

## THE MODULATORY ACTION OF DOPAMINE ON CRUSTACEAN FOREGUT NEUROMUSCULAR PREPARATIONS

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### SUMMARY

1. Effects of dopamine (DA) on contractile and electrical properties of decapod foregut neuromuscular preparations were examined.
2. Dopamine produced dramatic increases in nerve-evoked contractions and, in particular muscles, contractures and spontaneous contractions. These effects were observed at concentrations as low as  $5 \times 10^{-9}$  M-DA.
3. The DA-produced enhancement of nerve-evoked contractions was associated with an increase in amplitude of excitatory junctional potentials (EJPs).
4. The increase in EJPs resulted in part from an increase in muscle fibre membrane resistance that was particularly prominent over depolarized membrane potentials. A presynaptic action of dopamine cannot be as yet excluded.
5. In fibres from the muscle in which dopamine produced a contracture, dopamine also produced a depolarization.
6. In fibres from the muscle in which dopamine activated spontaneous contractions, dopamine also produced spontaneous rhythmic action potentials.
7. Dopamine also accelerated the half-time of muscle relaxation following muscle contraction.

### INTRODUCTION

Biogenic amines frequently produce effects on both vertebrate and invertebrate preparations that involve prolonged alterations in cellular excitability or metabolism, possibly mediated by cyclic nucleotides (Moore & Bloom, 1978, 1979; Kupfermann, 1979). These effects are often termed modulatory when they modify the efficacy of some other process. Three biogenic amines, serotonin, octopamine and dopamine, have each been shown to modulate muscular contractions in molluscan and arthropod neuromuscular preparations, but the cellular mechanisms of these effects are not yet well defined. In *Aplysia* buccal musculature (Weiss, Cohen & Kupfermann, 1975, 1978) a direct action of serotonin on contractile mechanisms has been proposed, while

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at some crustacean neuromuscular junctions (Dudel, 1965; Glusman & Kravitz, 1978) serotonin causes a presynaptic increase in evoked transmitter release. In the locust it has been suggested that octopamine increases transmitter release from some pre-synaptic terminals and may also affect the contractile process directly (Evans & O'Shea, 1978; O'Shea & Evans, 1979). In *Aplysia* gill musculature, dopamine increases the amplitude of extracellularly recorded junction potentials, consistent with either a pre- or postsynaptic mechanism of action (Swann, Sinback & Carpenter, 1978). As yet, there is no direct evidence that biogenic amines can modulate invertebrate neuromuscular function by an alteration of muscle fibre electrical properties. This is perhaps surprising, since these amines are capable of affecting conductances in a variety of invertebrate neurones (Ascher, 1972; Gerschenfeld & Paupardin-Tritsch, 1974; Pellmar & Wilson, 1977).

The present report describes modulatory effects produced by dopamine on some neuromuscular preparations from the stomatogastric system of the crab, *Cancer magister*, and the spiny lobster, *Panulirus interruptus*. A preliminary report of some of these results has appeared in abstract form (Lingle, 1979).

#### METHODS

Neuromuscular preparations were prepared from the foregut of the crab, *Cancer magister*, and the spiny lobster, *Panulirus interruptus*. In a few experiments, opener muscles from the walking legs of *Panulirus* were used. *C. magister* was obtained from the Oregon Institute of Marine Biology, Charleston, Oregon; and *Panulirus* from Pacific Biomarine, Venice, CA. Animals were maintained in sea-water tanks at appropriate temperatures.

Muscles and attached nerves (identified according to the nomenclature of Maynard & Dando (1974)) were isolated from the foregut and pinned out in Sylgard-lined 1–3 ml perfusion chambers. Preparations were superfused continuously with a gravity-fed system at 5–10 ml/min. Inflowing saline was cooled by circulation through a plexiglass heat exchange plate sitting on a Peltier cooling plate. Temperature was monitored by a small thermometer in the muscle chamber. Experiments were done at 12–16 °C for *Panulirus* and 8–14 °C for *Cancer*. Within a given experiment, temperature varied less than 1 °C. Bath applications of drugs were accomplished by switches in the perfusion line. The precise time of drug entry into the perfusion chamber was not determined, but was less than 30 s after switching perfusion lines. Similarly, the time of complete removal of a drug from the bath is not known. However, several bath volumes were exchanged each minute. Thus, bars on figures correspond to the time during which the drug-containing perfusion line was open. In most cases a standard drug application of 3 min was used, a time found to yield maximal responses of nerve-evoked contractions at a given drug concentration.

Muscles of the decapod stomatogastric system are anatomically and physiologically similar to other crustacean striated muscles and receive a distributed excitatory innervation from identifiable motor neurones within the stomatogastric ganglion (Govind, Atwood & Maynard, 1975; Maynard & Dando, 1974; Selverston *et al.* 1976). For this study muscle gm6 of *Cancer* and the gm6b and dorsal dilator (cpv 1a, 1b) muscles of *Panulirus* were examined. The gm6 muscles receive single excitatory innervation from

The LG neurone which is likely to be glutamatergic (Lingle, 1979, 1980). The cpv 1a, 1b muscle, consisting of two bundles, is innervated by two electrically coupled cholinergic neurones (Marder, 1976). Neither muscle receives inhibitory innervation. Muscles were prepared for either tension or intracellular recordings. Tension was measured using a Grass FT 03 force displacement transducer. Intracellular recordings and injection of current were made conventionally. Microelectrodes were filled with

