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EXCITATORY EFFECTS OF DOPAMINE ON THE CARDIAC GANGLIA OF THE CRABS *PORTUNUS SANGUINOLENTUS* AND *PODOPHTHALMUS VIGIL*

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SUMMARY

1. Dopamine, a cardioexcitor in decapod crustaceans, increased the frequency and/or duration of bursts of action potentials in the semi-isolated cardiac ganglia of two species of crabs. The number of motoneurone action potentials in each burst was increased, which in the intact heart would increase the force and amplitude of heart contraction.

2. The effects were concentration-dependent, with a threshold concentration of 10^{-8} M or lower when dopamine was applied by continuous perfusion. At 5×10^{-6} M, dopamine increased burst frequency by 200%.

3. The main site of dopamine action was the group of four posterior small interneurones which normally function as the pacemaker for the cardiac ganglion system. Effects on the five large motoneurones occurred at higher concentrations. This regional difference in sensitivity was demonstrated by selective applications of dopamine to different parts of the cardiac ganglion and by the use of preparations in which the two ends of the ganglion had been functionally separated by a ligature around the ganglionic trunk.

4. In the small neurones, dopamine was found to stimulate the slow tetrodotoxin-resistant regenerative depolarizations known as driver potentials. The effects on driver potential frequency and train duration were concentration dependent. In one of the two species of crabs, in which electrotonic connections between small and large neurones are strong, large neurone driver potentials were indirectly induced by dopamine.

5. In the tetrodotoxin-treated large motoneurones, dopamine, at a concentration about ten-fold higher than needed to activate the small neurones, decreased the threshold for current-induced driver potentials, and slightly reduced membrane resistance.

6. We suggest that the excitatory action of dopamine on the untreated cardiac ganglion can in large part be accounted for by its action on driver potential production in the small neurones.

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INTRODUCTION

In crustaceans, the heart beat is generated by endogenous rhythmic electrical activity in the small network of neurones comprising the cardiac ganglion (Alexandrowicz, 1932). These neurones continue their coordinated generation of bursts of action potentials when the ganglion is isolated from the myocardium and from the central nervous system (Welsh & Maynard, 1951). However, the endogenous activity of the ganglion is subject to modulation *via* acceleratory and inhibitory neurones originating in the central nervous system (Maynard, 1953; Terzuolo & Bullock, 1958; Shimahara, 1969) and by cardioactive substances released into the haemolymph from neurohaemal structures known as pericardial organs (Alexandrowicz, 1953; Alexandrowicz & Carlisle, 1953; Maynard & Welsh, 1959; Cooke, 1964; Sullivan, Friend & Barker, 1977). Dopamine is one of several monoamines and peptides which have been found in the pericardial organs of Crustacea (Cooke & Goldstone, 1970; Sullivan, Friend & McCaman, 1976; Sullivan *et al.* 1977). It has been shown to increase the force and frequency of heart beat in several macruran and brachyuran crustaceans (Berlind, Cooke & Goldstone, 1970; Florey & Rathmayer, 1978; Cooke & Sullivan, 1982) at concentrations which are consistent with a role as a neurohormone or transmitter. In this paper, we analyse a mechanism of action of dopamine on the endogenous activity of the semi-isolated cardiac ganglia of two crabs, *Portunus sanguinolentus* and *Podophthalmus vigil*. The effects on the different types of neurone in the ganglion are also analysed.

There are nine neurones in the crustacean cardiac ganglion (Alexandrowicz, 1932), five large motoneurones which innervate the heart muscle, and four small neurones which normally initiate and sustain rhythmic activity in the network as a whole (Maynard, 1955). In *Portunus sanguinolentus*, all the cardiac ganglion neurones are electrically coupled to one another, and the small posterior neurones also excite the two posterior and three anterior motoneurones *via* chemical synapses (Tazaki & Cooke, 1979*a*). Similar interneuronal interactions appear to occur in the less completely analysed cardiac ganglion of *Podophthalmus vigil* (Berlind, 1982). In Crustacea generally, intracellular recording from small neurones has been hindered by their small size and lack of visibility in living preparations (e.g. Tazaki & Cooke, 1979*a*). The large motoneurones can be impaled readily (Hagiwara & Bullock, 1955), and in *Portunus* have yielded considerable information on the capacity of individual neurones to generate not only action potentials, but also a slow, depolarizing local potential termed a driver potential (Tazaki & Cooke, 1979*b*,*c*). This endogenous event plays a major role in the organization of action potentials into bursts, and may also be important in the maintenance of network rhythmicity (Benson, 1980; Berlind, 1982). Results reported by Cooke & Sullivan (1982) suggest that dopamine might act on the driver potential mechanism in the small neurones of the crab cardiac ganglion. Our results provide evidence to support this suggestion. We show that dopamine enhances driver potential generation strongly in the small neurones and has weak excitatory effects on the motoneurones. A brief report of this work has appeared previously (Miller, Sullivan, Benson & Berlind, 1981).

MATERIALS AND METHODS

Semi-isolated cardiac ganglia were prepared from the hearts of male and female specimens of two species of crab, *Portunus sanguinolentus* and *Podophthalmus vigil*. The crabs were collected locally and were maintained in a circulating, natural sea water system. All experiments were performed at 21–24 °C.

The dissection was carried out as described previously (Tazaki & Cooke, 1979*a*; Benson, 1980). The ganglion was pinned out in a Sylgard recording chamber through small muscle blocks which were left intact in order to minimize damage to fine neuronal processes originating in the ganglion. In a small number of experiments, muscle was completely removed from the ganglion. These preparations showed dopamine effects identical to those of less completely dissected ganglia. The recording chamber (total capacity 1 ml) was perfused continuously with crab saline (Tazaki & Cooke, 1979*a*), and a multichannel system allowed the composition of the bathing saline to be changed without interrupting the flow.

Dopamine stock solution was prepared immediately prior to the experiment. Dilution in saline was carried out just before introduction of samples into the recording chamber. Dopamine was applied in two ways. For the dose-response curve of burst frequency (Fig. 1), the bathing saline contained the concentration indicated and perfusion was continued until a steady-state response was achieved. The ganglion was then superfused with normal, dopamine-free saline for 30–60 min before the next concentration was applied. For most other experiments, 50–200 μl aliquots of saline containing dopamine were added to the perfusion stream at the entrance to the experimental chamber. Dilution within the chamber reduced the peak effective concentration at the ganglion surface by a factor of ten to fifty. The concentrations cited in the Figures and Results section for this type of application are those in the added sample.

Conventional recording techniques were used, with microelectrodes impaling one or more large neurones. WPI model 707 amplifiers allowed current-passing through the recording electrodes. All records presented here were obtained from a passive electrode, which in some experiments was in the same cell as the current-passing electrode, and in others was in an electrotonically coupled neurone.

Two modified types of preparations were used to determine the primary site of dopamine action. In 'two-pool' experiments, a ganglion was placed across a Sylgard bridge with its anterior end (with three large neurone somata) in one chamber and the posterior end (four small neurone somata and two large) in the second chamber. A Vaseline dam was laid over the bridge, effectively separating the two pools, which were perfused independently. Dopamine could be added to one or both chambers. In two-pool experiments, all neuronal connections within the ganglion were intact, and anterior and posterior neurones retained electrotonic coupling with one another.

For the second altered preparation, a ligature of fine silk was placed around the trunk of the ganglion between anterior and posterior neurone groups (Tazaki & Cooke, 1983*a*). When pulled tight, the ligature functionally isolated the two groups, and the normally strong electrotonic connections between anterior and posterior neurones were lost. These experiments were carried out in a single perfusion pool, with records taken from large neurones on opposite sides of the ligature. The posterior two large neurones in such a preparation retained connections to the four small

neurones, and the small neurone activity was reflected in the large neurone recording *via* electrotonic and chemical synaptic connections (see Results). Although the small neurone axons remained in the isolated anterior end of the ganglion, no discrete activity in these ligatured axons was detected.

Apparent membrane resistant (R_m) was measured with two electrodes in a single large neurone soma. The peak voltage change in response to 1- to 5-s hyperpolarizing or depolarizing current pulses was monitored with a passive electrode. Applied current was monitored with a WPI 180 virtual ground. The peak voltage change was a linear function of current in the hyperpolarizing direction, but was distorted in the depolarizing direction by the activation of voltage-dependent transmembrane currents.

