Journal of Physiology (1988), **407**, *pp.* 199–213
With 10 *text-figures*
Printed in Great Britain

DOPAMINE-INDUCED DEPOLARIZING RESPONSES ASSOCIATED WITH NEGATIVE SLOPE CONDUCTANCE IN LB-CLUSTER NEURONES OF *APLYSIA*

BY M. MATSUMOTO, K. SASAKI*, M. SATO, M. SHOZUSHIMA AND K. TAKASHIMA

From the Department of Physiology, School of Medicine, Iwate Medical University, 19-1 Uchimaru, Morioka-City, Iwate-ken 020, Japan

(*Received 29 March 1988*)

SUMMARY

1. Current- and voltage-clamp methods were used to evaluate the intracellular and ionic mechanisms involved in dopamine-induced slow depolarizations recorded from neurones of the LB cluster in the abdominal ganglion of *Aplysia kurodai*.

2. In voltage-clamped cells, dopamine induced a slow inward current that, over the range studied (−40 to −110 mV), decreased in amplitude with hyperpolarization of the cell, but failed to invert when the cell was hyperpolarized beyond the reversal potential for K^+, E_K.

3. Bathing the ganglion in 3-isobutyl-1-methylxanthine (IBMX) caused a significant increase in the dopamine response.

4. Most of the responses to dopamine were markedly augmented in Ca^{2+}-free media, but were depressed in Na^+-free media.

5. An intracellular injection of cyclic adenosine 3′,5′-monophosphate (cyclic AMP) into the same cell type produced an inward current which, like the response to dopamine, diminished in amplitude with hyperpolarization of the cell.

6. Like the dopamine response, the cyclic AMP response increased in the presence of IBMX, was enhanced in Ca^{2+}-free media, was depressed in Na^+-free media, and was unaffected by changes in external potassium.

7. In a few cells, although the cyclic AMP-induced responses disappeared in Na^+-free media, the dopamine-induced slow inward current responses did not. However, these Na^+-free resistant responses disappeared completely in Na^+- and Ca^{2+}-free media.

8. It was concluded that most of the dopamine-induced inward current responses were produced by an increase in permeability, mainly to Na^+, triggered by a receptor-controlled increase in intracellular cyclic AMP.

Authors' names appear in alphabetical order.
* To whom requests for reprints should be addressed.

INTRODUCTION

Dopamine (DA) induces either depolarization or hyperpolarization of the resting membrane in the neurones of both invertebrates and mammals depending on the receptor type of the cell membrane (Ascher, 1972; Boudier, Gielen, Cools & Van Rossum, 1974; Berry & Cottrell, 1975; Kitai, Sugimori & Kocsis, 1976; Gallagher, Inokuchi & Gallagher, 1980; Herrling & Hull, 1980; Bernardi, Cherubini, Marciani, Mercuri & Stanzione, 1982; Paupardin-Tritsch, Colombaioni, Deterre & Gerschenfeld, 1985). Among these receptors, there is the so-called D_1 type which is positively coupled with a membrane adenylate cyclase (Kebabian & Calne, 1979; Seeman, 1981; Stoof & Kebabian, 1984; Stoof, Werkman, Lodder & de Vlieger, 1986). Binding of DA to this type of receptor stimulates adenylate cyclase and increases the concentration of the intracellular cyclic adenosine 3′,5′-monophosphate (cyclic AMP).

In certain molluscan neurones, intracellularly applied cyclic AMP has been shown to cause an excitatory response that has an atypical voltage dependence, is enhanced by Ca^{2+}-free media, and appears to be Na^+ dependent (Aldenhoff, Hofmeier, Lux & Swandulla, 1983; Kononenko, Kostyuk & Shcherbatko, 1983; Green & Gillette, 1983; Swandulla & Lux, 1984; Connor & Hockberger, 1984; Hara, Sawada & Maeno, 1985; Kehoe, 1985).

In the LB cluster of the abdominal ganglion of *Aplysia kurodai*, we have observed a slowly depolarizing response to DA that has similar properties to the cyclic AMP response referred to above. These data suggest that in the LB neurones DA binds to a D_1 type of receptor, which in turn activates adenylate cyclase and produces a cyclic AMP-dependent inward current. Detailed analysis, however, revealed a few neurones whose DA-induced slowly depolarizing responses could not be explained solely by an increase in intracellular cyclic AMP.

The purpose of this paper is to characterize these responses electrophysiologically and to clarify their underlying intracellular and ionic mechanisms.

Preliminary accounts of this work have previously been reported (Sasaki, Matsumoto, Shozushima, Takashima & Sato, 1986*a*, *b*).

METHODS

Preparation and 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, Austin, Yai & Maruhashi, 1968): Na^+, 587; K^+, 12; Cl^-, 671; Ca^{2+}, 14; Mg^{2+}, 52 mM. The pH was adjusted to 7·4 with Tris and HCl, and the cells were continuously perfused at a constant flow rate of 5 ml/min successively with each of the perfusing media which were maintained at 23 ± 1 °C. The effective perfusing volume of the chamber was 0·2 ml. Na^+-free media were prepared by replacing NaCl with Tris hydrochloride. Modification of the K^+ concentration was made simply by adjusting the quantity of KCl in the artificial *Aplysia* medium. A Ca^{2+}-free medium was made by replacing $CaCl_2$ with equimolar $MgCl_2$.

Evaluation of dopamine-induced response

Two microelectrodes filled with 1·8 M-potassium citrate with a resistance of 2–3 MΩ were introduced into a single cell of the LB cluster (Frazier, Kandel, Kupfermann, Waziri & Coggeshall,

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.

Both current and voltage clamp methods were used to evaluate the response of the LB cells to bath-applied or pressure-injected DA, or to intracellularly applied cyclic AMP.

In voltage clamp, the responses to these agents were evaluated at various clamped 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 mode, 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 dopamine (DA), cyclic adenosine 3′,5′-monophosphate (cyclic AMP), 3-isobutyl-1-methylxanthine (IBMX), apomorphine (Apo), perphenazine (Per), and lysergic acid diethylamide (LSD). Cyclic AMP was dissolved in distilled and deionized water, and intracellularly applied to the neurone by ionophoresis. The other drugs were dissolved in the normal artificial *Aplysia* medium and applied to the neurone at a constant flow as described above. DA-induced responses were evaluated by the maximal current flow produced at each concentration of DA. The duration of DA application was adjusted so as to obtain just the beginning of the maximal plateau response, for example 15 s at 1 mM-DA and 20 s at 0·1 mM-DA. After each application of a drug, the cells were washed with the normal *Aplysia* medium for at least 10–20 min before any subsequent examination. Dopamine was dissolved immediately before application in order to minimize its oxidative degradation. In analysing both the effects of drugs and the effects of alteration of the external ionic environment on the DA-induced response, either the drug medium or the appropriate artificial *Aplysia* medium was perfused 4 min before an application of DA unless otherwise specified.

