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EFFECTS OF PERFUSION PRESSURE ON THE ISOLATED HEART OF THE LOBSTER, *PANULIRUS JAPONICUS*

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SUMMARY

1. Systolic contractions of the isolated heart were usually composed of a first and second systolic contraction (FSC and SSC) which corresponded to the first and second large potentials (FLP and SLP), respectively, recorded in the cardiac muscle cells and represented excitatory junction potentials produced by impulse bursts of the large cells of the cardiac ganglion.

2. Under constant pressure, the magnitudes of the FSC and the SSC appeared to change according to the amplitudes and durations of the FLP and the SLP. Further, the total amplitude of systole was often affected by the time of occurrence of the SSC (or the SLP).

3. Internal perfusion pressure and heart tonus were linearly related over a considerable range. With an increase in heart tonus (0–30 mg), the magnitude of the FSC was enhanced markedly in the absence of equivalent increases in the amplitude and duration of the FLP. The elevation of heart tonus was also related to an increase in beat frequency.

4. The SSC decreased in magnitude and disappeared when the beat frequency exceeded approx. 1 Hz under high perfusion pressure. Further, the abolition of the SSC resulted in a steeper slope in the curve relating beat frequency to tonus. The SSC was absent during the decline of beating caused by rapid reduction of the pressure.

5. The SSC was abolished by transverse cuts of the ganglionic trunk between the 4th and 5th large cells or between the 5th large and the 6th small cells, but the SSC often remained after the trunk was severed at the region between the 6th and 7th small cells. After severing the trunk, the heart still had the ability to respond to pressure as detected by a change of the beat frequency.

6. Spontaneous slow contractions of the cardioarterial valves were often observed.

INTRODUCTION

The lobster heart is a single-chambered structure made up of striated muscle fibres and is suspended in the pericardial cavity by its arteries and by elastic bands of connective tissue, called suspensory ligaments (Maynard, 1960). Haemolymph enters

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the heart through ostia and is driven by contractions of the heart into the anterior and posterior arteries. Cardiac contractions follow periodic bursts of impulses of the cardiac ganglion (Matsui, 1955; Maynard, 1955; Anderson & Cooke, 1971), which lies on the inside of the dorsal wall of the heart (Alexandrowicz, 1932). In *Panulirus*, nine neurones (five large anterior and four small posterior) are dispersed along a straight trunk of the ganglion. Axon impulses of the four small cells evoke synaptic potentials in the large cells (Tazaki, 1973; Freisen, 1975; Tameyasu, 1976). The firing of each of the five large cells provides motor output to the cardiac muscle (Kuramoto & Kuwasawa, 1980). Synaptic and electrotonic connections among the nine cells, including endogeneously bursting neurones, have been examined physiologically in both *Panulirus* and *Homarus* (Hagiwara & Bullock, 1957; Watanabe, 1958; Watanabe & Bullock, 1960; Conner, 1969; Hartline & Cooke, 1969; Tazaki, 1971, 1972; Mayeri, 1973; Matsui, Kuwasawa & Kuramoto, 1977; Hartline, 1979). More recently, slow depolarizing responses of the cardiac ganglion cells (Tazaki & Cooke, 1979*a*,*b*,*c*) and basic properties of oscillators in the cardiac ganglion (Benson, 1980) have been characterized in the crab. Mechanisms by which the spontaneous activity of neurones in cardiac ganglia is integrated have also been established. Further, some investigators have reported on the relationship between the nerve impulse pattern of the large cells and the muscle electrical responses (Van der Kloot, 1970; Anderson & Cooke, 1971; Kuramoto & Kuwasawa, 1980). The periodic discharges of cardiac muscle cells are usually made up of two phases, which originate from the burst discharge pattern of the large cells. Anderson & Cooke (1971) have also presented simultaneous recordings of muscle membrane potential and heart tension. Internally perfused lobster hearts are sensitive to small increases in perfusion pressure (Maynard, 1960; Cooke, 1966) and to decreases in flow volume (Kuramoto & Kuwasawa, 1980). Alexandrowicz (1932) speculated that each of the ganglion cells may have stretch-sensitive dendrites whose arborescent branches extend into the cardiac muscle.

First, we investigated the characteristic changes of the heart beat produced by pressure. Then, the relationship between the cardiac muscle discharges and heart contractions was analysed by simultaneous recording under high and low pressure. Neuronal mechanisms that change the rhythm and form of the heart contractions were also examined by severing the ganglionic trunk. It is clear that an increase or decrease of pressure alters the heart tonus. This, in turn, causes changes of periodic activity of the large and small cells and, at the same time, of the magnitude of the heart contractions in the absence of equivalent changes of muscle membrane depolarization.

MATERIALS AND METHODS

All experiments were performed on the marine spiny lobster, *Panulirus japonicus*. The walking legs and antennae were removed at their bases from 56 animals of both sexes (weight about 200 g) and were allowed to bleed. The heart was exposed and removed carefully from the body by cutting off the ligaments, the arterial vessels and the other connective tissue. The isolated heart was suspended by its ligaments, and the connective tissue of the vessels, dorsal-side-up in a perfusion chamber containing a saline bath. A branched tube consisting of a pair of outlets (1 mm i.d.) was utilized

to perfuse the heart. The saline flowed from the outlets of the branched tube into the left ostia. It was pumped out by cardiac contractions through the left antennary artery and the dorsal abdominal artery (the others were closed). This limitation of outflow from the heart was necessary to inflate the heart properly. The perfusion medium was a modified Pantin's solution (530 mM-NaCl, 10·7 mM-KCl, 18·0 mM-$CaCl_2$, 24·6 mM-$MgCl_2$, 2·3 mM-$NaHCO_3$ and 3 mM-glucose). In some instances, fresh natural sea water was used. The temperature during the experiments was 18–22 °C.

Perfusion pressure was changed by raising or lowering the perfusion bottle (100 ml) which was continuously filled with the saline. The pressure was monitored by a pressure transducer (National LX 1601D) put into a bypass of the perfusion tube located at the same height as the heart. Thereby, the applied pressure could be changed in a reproducible manner. In some instances (15 hearts), the pressure in the heart or the sternal artery was measured by a small sensor for pressure (Kulite XT-190) plugged into one side of a Teflon tube (1 mm i.d.) which was filled with silicone oil (Toray SH200) and inserted into the ventricle through one of the right ostia or into the artery.

A mechanogram of heart or valve contraction was obtained with a strain gauge, a lever of which was attached to the ophthalmic artery because the magnitude of heart contractions was largest at the anterior of the heart. Nearly isometric contractions of the antero-posterior axis were recorded as representative of heart contractions.

A suction glass capillary electrode (100 μm tip diameter) was used for extracellular recording from the cardiac muscle. Intracellular recordings were also made from cardiac muscle cells using glass microelectrodes filled with 3 M-KCl solution (20–30 MΩ).

Experiments in intact hearts were followed by severing the ganglionic trunk. This was accomplished by slicing off a small area on the surface of the dorsal heart wall and then cutting it transversely through a narrow crevice between the muscle bundles. After the operation, the heart wall remained relatively intact, so that the effect of the procedure on the internal pressure was insignificant. After the physiological experiments, the cut region of the trunk was stained with methylene blue (Alexandrowicz, 1932). The nine ganglion cells are numbered from anterior to posterior along the trunk.

Muscle discharges, heart contractions and the pressures were monitored through d.c.-amplifiers on a storage oscilloscope (Sony-Tektronix 5113) and simultaneously recorded on a tape recorder (d.c.-625 Hz). Interbeat intervals (reciprocals of beat frequency) were measured with an electronic counter which determined the time from one systole to the next. Thus, the cardiac muscle discharges, the heart contractions, the interbeat intervals and the pressures were simultaneously displayed at a given speed on a pen recorder chart. The amplitude of the potentials was not attenuated by either the tape or the pen recorder.