RESULTS

Changes in large neurone activity in response to dopamine

Dopamine had excitatory effects on isolated cardiac ganglia. All preparations on which it was tested responded with concentration-dependent changes in burst frequency (Figs 1, 2), burst duration (Figs 2, 3) and number of action potentials per burst (Fig. 3). The detailed nature of the response and threshold concentration for a dopamine effect varied from one preparation to another. Threshold was usually less than 10^{-8} M when dopamine was perfused continuously and between 10^{-7} M and 10^{-6} M with pulse applications. Near threshold, burst length typically increased, an

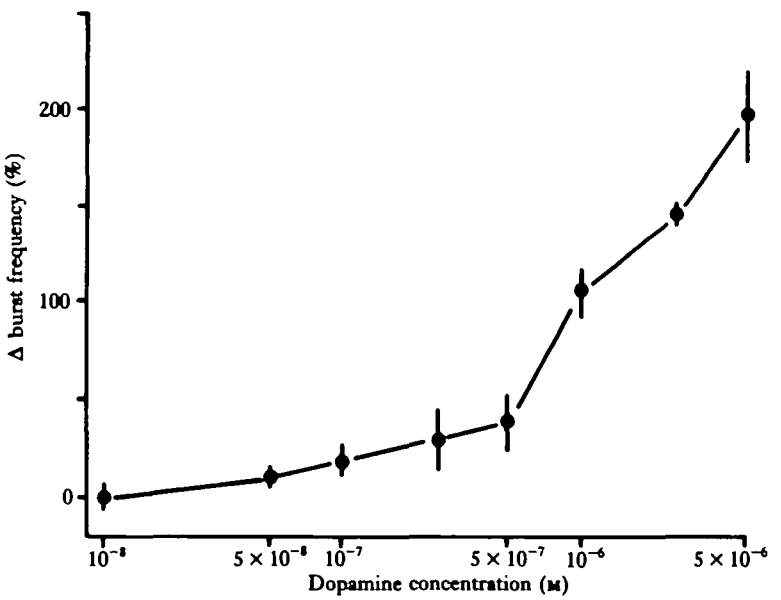

Fig. 1. Concentration dependence of the change in frequency of bursting induced by dopamine in isolated cardiac ganglia from *Portunus sanguinolentus*. The vertical axis shows the steady-state change in frequency in response to dopamine perfused at the indicated concentration. Frequency was calculated from the number of bursts occurring during 2 min of steady state activity. The data were obtained from eight preparations and each point shows the mean and standard deviation ($N = 3$ to 5).

effect which was often, but not always, accompanied by a slight decrease in burst frequency (second trace, Fig. 2; Fig. 3). At higher concentrations, the predominant effect during steady state perfusion or at the peak response to a pulse application was usually a clear increase in burst frequency (Fig. 2, bottom traces). Effects on burst duration at high dopamine concentrations varied considerably from ganglion to ganglion. In some preparations burst duration was significantly shorter during the period of accelerated bursting (Fig. 2), while in others bursts were slightly prolonged as compared to control periods. Whether or not the burst duration was increased by high dopamine levels, the number of action potentials per burst increased, due to an increase in intraburst action potential frequency. Evidence presented in more detail below suggests that the action potential discharge of the small neurones during each burst was accelerated in almost all preparations and at all concentrations, and was often greatly prolonged. This effect was seen in extracellular records which include small neurone action potentials (Fig. 4). In intracellular records from large neurones

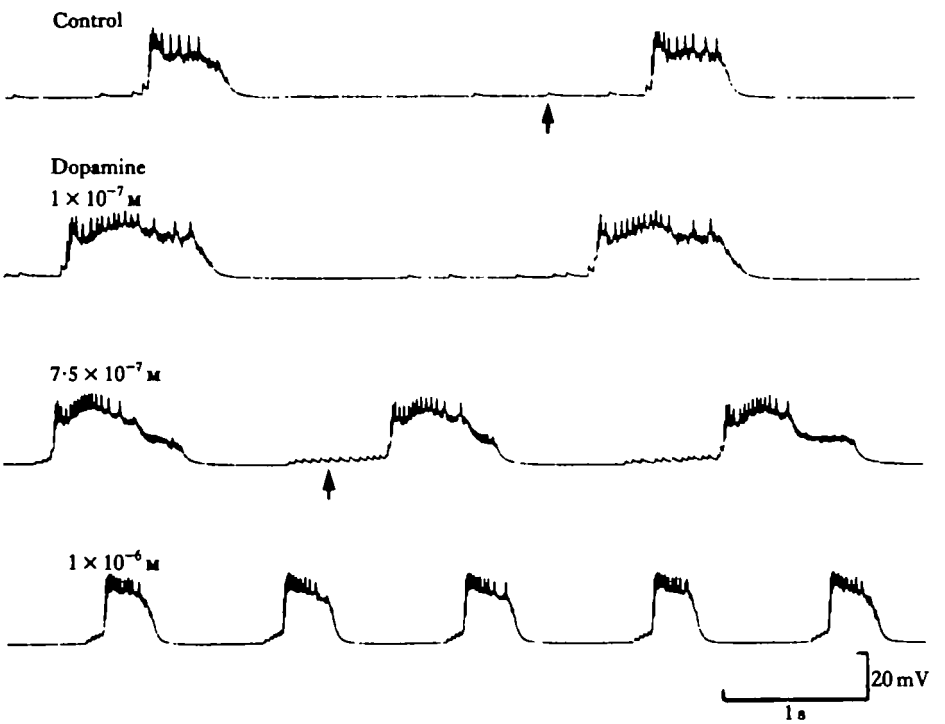

Fig. 2. Intracellular recordings from an anterior large neurone in a *Portunus sanguinolentus* ganglion, showing altered patterns of activity following dopamine application. In the bottom three traces, records show the peak response following a 50-μl pulse of dopamine at the indicated concentration. Action potentials in large neurone records ride on a slow depolarization derived in part from synaptic potentials and in part from an endogenous large neurone driver potential. At a low concentration of dopamine (10^{-7} M applied) this ganglion responded with an increase in burst duration and a slight decrease in frequency. A ten-fold higher concentration caused an increase in frequency and decrease in duration. At all concentrations, the number of action potentials per burst increased. Note the increase in frequency of unitary excitatory postsynaptic potentials preceding the action potential burst (arrows). This change reflects an acceleration of discharge of the small neurones presynaptic to the recorded cell.

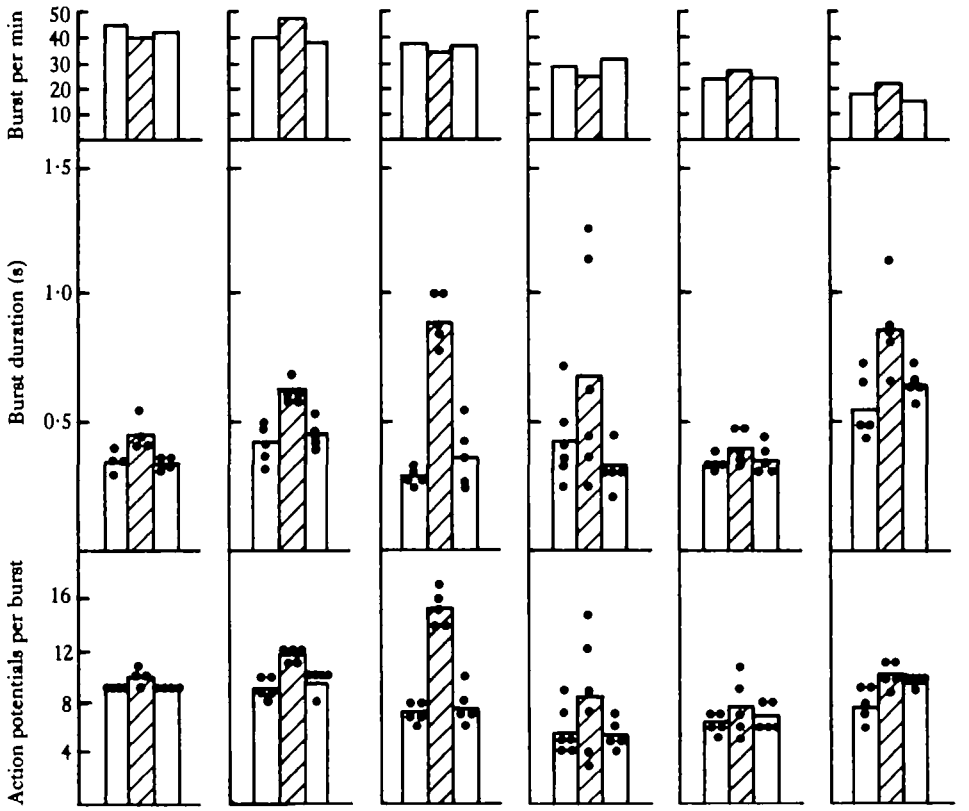

Fig. 3. Response of six *Portunus sanguinolentus* cardiac ganglia to a pulse of dopamine (0·2 ml, 10^{-6} M). Burst frequency, burst duration and number of large neurone action potentials per burst (top to bottom) are plotted for each preparation. Each group of three bars shows (left to right) the value just before dopamine application, at the time of maximal response to dopamine (cross-hatched), and after a 5-min recovery period. Average values for burst frequency are derived from two consecutive 30-s periods. Burst durations and number of action potentials per burst are averages derived from four to six consecutive bursts (each dot shows the value from a single burst). Note that at this dopamine concentration, which, in most preparations is slightly above threshold for the pulse method of application, the effect on burst frequency was variable (three increased and three decreased), but that all six preparations showed increases in average burst duration and number of action potentials per burst.

enhancement of small neurone discharge was reflected by an acceleration of synaptic potentials impinging on the large neurone, and a prolonged phase of synaptic input after the main part of the large neurone burst (best illustrated in Fig. 2, by a comparison of the 'control' trace and the response to $7{\cdot}5\times10^{-7}$ M dopamine).