Application of cyclic AMP

When cyclic AMP was ionophoretically injected into the cells, a triple-barrelled microelectrode replaced one of the single-barrelled electrodes. One barrel (filled with 2 M-potassium citrate) was connected to the current output of the clamp unit and was used for passing the current across the cell membrane in both the current clamp method and the voltage clamp method. The second and third barrels, filled with 0·2 M-cyclic AMP and 2 M-potassium citrate, respectively, were connected to the outputs of the WPI M160 Microiontophoresis Programmer. Cyclic AMP was injected by repetitive current pulses, with a pulse duration of 200 ms and a repetition rate of 2·5 Hz. The intracellular concentration of cyclic AMP was estimated according to the method of Connor & Hockberger (1984) and calculated to be in the range of 10–300 μM.

RESULTS

Characteristics of dopamine-induced responses

In current clamp experiments, an application of 0·01–0·1 mM-DA to the LB cell induced a depolarizing response of approximately 10–15 mV, leading to the generation of action potentials. The response measured at resting potential was always associated with an apparent increase in membrane resistance, as shown in Fig. 1*A*. The time course of this response was much slower than that of depolarizing responses of other cells with the fast type of DA receptor.

The current response of a similar LB cell to DA recorded under voltage clamp at −55 mV is shown in Fig. 1*B*. This response is often associated with the reverse polarity of the conductance-measuring pulses, indicating the appearance of a negative conductance, as shown in Fig. 1*B*.

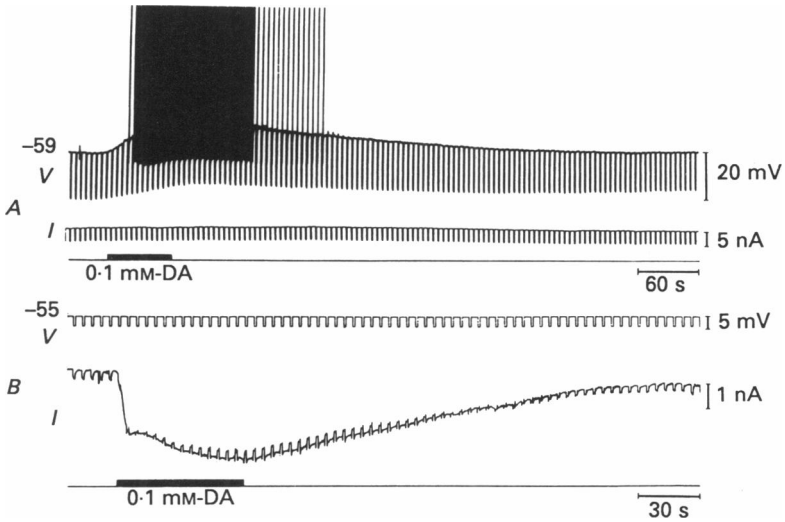

Fig. 1. DA-induced response in a neurone of the LB cluster of an *Aplysia* abdominal ganglion. *A*, bath application of 0·1 mM-DA induced a depolarizing response in the current-clamped cell. Periodic downward deflections in the voltage record (*V*) show the electrotonic potentials induced by constant 800 ms inward current pulses (*I*) delivered every 5 s, indicating the changes in effective membrane resistance. *B*, DA-induced slow inward current (*I*) in a similar cell voltage-clamped at −55 mV.

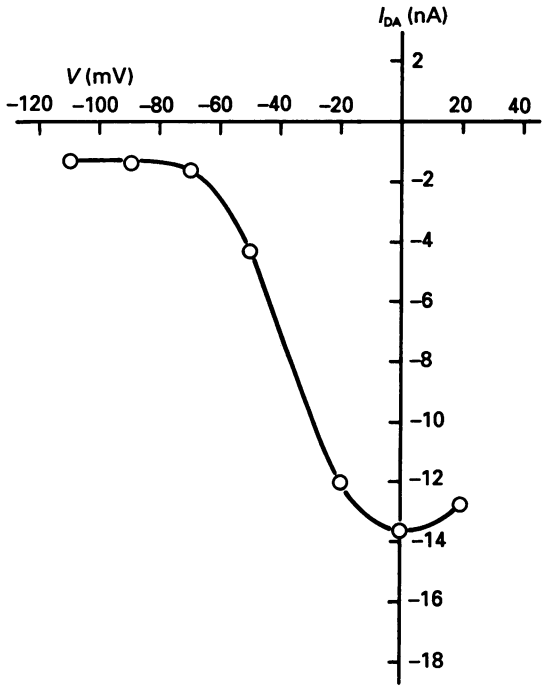

Fig. 2. Voltage dependence of the slow inward current induced by 0·1 mM-DA. DA-induced current (I_{DA}) recorded under the voltage clamp was plotted against the membrane potential (*V*). Resting potential −57 mV. Note that I_{DA} increases with membrane depolarization and decreases with hyperpolarization, but does not cross the voltage axis even at the voltage more hyperpolarized than −100 mV.

Effect of the changes in holding potential. The cell membrane was first clamped at a given potential level, then 0·1 mM-DA was applied to the cell in order to record the DA-induced inward current. The inward current responses to 0·1 mM-DA were measured at various potential levels on either side of the resting level (−57 mV). The maximum amplitude of the DA-induced current (I_{DA}) at each membrane potential was plotted against the membrane potential (V) as shown in Fig. 2. The DA-induced

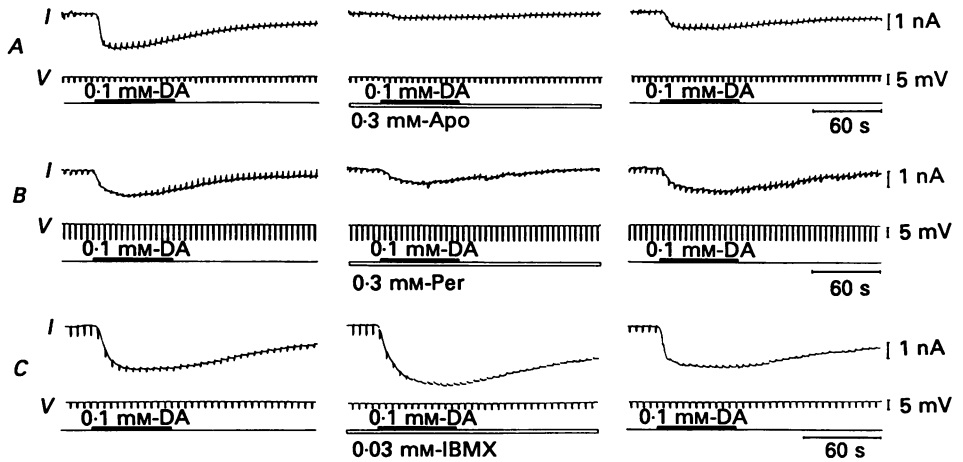

Fig. 3. Blocking effects of 0·3 mM-apomorphine (Apo, *A*) and 0·3 mM-perphenazine (Per, *B*), and facilitatory effect of 0·03 mM-IBMX (*C*) on the DA-induced slow inward current. Application of each drug started 4 min prior to DA. Left and right are the controls taken before drug application and after 15 min washing. Neurones were voltage-clamped at −55 mV.