RESULTS

Characteristic responses of the lobster heart to change in perfusion pressure

The beat frequency and amplitude (maximal tension) under the initial conditions were about 0·5 Hz and 150 mg, respectively. The frequency and amplitude of the beat

were enhanced in response to increases in perfusion pressure (Fig. 1). The beat gradually resumed the initial frequency and amplitude on reducing perfusion pressure (e.g. Fig. 2). The frequency and the amplitude slowly (Fig. 1A) or rapidly (Fig. 1B) increased and reached a plateau in response to each amount of applied pressure. Heart tonus, indicated by a raised baseline on the strain gauge recording contractions, was also developed by the applied pressure. However, even at the highest pressure examined (50 g cm^{-2} in the perfusion line), some preparations stayed at a lower rate (Fig. 1A) and others reached high rates (Fig. 1B). The higher rate of beat frequency and the greater amplitude of systole seemed to be a function of the increase in heart tonus. The responses to pressure were characterized by fluctuations in the amplitude or interval of the beat, which took place when a high perfusion pressure of about 30 g cm^{-2} was applied (Fig. 2). Two systole types, one large and one small, alternated and a steady pattern was maintained after a few minutes (Fig. 2A). Corresponding to alternation of the large and small systoles, interbeat intervals increased and decreased. In other preparations, the systole changed from large to a small or intermediate size several minutes after the pressure rise (Fig. 2B). Although the beating pattern in Fig. 2B is irregular as compared with that in Fig. 2A, the large and small systoles also alternate in the middle part of the response (i.e. about 2 min in Fig. 2B). After the systole had changed from large to small, the large systole sometimes occurred transiently (Fig. 2C). The large systole was closely related to the fluctuation in interbeat interval, being followed by a longer interval. Thus, it seems that the heart could beat

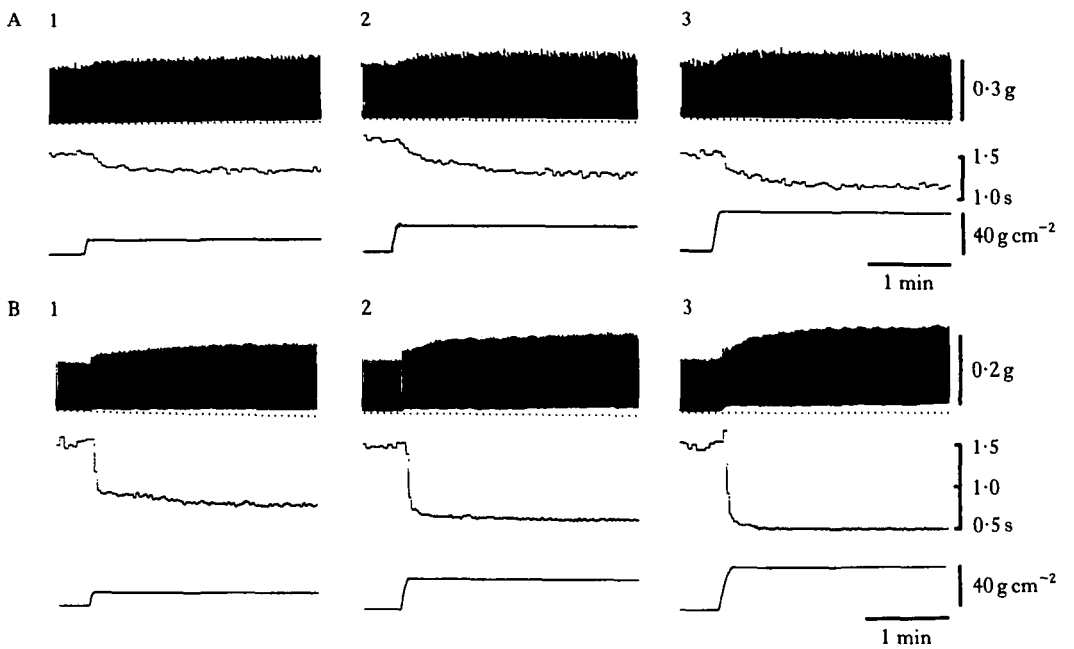

Fig. 1. Two examples of the responses of the lobster heart to perfusion pressure. The heart contracts with two strokes (A) or one stroke (B) for each beat (upper traces). The interbeat interval (middle traces) slowly (A) or rapidly (B) decreases with each of the pressure rises (13, 26 and 40 g cm^{-2}, lower traces). The changes appear to be correlated with an elevation of heart tonus indicated by a raised baseline of the heart contractions. The original baseline of heart contractions is shown with a dotted line.

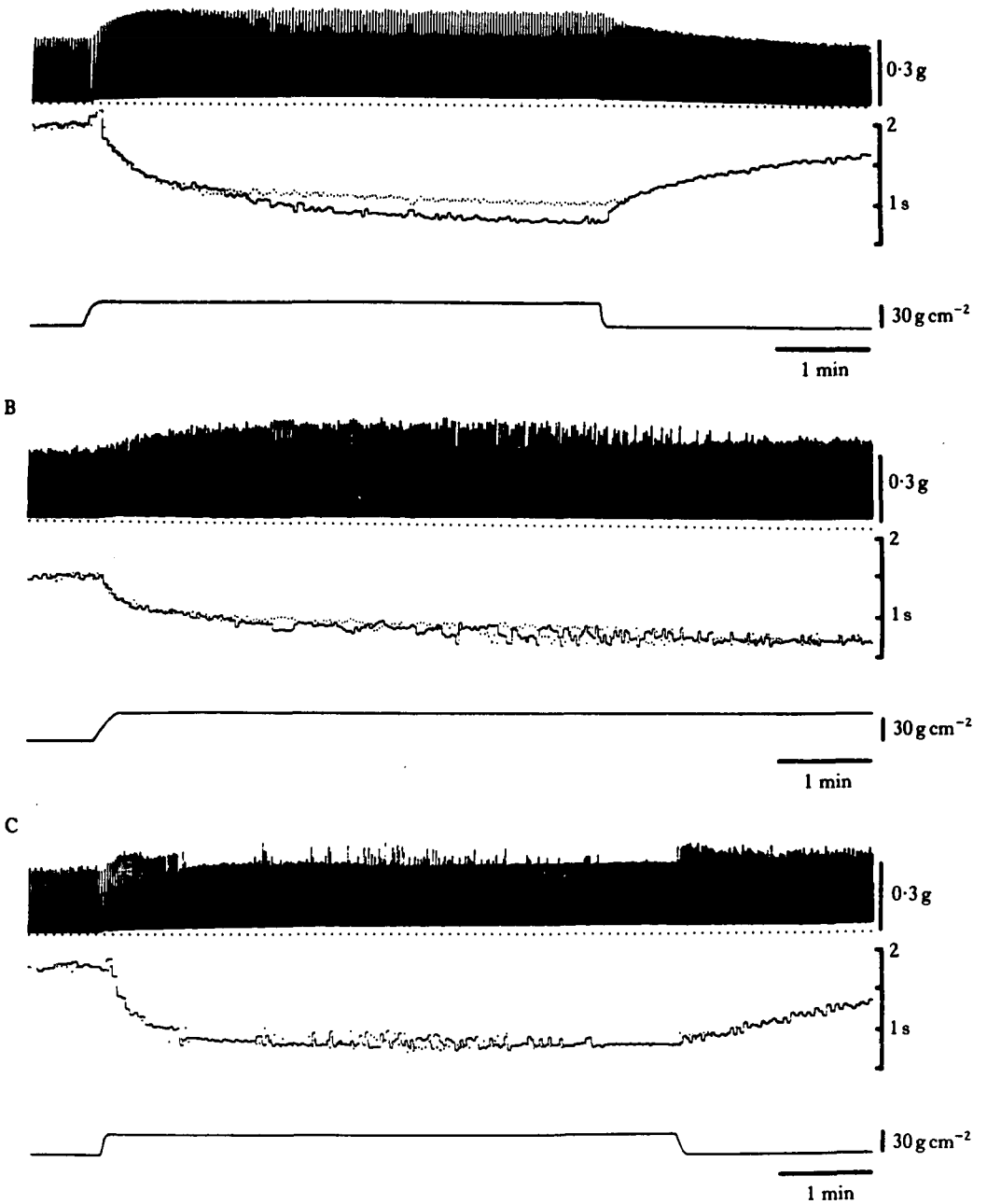

Fig. 2. Response patterns of the lobster heart produced by a high perfusion pressure. (A) A response pattern of the heart characterized by alternation of large and small systoles (upper trace) or of long and short interbeat intervals (middle trace, bars and dots) after an increase of pressure (lower trace, 30 g cm^{-2}). (B) Another response pattern of the heart in which the systole converts from a large to small one. (C) One more example of the response pattern characterized by a rapid change of the systole size from a large to small one. In A and C, the changes in amplitude and interval of the beat produced by the high pressure reverse on return to the initial low pressure. The original baseline of heart contractions is shown with a dotted line.