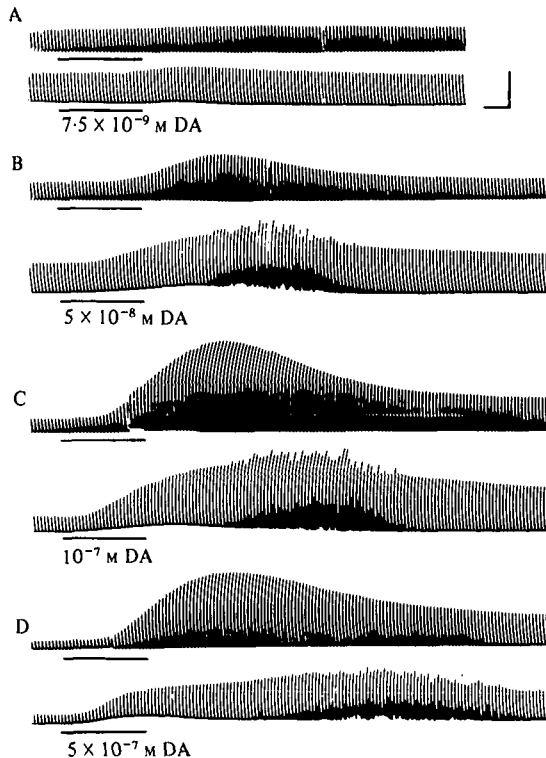


Fig. 1. Dopamine effects on contractile properties of stomatogastric neuromuscular preparations from *Panulirus interruptus*. The records show the effects of increasing DA concentrations (A-D) on a pair of muscles, the gm6b (top trace in each pair) and the cpv 1a, 1b (bottom). Each deflexion corresponds to a contraction resulting from stimulation of the excitatory motor nerve(s) to the muscles with trains of pulses at 40 Hz for 500 ms. DA at the concentrations indicated was applied for the duration of the bars. Vertical calibration is 1.1 g except for the top trace in A and B and both traces in D which are 2.2 g. Horizontal calibration is 1 min.

3 M-KCl and had resistances of 10–30 M $\Omega$ . Innervating nerves were stimulated via trains of pulses applied through a suction electrode. Similar procedures were used for experiments on opener muscles except that the walking leg shell was used as the perfusion chamber.

Saline compositions were the following (in mM): *Panulirus* (Marder, 1976): NaCl, 479; KCl, 12.7; MgSO<sub>4</sub>, 10; CaCl<sub>2</sub>, 13.7; NaSO<sub>4</sub>, 3.9; Tris base, 8.3; maleic acid, 3.6. *C. magister* (Auerbach, 1978): NaCl, 466; KCl, 11.3; MgSO<sub>4</sub>, 19.5; CaCl<sub>2</sub>, 12.6; Tris base, 11; maleic acid, 4.8. The pH of all solutions was 7.3–7.6. Dopamine, D, L-octopamine, and serotonin-creatinine sulphate complex were obtained from Sigma Chemical Company. Dopamine solutions were made up fresh before each application from a stock solution acidified with HCl.

## RESULTS

*Effects of dopamine on resting and evoked tension in foregut muscles*

Dopamine (DA) had 3 excitatory effects upon the contractile properties of foregut nerve-muscle preparations, as illustrated in Fig. 1 for a gm6b and the cpv 1a, 1b muscles of *Panulirus*. First, in both muscles DA produced a dose-dependent enhancement of the contractions evoked by stimulation of the excitatory motor nerves. Secondly, in the cpv 1a, 1b muscle, DA produced a slow contracture. Third, DA evoked

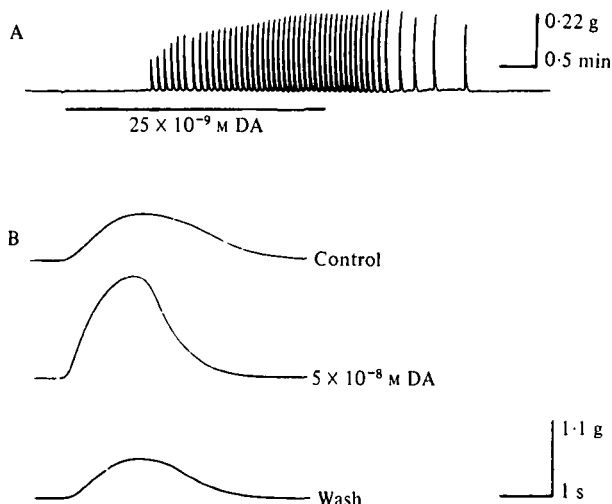


Fig. 2. Dopamine effects on spontaneous contractions and muscle relaxation. In A, DA activated rhythmic spontaneous contractile activity in the absence of nerve stimulation in cpv 1a, 1b muscle.  $2.5 \times 10^{-8}$  M-DA was applied for the duration of the bar. In B, expanded sweeps of nerve-evoked contractions in muscle cpv 1a are displayed.  $5 \times 10^{-8}$  M-DA increases the amplitude and rate of muscle relaxation in the middle trace. Nerves to muscle were stimulated with a 40 Hz train for 500 ms.

spontaneous contractile activity in fibres within the cpv 1a, 1b muscle. The enhancement of the neurally evoked contractions was reliably produced in the gm6 muscles at concentrations down to  $5 \times 10^{-8}$  M, and in the cpv 1a, 1b down to  $5 \times 10^{-9}$  M. The lowest concentrations required to produce responses were  $5 \times 10^{-9}$  M and  $10^{-9}$  M in the gm6b and cpv 1a, 1b, respectively. The DA-produced contractures and spontaneous contractions also occurred at concentrations around  $10^{-8}$  M. In the gm6 muscles DA concentrations above  $1 \mu\text{M}$  occasionally produced a small contracture, but spontaneous contractions were never observed in the gm6 muscles.

