207–4211.

DETERRE, P., PAUPARDIN-TRITSCH, D., BOCKAERT, J. AND GERSCHENFELD, H. M. (1981). Role of cyclic AMP in a serotonin-evoked slow inward current in snail neurones. *Nature* **290**, 783–785.

DRUMMOND, A. H., BENSON, J. A. AND LEVITAN, I. B.(1980). Serotonin-induced hyperpolarization of an identified *Aplysia* neurone is mediated by cyclic AMP. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5013–5017.

ECKERT, R. AND LUX, H. D. (1975). A non-inactivating inward current recorded during snail depolarizing voltage steps in snail pacemaker neurones. *Brain Res.* **83**, 486–489.

EPSTEIN, I. R. AND MARDER, E. (1990). Multiple modes of a conditional neural oscillator. *Biol. Cybernetics* **63**, 25–34.

FUNASE, K. (1990*a*). Oxytocin-induced sodium current is mediated by cyclic AMP-dependent protein phosphorylation in an identified snail neurone. *Brain Res.* **517**, 263–268.

FUNASE, K.(1990*b*). The ionic mechanism of two negative slope resistance regions evoked by serotonin in snail neurones. *Jpn J. Physiol.* **40**, S122.

GORMAN, A. L. F., HERMANN, A. AND THOMAS, M. V. (1982). Ionic requirements for membrane oscillations and their dependence on the calcium concentration in a molluscan pacemaker neurone. *J. Physiol., Lond.* **327**, 185–217.

GREENBERG, S. M., BERNIER, L. AND SCHWARTZ, J. H. (1987). Distribution of AMP and cyclic AMP-dependent protein kinase in *Aplysia* sensory neurones. *J. Neurosci.* **7**, 291–301.

GREENGARD, P. (1976). Possible role for cyclic nucleotides and phosphorylated membrane proteins in postsynaptic actions of neurotransmitters. *Nature* **260**, 101–108.

ICHINOSE, M. AND MCADO, D. J. (1988). The voltage-dependent, slow inward current induced by neuropeptide FMRFamide in *Aplysia* neurone R14. *J. Neurosci.* **8**, 3891–3900.

ICHINOSE, M. AND MCADO, D. J. (1989). The cyclic GMP-induced inward neurone R14 of *Aplysia californica*: Similarity to a FMRFamide-induced inward current. *J. Neurobiol.* **20**, 10–24.

JOHNSTON, D. (1976). Voltage clamp reveals basis for calcium regulation of bursting pacemaker potentials in *Aplysia* neurones. *Brain Res.* **107**, 418–423.

KANDEL, E. R. AND SCHWARTZ, J. H. (1982). Molecular biology of learning modulation of transmitter release. *Science* **218**, 433–443.

KIRK, M. D., RAUSSING, R. AND SCHELLER, R. H. (1988). Egg-laying hormone, serotonin and cyclic nucleotide modulation of ionic currents in the identified motor neurone B16 of *Aplysia. J. Neurosci.* **8**, 1181–1193.

KIRK, M. D. AND SCHELLER, R. H. (1986). Egg-laying hormone of *Aplysia* induces a voltage-dependent slow inward current carried by Na^+ in identified motor neurone. *Proc. natn. Acad. Sci. U.S.A.* **83**, 3017–3021.

KLEIN, M., CAMARDO, J. AND KANDEL, E. R.(1982). Serotonin modulates a specific potassium current in

the sensory neurones that show presynaptic facilitation in *Aplysia*. *Proc. natn. Acad. Sci. U.S.A.* **79**, 5713–5717.

KOSTYUK, P. G. (1986). Cyclic nucleotides as modulators and activators of ionic channels in the nerve cell membrane. *Prog. Brain Res.* **69**, 133–137.

KREBS, E. G. AND BEAVO, J. A. (1979). Phosphorylation-dephosphorylation of enzymes. *A. Rev. Biochem.* **48**, 929–959.

LEMONS, J. R., NOVAK-HOFER, N. AND LEVITAN, E. S. (1986). Regulation of ion channel activity by protein phosphorylation. *Prog. Brain Res.* **69**, 107–118.

LEVITAN, E. S., KRAMER, R. H. AND LEVITAN, I. B. (1987). Augmentation of bursting pacemaker activity by egg-laying hormone in *Aplysia* neurone R15 is mediated by a cyclic AMP-dependent increase in Ca^{2+} and K^+ currents. *Proc. natn. Acad. Sci. U.S.A.* **84**, 6307–6311.