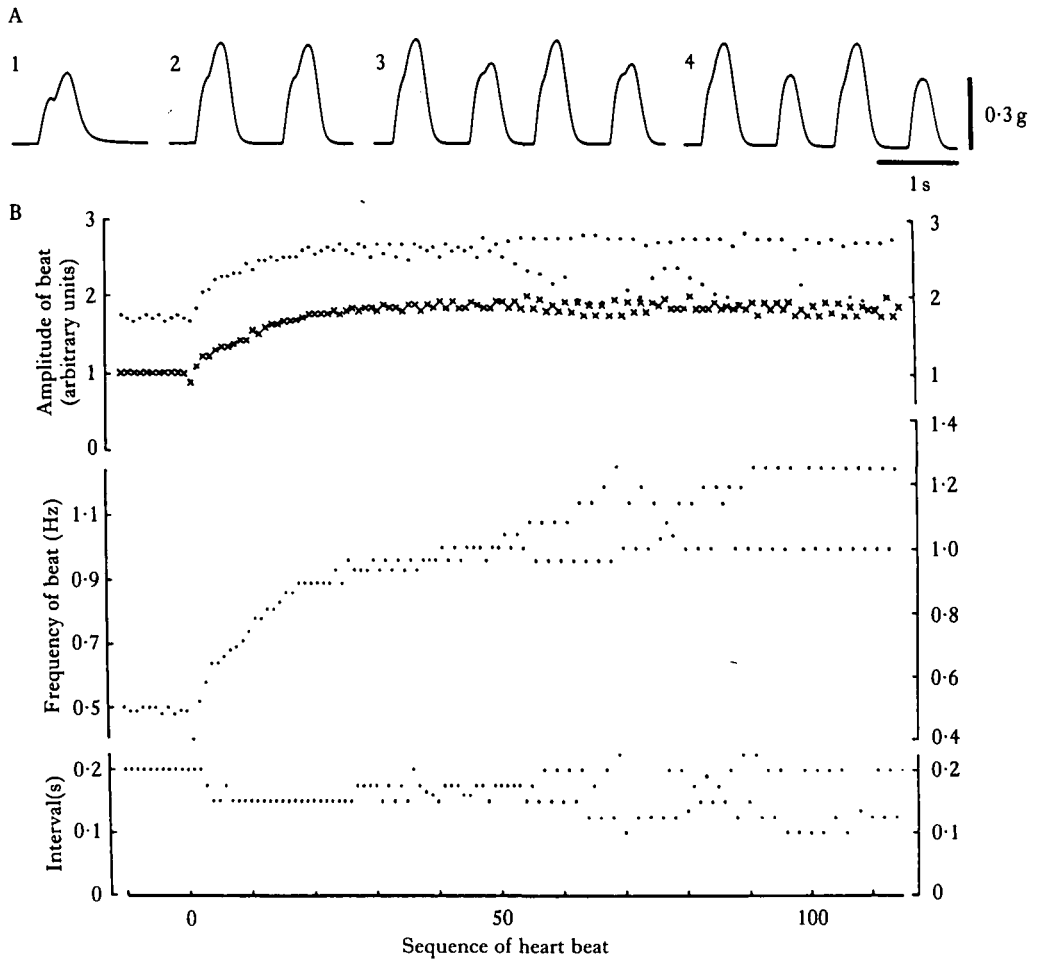

Fig. 3. Beat pattern change of the lobster heart. (A) Contraction waves of the heart before (1) and after (2–4) an increase of the pressure (50 g cm^{-2}). The larger systoles (2 and 3) are composed of a first and second systolic contraction (FSC and SSC) as judged by single notches on the waves. The small systoles (4) have no detectable notch; being single heart contractions. (B) Amplitude and frequency of the beat and interval between FSC and SSC plotted against each time of the beat. The peak portions of FSC (x) are plotted in the upper graph with total amplitude of the beat. The changes in amplitude, frequency and interval appear to occur in close correlation with each other.

regularly if the systole were either large or small (Fig. 1). However, usually the large type of systole predominated under low perfusion pressure (less than 20 g cm^{-2}) and the small type under high pressure (more than 30 g cm^{-2}). The change in systole size or the fluctuation in the interbeat interval produced by the high pressure reversed when the pressure returned to the initial low state (Fig. 2A, C). Thus, the changes in beat pattern under high pressure were reversible.

In most of our preparations (84 %), a notch was clearly visible in the contraction record of the heart as illustrated in the typical records shown in Fig. 3A. The single notch indicates a double-contraction systole. The two contractions can be called the first systolic contraction (FSC) and the second systolic contraction (SSC). In the early phase after the pressure was raised, FSC and SSC increased in magnitud

(Fig. 3A1, A2). Large and medium or small systoles alternately repeated in the late phase (Fig. 3A3, A4). The systoles of large and medium sizes with single notches were double-contraction systoles composed of FSC and SSC. The small systole on which no notch could be detected might be a single component of FSC (Fig. 3A4). After an increase of pressure ($50\,g\,cm^{-2}$), the beat frequency gradually increased to approximately 1 Hz (the early phase), and then the beat frequency increased alternately (the late phase) (Fig. 3B). In the late phase, the average beat frequency slowly increased with time and reached a plateau. The interval between FSC and SSC decreased from 200 to 160 ms in the early phase and then fluctuated alternately within a range of 160–200 ms. The interval fluctuated with changes in frequency and amplitude of the beat in the late phase (Fig. 3B). The total amplitude of the beat and FSC magnitude gradually increased in the early phase. On the other hand, the total beat amplitude decreased in the late phase while the FSC magnitude appeared to be almost constant; that is, the decrease of beat amplitude was largely due to decreases of the SSC magnitude. It is therefore clear that in the extreme case, the SSC disappeared resulting in a systole of the single-contraction type. An example of a change of systole from the double-contraction type (FSC and SSC) to the single-contraction type (FSC) is shown in Fig. 2B,C. In most of the hearts (75%), systoles of only FSC became abundant as the beat frequency increased from 1 to 2 Hz. The heart response shown in Fig. 1A was approximately 9% and the hearts as shown in Fig. 1B were approximately 16%.

A variety of responses may be attributed to differences in internal perfusion pressure in the heart. We have five measurements of the internal heart pressure (Fig. 4A). These were, in three cases, distorted by the presence of the cannulae. In ten preparations the transducer recorded from the sternal artery. Thus, we obtained simultaneous records of the internal pressure and its changes during the heart beat. The systolic pressure measured in the heart itself appeared to increase in proportion to the applied pressure (e.g. curve c in Fig. 4B). The aortic pressure also appeared to increase with the applied pressure within the range of $10–40\,g\,cm^{-2}$ (e.g. curves a, b and d in Fig. 4B). However, the maximum internal pressure varied considerably from preparation to preparation and ranged from $2{\cdot}5–15\,g\,cm^{-2}$ for an applied pressure of $60\,g\,cm^{-2}$. The increments in internal heart pressure were approximately linearly proportional to each increment of applied pressure.