Spontaneous rhythmic contractions were often observed in the cpv 1a, 1b preparations (16 of 23) immediately following removal of this muscle from the animal. These contractions usually lasted for no more than the initial 30 min, and were usually confined to the cpv 1a bundle. Following the application of DA (Fig. 2A), spontaneous contractions characteristically occurred at some time after the peak of the DA-produced contracture (as in Fig. 1 and Fig. 4). During prolonged DA application, spontaneous contractions persisted for hours with only slight desensitization to a given DA concentration. Such contractions were not blocked by agent

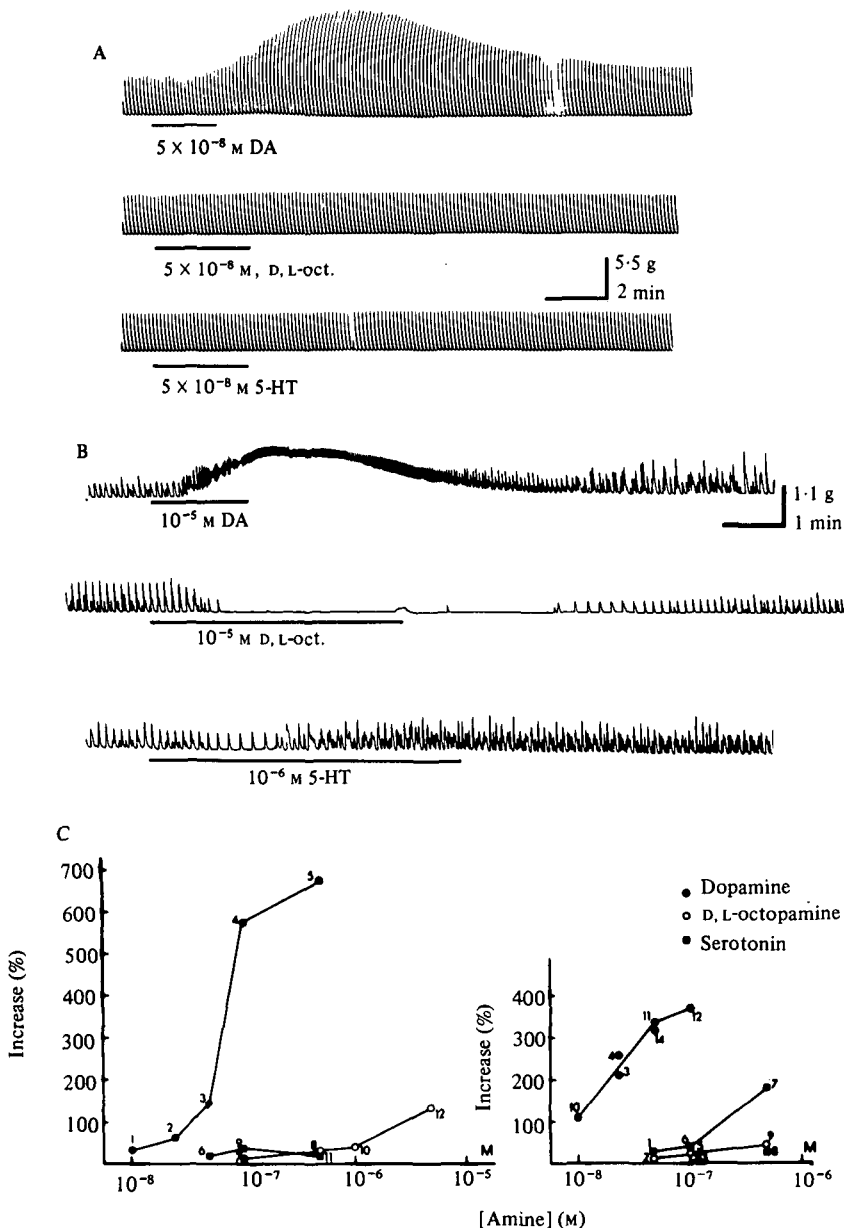


Fig. 3. Comparison of the effects of dopamine to octopamine and serotonin. In (A) the effects of  $5 \times 10^{-8}$  M-DA on nerve-evoked contractions is muscle cpv 1a, 1b are compared to the effects of  $5 \times 10^{-8}$  M serotonin and  $5 \times 10^{-8}$  M octopamine. Nerve stimulation was with 1 s trains at 30 Hz. The order of amine application did not affect the results. In some preparations these concentrations of serotonin and octopamine did produce some enhancement. In (B) the effects of  $10^{-5}$  M-DA,  $10^{-5}$  M octopamine and  $10^{-6}$  M serotonin on spontaneous contractions of muscle cpv 1a, 1b are compared. In this case spontaneous contractions were present following removal of the muscle from the animal. Neither octopamine nor serotonin activated spontaneous contractions. In (C) partial dose-response curves of the effects of DA, octopamine and serotonin on nerve-evoked contraction amplitude on a gm6b (left) muscle and cpv 1a, 1b (right) muscle from *Panulirus*. Ordinate corresponds to the percentage increase in nerve-evoked contraction amplitude produced by a given amine concentration over the amplitude prior to amine application. Contractions were elicited by 40 Hz trains of pulses to the excitatory nerves. Numbers correspond to the order of drug application.

which block the cholinergic excitatory synaptic potentials in this muscle, suggesting that the mechanism of spontaneous contractile activity results from a direct action of dopamine on the muscle fibres.

In addition to its effects on the amplitude of nerve-evoked contractions, DA also increased the half-time of muscle relaxation following nerve-evoked contractions at concentrations as low as  $10^{-8}$  M. It may also increase the half-time of contraction, but this is difficult to assess since stimulation of the excitatory nerve persists for most of the contraction time.