current gradually decreased with membrane hyperpolarization but no reversal in its polarity was observed even when the membrane was clamped at potentials more negative than −100 mV. At more depolarized levels than the resting potential, the DA-induced current markedly increased. Thus the current–voltage relationship showed a characteristic negative slope. Similar results were obtained with eleven LB cells.

Effects of apomorphine, perphenazine, sulpiride and LSD. Apomorphine is known to act as a partial agonist to the D_1 type of DA receptor (Kebabian & Calne, 1979). An application of 0·3 mM-apomorphine alone caused only a negligible inward current; however, it depressed the DA-induced response by as much as 70% (Fig. 3*A*).

Perphenazine, a D_1 antagonist (Miller & McDermed, 1979), also blocked the DA-induced inward current as shown in Fig. 3*B*, while 0·03 mM-sulpiride, commonly known as a D_2 antagonist (Kebabian & Calne, 1979), failed to block the DA-induced inward current. This concentration of sulpiride was high enough to block completely the DA-induced hyperpolarizing responses of the D_2-type receptors recorded from the cells in the RB cluster (data not shown). The DA-induced current was also significantly depressed in the presence of 10 μM-LSD (data not shown).

Effect of IBMX. The DA-induced response was potentiated in the presence of

0·03 mM-isobutylmethylxanthine (IBMX), a phosphodiesterase inhibitor, as shown in Fig. 3*C*.

Effects of changes in extracellular ionic concentrations. In an attempt to determine the ionic mechanisms underlying the DA-induced response, experiments were performed in which the ionic gradients for K^+, Ca^{2+}, and Na^+ were altered, independently, by appropriate changes in the extracellular medium bathing the ganglia.

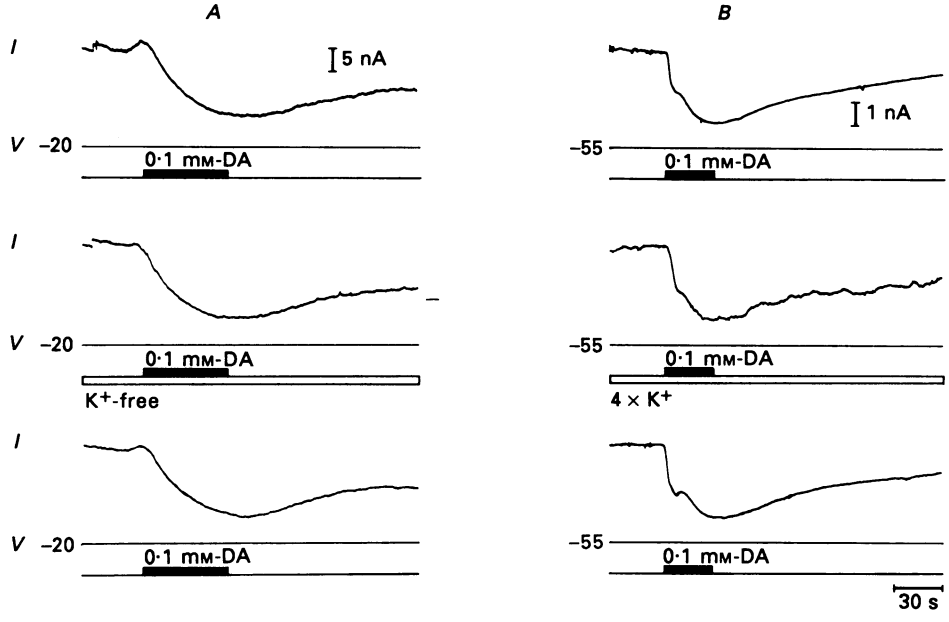

Fig. 4. Alteration of the external K^+ concentration ($[K^+]_o$). *A*, no significant effects of K^+-free medium on the DA-induced inward current. Top and bottom traces are controls taken before and after the test. The membrane potential was clamped at −20 mV in order to exaggerate the changes in inward current due to a decrease in K^+ permeability, if it occurred. *B*, no appreciable effects of 4 times normal K^+-medium ($4 \times K^+$) on the DA-induced inward current.

When the external K^+ concentration ($[K^+]_o$) was reduced to zero (K^+-free medium), the amplitude of the DA-induced inward current did not change significantly in cells clamped at their resting potentials (−50 ~ −60 mV). This was also true even when the driving force was increased by clamping the resting membrane at −20 mV in order to exaggerate any change in the inward current due to changes in permeability to K^+ (see Fig. 4*A*). When $[K^+]_o$ was increased to 4 times normal ($4 \times K^+$), the DA-induced current was not altered significantly as shown in Fig. 4*B*. Similar results were obtained from twelve cells in the LB cluster. These results, together with the absence of a reversal in sign of the response near the K^+ equilibrium potential (−70 to −90 mV), suggest that the decrease in K^+ permeability was not a major contributor to the DA-induced response in our preparation.

When the ganglion was bathed in a Ca^{2+}-free medium for 4 min, there occurred a slight depolarization of the resting membrane (not shown). The inward current

response to DA was, on the other hand, 2–3 times larger in Ca^{2+}-free medium than that seen in normal *Aplysia* medium. An example is shown in Fig. 5*A*. The size of this augmentation remained unchanged during a prolonged perfusion with Ca^{2+}-free medium even for 30 min. This seemed to exclude a possibility of the involvement of any excitatory interneuronal activities in the DA-induced response since all interneuronal input activities would be blocked after 30 min perfusion with Ca^{2+}-free medium.