The more significant responses to pressure seem to be related to greater increases of heart tonus (Fig. 1). When the strain gauge recordings on the tape were amplified ten times to measure the increases of heart tonus, we found that the baseline of the records was drifted very slowly in a regular pattern by the change of room temperature ($20 \pm 1\,°C$). The drifted line could be traced easily. Therefore, the measurements of tonus tension were performed along the baseline drifted by the temperature change. The maximal change of heart tonus developed by the applied pressure was obtained with the magnified scale. Increases of the tonus tension plotted as a function of the applied pressure or the aortic pressure show that the curves rose with pressure increases (Fig. 5). The heart tonus was elevated progressively by the pressure. The extent of the elevation differed from preparation to preparation (17·7 mg in curve a and 47 mg in curve d, Fig. 5). The curves for the internal pressure (Fig. 5B) are closer to each other than the curves of applied

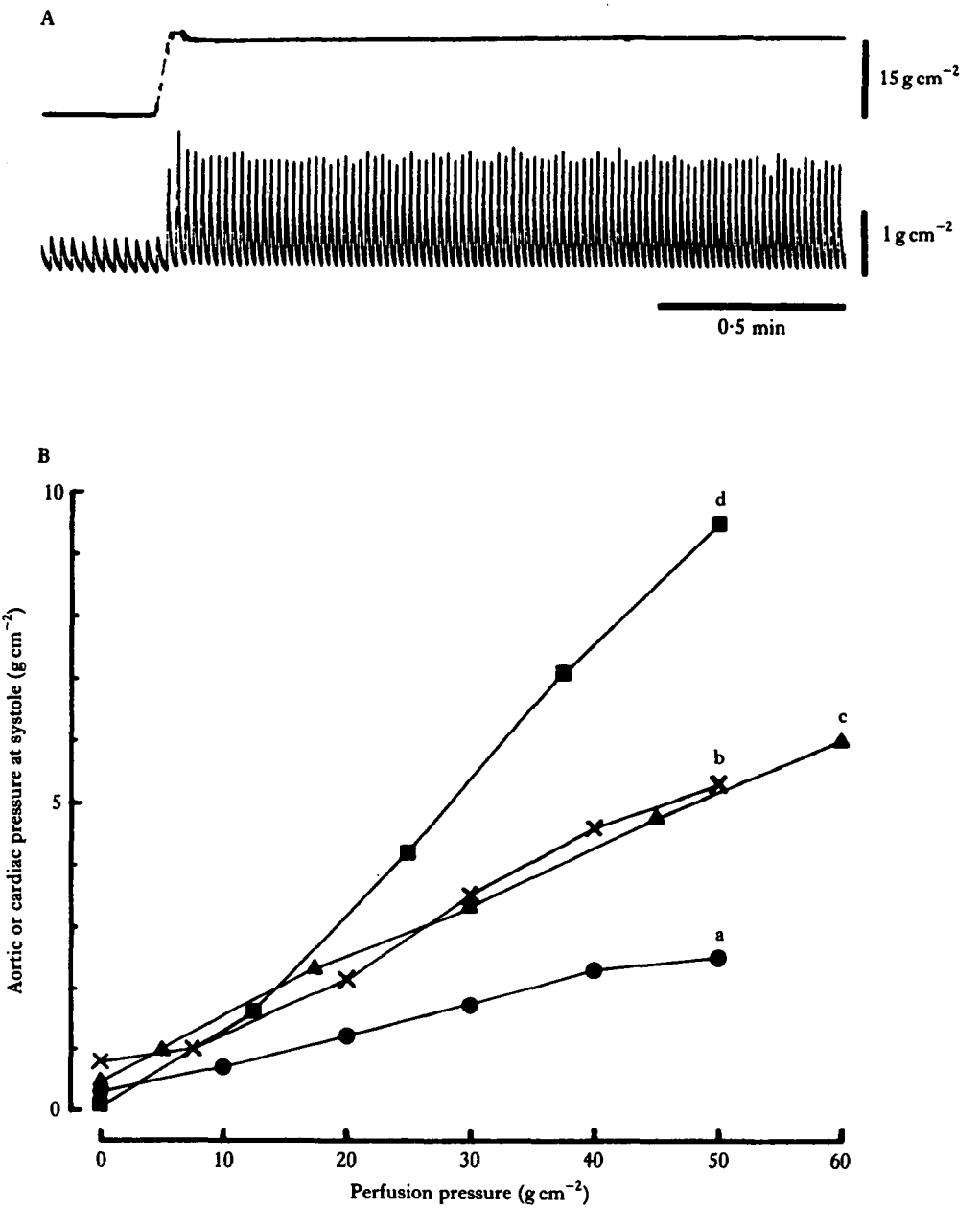

Fig. 4. Relationship between the pressure applied to ostia and the internal pressure of the heart or the sternal artery of the lobster. (A) A simultaneous record of the applied pressure (upper) and the internal cardiac pressure (lower). (B) Increases of the internal and systolic pressure plotted against the amount of perfusion pressure applied to ostia. Curves a, b and d are examples of the aortic pressure and curve c is an example of the cardiac pressure.

pressure (Fig. 5A). The tonus and internal pressure are directly and linearly related over a considerable range.

Increase in beat frequency is seen to be linearly related to the tonus tensio

(Fig. 6A). The slope of the curves for beat frequency appeared to be divided into two groups. In one group, the beat frequency (curves a, b and c) increased gradually with increase of the tonus tension in the range of 0–30 mg, while in the others the frequency (curve d) increased rapidly showing a linear relation to the tonus up to 47 mg. The curves of b and c are interesting because, when the tonus tension exceeded 30 mg, a rapid increase in beat frequency was associated with the disappearance of SSC

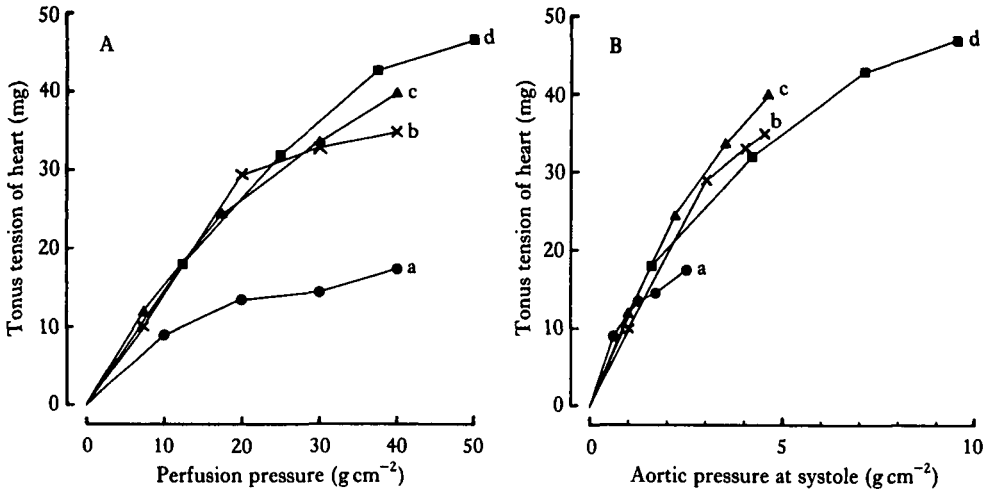

Fig. 5. Relationship between the maximal tension of heart tonus developed and the perfusion pressure applied to ostia (A) or the systolic pressure in the sternal artery (B). The curves marked a, b, c and d are obtained from the preparations shown in Figs 1A, 2A, 2C and 1B, respectively.