The effects of dopamine were exerted rapidly after the introduction of pulses into the chamber. With long-term perfusion of dopamine, desensitization did not occur over the period of exposure. Similar results have previously been reported for dopamine action on the intact isolated hearts of another crab species (Berlind *et al.* 1970), which do show declining responsiveness to other monoamines during continuous perfusion.

Neuronal target of dopamine

Experiments using the two-pool protocol and application of pulse doses of

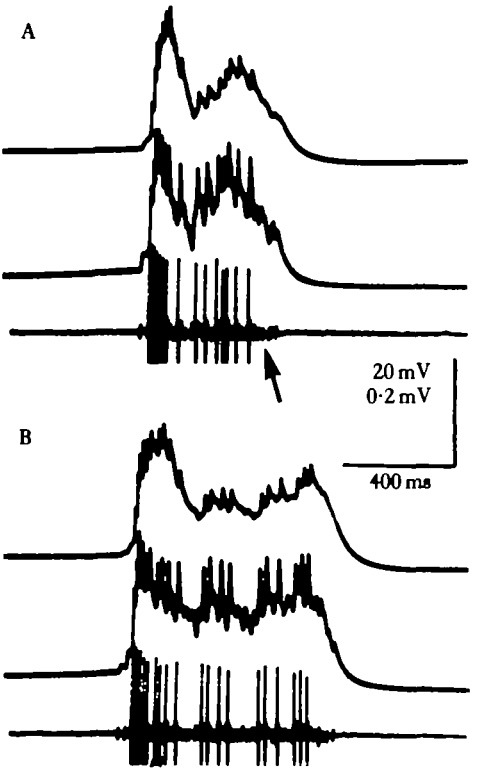

Fig. 4. Records from a *Portunus sanguinolentus* cardiac ganglion, showing prolongation of both small neurone and large neurone bursts by dopamine. Traces are recorded intracellularly from a posterior large neurone (top), and anterior large neurone (middle) and extracellularly from the trunk of the ganglion (bottom). The records in A are simultaneously recorded just before application of a pulse of dopamine (0·2 ml, 10^{-6} M) and in B during the peak of the dopamine-induced response. In the extracellular record, small neurone action potentials are indicated by the arrow in A; the lower parts of the large neurone action potentials are cut off in the photograph. The top voltage calibration refers to the intracellular traces, and the bottom to the extracellular traces.

dopamine showed that the main effect of dopamine was exerted on the small neurones. Dopamine evoked responses typical of the whole ganglion when applied to the posterior chamber containing both small and large neurones (Fig. 5), but, when applied to the anterior chamber (which contained large neurone somata only), it had little or no effect even at high concentrations. Simultaneous application of dopamine to both chambers induced a response that was usually indistinguishable from the response seen when only the posterior end of the ganglion was exposed.

Results from ligatured preparations support the conclusion that the action of dopamine is primarily on small neurones. In a ligatured preparation, the three anterior large neurones, isolated from small neurone somata, continued to produce coordinated bursts (Fig. 6). A recording from a large neurone posterior to the ligature (and still coupled to the small neurones) usually showed small amplitude (2–5 mV) rhythmic depolarizations. These recurrent potentials, also noted by Tazaki & Cooke (1983*a*), represent the summed activity of the small neurone group as recorded *via* the electrotonic and/or chemical synaptic connections between small and large neurones. The form of these potentials suggests that the sites of driver potential

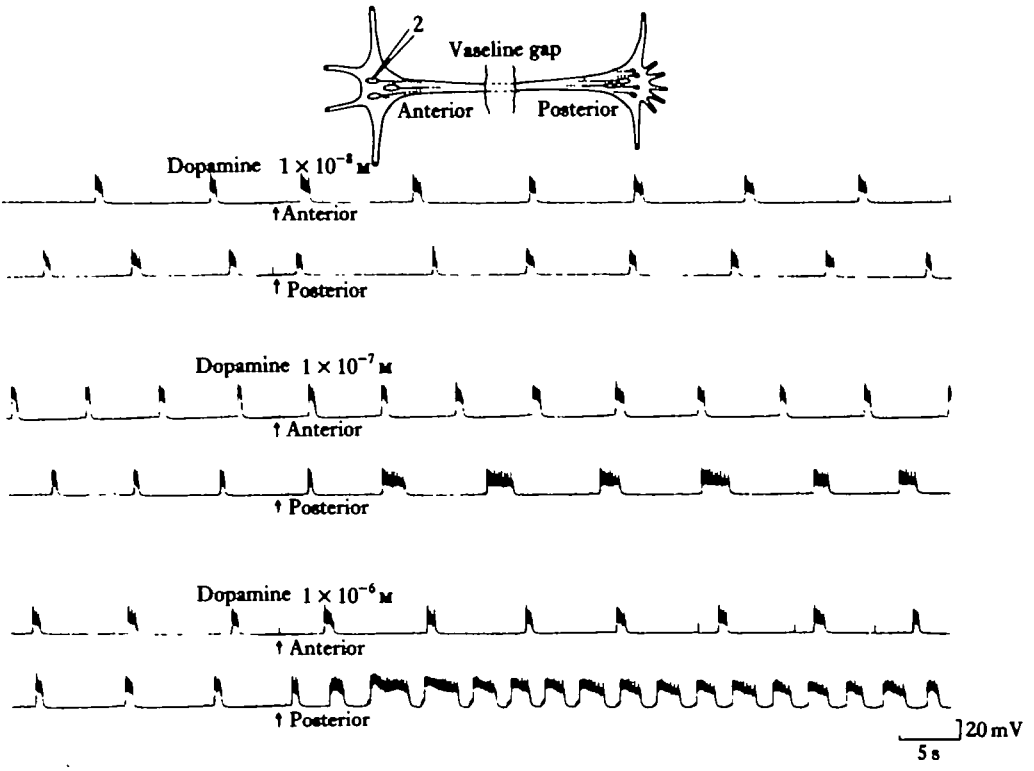

Fig. 5. A two-pool experiment on a *Portunus sanguinolentus* ganglion. All traces were recorded from a cell body at the anterior end of the ganglion, but any of the five large neurones would have shown a similar pattern of activity and an identical record of action potentials. Each pair of traces shows the response to application of 50-μl pulses of dopamine (arrows) to the anterior end of the ganglion (top trace of each pair) only or to the posterior end only (bottom). Dopamine alters activity significantly when applied to the region which includes the small neurone somata.

generation in the small neurones are posterior to the ligature. In *Portunus*, the two large neurones remaining posterior to the ligature failed to develop an active slow membrane depolarization (driver potential) and therefore did not generate bursts of action potentials. In *Podophthalmus* they were capable of doing so (see below).

When dopamine was applied uniformly to a ligatured *Portunus* preparation it altered activity posterior to the ligature only. Dopamine greatly prolonged the small cell discharge (evoking small neurone bursts lasting up to several seconds) and increased the frequency of small neurone impulse generation as monitored through the synaptic connection to the posterior large neurones (upper traces in Fig. 6). The amplitude of the depolarization was also significantly increased. Despite this enhancement of small neurone discharge, which probably reflects the generation of longer and larger driver potentials in the small neurones, the input was still unable to trigger active responses in the posterior large cells in *Portunus*. The activity of the functionally isolated anterior large neurone group was usually unaltered, even at high concentrations (lower traces, Fig. 6).