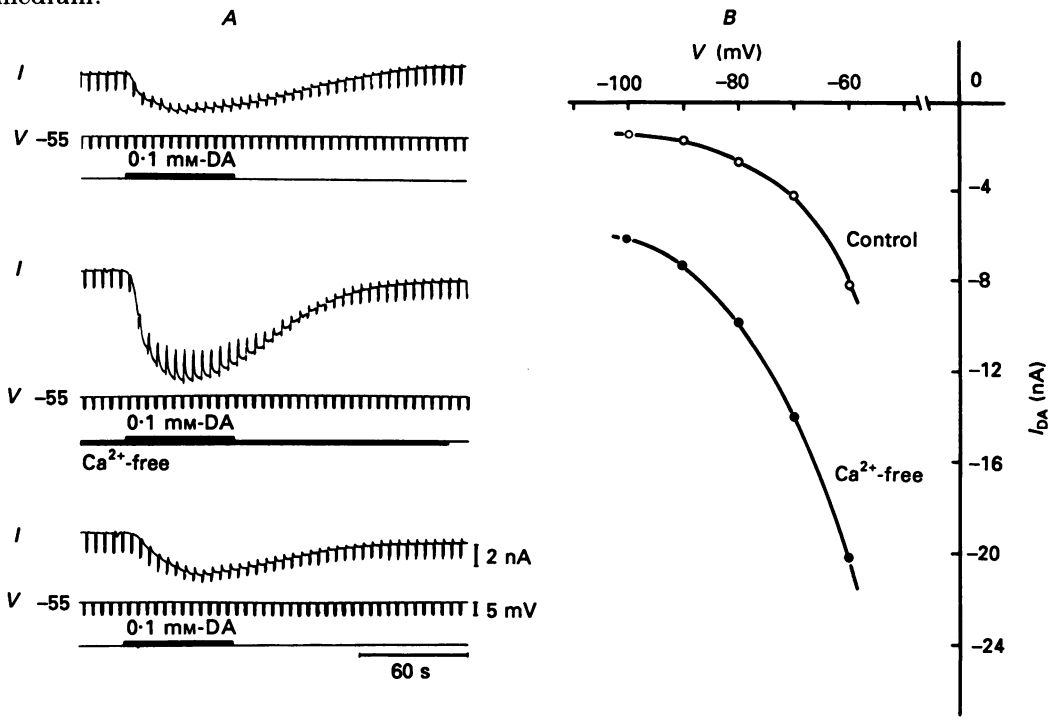

Fig. 5. Effect of Ca^{2+}-free medium on the DA-induced current. *A*, potentiating effect of Ca^{2+}-free medium on the DA-induced inward current. Top and bottom traces are controls taken before and after the test. *B*, effects of Ca^{2+}-free medium on I_{DA}-V relationship. Method of plotting the I_{DA}-V curve is same as in Fig. 2. Inward is down.

The peak amplitude of the current response to 0·1 mM-DA was measured at different membrane potentials during the perfusion of Ca^{2+}-free medium, and plotted against the membrane potential. The DA-induced inward current was markedly augmented throughout the entire range of membrane potentials keeping the characteristic negative slope. The augmenting effect of Ca^{2+}-free media appeared to be voltage dependent since the size of the augmentation was greater at potentials more negative than the resting potential as seen in Fig. 5*B*. A similar augmenting effect of Ca^{2+}-free medium was confirmed with all cells examined ($n = 20$).

In order to see if an increase in the affinity of the receptor for DA contributes to the augmentation during the perfusion of the Ca^{2+}-free medium, dose–response curves for DA in the control medium and in the Ca^{2+}-free medium were compared. In the control medium, the minimum concentration of DA inducing an inward

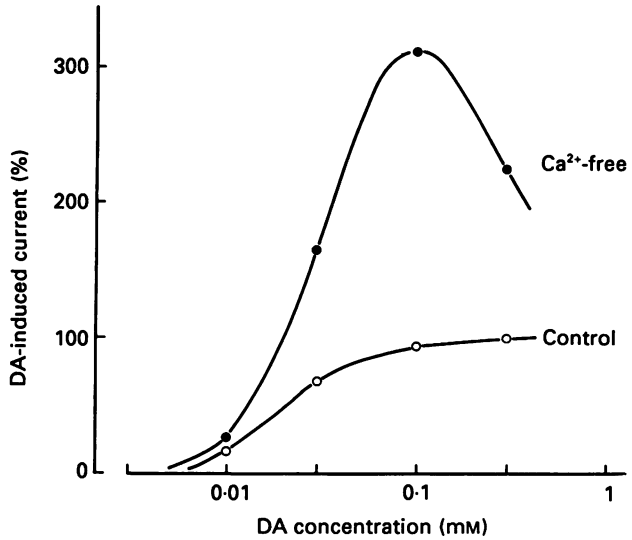

Fig. 6. Dose–response curves obtained for the normal artificial medium of *Aplysia* and for the Ca^{2+}-free medium. DA-induced currents were normalized by the value obtained in 0·1 mM-DA in the calcium-containing medium. The relative values of the response were plotted against the DA concentrations on a logarithmic scale. Each point represents mean obtained from five cells voltage-clamped at −55 mV.

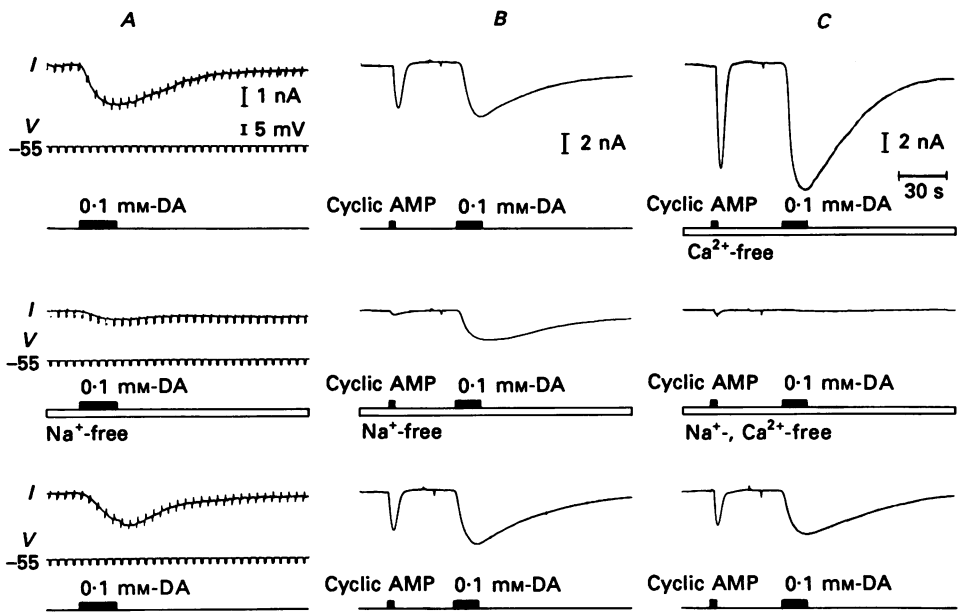

Fig. 7. *A*, effects of Na^+-free medium. Top and bottom traces are controls taken before and after the test. *B*, same as in *A* but records are taken from a different cell. DA-induced response of this cell was not depressed as much as seen in *A*. *C*, effects of Ca^{2+}-free, and Na^+- and Ca^{2+}-free media on the cyclic AMP-induced and DA-induced responses of the same cell shown in *B*.

current ranged between 0·003 and 0·01 mM (Fig. 6). Since the DA-induced inward current saturated at concentrations greater than 0·1 mM-DA, the apparent dissociation constant was estimated to be 0·02–0·03 mM in the normal *Aplysia* medium. The Ca^{2+}-free medium did not appreciably change this apparent dissociation constant.