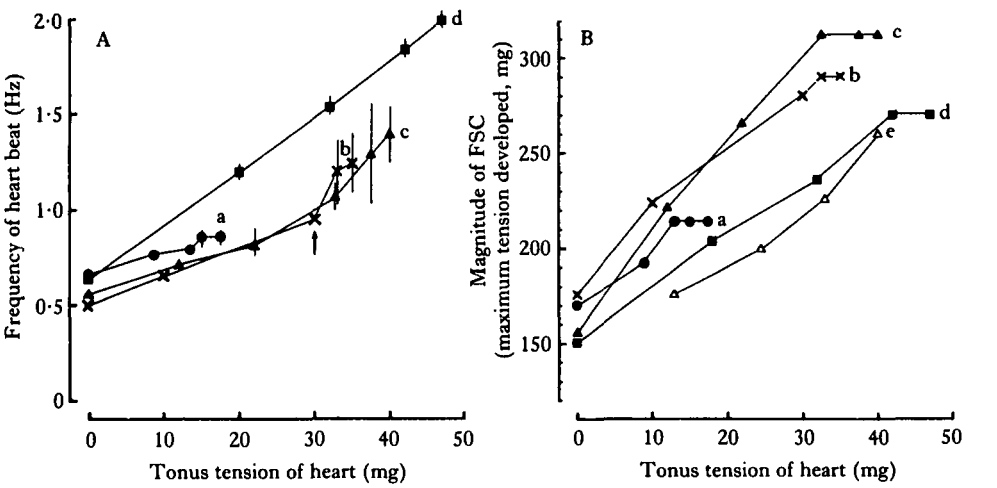

Fig. 6. Relationship between the heart tonus and the frequency (A) and magnitude (B) of the heart beat obtained from the same preparations as shown in Fig. 5. (A) Curves of a, b and c exhibit gradual increases while curve d shows a rapid increase with elevation of the tonus tension. The former group is obtained from hearts having double-contraction systole (Figs 1A, 2A, 2C). The latter is from a heart having single-contraction systole (Fig. 1B). The arrow indicates a critical point where the SSC is abolished or not. Vertical bars are variation ranges of the beat frequency. (B) Increases of the FSC magnitude plotted as a function of the tonus tension. Curve e is obtained from the preparation shown in Fig. 2B. Curve c shows FSC increasing linearly with elevation of heart tonus over the widest range (156–312 mg) of the hearts examined.

(Fig. 3B). Therefore, the presence of SSC reduces the frequency response to increased tonus tension.

The extent of increase of the FSC (i.e. maximum tension developed) also appears to be a function of tonus tension (Fig. 6B). The plots indicate that the FSC magnitude increases linearly with the elevation of the tonus tension over a range which varies for different hearts. The FSC magnitude reached a maximum value and showed a maximum tension at different values from preparation to preparation (i.e. 214 mg in curve a and 312 mg in curve c). It is apparent that the plateau levels are related to the internal pressure and tonus tension increase by perfusion. In general, all the curves rose with a similar slope. Therefore, an increase in FSC seems to be linearly related to an increase in the tonus tension until the FSC reaches a maximal value.

Correspondence between cardiac muscle discharges and heart contractions

Transmembrane potential changes of the cardiac muscle cell and contractions of the heart were simultaneously recorded while the perfusion pressure was increased or decreased (Figs 7, 8, 10). Heart contraction was accomplished by synchronous contractions of cardiac muscle fibres. Muscle membrane depolarizations were made up of excitatory junction potentials (EJPs), which were responses to nerve impulses generated in the large ganglion cells. Under low pressure perfusion, the periodic depolarizations of a muscle cell showed two major phases, each being composed of summed EJPs (Fig. 7). The first large potential (FLP) was followed by the FSC and the second large potential (SLP) by the SSC. Under high pressure perfusion, the periodic discharges became single large peaks, that is, the SLP disappeared (Figs 7A3, 8). Under conditions in which amplitude of the FLP or the SLP was nearly constant, magnitude of the systole showed marked increase with a prolonged duration of sustained depolarization of the muscle membrane (Fig. 7B). In the prolonged depolarizing potentials, a tonic train of small EJPs associated with the large EJPs was often observed (Fig. 7B, lower records). By contrast, when the duration of the FLP was almost constant, changes in the FSC magnitude were not striking (cf. Fig. 9). With regard to the SLP, both the amplitude and duration of the large EJPs often exhibited large variations from beat to beat (Fig. 7A, B, C). Apparently in parallel with the variations of the large EJPs, the SSC magnitude also exhibited comparable variation. These correlated changes suggested that the magnitude of the heart contractions changed in relation to the amplitude and duration of the large EJPs in the cardiac muscle cells.

In some instances, the maximal amplitude of a systole decreased with an increase in the interval between the FLP and SLP (e.g. the 2nd beats in Fig. 7C1, C2). The interval between the FLP and the SLP was a factor in the summation of the FSC and the SSC. The observations indicated that the largest amplitude of systole was produced when the SLP was generated with an appropriately short delay following the FLP (Sugi & Kosaka, 1964).

The magnitude of the FSC and the SSC increased markedly when external perfusion pressure was increased but the FLP and the SLP were not markedly enhanced (Fig. 8). The resting membrane potential of the muscle cell did not change significantly, either. In some instances, we observed that the duration of the FLP was not clearly altered by an increase in the perfusion pressure. The effect of pressure

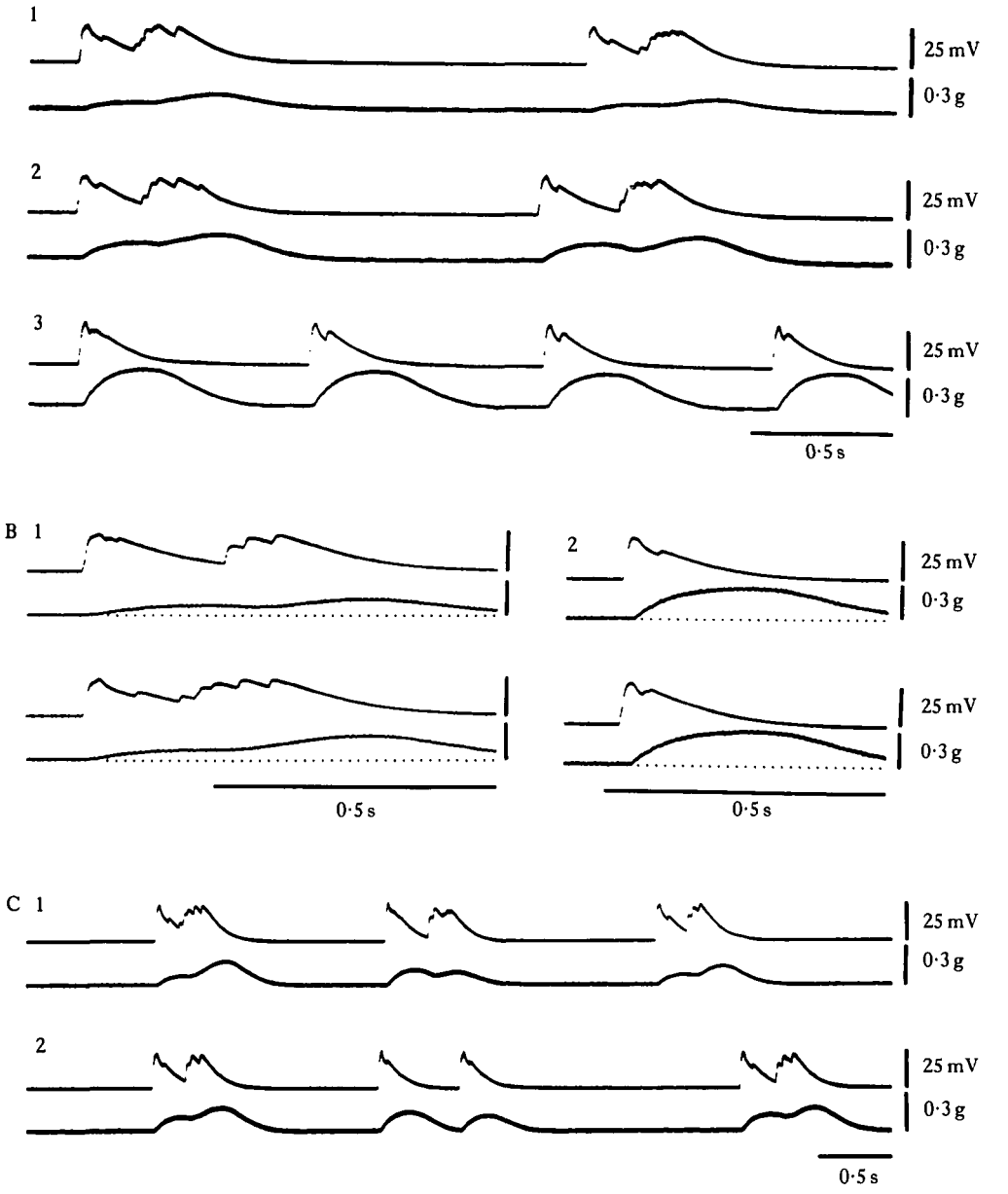

Fig. 7. Cardiac muscle electrical activity and the heart contractile activity under a low or high constant perfusion pressure. (A) 1 and 2, FLP and SLP in the muscle cell (upper traces) are respectively followed by FSC and SSC of the heart (middle traces) under low pressures (10 and 20 g cm^{-2}, lower traces). (A) 3, SLP and SSC disappear under high pressure (30 g cm^{-2}). (B) A role of small EJPs for sustained depolarization of the muscle membrane (upper traces). The prolonged and sustained depolarizations result in greater amplitude of systoles (lower traces) under a low (1) or high (2) constant perfusion pressure. (C) Total magnitude of the systoles is considerably swayed by the starting point of SLP (therefore SSC) succeeding FLP (therefore FSC) as well as by changes of magnitude in each of FSC and SSC. The perfusion medium was fresh natural sea water in this experiment. The records were displayed on the oscilloscope and photographed.