The action of dopamine on ligatured *Podophthalmus* preparations was similar to that observed in *Portunus*, in that small neurone discharge was enhanced while the

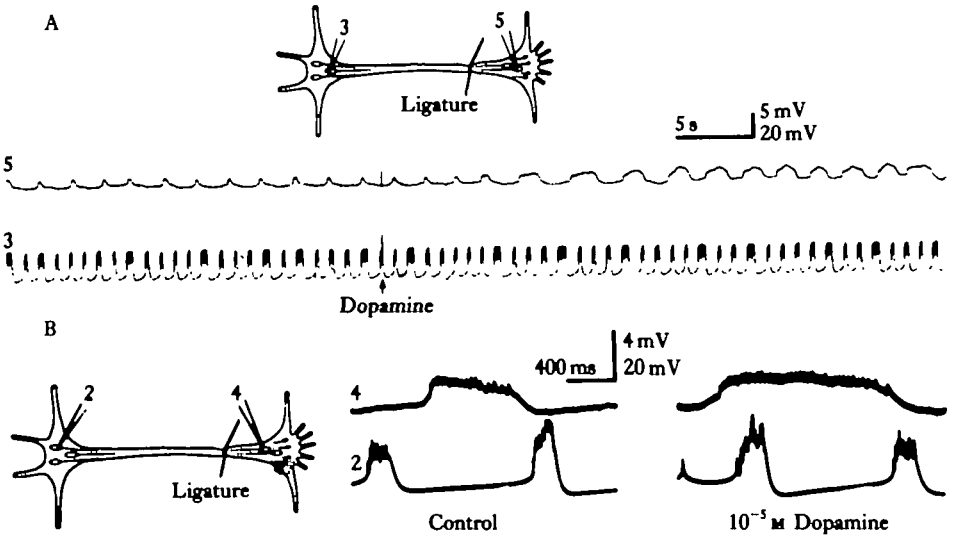

Fig. 6. Responses to dopamine recorded in posterior (top trace of each pair) and anterior large neurones in ligatured *Portunus sanguinolentus* preparations. Note that the traces from the posterior end are amplified more than those from the anterior end (in each record, the upper voltage calibration refers to the top traces). The total amplitude of the depolarizing potentials is only about 3 mV when recorded posteriorly. (A) Application of a 50-μl pulse of dopamine at 10^{-6} M to the whole ganglion did not alter anterior activity, but increased the amplitude and duration of the posterior depolarizing potentials. (B) A high speed, high gain record from a different ligatured preparation shows (upper traces) the individual synaptic potentials in a posterior large neurone which reflect small neurone action potentials. Following dopamine application, the small neurone action potential train was prolonged and accelerated. Activity in the functionally isolated anterior cell group was not significantly altered (lower traces).

activity of the isolated anterior large neurone group was not altered. However, in most *Podophthalmus* ganglia the posterior large neurones, which retained connections to the small neurones, were also activated following dopamine application. This activation is probably indirect, resulting from larger synaptic depolarization of the large neurone membrane, with a consequent triggering of slow regenerative responses (driver potentials) in the large neurones (Fig. 7). The different responses of the two species might be due to a more posterior location of driver potential sites in posterior large neurones of *Podophthalmus* or to stronger coupling between small and large neurones in this species (Berlind, 1982).

Driver potentials as a target of dopamine action

When *Portunus* cardiac ganglia were treated with tetrodotoxin (TTX) at a concentration of 3×10^{-7} M, spontaneous activity in the ganglia ceased but full large neurone driver potentials, 24–28 mV in amplitude, could be evoked by passing brief depolarizing current pulses across the membrane (Tazaki & Cooke, 1979*b*,*c*). When a TTX-treated ganglion was exposed to a pulse of dopamine (10^{-6}–10^{-5} M applied concentrations), recordings from a large neurone exhibited a series of small amplitude (2–4 mV) depolarizations, devoid of spikes, which repeated spontaneously at a rate which increased with concentration (Figs 8, 9). The effect lasted for several minutes, and the duration of individual potentials sometimes exceeded 1 s. These small

depolarizations, which have also been observed by Cooke & Sullivan (1982), most probably represent driver potentials in the small neurones, conducted through the electrotonic junctions to the recording site in the large neurones. The amplitude of the electrotonically conducted depolarizations was too small to trigger the active responses of the large neurone membrane. In ligatured preparations, these small potentials were recorded posterior to the ligature only (Fig. 8) where small neurone somata and presumably sites of driver potential generation by these cells are located. In two-pool experiments, these small oscillations of the membrane potential could be recorded from any of the five large neurone somata, but only when dopamine was applied to the posterior pool, containing small neurone cell bodies (Fig. 9). Application of even the highest concentrations to anterior large cells only did not elicit similar responses.

In TTX-treated *Podophthalmus* cardiac ganglia a different response was observed. Dopamine pulses at the same concentrations tested in *Portunus* generally evoked a train of driver potentials of large amplitude (25–30 mV) which sometimes alternated with smaller potentials (4–12 mV) (Fig. 10). The larger potentials were driver potentials generated by the large neurones, while the smaller events were probably electrotonically conducted small neurone driver potentials which failed to trigger the large neurone active response. As the concentration of dopamine was increased, the evoked train increased in duration, the driver potential frequency increased, and the percentage of small neurone driver potentials which successfully triggered an active response in the large neurones increased (Fig. 10A, B). The activation by dopamine

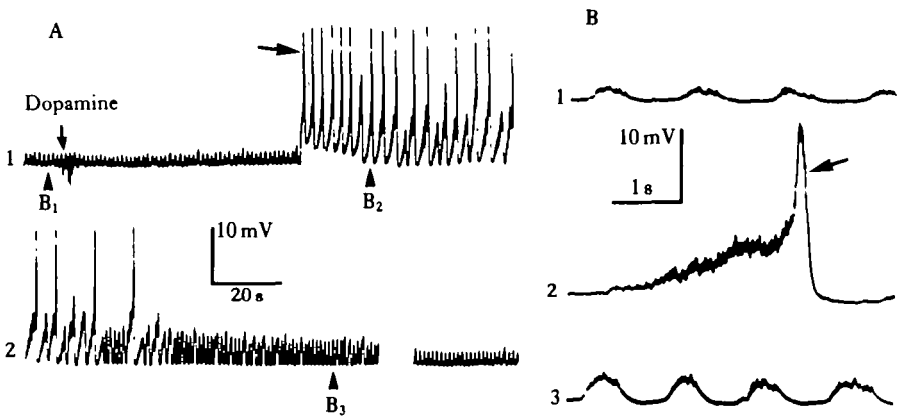

Fig. 7. Responses of a ligatured *Podophthalmus vigil* cardiac ganglion to a pulse of dopamine (0·2 ml, 10^{-5} M). Traces are recorded intracellularly from a large neurone posterior to the ligature; the recorded neurone remains connected physiologically to the small neurones. Top and bottom chart records in A are continuous, except for a 2-min gap toward the end of A_2. Expanded oscilloscope traces from the three points indicated by the arrowheads are shown in B. Before dopamine application (beginning of A_1, B_1) small amplitude bursts recorded in the large neurones represent electrotonically-recorded small neurone discharge. Following application of dopamine the small neurone discharge is enhanced and prolonged so that it is sometimes sufficient to trigger the full regenerative response (driver potential) of the posterior large neurone (arrows in A_1, B_2). Later during the dopamine response (middle of A_2, B_3) regenerative responses of the large neurone are no longer evoked, but the electrotonically recorded small neurone burst is larger in amplitude and faster than during control periods before dopamine application and after recovery. Similar indirect activation of posterior large neurones occurred in most ligatured *Podophthalmus vigil* preparations, but was never observed in *Portunus sanguinolentus* ganglia (see text). Activity of anterior large neurones in this preparation was not altered by dopamine (not illustrated).

of large neurone driver potentials in *Podophthalmus* appears to be indirect. In ligatured preparations, full driver potentials were evoked by dopamine posterior to the ligature only, while functionally isolated anterior large neurones remained silent even at high concentrations (Fig. 10C). We suggest, therefore, that the main direct target for dopamine in *Podophthalmus* appears to be the mechanism generating driver potentials in small neurones, as it is in *Portunus*. The indirect activation of the large neurones in *Podophthalmus* most probably reflects stronger coupling between small and large neurones in this species (see Berlind, 1982).