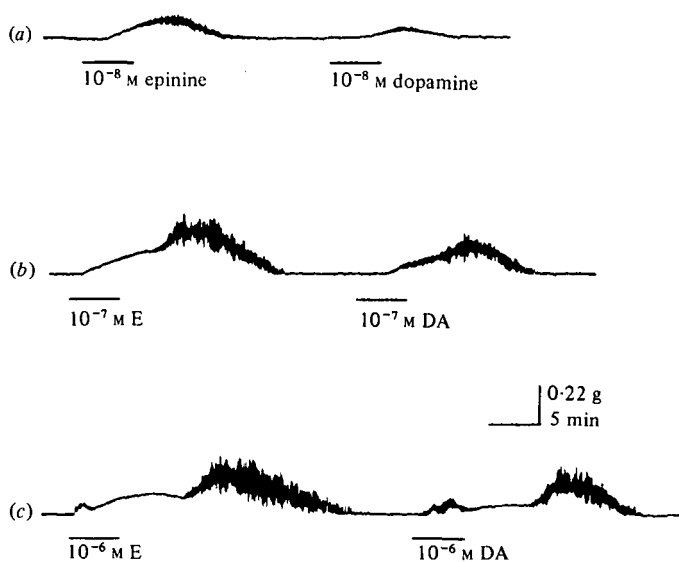


Fig. 4. Comparison of the effects of epinine and dopamine on muscle cpv 1a, 1b. The dopamine receptor agonist, epinine, and dopamine were applied sequentially to a cpv 1a, 1b muscle. Both agents produced muscle contraction and spontaneous contractions. Horizontal calibration is 5 min. Agents were introduced into the perfusion line during the bars. Vertical calibration is 0.22 g.

#### *Comparison of actions of dopamine, serotonin and octopamine*

Effects of serotonin and octopamine were compared to those of DA because there is some evidence for pharmacologically distinct receptors to serotonin and octopamine which produce an enhancement of nerve-evoked contractions in *Homarus* (Kravitz *et al.* 1976).

Both serotonin and octopamine produced an enhancement of nerve-evoked contractions in the foregut preparations. However, the thresholds of the responses to serotonin and octopamine were quite variable and the magnitudes of the responses were much smaller than those evoked by DA. In contrast to dopamine, both serotonin and octopamine did not usually enhance contractions at  $5 \times 10^{-8}$  M (Fig. 3A) and required concentrations as high as  $1 \mu\text{M}$  (Fig. 3C). The order of amine application did not affect the relative effectiveness of the three amines, although some refractoriness to repeated applications of octopamine or serotonin was evident. In addition, neither serotonin or octopamine produced contractures or the appearance of spontaneous

contractions in the cpv 1a, 1b muscle. On the contrary, octopamine inactivated spontaneous contractions while serotonin increased the frequency and amplitude of the contractions (Fig. 3 B). Thus, at least some of the actions of dopamine on these foregut nerve-muscle preparations appear to be mediated by a receptor specific for dopamine. This is further supported by the finding that the putative dopamine receptor agonist, epinine, is comparable in potency with dopamine in producing enhancement of evoked contractions, contracture, and spontaneous contractions in foregut preparations. This is illustrated for the latter two phenomena in Fig. 4.

The above effects of the three amines differ from the effects upon the limb muscles of *Homarus* (Kravitz *et al.* 1976). They were also found to differ from the effects upon the opener muscle of *Panulirus*. As in *Homarus*, serotonin and octopamine increased the evoked contractions (Fig. 5). However, DA at concentrations up to  $50\text{ }\mu\text{M}$  had little or no effect upon resting tension or nerve-evoked contractions in the *Panulirus* opener muscle.

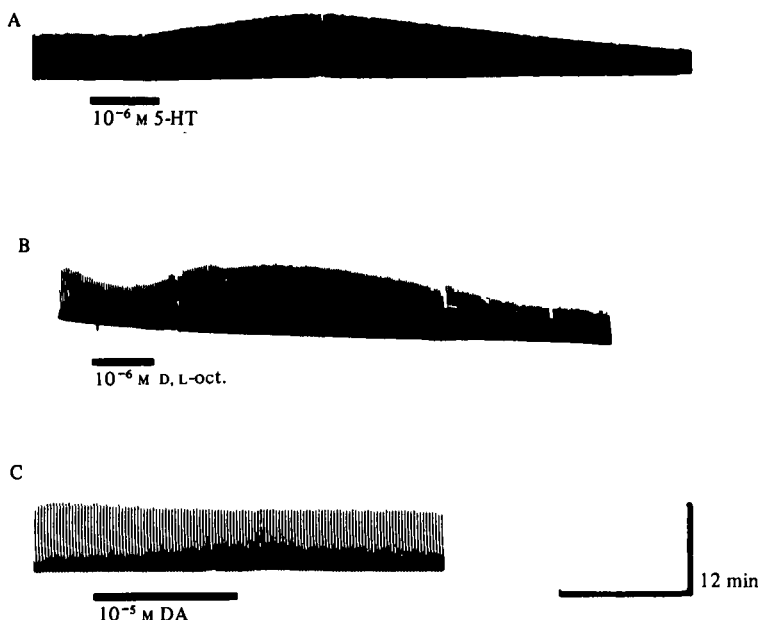


Fig. 5. Comparison of effects of dopamine, serotonin and octopamine on walking leg opener muscles of *Panulirus*. Trains of pulses were applied to the opener muscle excitatory nerve to elicit muscle contraction. Amines were introduced into the perfusion lines to the muscle for the duration of the bars. In (A)  $10^{-6}\text{ M}$  serotonin was applied; in (B)  $10^{-6}\text{ M}$  D,L-octopamine; in (C)  $10^{-5}\text{ M}$  dopamine. Horizontal calibration is 12 min and vertical calibration is 1.1 g in a and b and 2.2 g in c.

#### *Effects of DA on foregut muscle electrical properties*

To help determine the mechanisms of dopamine action on foregut preparations, effects of DA on resting membrane potential, excitatory junctional potentials (EJPs) and membrane resistance were examined.

