Polysensory response characteristics of dorsal root ganglion neurones that may serve sensory functions during myocardial ischaemia

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Abstract

Objective: To determine the response characteristics of dorsal root ganglion neurones that may serve sensory functions during myocardial ischaemia. Methods: Extracellular recordings were made from 54 spontaneously active and 5 normally quiescent dorsal root ganglion neurones (T\textsubscript{2}-T\textsubscript{5}) in 22 anaesthetized open-chest dogs under control conditions and during epicardial mechanical or chemical stimulation and myocardial ischaemia. Results: The activity of 78\% of spontaneously active and all quiescent neurones with left ventricular sensory fields was modified by left ventricular ischaemia. Forty-six spontaneously active neurones (85\%) were polysensory with respect to mechanical and chemical stimuli. The 5 quiescent neurones responded only to chemical stimuli. Spontaneously active neurones associated with left ventricular mechanosensory endings (37 neurones) generated four different activity patterns in response to similar mechanical stimuli (high or low pressure active, high–low pressure active, high–low pressure inactive). A fifth group generated activity which was not related to chamber dynamics. Adenosine, adenosine 5'-triphosphate, substance P and bradykinin modified 72, 61, 65 and 63\% of the spontaneously active neurones, respectively. Maximum local mechanical or chemical stimuli enhanced activity to similar degrees, as did ischaemia. Each ischaemia-sensitive neurone displayed unique activity patterns in response to similar mechanical or chemical stimuli. Conclusions: Most myocardial ischaemia-sensitive dorsal root ganglion neurones associated with epicardial neurites sense mechanical and multiple chemical stimuli, a small population sensing only mechanical or chemical stimuli. Activity patterns generated by these neurones depend on their primary sensory characteristics or those of other neurones that may converge on them, as well as the type and magnitude of the stimuli that impinge upon their sensory fields, both normally and during ischaemia.

Keywords: Myocardial ischemia; Neurotransmission; Adenosine; Bradykinin; Dog, anesthetized

1. Introduction

Most cardiac sensory neurites that are associated with afferent axons in sympathetic nerves display polymodal behaviour, responding to mechanical and chemical stimuli \[1-3\]. Few respond only to chemical stimuli \[3\]. Myocardial ischaemia is known to activate the sensory nerve endings of some of these axons \[4\]. Myocardial ischaemia enhances myocardial release of adenosine and adenosine 5'-triphosphate (ATP) \[5,6\]. Adenosine has been implicated in the genesis of pain elicited by myocardial ischaemia in man \[7,8\]. Although adenosine has been reported to induce minor \[9\] or no \[10\] change in activity generated by cardiac afferent axons in feline thoracic rami, the epicardial sensory endings of some canine dorsal root ganglion afferent neurones are sensitive to this chemical \[11\].

Another endogenously released algesic agent, substance P, exerts no effect on cultured mouse dorsal root ganglion cardiac afferent neurones \[12\] or the sensory endings of cardiac afferent axons in feline thoracic rami \[3\]. However, it can enhance nociceptive symptoms in man induced by intracoronary administration of adenosine \[7\]. The peptide bradykinin activates the sensory fields of nodose ganglion
epicardial afferent neurones [13] and the sensory endings of afferent axons in sympathetic nerves [1,10]. We sought to determine the response characteristics of ischaemia-sensitive dorsal root ganglion neurones with epicardial neurites to myocardial ischaemia and to various mechanical and chemical (adenosine, ATP, substance P and bradykinin) stimuli.

Activity generated by neurones in situ can be recorded for a number of hours using extracellular recording techniques, thereby permitting multiple stimuli to be applied to their sensory endings [13]. Thus, we used extracellular recording techniques to determine the response characteristics of individual ischaemia-sensitive dorsal root ganglion epicardial afferent neurones to a variety of stimuli.