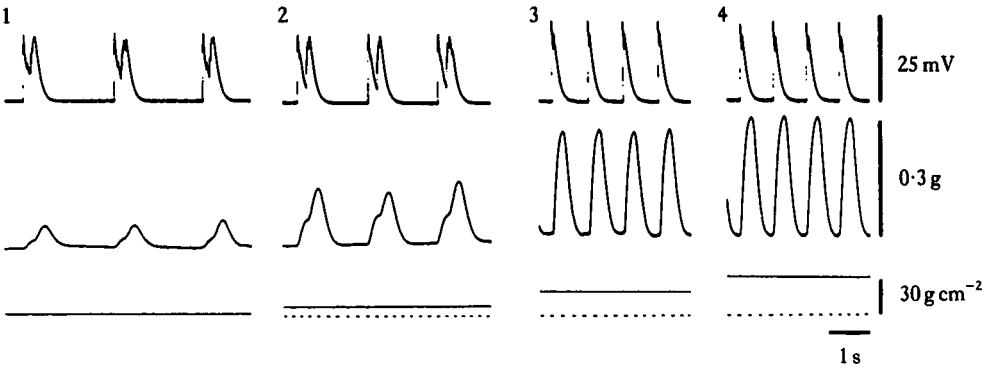

Fig. 8. Alteration of the muscle electrical activity and the heart contractile activity produced by increases of perfusion pressure. The resting membrane potential (60 mV) is almost constant for increases of perfusion pressure (upper traces). Magnitude of the systoles (middle traces) increases as the pressure is raised (lower traces) but the increases appear to occur in the absence of equivalent changes of the muscle membrane depolarization. The perfusion medium was fresh natural sea water.

the magnitude of the FSC was examined quantitatively and repetitively by the use of a heart in which the systole was predominantly of the single-contraction type. The field potentials of EJPs (Fig. 9A, B) are produced synchronously in a large number of cardiac muscle fibres. They were similar in shape to the FLP recorded intracellularly from a single muscle cell (Figs 7, 9B). The amplitude of the extracellular FLPs might have been affected by an alteration in resistance between the suction electrode and the muscle tissue. However, observed voltage of the baseline did not change significantly (Fig. 9A, upper trace). Therefore, it is likely that the electrode seal resistance was constant during the series of recordings. Increases of the FLP amplitude were not obvious (Fig. 9A and B, upper trace) while the FSC magnitude increased gradually and progressively with an elevation in perfusion pressure (Fig. 9A, C curve b). The duration of the FLP is almost constant (Fig. 9B). Therefore, increases of the ratio indicated that the magnitude of the FSC enhanced by pressure might not have been attributable to changes of the FLP. The small difference between curves a and b indicated that the FSC magnitude was enhanced by a small increase of the FLP amplitude. The increase of the single heart contractions was closely related to the elevation of tonus tension (Figs 6B, 9C curve c). Therefore, the magnitude of the heart contraction was enhanced in the absence of equivalent changes of muscle membrane depolarization. The important factor may be the increase in heart tonus.

For several minutes after rapidly reducing the perfusion pressure from a high level to zero, the amplitude and frequency of the heart beat decreased markedly and then

Fig. 9. Increases of FLP and FSC depending on elevation of perfusion pressure and heart tonus. (A) FLP amplitude (approx. 0·1 mV) recorded with a suction electrode is not significantly increased (upper trace) while FSC magnitude markedly increases (middle trace) with increases of perfusion pressure (lower trace). (B) Four examples are derived from the record in A and shown with a magnified time scale of 1 s frame^{-1}. Their positions correspond respectively to the pressure heights shown in A. The duration of FLP (upper traces) is almost constant for increases of perfusion pressure. (C) Percentage changes in the FSC magnitude (a) and in the ratio of the FSC magnitude and FLP amplitude (b) are shown as a function of the amount of perfusion pressure. Maximum tension of the heart tonus (c) is elevated in proportion to the pressure rises (0–30 g cm^{-2}) with a similar curve to b.

stopped. After some time it began to beat again and gradually returned to the normal beat. The arrest and reinitiation of heart contractions followed the disappearance and reappearance of EJPs (Fig. 10A). The SLP and the SSC disappeared in the early part of the heart beat decline and reappeared later during recovery than did the FLP and