In gm6 fibres, dopamine had little or no effect on resting potential ( $-60$  to  $-85\text{ mV}$ ) at concentrations up to  $5\text{ }\mu\text{M}$ , but usually produced definite increases in the amplitude

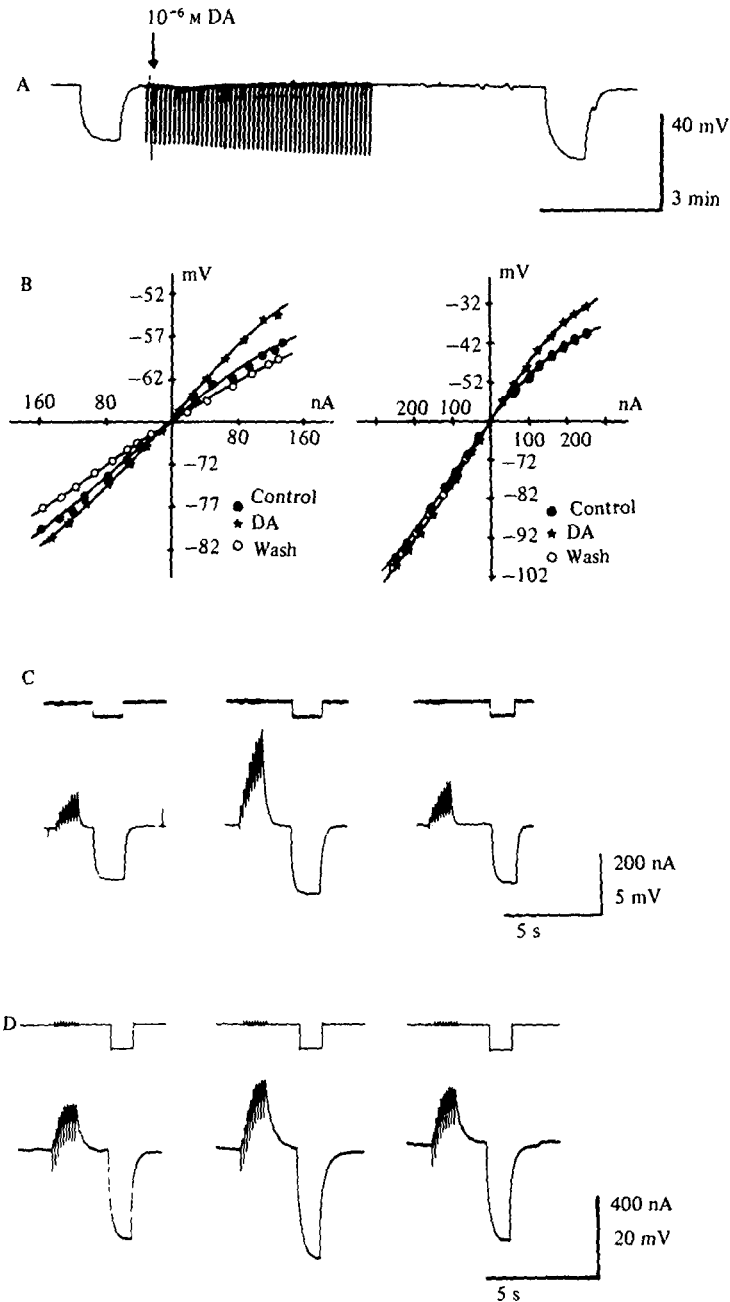


Fig. 6. Effects of dopamine on synaptic transmission and electrical properties of gm6 muscle fibres of *Cancer* and *Panulirus*. In A, membrane potential of a gm6b fibre of *Panulirus* was monitored during current injection.  $10^{-6}$  M-DA was applied at the arrow. Resting potential was -72 mV. The recorder was accelerated (horizontal calibration is 3 s) to show hyperpolarizing voltage responses. In B, the effect of DA on current-voltage relations of a gm6 fibre of *Cancer* and a gm6b fibre of *Panulirus* are shown. Plots were obtained from maximal voltage responses to current pulses of different amplitudes. Origin corresponds to resting potential. Plots were generated during peak of response to DA.  $2 \times 10^{-6}$  M-DA was used on gm6 (left) and  $10^{-6}$  M-DA for gm6b (right). In C, the effect of DA on excitatory junctional potentials

of constant hyperpolarizing current pulses (Fig. 6A). Since constant current pulses are frequently inadequate to reveal alterations in membrane conductance with voltage-dependent characteristics, complete current-voltage relations were examined. The results showed that DA produces a resistance increase that is particularly prominent at depolarized membrane potentials. DA also produced a large enhancement of EJPs in both species (Fig. 6C, D). This enhancement was of prolonged time course, similar to that observed for the enhancement of evoked contractions. Both the increase in EJP amplitude and resistance increases occurred at concentrations as low as  $5 \times 10^{-8}$  M, the lowest concentration examined. However, as discussed in more detail below, it is not possible to determine what percentage of the EJP enhancement is the result of the increase in membrane resistance, since EJPs are generated from distributed sites along the muscle fibre while the current electrode generates current at a single site close to the recording electrode. Yet the large increase in EJP amplitude relative to the resistance changes observed may be indicative of an action of DA on presynaptic nerve terminals (as in Fig. 6C). The discrepancy between increases in EJP amplitude and resistance increases was more pronounced at dopamine concentrations above  $1 \mu\text{M}$ .