LEVITAN, E. S. AND LEVITAN, I. B. (1988). Serotonin acting *via* cyclic AMP enhances both the hyperpolarizing and depolarizing phases of pacemaker activity in the *Aplysia* neurone R15. *J. Neurosci.* **8**, 1152–1161.

MATSUMOTO, K., SASAKI, K., SATO, M., SHOUZUSHIMA, M. AND TAKAHASHI, K. (1988). Dopamine-induced depolarizing responses associated with negative slope conductance in LB-cluster neurones of *Aplysia*. *J. Physiol., Lond.* **407**, 199–233.

ONOZUKA, M., FURUICHI, H. AND FUKAMI, F.(1991*a*). Cyclic AMP-dependent protein phosphorylation is involved in the development of negative slope resistance and a reduction of the potassium current induced by pentylenetetrazole in identified snail neurones. *Neurosci. Lett.* **123**, 257–260.

ONOZUKA, M., FURUICHI, H., KISHII, K. AND IMAI, S. (1986). Membrane properties and intracellular biochemical processes during vasopressin-induced bursting activity in snail neurones. *Neurosci. Res.* **4**, 37–50.

ONOZUKA, M., IMAI, S., DEURA, S., NISHIYAMA, K. AND OZONO, S. (1988*a*). Stimulation of sodium current by cyclic AMP is mediated through protein phosphorylation in *Euhadra* neurones. *Experientia* **44**, 996–998.

ONOZUKA, M., IMAI, S., FURUICHI, H. AND OZONO, S. (1991*b*). A specific 70K protein found in epileptic rat cortex: Induction of bursting activity and negative resistance by its intracellular application in *Euhadra* neurones. *J. Neurobiol.* **22**, 287–297.

ONOZUKA, M., IMAI, S., KUBO, K., DEURA, S., NISHIYAMA, K. AND OZONO, S. (1988*b*). Cyclic AMP-dependent protein phosphorylation is involved in activation of the potassium current associated with endogenous cellular calcium in *Euhadra* neurones. *Brain Res.* **473**, 401–405.

ONOZUKA, M., IMAI, S. AND OZONO, S. (1990). The 70-KDa epileptic cortical protein elicits bursting activity accompanied by reduction of outward current in *Euhadra* neurones which is inhibited by an antibody against this protein. *Brain Res.* **531**, 276–279.

ONOZUKA, M. AND TSUJITANI, M. (1991). Pentylenetetrazole suppresses the potassium current in *Euhadra* neurones which is coupled with Ca^{2+}/calmodulin-dependent protein phosphorylation. *Neurosci. Res.* **11**, 146–153.

SHUSTER, M. J., CAMARDO, J. S., SIEGELBAUM, S. A. AND KANDEL, E. R. (1986). Modulation of the S K^+ channel by cyclic AMP-dependent protein phosphorylation in cell-free membrane patches. *Prog. Brain Res.* **69**, 119–132.

SIEGELBAUM, S. A., CAMARDO, J. S. AND KANDEL, J. S. (1982). Serotonin and cyclic AMP in *Aplysia* sensory neurones. *Nature* **299**, 413–417.

SMITH, T. G., BARKER, J. L. AND GAINER, H. (1975). Requirements for bursting pacemaker potential activity in molluscan neurones. *Nature* **253**, 450–452.

SWANDULLA, D. AND LUX, H. D. (1984). Changes in ionic conductance induced by cyclic AMP in *Helix* neurones. *Brain Res.* **305**, 115–122.

SWEATT, J. D., VOLTERRA, A., EDMONDS, B., KARL, K. A., SIEGELBAUM, S. AND KANDEL, E. R. (1989). FMRF amide reverses protein phosphorylation produced by 5-HT and cyclic AMP in *Aplysia* sensory neurones. *Nature* **342**, 275–278.

TAUSSIG, R., SWEET-CORDERO, A. AND SCHELLE, R. H. (1989). Modulation of ionic currents in *Aplysia* motor neurone B15 by serotonin, neuropeptides and second messengers. *J. Neurosci.* **9**, 3218–3229.

WATANABE, K. AND FUNASE, K. (1991). Cyclic AMP elicits biphasic current whose activation is mediated through protein phosphorylation in snail neurones. *Neurosci. Res.* **10**, 64–70.

WILSON, W. A. AND WACHTEL, H. (1978). Prolonged inhibition in burst firing neurones: synaptic inactivation of the slow regenerative inward current. *Science* **202**, 772–775.