Perfusion of the ganglion with a Na^+-free medium caused, in a current-clamped cell, a membrane hyperpolarization of 5–10 mV, which was not accompanied by a

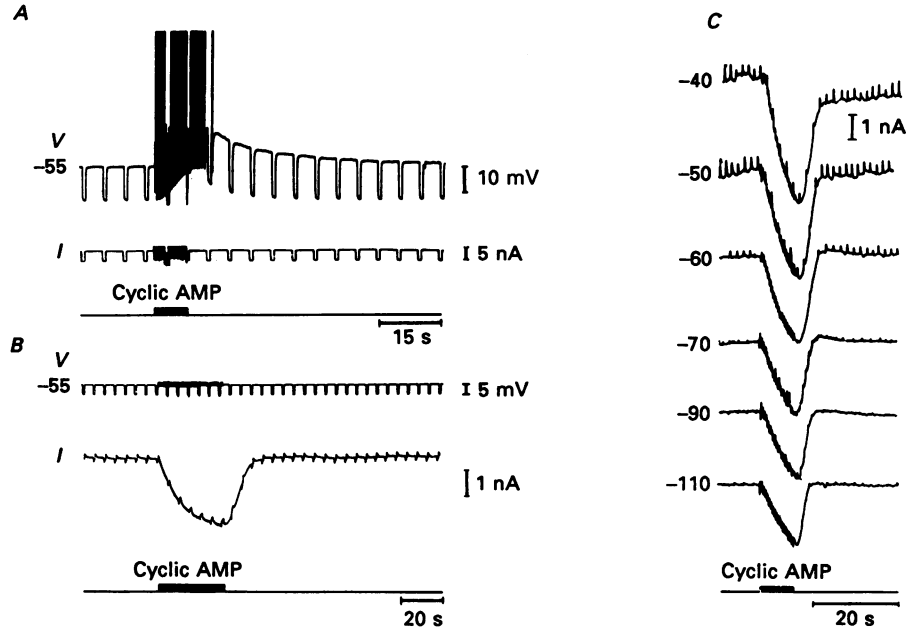

Fig. 8. *A*, cyclic AMP-induced depolarization in a current-clamped LB neurone. The periodic downward deflections are for measuring the membrane resistance. Cyclic AMP was ionophoretically injected into the cell. Refer to Methods for the intracellular concentration of cyclic AMP shown hereafter. *B*, cyclic AMP-induced inward current recorded from a voltage-clamped LB neurone at −55 mV. *C*, voltage-dependent nature of the cyclic AMP-induced inward current. The resting membrane was clamped at varying potentials shown in the left in millivolts. The slow recovery shown in *C* at −40 mV was an artifact. The rapid upward deflections of the baseline are spontaneous synaptic activities, probably IPSPs.

change in membrane resistance. In the Na^+-free medium, the DA-induced inward current responses of most cells (nineteen out of twenty-four) disappeared almost completely (see Fig. 7*A*), whereas the responses of other cells (five out of twenty-four) were depressed to 30–50% of the control (see Fig. 7*B*).

This residual component of the DA-induced inward current was not further depressed even with a prolonged Na^+-free perfusion (40 min). Such a Na^+-free-resistant response was markedly augmented in Ca^{2+}-free medium but disappeared completely in Na^+- and Ca^{2+}-free medium as shown in Fig. 7*C*. It should be noted that the cyclic AMP-induced inward current response of the same cell disappeared completely in Na^+-free medium.

Characteristics of cyclic AMP-induced responses

Cyclic AMP has been shown to induce in molluscan neurones an inward current bearing marked similarity to the DA-induced inward current observed here (Aldenhoff *et al.* 1983; Kononenko *et al.* 1983; Green & Gillette, 1983; Swandulla & Lux, 1984; Connor & Hockberger, 1984; Hara *et al.* 1985; Kehoe, 1985). This similarity, coupled with the finding that the phosphodiesterase inhibitor IBMX

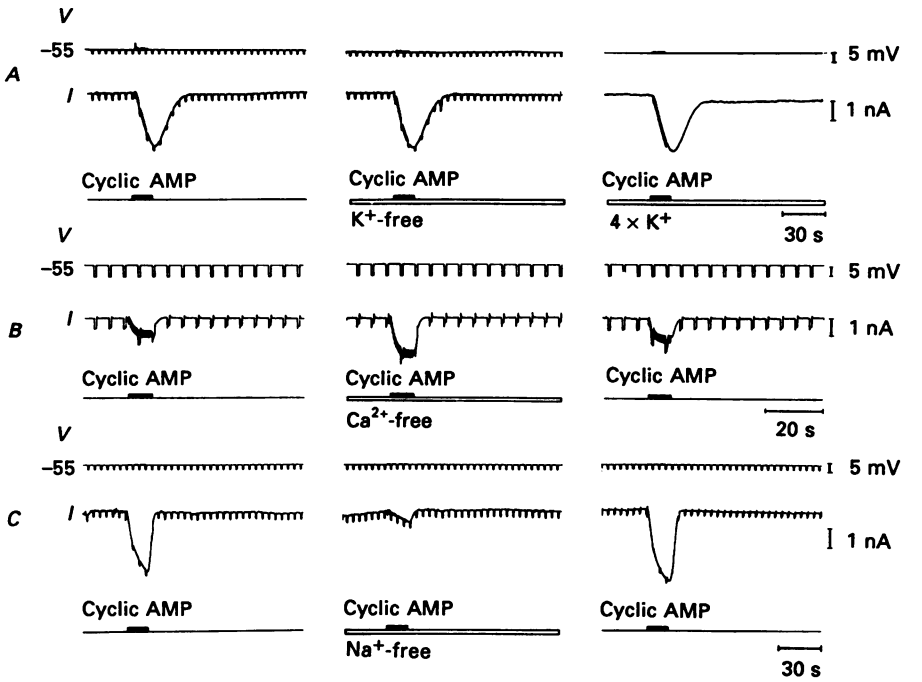

Fig. 9. *A*, perfusions with K^+-free (middle), or 4 times normal $[K^+]_o$ medium ($4 \times K^+$) (right) showed no effect on the cyclic AMP-induced response. *B*, the cyclic AMP-induced response shown on the left was markedly augmented in Ca^{2+}-free medium (middle), but returned to original after washing with normal $[Ca^{2+}]_o$ medium (right). *C*, the cyclic AMP-induced response shown on the left disappeared in Na^+-free medium (middle), but returned to original after washing with normal $[Na^+]_o$ medium (right).

enhances the DA-induced current in the LB cells (see Fig. 3), led us to test the effects of an intracellular injection of cyclic AMP on the same cells.