With the cpv 1a muscle bundle, DA enhanced the evoked contractions, and produced contracture and spontaneous contractions. In contrast to the gm6 muscle fibres, resting potential ( $-45$  to  $-65$  mV) was consistently depolarized by up to 12 mV by  $2.5\text{--}10 \times 10^{-8}$  M-DA (Fig. 7A). In addition to the DA-produced depolarization, a prolonged apparent resistance increase was seen with constant current pulses. Although the time course of the depolarization corresponded approximately with the time course of DA application, the resistance change persisted for some time after removal of DA and the return to normal resting potential. As in the gm6 muscles, DA produced a large enhancement of EJPs of prolonged time course in bundle cpv 1a (Fig. 7B). Complete current-voltage plots generated in muscle cpv 1a also showed greater apparent resistance increases at depolarized potentials in the presence of DA than were revealed by hyperpolarizing current pulses alone. The vigorous spontaneous contractile activity that occurs during the application of DA often made such recordings difficult to maintain in this muscle, so some plots were obtained in the presence of 20 mM manganous ions to minimize muscle movement. A substantial resistance increase at depolarizing potentials was still observed (Fig. 7E). The effect of DA on the input resistance of cpv 1a fibres was consistently greater than in gm6 fibres. This may in part be accounted for by the larger size of gm6 fibres and a concomitantly larger resting input conductance. In a few cases in normal saline, electrogenic activity was observed in the cpv 1a fibres in response to DA. In the records shown in Fig. 7C, DA activated a slow wave process that led to single spikes or trains of spikes. In addition, electrogenic responses could be elicited by release from hyperpolarizations (Fig. 7D) or by depolarizing current pulses. Although 20 mM  $\text{Mn}^{2+}$  did not block the

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from the same gm6 fibre of *Cancer* shown in B is illustrated. The bottom trace monitors EJPs resulting from a train of pulses to the excitatory motor nerve, the top trace monitors hyperpolarizing current pulses injected into the fibre. DA was applied for 4 min and traces were taken at 0, 14 and 59 min; 0 min is the control. In (D) the effect of  $2 \times 10^{-7}$  M-DA on EJPs from a gm6b fibre of *Panulirus* is illustrated. The bottom trace monitors EJPs resulting from a train of pulses to the excitatory motor nerve, the top trace monitors current. DA was applied for 5 min and traces were taken at 0, 11 and 70 min.