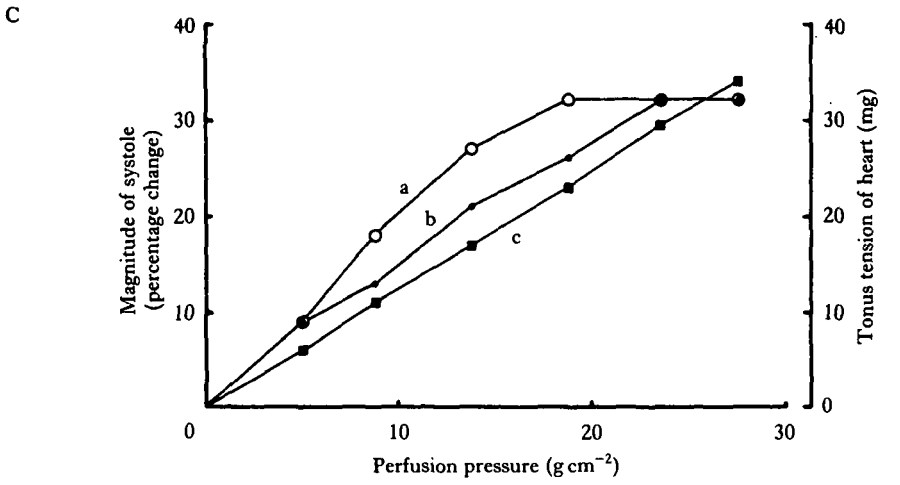

Fig 9

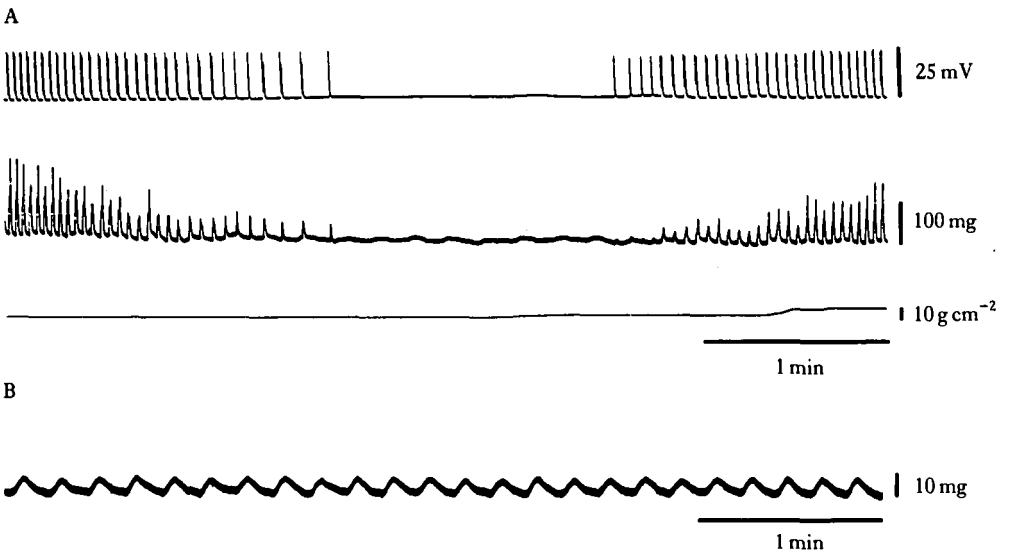

Fig. 10. Arrest and reinitiation of heart contractions after a rapid drop of perfusion pressure. (A) The simultaneous record starts at 3 min after dropping the pressure from 50 to 0 g cm^{-2}. Disappearance and reappearance of EJPs (approx. 25 mV, upper trace) are followed by arrest and reinitiation of the heart contractions (middle trace). During beat stoppage, slow undulating waves continue (middle trace). Potential changes corresponding to them (upper trace) are not detected in the muscle cell. (B) Spontaneous slow contractions of the cardioarterial valve. The valve recorded was situated at the gate from the ventricle to the antennary artery and isolated from the heart together with the right antennary artery.

the FSC. Slow undulating waves of small contractions seen prominently during the period of heart stoppage (Fig. 10A), were also observed during the normal heart beat (Fig. 1B). These slow contractions appeared to correspond to the movement of the valves which are situated at a gate from the ventricle to each of the arteries. Slow contractions recorded from a preparation consisting of the cardioarterial valves isolated together with the antennary artery were comparable in time course to these slow undulating waves (Fig. 10A, B). They are slower than the heart beat.

Role of the posterior ganglion cells

In the ganglionic trunk of the heart, the four large anterior cells and the four small posterior cells are close to each other while the 5th cell is situated at some distance from the others. It was easy to sever the trunk anterior and posterior to the 5th cell

Fig. 11. Effects of severing the ganglionic trunk on the systole form and the beat rhythm of the lobster heart. The heart originally beats double-contraction systole (FSC and SSC). The trunk is transversely cut (arrows) producing an abrupt decrease in interbeat interval (lower traces) which is gradually restored. The records in A, B, C and D are continuous with a 3-min intermission. (A) SSC is maintained (upper trace) after cutting the trunk between the 6th and 7th cells. (B) SSC disappears (upper trace) after a second cut of the trunk between the 5th and 6th cells, i.e. the systole form turns to the single-contraction systole. (C) The systole form of single contraction is unchanged (upper trace) after a third cut of the trunk between the 4th and 5th cells. The decreased interval of beat (lower trace) continues for longer than 4 min (D1) but is restored about an hour after the cut (D2). (E) A response of the heart to the perfusion pressure in the same preparation as D. With increases of the pressure (upper trace), the beat amplitude (middle trace) and the interbeat interval (lower trace) are changed.

body without causing marked changes in the recordings of the heart beat. After cutting these trunk regions, the heart beat did not stop if normal perfusion continued (Fig. 11). The frequency of the beat was higher than 1 Hz just after the cut and then in a few minutes recovered gradually to the level observed before the cut (0·5–0·7 Hz) (Fig. 11A,B,C), after which a steady beat continued for more than 1 h (Fig. 11D,E).

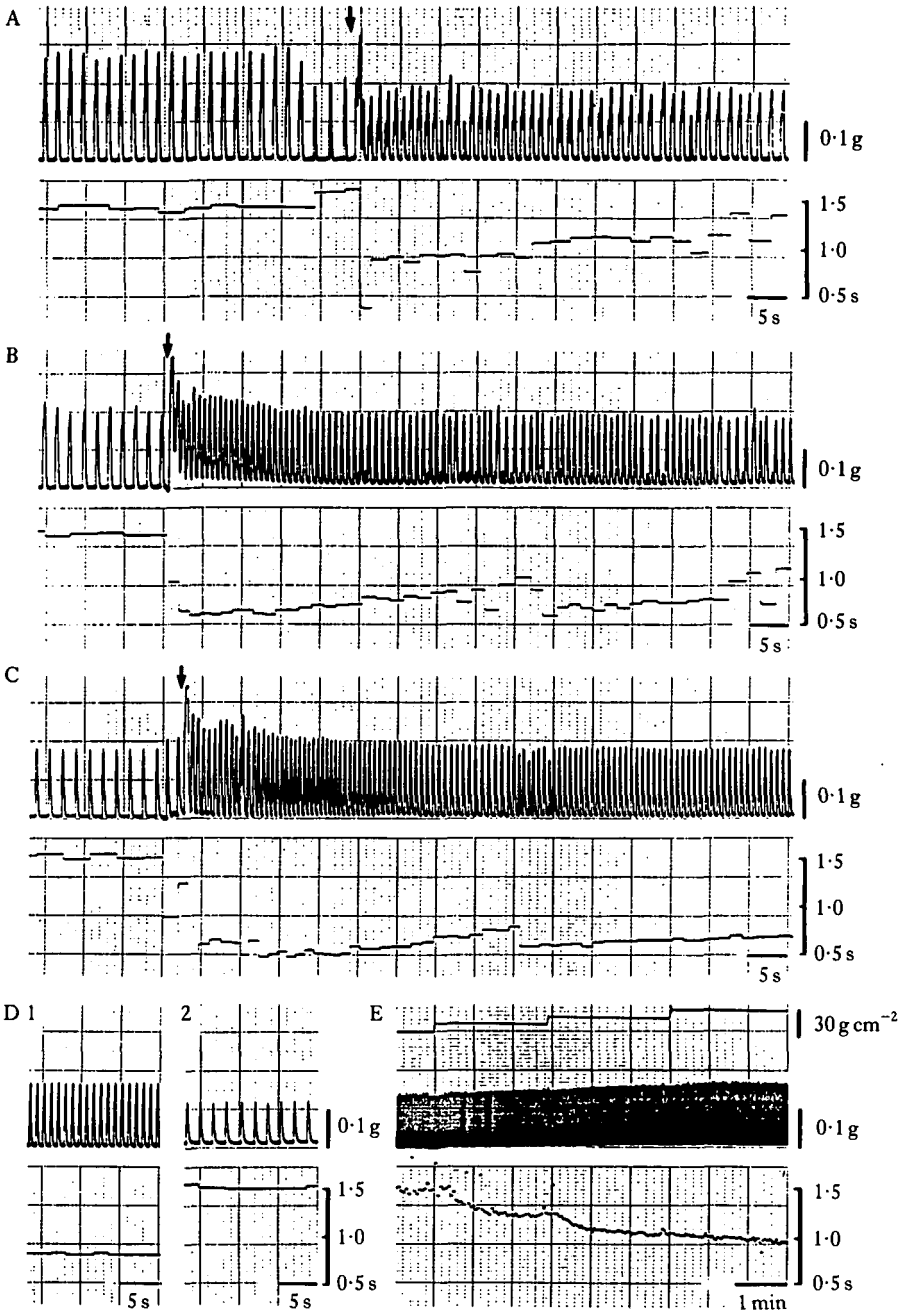

Fig 11

Methylene blue staining confirmed that axons in the trunk were intersected in the cut regions.

After the trunk regions between the 6th and the 7th small cells were cut, systoles showing double contractions (FSC and SSC) were still present although the SSC magnitude became smaller than normal (Fig. 11A, B). On the other hand, double-contraction systoles became single-contraction systoles when the trunk was cut between the 5th large and the 6th small cells or between the 4th and 5th large cells (Fig. 11B, C). This implies that the anterior large cells are capable of the regular production of a single systolic contraction (FSC) in the absence of interaction with the posterior large and small cells. In three hearts in which the SSC was rarely produced (Fig. 1B), no significant changes in the form of the systole were observed following severance of the trunk posterior to the 5th cell (e.g. Fig. 11C). In some of the trunk cutting experiments (10%), the FSC was not maintained but deteriorated to asynchronous small magnitude contractions. This may be due to experimental damage to the cardiac ganglion.