Ionophoretic pulses of 200 ms duration were passed at a repetition rate of 2·5 Hz through a microelectrode filled with 0·2 M-cyclic AMP (sodium salt). This caused a 5–10 mV membrane depolarization accompanied by an apparent increase in membrane resistance measured by the constant-current pulses as shown in Fig. 8*A*. When the resting membrane potential of the same cell was clamped at −55 mV, cyclic AMP induced an inward current associated with the decreased or negative conductance as seen in the DA-induced response (Fig. 8*B*). The cyclic AMP-induced response was augmented when the resting membrane was depolarized, showing

characteristic voltage dependence similar to that observed with the DA-induced response (Fig. 8 *C*, see also Fig. 2).

Effects of the changes in extracellular ionic concentrations. When $[K^+]_o$ was increased to 4 times that of normal medium or decreased to K^+-free, the amplitude of the cyclic AMP-induced inward current was not significantly affected as shown in Fig. 9*A*. However, the cyclic AMP-induced inward current was approximately doubled during the perfusion with Ca^{2+}-free medium (Figs 7*C* and 9*B*). Furthermore, it was

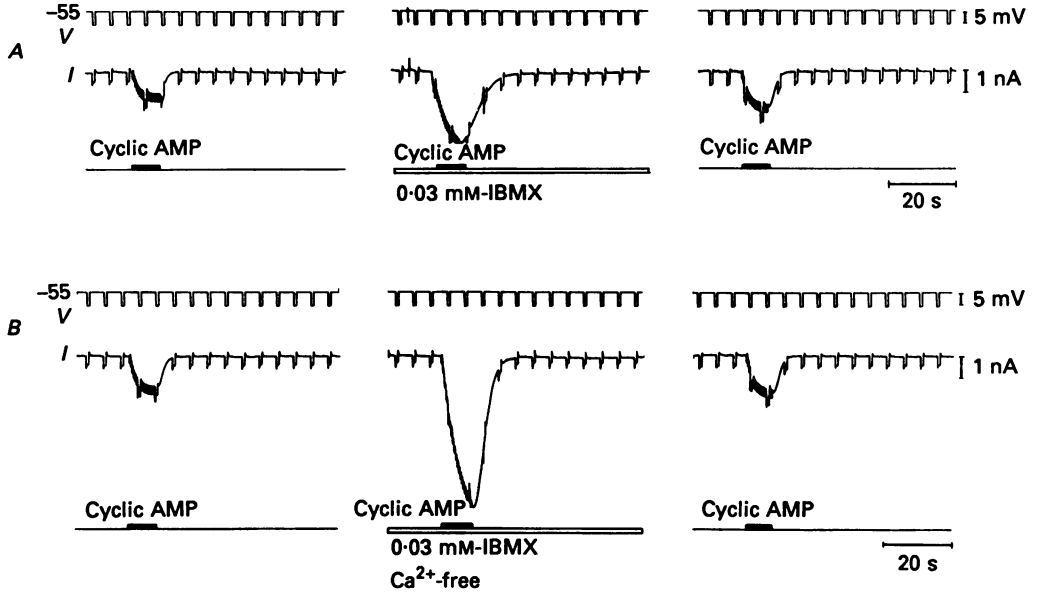

Fig. 10. Effect of IBMX on the cyclic AMP-induced inward current. *A*, perfusion of 0·03 mM-IBMX markedly augmented the cyclic AMP-induced current response shown on the left but returned to original after washing (right). *B*, combined effects of IBMX and Ca^{2+}-free medium on the cyclic AMP-induced response of the same cell as shown in *A*.

almost completely eliminated during the perfusion of a Na^+-free medium as shown in Figs 7*B* and 9*C*. Such a marked depressing effect of Na^+-free medium on the cyclic AMP-induced response was confirmed with all cells ($n = 21$) examined in the LB cluster.

Effects of IBMX. We further examined the effects of IBMX, a phosphodiesterase inhibitor, on the cyclic AMP-induced inward current as shown in Fig. 10. In the presence of 0·03 mM-IBMX, the cyclic AMP-induced inward current was doubled (Fig. 10*A*). The effect of IBMX was reversible since the cyclic AMP-induced current returned to almost the same level as the control after 10 min of washing with normal *Aplysia* medium. The augmenting effect of IBMX was confirmed in Ca^{2+}-free media as shown in Fig. 10*B*. This indicated that the augmenting effect of Ca^{2+}-free media previously observed in the absence of IBMX was not due to the change in phosphodiesterase activity.

DISCUSSION

Pharmacology

In the present study, it has been shown that DA induces a slow inward current in the LB-cluster neurones in *Aplysia* abdominal ganglia. This inward current was blocked in the presence of LSD, apomorphine and perphenazine, but not by sulpiride. In snail neurones, apomorphine is known to mimic DA-induced depolarization (Van Rossum, 1978). However, in our preparation, 0·3 mM-apomorphine caused only a slight depolarization but blocked the DA-induced slow inward current almost completely. Apomorphine might have acted in our preparation as a weak partial agonist with strong antagonist properties, as suggested by Kebabian & Calne (1979). A phenothiazine derivative such as perphenazine is known to be an antagonist to the D_1 type of DA receptor (Miller & McDermed, 1979) while sulpiride is a typical antagonist to the D_2 type of DA receptor (Kebabian & Calne, 1979). In our preparation 0·3 mM-perphenazine substantially blocked the DA-induced inward current. A similar blocking effect of fluphenazine, another phenothiazine derivative, on the DA-induced slow depolarization has also been reported in *Planorbis* neurones (MacDonald & Berry, 1978). All these results indicated that the pharmacology of the receptor responsible for the slow inward current shown in the present study resembles that of the D_1 type, which has been shown in other preparations to be coupled with adenylate cyclase. The potentiating effect of IBMX on the DA-induced inward current studied here further supports this postulation.

Ionic mechanisms in the dopamine-induced inward current

The DA-induced inward current described in this paper displays an atypical current–voltage relationship, since between -40 and -110 mV, the current remains inward, and shows a negative slope conductance; that is, the more hyperpolarized the cell, the smaller the response.