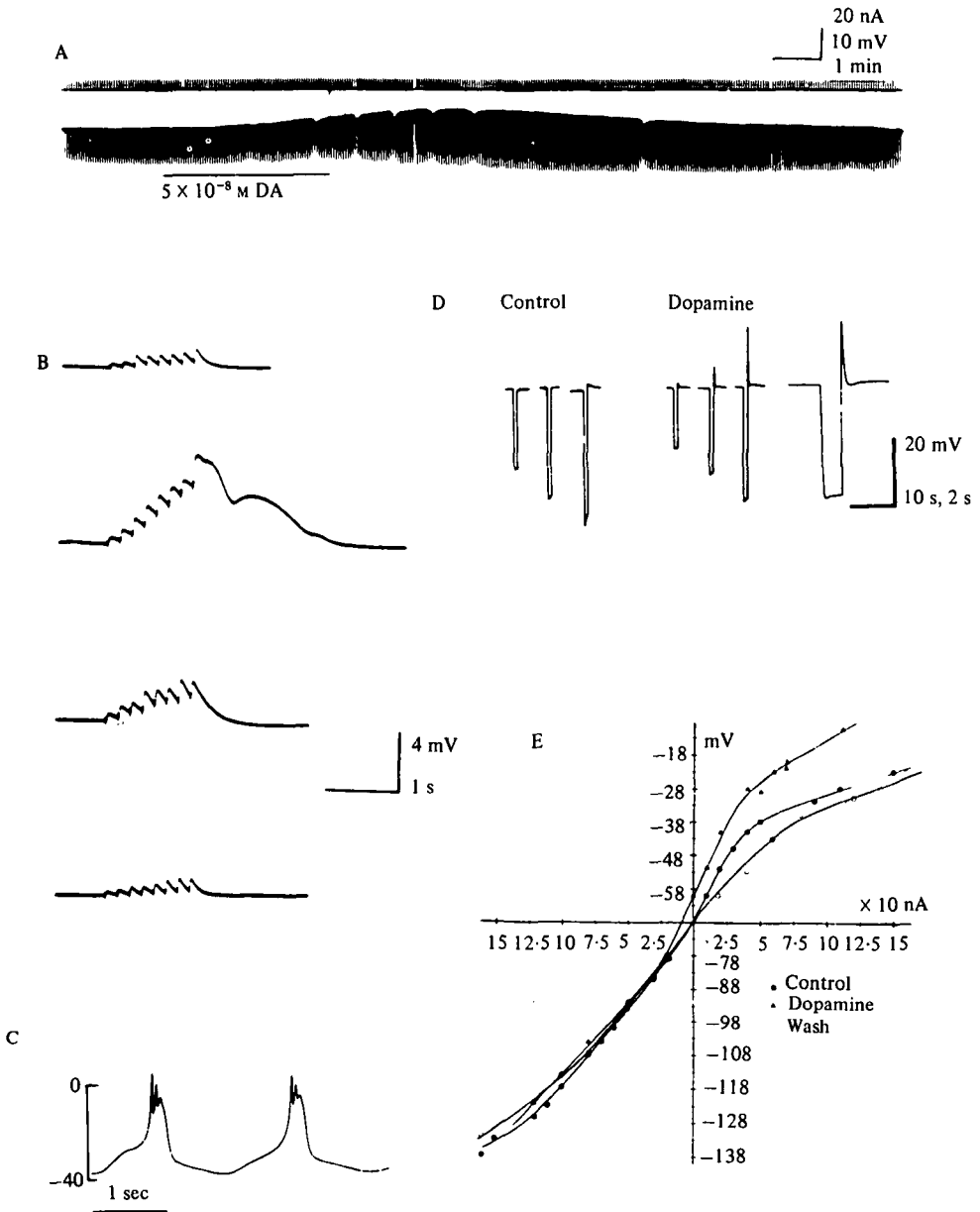


Fig. 7. Effects of dopamine on synaptic transmission and electrical properties of cpv 1a, 1b fibres from *Panulirus*. In A, membrane potential (bottom trace) of cpv 1a fibre is monitored, during injection of constant hyperpolarizing current pulses (top trace).  $5 \times 10^{-8}$  M-DA was applied for the duration of the bar. In B the effects of DA on EJPs of a cpv 1a fibre are shown.  $10^{-8}$  M-DA was applied for 5 min and traces were taken at 0, 7, 11 and 28 min. Resting potential of cell was  $-54$  mV and cell was depolarized  $5$  mV in DA. In C, rhythmic action potentials produced by  $5$  nM-DA in a cpv 1a fibre during spontaneous contractile activity are shown. In D current pulses injected for each response were, from left to right,  $240$  nA,  $330$  nA,  $410$  nA,  $145$  nA,  $240$  nA,  $320$  nA. In E, current-voltage relations of a cpv 1a fibre of *Panulirus* in the presence and absence of  $10^{-7}$  M dopamine is plotted.  $20$  mM manganese was included in the saline to minimize muscle movement. Although manganese increased muscle fibre resistance, the effect was similar at all membrane potentials.

dopamine-produced resistance changes, it did block the rhythmic spikes in the cpv 1 a muscle fibres, suggesting that the DA-activated spikes may be calcium spikes.

Although the cpv 1 a bundle of this muscle is highly sensitive to DA, the cpv 1 b is largely unresponsive to DA. Concentrations up to  $1\ \mu\text{M}$  DA produced little or no effect on membrane potential, membrane resistance, or EJPs. However, spontaneous contractions were occasionally observed in the cpv 1 b, indicating that DA can have some effect on this bundle. The difference in sensitivity to DA between the cpv 1 a and the cpv 1 b bundles points out that muscle fibres innervated by the same motor neurones may be differentially sensitive to DA. However, since only surface fibres of these muscles were examined intracellularly, it is not clear to what extent fibres within a given bundle are homogeneous with respect to sensitivity to DA.

#### DISCUSSION

##### *Correlation of effects of DA on tension and electrical properties of foregut muscles*

Dopamine has three primary excitatory effects on foregut muscle: (1) the enhancement of evoked contractions, (2) contracture, and (3) spontaneous contractions. These effects can be in part accounted for by the direct effects of DA on EJP amplitude, on membrane resistance, membrane potential, and activation or unmasking of electrogenicity in muscle fibres. First, dopamine produced a prolonged increase in membrane resistance in both the gm6 and cpv 1 a muscles that is most prominent at depolarized membrane potentials. This resistance change clearly contributes to the prolonged EJP enhancement and that of evoked contractions. However, as discussed below, other factors may also affect the EJP and evoked contraction enhancement. Secondly, DA produced a contracture in the cpv 1 a muscle but not in the gm6 muscles. This contracture can be correlated with the DA-produced depolarization that occurs only in the cpv 1 a. Both the time course and magnitude of the depolarizations are qualitatively sufficient to account for the observed muscle contracture. Finally, spontaneous rhythmic contractions are activated by DA in muscle cpv 1 a. The occurrence of the spontaneous rhythmic spikes in the cpv 1 a muscle but not the gm6 muscle is consistent with this spontaneous rhythmic contractile activity.

It remains possible that some of the enhancement of EJPs may result from either a presynaptic component of DA action or a change in postsynaptic sensitivity to the action of the excitatory transmitter. However, the present data are not sufficient to address these questions. Both the EJP enhancement and resistance increase are of prolonged time course and occur at comparable concentrations of DA. Although EJP enhancement is consistently greater than the increases in membrane resistance particularly at higher DA concentrations (see Fig. 6C and D), this comparison is inadequate. First, resistance changes are substantially greater at depolarized membrane potentials. This means that hyperpolarizing current pulses underestimate the contribution of resistance effects to the EJP increase. Secondly, EJPs are generated by synaptic currents at many sites distributed along these muscle fibres. Thus, during alteration of membrane cable properties by dopamine the contribution of more distant synaptic sites will be increased. In contrast, the current pulses generated from a current electrode adjacent to the recording electrode would only be expected to reflect synaptic currents generated at similar distances close to the recording electrode.

It is also possible that the DA-produced increase in EJP amplitude is not the only factor contributing to the increase in nerve-evoked contractions. For example, an increase in calcium influx during a given depolarization of the muscle fibres could produce an enhancement of nerve-evoked contractions that would not be detected electrophysiologically.

Despite the above qualifications, the effects of dopamine described here clearly show that a modulatory effect produced by an amine on neuromuscular function can involve specific alterations in membrane electrical properties and raise the possibility that a property as basic as electrogenicity may be strongly controlled by neurohumoral influences. The alteration of muscle fibre electrical properties that contributes in part to the potent neuromodulatory effects produced by DA on the decapod foregut muscles is in contrast to the reported effects of amines on other invertebrate neuromuscular preparations (Weiss, Cohen & Kupfermann, 1978; Kravitz *et al.* 1976; Evans & O'Shea, 1978). On the other hand, in many neuronal preparations and in vertebrate heart muscle there are indications that some of the actions mediated by both biogenic amines and other 'non-classically' acting substances involve voltage-dependent membrane conductances (Pellmar & Wilson, 1977; Brown & Adams, 1980; Mudge, Leeman & Fischbach, 1979; Klein & Kandel, 1978; Noble, 1979). The apparent absence of direct effects of amines on muscle fibre electrical properties in invertebrate neuromuscular preparations is somewhat curious, although effects on presynaptic neuronal conductances have been supposed. Since some voltage-dependent conductances are not apparently activated until around  $-30$  mV (Pellmar & Wilson, 1977; Pellmar & Carpenter, 1979), a membrane potential range over which it is generally difficult to work on muscle preparations, effects of amines on invertebrate muscle electrical properties may have been overlooked. Alternatively, the modulation of neuromuscular function in different invertebrate organisms may involve quite different sites and mechanisms of action. Certainly, it is unlikely that the acceleration of muscle relaxation produced by DA on foregut muscles can be accounted for solely by an alteration of electrical properties, and this aspect of the action of DA is similar to one of the actions of octopamine on relaxations from twitch tension of a locust muscle preparation (O'Shea & Evans, 1979).