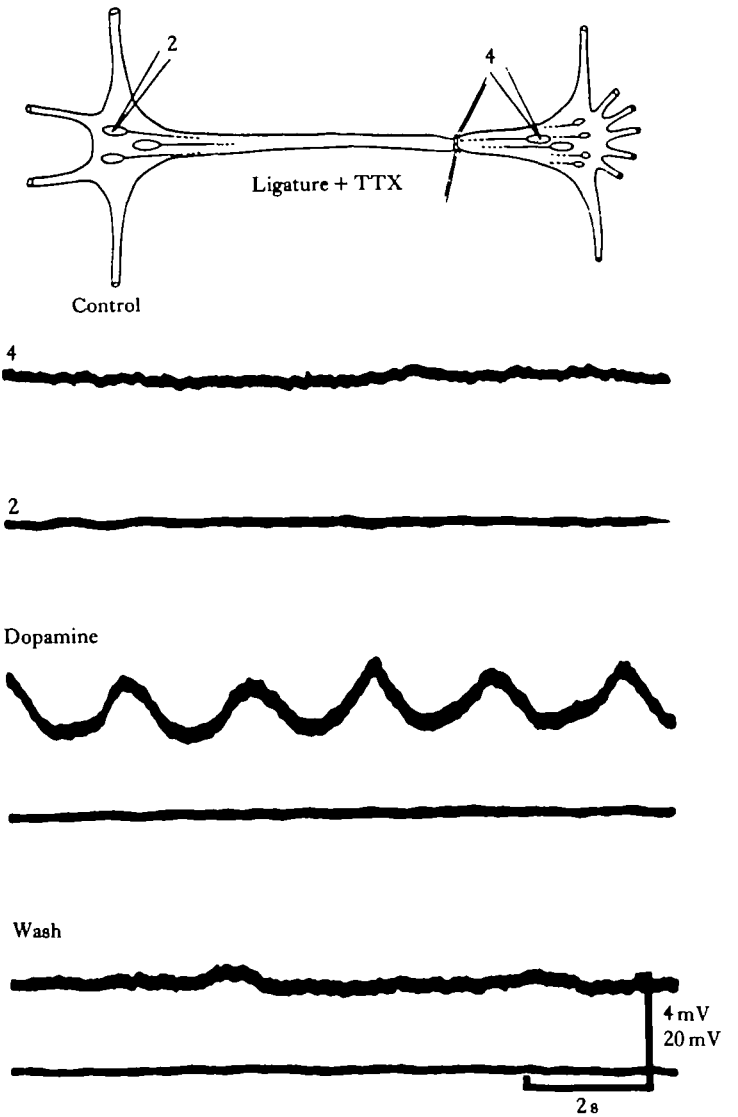

Fig. 8. Recordings from anterior (2) and posterior (4) large neurones in a *Portunus sanguinolentus* preparation perfused with 3×10^{-7} M tetrodotoxin (TTX) to suppress action potentials and spontaneous activity. Application of a pulse of dopamine (10^{-6} M) induced 2 mV depolarizing potential which could be recorded only posterior to the ligature. Upper voltage calibration refers to the top trace of each pair.

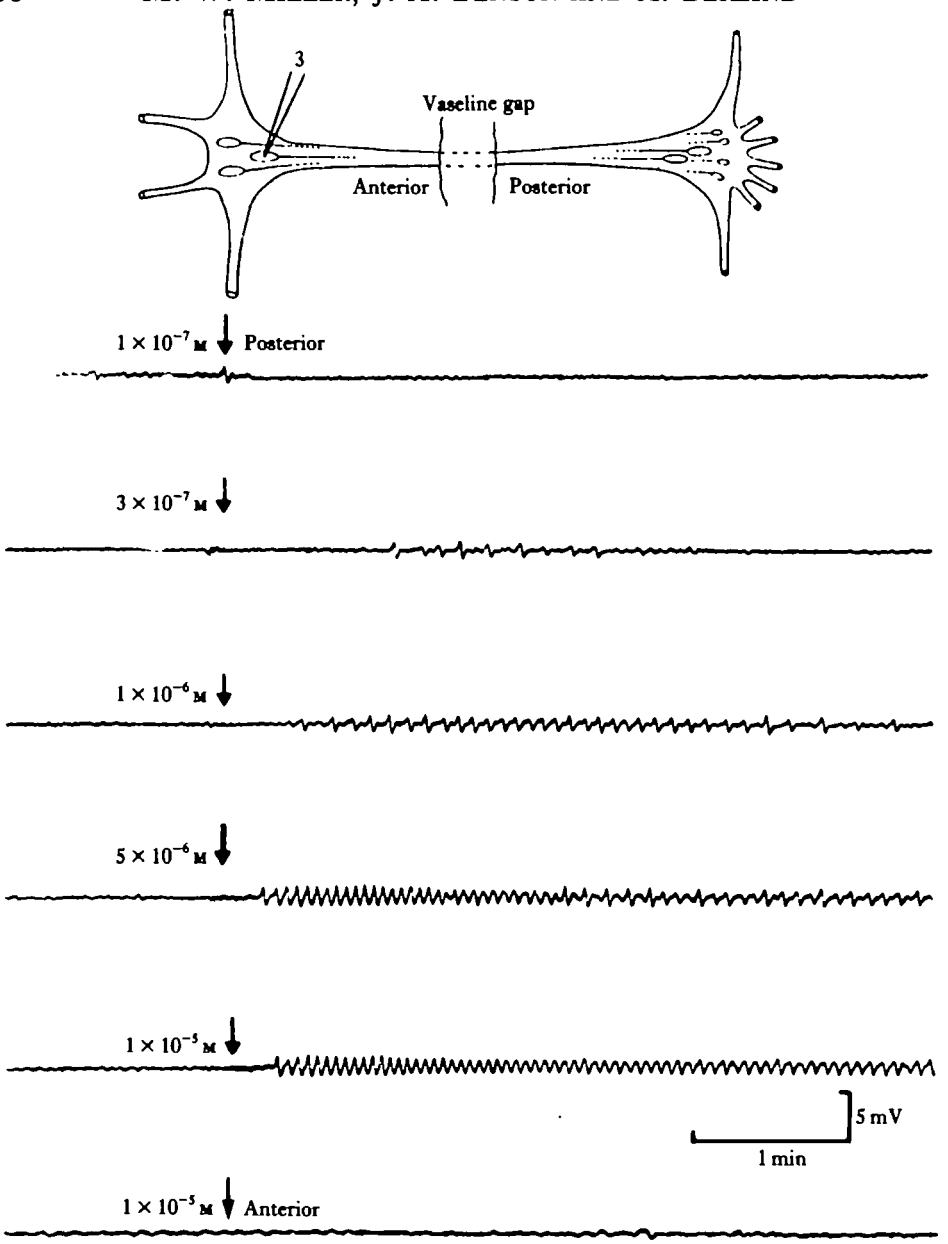

Fig. 9. Recording from an anterior large neurone in *Portunus sanguinolentus* in TTX (3×10^{-7} M) showing concentration-dependence of the response of small neurone driver potentials to dopamine pulses applied to the posterior chamber in a two-pool experiment. The bottom trace shows that application to anterior neurones only of the highest concentration tested did not directly induce a driver potential response. Note that the duration of the individual small neurone potentials was not significantly different at different dopamine concentrations.

Evidence for burst-enhancement by dopamine in TTX-free saline

The work of Tazaki & Cooke (1979*b*,*c*) has suggested that driver potentials are critically important in the organization of the bursting pattern of discharge of cardiac ganglion neurones in the absence of TTX. Since our results described in the preceding