Other investigators (in molluscs, Deterre, Paupardin-Tritsch, Bockaert & Gershenfeld, 1981, 1982; Siegelbaum, Camardo & Kandel, 1982; Cottrell, Davies & Green, 1984; Paupardin-Tritsch *et al.* 1985; Kehoe, 1985; Walsh & Byrne, 1985; in rat sympathetic ganglia, Brown & Adams, 1980; in hippocampal cells, Halliwell & Adams, 1982) have described transmitter-induced inward current responses that, like the response studied above, diminish with hyperpolarization of the cell. However, the inward-going currents, described by those authors invert around the reversal potential for K^+, and are affected by changes in E_K in a way to be expected for a diminution in K^+ conductance. In contrast, although the response studied here decreases with hyperpolarization of the membrane, it does not invert even when the cell is held at 20–30 mV more negative than E_K. Furthermore, changes in external K^+ concentration have no effect on the voltage dependence of the response.

A few other cases of transmitter-induced inward currents showing negative slope conductances that are not manifestations of diminutions in K^+ conductances have been observed. Pellmar (1981*a*) observed such an atypical voltage dependence in response to DA in *Aplysia* ganglion cells, and in view of its block by the calcium channel blocker, Cd^{2+}, concluded that the response she observed was carried by

Ca^{2+}. Inward currents showing a negative slope conductance were also observed in cell B16 of the buccal ganglion of *Aplysia* by Kirk & Scheller (1986) in response to 5-hydroxytrypatamine (5-HT) or egg-laying hormone (ELH). In the latter case an involvement of K^+ conductance changes cannot be excluded, since these authors neither studied the transmitter-induced response at potentials more negative than E_K, nor altered external K^+ concentration. However, like the DA-induced current described here, the responses studied by Kirk & Scheller (1986) disappear in Na^+-free media and are enhanced in Ca^{2+}-free media.

However, the current that most resembles the DA-induced response studied in this paper is that previously seen in molluscan neurones in response to an intracellular injection of cyclic AMP. In addition to causing inward-going currents in molluscan neurones that can be attributed to a diminution in K^+ conductance (see Deterre *et al.* 1981, 1982; Siegelbaum *et al.* 1982; Walsh & Byrne, 1985; Kehoe, 1985), cyclic AMP has been shown to elicit an inward current that fails to invert beyond E_K. The cyclic AMP-induced current with such an atypical current–voltage relationship and a dependence on Na^+ has been described by many investigators (Aldenhoff *et al.* 1983; Kononenko *et al.* 1983; Green & Gillette, 1983; Swandulla & Lux, 1984; Connor & Hockberger, 1984; Hara *et al.* 1985; Kehoe, 1985), as has its enhancement in low external Ca^{2+} (Aldenhoff *et al.* 1983; Swandulla & Lux, 1984; Hara *et al.* 1985; Kehoe, 1985; Gillette & Green, 1987) or in the presence of phosphodiesterase inhibitors. In all of these respects this cyclic AMP-induced current resembles the DA-induced inward current observed here. Furthermore, our own experiments testing the effect of cyclic AMP injections in the same cells used for the DA experiments revealed that the cyclic AMP-induced current may be dominant in these cells.

For example, the cyclic AMP-induced inward current recorded from the cells with DA receptors of this type showed atypical voltage dependence, marked depression in low-Na^+ medium, enhancement in low-Ca^{2+} medium and augmentation by IBMX. These characteristics were quite similar to those of the DA-induced inward current recorded from the same cells. In addition, Gillette & Green (1987) reported most recently that the cyclic AMP-induced responses recorded from the neurones of *Pleurobranchaea* were also augmented in low-Ca^{2+} media, and that the greater rate of augmentation was observed at potentials more negative than the resting potential. This voltage-dependent effect of low Ca^{2+} on the size of augmentation is consistent with that observed with DA-induced responses in the present experiment. Accordingly, we can conclude that most of the DA-induced responses studied here are produced by a receptor-controlled increase in intracellular cyclic AMP. However, there were a few exceptional neurones of which DA-induced responses were not markedly depressed in Na^+-free medium whereas the cyclic AMP-induced responses of the same cells disappeared completely in Na^+-free medium as shown in Fig. 7*B*. Pellmar (1981*a*) has analysed the ionic mechanism underlying the DA-induced slow inward current recorded from ganglion cells of *Aplysia*. She concluded that the ions carrying this inward current were not Na^+ but Ca^{2+}, though she did not examine the effect of Ca^{2+}-free medium. It should be noted that the DA-induced inward current responses resistant to low Na^+ were markedly augmented in Ca^{2+}-free medium, but completely disappeared in Na^+-free, Ca^{2+}-free medium. Our interpretation is that

this type of DA-induced slow inward current was produced by opening of voltage-dependent ion channels through which Na^+ is more readily permeable than Ca^{2+}, and that Ca^{2+} is normally inhibiting Na^+ influx. At any rate, we can say that this type of DA response is not produced as the result of an increase in intracellular cyclic AMP, as Pellmar (1981*b*) later suspected. Further experiments are needed to determine the intracellular messenger mediating this Na^+-free-resistant response.

The authors wish to thank Mr Itoh, Mr Kawasaki, Mr Mito and the Osawa Fishermen's Union for a constant supply of *Aplysia kurodai*. They also wish to thank Professor P. Langman for his kind assistance with English usage. This study has been partly supported by the grants provided by The Ichiro Kanehara Foundation, and by The Narishige Neuroscience Foundation to K. S. It was also supported by Iwate Medical University Keiryokai Grant No 26.

REFERENCES

ALDENHOFF, J. B., HOFMEIER, G., LUX, H. D. & SWANDULLA, D. (1983). Stimulation of a sodium influx by cAMP in *Helix* neurons. *Brain Research* **276**, 289–296.

ASCHER, P. (1972). Inhibitory and excitatory effects of dopamine on *Aplysia* neurones. *Journal of Physiology* **225**, 173–209.

BERNARDI, G., CHERUBINI, E., MARCIANI, M. G., MERCURI, N. & STANZIONE, P. (1982). Responses of intracellularly recorded cortical neurons to the ionophoretic application of dopamine. *Brain Research* **245**, 267–274.

BERRY, M. S. & COTTRELL, G. A. (1975). Excitatory, inhibitory and biphasic synaptic potentials mediated by an identified dopamine-containing neurone. *Journal of Physiology* **244**, 589–612.

BOUDIER, H. A. J. S., GIELEN, W., COOLS, A. R. & VAN ROSSUM, J. M. (1974). Pharmacological analysis of dopamine-induced inhibition and excitation of neurones of the snail *Helix aspersa*. *Archives internationales de pharmacodynamie et de thérapie* **209**, 324–331.

BROWN, D. A. & ADAMS, P. R. (1980). Muscarinic suppression of a novel voltage-sensitive K^+-current in a vertebrate neurone. *Nature* **283**, 673–676.