Responses to increases of perfusion pressure, consisting of increases in the frequency and amplitude of the beat without a change in the form of systole, were obtainable after the trunk had been severed at three points (Fig. 11E). The origin of beats was in the anterior trunk including the four large cells. However, about 60% of the hearts were not consistently responsive to pressure after severing the trunk. The surgical procedure may have seriously damaged the sensory portions of the ganglion cells. In successful operations (approx. 40%), the response was similar to those of intact hearts in which the SSC was not prominent (Fig. 1B).

DISCUSSION

Inflation of the isolated or *in situ* crustacean heart increases the amplitude and frequency of its beat (Maynard, 1960). In the isolated heart of *Panulirus japonicus*, reproducible responses to increase or decrease of the perfusion pressure were also obtained. The frequency of the normal beat of the isolated hearts ranged between 0·5–2 Hz. The method for perfusion employed in this study is somewhat different from those used by previous investigators (Matsui, 1955; Maynard, 1960; Cooke, 1966). The isolated heart was suspended in a saline bath and perfused orthodromically through a pair of ostia without cannulae. This method was suitable for recording the natural mechanical and electrical activities from the isolated heart although the pressure applied to ostia was not always the same as the internal perfusion pressure. This technical weak point was overcome by comparing pressure in the perfusion system with the internal pressure in the heart or an artery and with the heart tonus.

Spontaneous slow contractions were recorded in isolated hearts and cardioarterial valves (Fig. 10). In a previous study, we observed spontaneous and/or induced contractions of the cardioarterial valves (Kuramoto & Ebara, 1982). The slow rhythmic contractions were independent of the burst discharges of the cardiac ganglion. Therefore, the slow contractions recorded from the heart preparations in this study, especially when they are quiescent, can be attributed to the valve contractions.

The morphological organization of the cardiac ganglion of *Panulirus japonicus* has been described by Ohsawa (1972). It was possible to sever the trunk without spoilin

the bursting activity of the ganglion cells. After cutting the trunk, regular beating was maintained for several hours, although unusual beating was observed for a few minutes immediately after making the cut (Fig. 11). This suggests that the effects of the injury on the ganglion cell burst discharges declined in a few minutes, and further, that the heart can beat regularly under perfusion with only the anterior region of the ganglion containing the four large cells intact (Fig. 11E).

Almost all cardiac muscle cells of the lobster are activated synchronously by the five large cells (Kuramoto & Kuwasawa, 1980). The 1st–4th cell axons periodically generate an impulse train which may have a double bursting pattern. Each impulse produces large EJPs in the muscle cells. Therefore, the bursts generated in the four anterior cells play a major role in the production of a major depolarization in the muscle cells (the FLP and SLP). This study extends the observations to include the heart contractions. It is seen that the combination of the FLP and the SLP are followed by a similar combination of heart contractions, the FSC and the SSC (Figs 7, 8). Thus, it may be concluded that the heart beat rhythms follow the burst rhythms of the four anterior cells. The small EJPs produced by the 5th cell axons appear to be a minor component in production of large amplitude potentials in the muscle cells but contribute to sustained depolarization of the muscle membranes. The longer sustained depolarization of the muscle membrane was correlated to the larger systolic contraction (Fig. 7B).

The potentials recorded by the suction electrode were field potentials representing EJPs produced synchronously in a number of cardiac muscle cells. By reason of the polyneural and multiterminal innervation of all cardiac muscle fibres by the large cells, the large EJPs seen in a single muscle cell of the heart are roughly representative of the pattern of membrane potential changes in all parts of the heart (Kuramoto & Kuwasawa, 1980). Thus, we find that both the intracellular and extracellular potentials show a similar shape. A change in amplitude or duration of the large EJP recorded in a single muscle cell was indicative of and followed by a change in magnitude of the subsequent heart contraction (Fig. 7). This suggests that the magnitude of contraction of a single muscle fibres may depend on the amplitude and duration of its membrane depolarization as shown in the crayfish contractor epimeralis muscle fibres (Orkand, 1962). An important observation is that major increases in the FSC magnitude following an increase of the perfusion pressure occurred in the absence of equivalent changes of muscle membrane depolarization (Figs 8, 9C). The increases of FSC magnitude were in proportion to the elevation of the tonus tension of the heart within a certain range (0–30 mg in Fig. 9C). It is possible that the stretched muscle fibres produce greater tension for a given depolarization of the muscle membrane (Lang, Sutterlin & Prosser, 1970). Therefore, the magnitude of heart contraction is enhanced by the increased pressure through both the changes in the EJPs resulting from the response of the ganglion and from increased heart tonus representing the stretching of the cardiac muscle fibres. The direct effect on muscle contractile force was the more important.

The frequency of beat increased with the elevation of tonus tension of the neurogenic heart (Fig. 6A). The burst frequency of the cardiac ganglion cells may increase in proportion to stretch of the cardiac muscle fibres. On the other hand, the beat frequency slowed down and often stopped after the pressure was rapidly reduced to zero (Fig. 10A). The rapid drop of pressure might shrink the heart and influence the

burst frequency of the ganglion cells. We have no direct evidence in this species as to where and how the ganglion cells receive the stretch. However, the dendrites onto muscle fibres of the ganglion cells may be sensitive as suggested by Alexandrowicz (1932). Such dendrites are given off by each of the ganglion cells including the large cells. It has been suggested by several investigators that the internal pressure of the heart provides a sensory input to the small cells (Maynard, 1960; Cooke, 1966; Kuramoto & Kuwasawa, 1980). Here, we have shown that pressure can increase the burst frequency of the large cells in the anterior ganglionic trunk in the absence of input from the posterior trunk region, including the small cells (Fig. 11E). This shows that the large cells are also responsive to stretch of cardiac muscle. This being the case, then it is probable that the periodic impulse bursts of large and small cells are affected differently by a change in muscle stretch.

In isolated ganglia, the second bursts in the anterior large cells (represented by the SLP and the resulting SSC) are produced when the small cell burst continues long enough to overcome the relative refractory period for the initial large cell burst (Matsui, Ebara & Ai, 1972; Mayeri, 1973; Tazaki, 1973). Also, the second burst in the large cell is abolished, along with a reduction of the small cell synaptic potentials, after cutting the posterior trunk or after decreasing the perfusion pressure (Kuramoto & Kuwasawa, 1980). The SSC could be recorded in preparations having the anterior trunk including the 6th small cell and the five large cells (Fig. 11D). However, the SSC is abolished after a rapid drop in the pressure or a cut in the trunk which reduces synaptic input from the small cells to the anterior large cells.

The SSC was abolished when the beat frequency exceeded approximately 1 Hz under high tonus tension (more than 30 mg) (Figs 3B, 6A). This suggests that the large cells rarely produce the second burst when the burst frequency is higher than 1 Hz. The burst duration of the small cells may be decreased at high burst frequency and thus the second burst is not initiated in the large cells. In the isolated ganglia of *Homarus americanus*, the burst duration of the small cells is decreased by electrical stimulation (Mayeri, 1973). We observed that the small cell burst duration decreased markedly under high pressure when recorded from the posterior trunk by a suction electrode (Kuramoto & Ebara, 1979). The resting potential of a large cell soma decreased and increased over a range of 10 mV when the pressure was slowly increased and then decreased (unpublished observations). It is therefore probable that the large cell burst is affected not only by input from the small cells but also by the sensory portions of the large cells during changes of perfusion pressure.

The amount of increase in the beat frequency with increase of tonus appeared to be related to the presence or absence of the SSC (Fig. 6A). It has been shown that an endogenously bursting neurone in crustaceans has a linear relationship between burst duration and the interburst period during responses to various perturbations (Davis, 1971; Benson, 1980). For example, a longer burst duration of large cells is related to a smaller increase in burst frequency. When the burst duration of large cells is elongated by a long duration of the small cell burst, the SSC is produced. Therefore, the beat frequency would be expected to increase less for a given increase in tonus when the SSC is present than when it is not. This hypothesis is consistent with the data that the slopes of response curves are divided into two groups according to the presence or absence of the SSC (Fig. 6A).

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