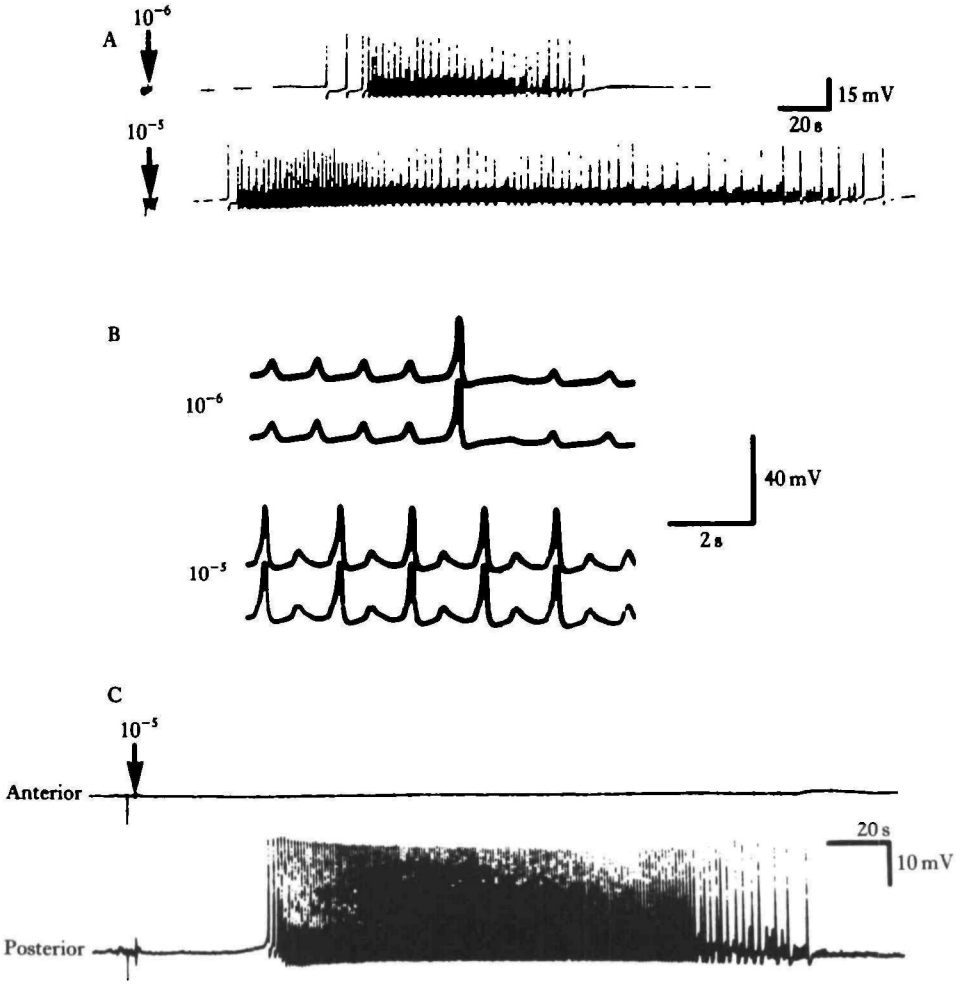

Fig. 10. (A) Responses to dopamine recorded in an anterior large neurone of a *Podophthalmus vigil* cardiac ganglion perfused continuously with TTX. In contrast to the situation in *Portunus sanguinolentus*, the *Podophthalmus* ganglion generated large amplitude driver potentials reflecting active responses of the large neurones, and interspersed smaller potentials which were probably electrotonically transmitted small neurone driver potentials. Note that the individual driver potentials during a train were triggered at membrane potentials several millivolts more hyperpolarized than the stable potential before the train. (B) Expanded oscilloscope records of the responses to dopamine from the same trials shown in A. Each pair of traces illustrates recordings from two different anterior large neurone somata, which showed synchronous activity. Top pair of traces, response to a pulse of 10^{-6} M dopamine; bottom pair, response to a pulse of 10^{-5} M dopamine. Both the frequency of oscillation and the percentage of responses which gave rise to large neurone driver potentials are higher following application of the large dopamine dose. (C) Response to dopamine of anterior (top) and posterior (bottom) large neurones in a ligatured *Podophthalmus* preparation. Large neurone driver potentials were evoked only posterior to the ligature, where small neurones had been directly activated.

section show only that dopamine treatment results in the activation of driver potentials in the presence of TTX, it is important also to demonstrate an enhancement of the mechanism responsible for organizing bursting activity in the absence of TTX. We have done so with two types of experiments.

When a *Portunus* ganglion was superfused with saline containing Mn^{2+} (5 mM), organized bursting was suppressed and the ganglion fired action potentials repetitively at a constant frequency (Fig. 11; see also Tazaki & Cooke, 1979*c*). This effect is due to partial blockade by Mn^{2+} of the Ca^{2+} channels utilized during the generation of driver potentials (Tazaki & Cooke, 1979*c*). In a Mn^{2+}-treated preparation, sustained hyperpolarizing current decreased the action potential frequency or silenced the ganglion, and depolarizing current could weakly activate bursting (Fig. 11). If dopamine

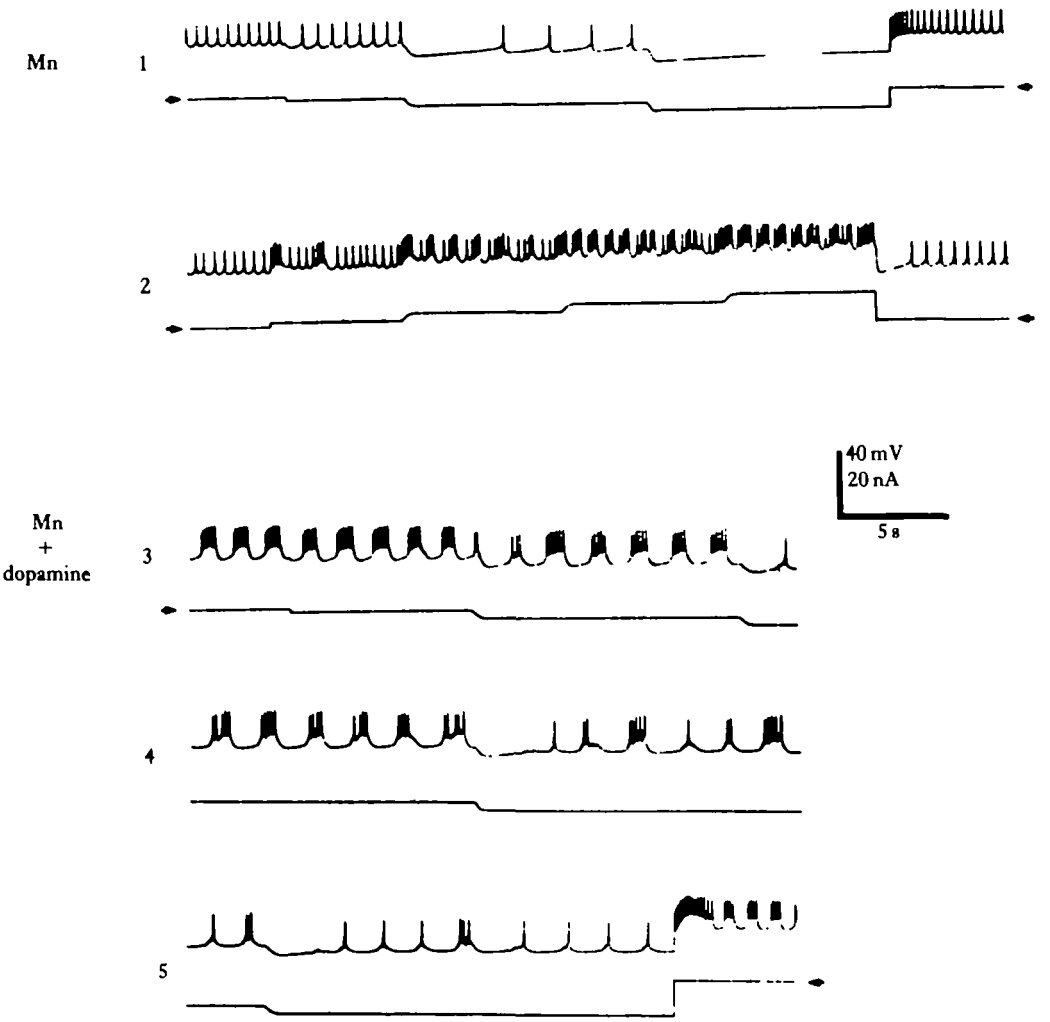

Fig. 11. Large neurone recording from a *Portunus sanguinolentus* preparation perfused with 5 mM-Mn^{2+} ions added to the saline. The lower trace in each pair is the current passed through a second electrode in the same cell, with the zero-current level indicated by arrows at the ends of the traces. Mn^{2+} reduced the activity to constant-frequency action potentials. The action potential frequency was decreased by hyperpolarizing current (trace 1). Depolarizing current could evoke weak bursts (trace 2). Dopamine (pulse application, 10^{-5} M) organizes strong bursts, which persisted to some degree when hyperpolarizing current was passed across the large neurone membrane (traces 3, 4 and 5 are continuous). With the strongest hyperpolarizing currents passed, which may prevent the large neurones from generating more than a single action potential, that potential clearly arises from a burst of synaptic potentials from small neurones that was not seen in the absence of dopamine.

was added to a ganglion in the presence of Mn^{2+}, strong well-organized bursting resulted, which persisted to some degree even when the neurones were hyperpolarized. The observation that bursting can occur even in the presence of Mn^{2+} probably reflects the fact that this ion does not totally suppress the Ca^{2+} current that is known to be primarily responsible for the depolarizing phase of driver potentials. Although Mn^{2+} almost completely eliminates electrically evoked driver potentials in the crab *Portunus* (Tazaki & Cooke, 1979*c*) and in lobsters, a partial regenerative response can be unmasked in Mn^{2+}-treated lobster large neurones if a competing K^+ current is simultaneously suppressed (Tazaki & Cooke, 1983*b*). Under voltage clamp conditions 4 mM-Mn^{2+} reduces the inward current in lobster large neurones to about 20% of normal (Tazaki & Cooke, 1983*c*).

Occasional recordings from weakly bursting preparations in normal saline also suggested enhancement of driver potentials by dopamine. In some preparations, recordings from large neurones showed very attenuated bursts, with fewer large neurone action potentials than usual. Lack of a large neurone burst was probably due to a failure to activate large neurone driver potentials completely. Application of dopamine to such preparations usually resulted in vigorous, apparently normal bursting, with a full discharge of the large neurones (Fig. 12).

Both the experiments on weakly bursting ganglia and those done in the presence of Mn^{2+} support the suggestion that dopamine activates or enhances mechanisms responsible for burst organization. This mechanism most probably involves the generation of driver potentials. It is possible that the results reported in this section represent, in part, a direct action of dopamine on the large rather than the small neurones.