CONNOR, J. A. & HOCKBERGER, P. (1984). A novel membrane sodium current induced by injection of cyclic nucleotides into gastropod neurones. *Journal of Physiology* **354**, 139–162.

COTTRELL, G. A., DAVIES, N. W. & GREEN, G. A. (1984). Multiple actions of a molluscan cardioexcitatory neuropeptide and related peptides on identified *Helix* neurones. *Journal of Physiology* **356**, 315–333.

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

DETERRE, P., PAUPARDIN-TRITSCH, D., BOCKAERT, J. & GERSHENFELD, H. M. (1982). c-AMP-mediated decrease in K^+ conductance evoked by serotonin and dopamine in the same neurone: A biochemical and physiological single cell study. *Proceedings of the National Academy of Sciences of the U.S.A.* **79**, 7934–7938.

FRAZIER, W. T., KANDEL, E. R., KUPFERMANN, I., WAZIRI, R. & COGGESHALL, R. E. (1967). Morphological and functional properties of identified neurons in the abdominal ganglion of *Aplysia californica*. *Journal of Neurophysiology* **30**, 1288–1351.

GALLAGHER, J. P., INOKUCHI, H. & GALLAGHER, S. P. (1980). Dopamine depolarization of mammalian primary afferent neurones. *Nature* **283**, 770–772.

GILLETTE, R. & GREEN, D. J. (1987). Calcium dependence of voltage sensitivity in adenosine 3′,5′-cyclic phosphate-stimulated sodium current in *Pleurobranchaea*. *Journal of Physiology* **393**, 233–245.

GREEN, D. J. & GILLETTE, R. (1983). Patch- and voltage-clamp analysis of cyclic AMP-stimulated inward current underlying neurone bursting. *Nature* **306**, 784–785.

HALLIWELL, J. V. & ADAMS, P. R. (1982). Voltage clamp analysis of muscarinic excitation in hippocampal neurones. *Brain Research* **250**, 71–92.

HARA, N., SAWADA, M. & MAENO, T. (1985). Influences of pressure-injected cyclic AMP on the membrane current and characteristics of an identified neuron of *Aplysia kurodai*. *Japanese Journal of Physiology* **35**, 985–1012.

HERRLING, P. L. & HULL, C. D. (1980). Iontophoretically applied dopamine depolarizes and hyperpolarizes the membrane of cat caudate neurons. *Brain Research* **192**, 441–462.

KEBABIAN, J. W. & CALNE, D. B. (1979). Multiple receptors for dopamine. *Nature* **277**, 93–96.

KEHOE, J. (1985). Synaptic block of a transmitter-induced potassium conductance in *Aplysia* neurones. *Journal of Physiology* **369**, 399–437.

KIRK, M. D. & SCHELLER, R. H. (1986). Egg-laying hormone of *Aplysia* induces a voltage-dependent slow inward current carried by Na^+ in an identified motoneuron. *Proceedings of the National Academy of Sciences of the U.S.A.* **83**, 3017–3021.

KITAI, S. T., SUGIMORI, M. & KOCSIS, J. D. (1976). Excitatory nature of dopamine in the nigro-caudate pathway. *Experimental Brain Research* **24**, 351–363.

KONONENKO, N. I., KOSTYUK, P. G. & SHCHERBATKO, A. D. (1983). The effect of intracellular cAMP injections on stationary membrane conductance and voltage- and time-dependent ionic currents in identified snail neurons. *Brain Research* **268**, 321–338.

MACDONALD, J. F. & BERRY, M. S. (1978). Further identification of multiple responses mediated by dopamine in the CNS of *Planobis corneus*. *Canadian Journal of Physiology and Pharmacology* **56**, 7–18.

MILLER, R. J. & MCDERMED, J. (1979). Dopamine-sensitive adenylate cyclase. In *The Neurobiology of Dopamine*, ed. HORN, A. S., KORF, J. & WESTERNIK, B. H. C., pp. 159–177. London: Academic Press.

PAUPARDIN-TRITSCH, D., COLOMBAIONI, L., DETERRE, P. & GERSCHENFELD, H. M. (1985). Two different mechanisms of calcium spike modulation by dopamine. *Journal of Neuroscience* **5**, 2522–2532.

PELLMAR, T. C. (1981*a*). Voltage-dependent current evoked by dopamine and octopamine in *Aplysia*. *Brain Research* **233**, 448–454.

PELLMAR, T. C. (1981*b*). Does cyclic 3′,5′-adenosine monophosphate act as second messenger in a voltage-dependent response to 5-hydroxytryptamine in *Aplysia*? *British Journal of Pharmacology* **74**, 747–756.

SASAKI, K., MATSUMOTO, M., SHOZUSHIMA, M., TAKASHIMA, K. & SATO, M. (1986*a*). Ionic mechanisms involved in dopamine induced slow depolarization. *Neuroscience Research Supplement* **3**, S142.

SASAKI, K., MATSUMOTO, M., SHOZUSHIMA, M., TAKASHIMA, K. & SATO, M. (1986*b*). Intracellular injection of cAMP produces inward current similar to the dopamine-induced response. *Journal of the Physiological Society of Japan* **48**, 208.

SATO, M., AUSTIN, G., YAI, H. & MARUHASHI, J. (1968). The ionic permeability changes during acetylcholine-induced response of *Aplysia* ganglion cells. *Journal of General Physiology* **51**, 321–345.

SEEMAN, P. (1981). Brain dopamine receptors. *Pharmacological Reviews* **32**, 229–313.

SIEGELBAUM, S. A., CAMARDO, J. S. & KANDEL, E. R. (1982). Serotonin and cAMP close single K^+ channels in *Aplysia* sensory neurons. *Nature* **299**, 413–417.

STOOF, J. C. & KEBABIAN, J. W. (1984). Two dopamine receptors: Biochemistry, Physiology, and Pharmacology. *Life Sciences* **35**, 2281–2296.

STOOF, J. C., WERKMAN, T. R., LODDER, J. C. & DE VLIEGER, TH. A. (1986). Growth hormone producing cells in *Lymnaea stagnalis* as a model system for mammalian dopamine receptors? *Trends in Pharmacological Sciences* **7**, 7–9.

SWANDULLA, D. & LUX, H. D. (1984). Changes in ionic conductances induced by cAMP in *Helix* neurons. *Brain Research* **305**, 115–122.

VAN ROSSUM, J. M. (1978). Two types of dopamine receptors in behavioural regulation. *Federation Proceedings* **37**, 2415–2421.

WALSH, J. P. & BYRNE, J. H. (1985). Analysis of decreased conductance serotonergic response in *Aplysia* ink motor neurons. *Journal of Neurophysiology* **53**, 590–602.