#### *Nature of conductances modified by dopamine*

The voltage-dependent nature of the membrane resistance increases produced by DA could be explained either by a decrease in conductance, possibly to chloride or more probably potassium; or, alternatively, increases in a voltage-dependent conductance, possibly to calcium, might be involved. In experiments in which the saline included 20 mM  $Mn^{2+}$  (which is thought to block calcium currents) no reduction in dopamine-produced depolarizations or resistance changes was observed, although DA-activated spikes were blocked. This result supports the idea that the resistance increases do not involve an increase in a voltage-dependent influx of calcium. However, a small increase in a voltage-dependent calcium conductance cannot be excluded. Such an increase in a calcium current in response to a given level of depolarization of a muscle fibre might contribute substantially to an enhancement of nerve-evoked contractions.

Although further study is required to clarify the DA effects, three observations indicate the complex nature of the conductance changes: first, the different time courses of the membrane potential effects and the resistance changes in the cpv 1a muscle; secondly, the frequent absence of membrane potential effects during resistance changes in the gm6 fibres; and thirdly, the difference in the effect of DA on membrane potential between cpv 1a and gm6b fibres. However, the disparity in effects of DA on the two muscles does not necessarily preclude a common mechanism of action. For example, a decrease in a voltage-dependent potassium rectification conductance could account for some aspects of these results. Although only cpv 1a fibres are depolarized by DA, fibres of these two muscles have different resting potentials. In a cell with a low resting potential ( $-45$  to  $-65$  mV) rectification conductances may be tonically activated. Thus, an inactivation of such conductances would result in a depolarization of the cell with a resistance increase. In addition, an inactivation of such conductances coupled with depolarization might enable a voltage-dependent calcium conductance to generate action potentials. However, in a cell with a resting potential ( $-65$  to  $-85$  mV) at which rectification conductances are not activated, inactivation of those conductances would not be expected to affect resting potentials or have substantial effects on the amplitude of hyperpolarizing current pulses. Clearly, additional study is required to test the validity of this or other explanations.

#### *Comparison of dopamine action to the action of other amines*

In foregut preparations, the three amines, dopamine, serotonin and octopamine, were found to produce an enhancement of nerve-evoked contractions. However, the action of dopamine consistently occurred at concentrations as low as  $10^{-8}$  M and showed less variability from animal to animal and from dose to dose. Frequently, concentrations as high as  $1 \mu\text{M}$  octopamine or serotonin were necessary to produce an enhancement of evoked contractions. Additionally, neither octopamine nor serotonin produced a contracture or the occurrence of spontaneous rhythmic contractions in the cpv 1a muscle. The action of dopamine could also be mimicked by the dopamine receptor agonist, epinine. In contrast, limb muscles of *Panulirus* were more sensitive to serotonin and octopamine than to dopamine. This result is similar to the findings for the opener muscle of *Homarus* (Kravitz *et al.* 1976).

These results support the idea that the foregut contains at least one amine receptor which is a dopamine-preferring receptor. However, it is possible, particularly at the higher concentrations of DA, that some of the effects of DA might be mediated by an interaction with an octopamine or serotonin receptor. Alternatively, a second dopamine receptor of lower affinity might be present. Two observations indirectly support the idea that at least one other amine receptor is present. First, at higher DA concentrations the enhancement of EJPs shows a larger discrepancy from the increase in muscle fibre resistance. Secondly, octopamine and serotonin produce enhancement of nerve-evoked contractions, but not the contracture and spontaneous contractions activated by dopamine. Antagonists of high specificity will be required to assess the possibility that the foregut contains multiple amine receptors.

*Potential sources of dopamine*

Finally, if the effects of dopamine described here are functionally important, a source of dopamine that might mediate these effects must be identified. It is known that the hindgut of the crayfish contains a rich plexus of nerve fibres that display a catecholaminergic histofluorescence (Elofsson *et al.* 1968). Similarly, input nerves to the stomatogastric ganglion of the foregut are known to contain dopamine by both biochemical (Barker, Kushner, & Hooper, 1979; Kushner & Ono, 1978) and histochemical (Kushner & Maynard, 1977) criteria. However, there is no catecholamine histofluorescence in nerves of the stomatogastric ganglion running to the foregut muscles, and histofluorescence in the regions of the muscles themselves has not been reported. Additionally, foregut nerve-muscle preparations are unable to synthesize dopamine from radiolabelled precursors (C. Lingle, unpublished experiments). Although these experiments are not definitive, at present there is no evidence to support the idea that a local source of dopamine is available to mediate the actions of dopamine on foregut nerve-muscle systems.

Alternatively, dopamine is known to be present along with serotonin and octopamine in the pericardial neurosecretory structures of the decapod Crustacea and may function as a neurohormone (Sullivan, Friend & McCaman, 1976; Sullivan, Friend & Barker, 1977; Evans, Talamo & Kravitz, 1975; Evans *et al.* 1976; Evans, Kravitz & Talamo, 1976). The low concentrations of dopamine required for action on foregut muscles are consistent with likely effective concentration ranges of the other putative neurohumorally acting biogenic amines in Crustacea. Thus, at present, neurosecretory tissues must be considered a possible source of dopamine for the *in vivo* modulation of neuromuscular function in the decapod foregut.

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