Evidence for weak large neurone responses to dopamine

The results so far described suggest that the main direct action of dopamine is on the small neurones. Weak effects on the excitability and membrane properties of the large neurones were detected in preparations treated with TTX. All of the effects noted below were seen in both normal preparations and in the anterior neurones of

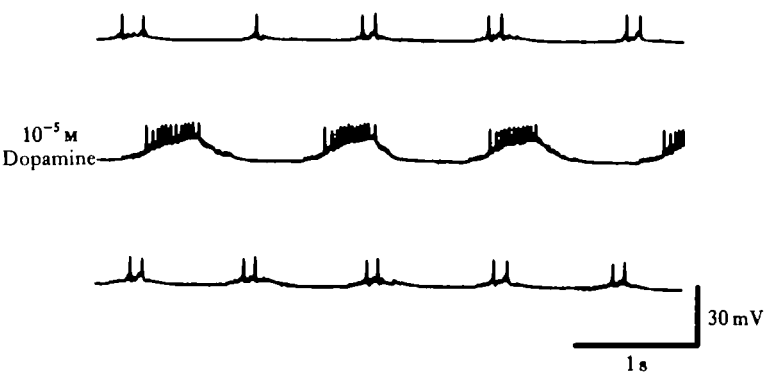

Fig. 12. Dopamine action on a weakly bursting *Podophthalmus vigil* preparation. Strong bursting was activated in the presence of dopamine (pulse application at 10^{-5} M) in the middle trace. This probably reflects full activation of large neurone driver potentials, which were absent before dopamine application (top trace) and after recovery (bottom).

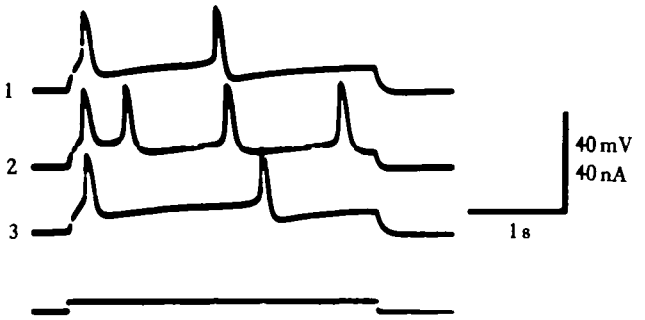

Fig. 13. Effect of dopamine on the response of an anterior large neurone in *Podophthalmus vigil* to depolarizing current passed through a second electrode in the same cell. The cell soma was separated from the posterior end of the ganglion by a ligature around the trunk, and the preparation was perfused continuously with TTX. The top three traces show the voltage response to a 3-s depolarizing constant current before (trace 1), during (trace 2) and after (trace 3) a pulse of 10^{-5} M dopamine. The applied current was the same in all three trials and is shown in the bottom trace. The repetitive driver potential responses of large neurones were enhanced by dopamine.

ligatured preparations, suggesting that a direct action on large neurones was being monitored. It should be stressed that these actions were generally seen only at dopamine levels ten to one hundred times higher than those required to alter small neurone excitability in the same preparation. (1) In both *Portunus* and *Podophthalmus* ganglia, the threshold current required to activate single large neurone driver potentials in TTX was reduced by up to 30% during exposure to a pulse (0·2 ml 10^{-5} M) of dopamine. This effect occurred, in most cases, with no change in resting membrane potential. In some experiments dopamine caused a 1–2 mV tonic depolarization. A similar depolarization induced by intracellular current injection had a much smaller effect on threshold than did dopamine. (2) In TTX-treated *Podophthalmus* ganglia, where large neurones respond with repetitive driver potentials during a prolonged depolarizing current pulse (Berlind, 1982), dopamine administration increased the number and frequency of driver potentials evoked (Fig. 13). Furthermore, *Podophthalmus* ganglia usually exhibited a single driver potential when long-duration (1–5 s) hyperpolarizing current pulses of sufficient intensity were turned off. In the presence of dopamine, two to four driver potentials were often seen following the turn-off of an identical current pulse. No consistent changes in form, amplitude or time course of individual large neurone driver potentials in either species

Fig. 14. Current-voltage relations of large neurones in *Portunus sanguinolentus*. In A and B the vertical axis shows the *peak* voltage obtained during 3-s hyperpolarizing current pulses of varying intensity. Dopamine (DA) induced a small decrease in the membrane resistance (decrease in slope) without a change in resting potential (RP). In the presence of Mn^{2+} (5 mM) (B) the input resistance was higher than in normal medium, but dopamine still decreases R_m. A and B are from the same neurone in a single preparation. (C) (solid lines) *Steady-state* voltage recorded at the end of 5-s hyperpolarizing and depolarizing current pulses. The *steady-state* I-V relationship is non-linear in both depolarizing and hyperpolarizing directions, reflecting the alteration of V-dependent conductances. The dotted line illustrates the *peak* voltage response in the same preparation showing a linear I-V relationship. Inset in C: recorded current and voltage traces, showing lack of a maintained voltage response (V) to a constant hyperpolarizing current pulse. The relaxation of the V-trace was seen in all preparations of both species, and was occasionally followed by a rebound driver potential at the turn-off of current. Arrowheads denote traces during treatment with dopamine at the two strongest current strengths.

were observed which could be attributed to dopamine action. (3) A small but highly reproducible decrease in large neurone membrane resistance (averaging −7% in response to an applied dose of 0·2 ml at a concentration of 10^{-5} M) was observed during dopamine action in both species (Fig. 14). The decrease in R_m due to dopamine was

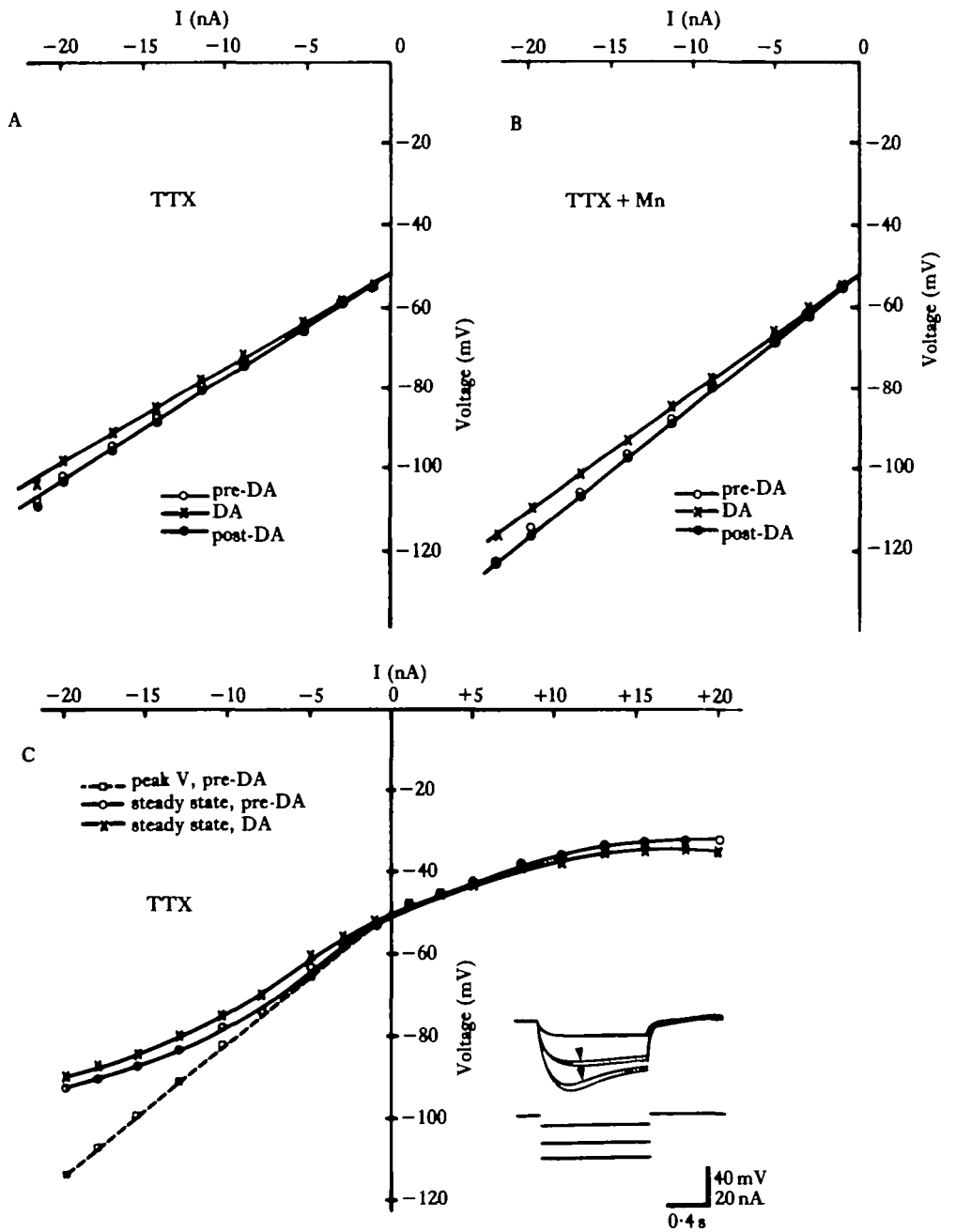

Fig. 14

seen whether or not the slight tonic depolarization occurred, and was also seen in preparations which were perfused with 5 mM-Mn^{2+} in addition to TTX.

DISCUSSION

The observations reported here show that dopamine has excitatory effects on the crab cardiac ganglion at concentrations which seem likely to be within the physiological range. Near threshold (less than 10^{-8} M during continuous perfusion) dopamine usually increased burst duration and slightly decreased burst frequency. At higher concentrations, frequency of bursting was increased in a dose-dependent manner. At all concentrations, dopamine increased the number of large neurone action potentials per burst by increasing burst duration and/or increasing intraburst action potential frequency. In the whole heart, an increased action potential output from the large motoneurones increases heart muscle depolarization (Benson, 1981). The higher frequency of bursts and increased muscle activation produced by dopamine thus correlate well with the larger and more rapid heart beats observed in dopamine-treated semi-intact hearts in other species (Berlind *et al.* 1970; Florey & Rathmayer, 1978; Cooke & Sullivan, 1982). Cardioexcitatory effects of dopamine on the heart of the horseshoe crab *Limulus polyphemus* also appear to be exerted primarily at the level of the cardiac ganglion (Augustine, Fetterer & Watson, 1982). In this species, from a different arthropod group, dopamine is present in the cardio-regulatory nerve and in the cardiac ganglion (O'Connor, Watson & Wyse, 1982), and might be utilized as a neurotransmitter by neurones regulating heart beat.

The results of our two-pool and ligature experiments suggest two major conclusions with regard to the neuronal target and mode of action of dopamine on the cardiac ganglion.

(1) Dopamine exerts its cardioexcitor action by interacting strongly with the small pacemaker neurones and has only weak direct effects on the five motoneurones.
(2) Dopamine specifically enhances driver potentials in small neurones.

The conclusion that dopamine has its primary effect on the small neurones comes from the observations showing an alteration of activity in the whole ganglion only when dopamine was applied to the posterior chamber in the two-pool experiments (Fig. 5) and from the observation that the activity in the posterior half of the ganglion only was altered when anterior cells were separated from small neurone somata by a ligature (Fig. 6). Although technical limitations preclude intracellular recording of small neurone activity, several types of evidence suggest a pronounced frequency increase, and/or a prolongation, of small neurone action potential discharge during each burst in the dopamine-activated ganglion (Figs 2, 4, 6, 7).

Our finding that the small neurones, which normally play a dominant role as the pacemaker neurones (Maynard, 1955), are the target of a cardioexcitor in this system was not entirely predictable. Although changes in burst frequency might be expected to be induced most readily by a direct influence on the pacemaker, the feedback from the large to the small neurones makes it possible for direct effects on large neurones to influence the pacemaking activity of the system as a whole (Benson, 1980). Indeed, the pentapeptide proctolin increases the frequency of bursting in the lobster (*Homarus americanus*) cardiac ganglion, *via* a direct effect on the large neurones

(Miller & Sullivan, 1981). Localized applications of 5-hydroxytryptamine, another monoamine cardioexcitor, to the lobster cardiac ganglion have also shown that frequency-enhancing effects can be exerted in part directly on the large neurones (Cooke & Hartline, 1975).

In the TTX-treated cardiac ganglion, dopamine initiated a train of driver potentials in the small neurones, and slightly lowered the threshold for activation of large neurone driver potentials. In one of the two species, *Podophthalmus*, electrotonic connections are strong enough that large neurone driver potentials typically occurred subsequent to small cell activation in TTX. In normal saline, chemical EPSPs supplement the electrotonic input from small neurones to evoke driver potentials in the large neurones. In *Portunus*, intact impulse-mediated chemical transmission might be a prerequisite for transfer of dopamine excitation from small neurones to large.

If, as we propose, the main action of dopamine is on driver potential generation, it is necessary to explain how the demonstrated effects of dopamine in TTX account for alteration of the bursting pattern in normal saline. During normal bursting, small neurone driver potentials are assumed to recur rhythmically in the absence of exogenous dopamine. Small neurone driver potentials in TTX recurred more rapidly with increasing concentrations of dopamine, and a similar increase in oscillatory rate of the small neurones in normal medium probably accounts for the increase in frequency of bursting.

It is likely that the observed increases in duration of the burst in large neurones are due primarily to a prolongation of synaptic input from small neurones to large, rather than to a direct effect of dopamine on the large neurone membrane. The bursts in small neurones in normal medium appeared to be significantly prolonged by dopamine in most preparations (Figs 6, 7), probably reflecting an increase in the duration of small neurone driver potentials. Although we have no direct information on the duration of small neurone driver potentials (in TTX) in the absence of dopamine, driver potentials which were evoked by dopamine in these neurones were very long, often exceeding 1 s. These depolarizing potentials are therefore very much longer lasting than electrically elicited driver potentials in large neurones, which are of the order of 150–250 ms (Tazaki & Cooke, 1979*b*; Berlind, 1982) and which are not altered by dopamine. It is likely, therefore, that late during a burst, action potentials in the large neurones of a dopamine-treated ganglion are driven primarily by synaptic potentials and depolarization conducted electrotonically from the small neurones, rather than directly by driver potentials of large neurones. Studies on the lobster cardiac ganglion have demonstrated that the late phase of the underlying depolarization recorded during each burst in large neurones is due primarily to discrete synaptic potentials (Hartline & Cooke, 1969). Pharmacological or neurohumoral enhancement of this phase can lead to a pronounced increase in duration of action potential bursts in the large neurones (A. Berlind, unpublished). There is not yet any clear indication of a dopamine-mediated increase of individual EPSPs recorded in large neurones but this possibility should be investigated more rigorously in view of the recent report of enhancement by dopamine of EJPs (Lingle, 1981) and nerve-evoked tension (Kravitz *et al.* 1976) in crustacean muscle.

We cannot yet evaluate the extent to which the observed effects of dopamine (at high concentration) directly on the large neurones might contribute to the altered

behaviour of the system. The results from two-pool experiments showing almost identical responses when dopamine was applied only to the posterior region or to the entire ganglion suggest that the effects exerted on the system as a whole *via* the large neurones are minimal. In addition, we have not observed any changes in the form of electrically-induced large neurone driver potentials which can be attributed to dopamine action, but the observed decrease in threshold and consequent enhancement of large neurone excitability could contribute to altered ganglionic activity when dopamine concentrations are high.

An action of dopamine similar to that on the cardiac ganglion has been reported for the crustacean stomatogastric ganglion, in which dopamine enhances or activates 'plateau potentials' (which appear to be functionally equivalent to driver potentials in the cardiac ganglion) in neurones of the pyloric system (Anderson & Barker, 1977, 1981; Raper, 1979). In the TTX-treated stomatogastric ganglion, where bursting has been suppressed, dopamine elicits slow oscillations of the membrane potential of some neurones, as it does in the cardiac ganglion. A putative cholinergic modulator interneurone (Nagy & Dickinson, 1983; Dickinson & Nagy, 1983) and muscarinic agonists (Marder & Paupardin-Tritsch, 1978; Benson, Nagy & Moulins, 1983) induce plateau potentials in the stomatogastric system by gating the activation of a voltage-dependent Ca^{2+} current in some stomatogastric neurones (F. Nagy & J. A. Benson, in preparation). Although it is possible that dopamine also alters a voltage-dependent current in the stomatogastric system, the identity of the current or currents remains unknown. In cardiac ganglion large neurones, a voltage-dependent calcium conductance is activated during the depolarizing phase of a driver potential, which is then terminated, in part, by activation of a voltage-dependent potassium conductance (Tazaki & Cooke, 1979*c*). Ionic mechanisms involved in the generation of small neurone driver potentials remain totally unexplored. It is premature to speculate on the ionic mechanisms of dopamine action on the cardiac ganglion, in view of the lack of direct information on the critical primary target cells, and the incomplete knowledge of the range of voltage-dependent conductances which might operate in the neurones of this system. The variety of voltage-dependent conductances in endogenously active neurones which are subject to transmitter or hormonal modulation precludes, at the present time, general statements about mechanisms (Adams & Benson, 1984).

The low threshold for the dopamine effects on the cardiac ganglion suggests the presence of specific dopamine receptors and hence a physiological role for dopamine as a modulator of the heart beat. Dopamine may be released from any of at least three sources: (a) the accelerator neurone terminals, (b) the pericardial organs, or (c) the synaptic terminals of intraganglionic neurones. A catecholamine, detected histochemically and biochemically, appears to be present within the lobster cardiac ganglion (Ocorr & Berlind, 1983), but evidence for intraganglionic release or physiological action has not been presented. The nature of the accelerator neurone transmitter in crabs is unknown. The pericardial organs of crabs and lobsters have been shown to contain dopamine (Cooke & Goldstone, 1970; Sullivan *et al.* 1976, 1977), which might be released into the haemolymph entering the pericardial cavity (Cooke & Sullivan, 1982) and function as a cardioexcitor hormone.

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