2. Methods

2.1. Animal preparations

Using techniques already described [11], we recorded the activity of neurones in dorsal root ganglia at the T2–T5 levels of the spinal cord, the intramyocardial pressures of the left and right ventricles, the chamber pressures of the left ventricle and left atrium, and the respiratory pressure in 22 mongrel dogs (9–15 kg and of either sex) that were tranquilized and anaesthetized as described before [13]. Following intubation, respiration was maintained by means of a Bird Mark 7 positive pressure respirator (Bird Corporation, Palm Springs, CA). Following a bilateral thoracotomy we made cranial to caudal slits in the left and right sides of the pericardial sac and used sutures to tether these edges to the thoracic wall, so that the ventricular and atrial surfaces could be visualized. We prevented the heart from drying by covering the epicardium with saline-soaked gauze; when we later removed the gauze, we used saline flushes to keep the epicardial surface moist.

So that we could occlude each major left coronary artery without mechanically distorting adjacent tissues and damaging the afferent nerves which accompany their origins, threads were placed around the left anterior descending and circumflex coronary arteries about 1 cm from their origins. Each thread was led through a short segment of polyethylene tubing. We also placed three pieces of umbilical tape, each led through a piece of polyethylene tubing, around the descending aorta and the superior and inferior vena cavae, so that we could occlude each of these vessels. One miniature solid-state pressure transducer (Konigsberg Instruments, model P19D, Pasadena, CA) was inserted into the right ventricular conus and another was inserted into the ventral wall of the left ventricle to record regional right and left ventricular intramyocardial pressures. Left ventricular and left atrial chamber pressures were measured via two catheters inserted into these chambers and connected to Bentley Trantec model 800 transducers (Bentley Trantec Inc., Irvine, CA).

Thereafter, we turned the dogs over and performed a dorsal laminectomy to expose the left (in 12 dogs) or right (in 10 dogs) dorsal root ganglia at the T2–T5 levels of the spinal cord, while leaving the dura of the spinal cord intact. To stabilize these ganglia, we secured the T1 and T6 vertebral processes, using a spinal cord stereotaxic apparatus, so that the dogs were suspended via their spinal processes. Tissues surrounding each ganglion were not disrupted, and no movement of the ganglion was detected. The exposed structures—the ganglia, their roots, and the dura—were covered with mineral oil.

At the end of each experiment, the dog was killed with an overdose of Euthanyl Forte, administered intravenously.

2.2. Neuronal recording

A tungsten microelectrode (10 mm diameter and exposed tip 50 mm; impedance of 9–11 MΩ at 1000 Hz) was driven into exposed dorsal root ganglia in micrometre increments; the indifferent electrode was attached to structures adjacent to the ganglia. Action potentials with signal-to-noise ratios of 3:1 or greater recorded by this means are derived from cell bodies and/or dendrites, not from axons of passage [11,13]. They were differentially amplified by an amplifier (Princeton Applied Research, model 113, Princeton, NJ, USA) that had bandpass filters set at 300 to 10 kHz and an amplification range of 100–500 × . The output of this device was further amplified (50–200 ×) and filtered (band width 200–10 kHz) by an optically isolated amplifier (Applied Microelectronics Institute, Halifax, NS, Canada). The lack of motion of the ganglia made it possible to record action potentials from a site for several hours. Action potentials were deemed to be generated by a single neurone if they (1) displayed the same configuration and amplitude for several hours and (2) maintained the same wave form, albeit with a different amplitude, when the microelectrode was moved micrometres away from the site where maximal activity was recorded. At the end of the experiments, we determined the conduction time of afferent axons connected to the studied neurones by delivering electrical stimuli (1–4 V, 1 ms, 0.1–5 Hz) to associated epicardial sensory fields (see below), using a unipolar ball electrode and an indifferent electrode attached to the thoracic wall. The neurone’s latency of activation was considered to be the time visualized on the oscilloscope between the stimulus artifact and the beginning of the generated action potential. We also estimated the axons’ conduction velocities, using an estimate of the anatomical distance between the stimulating and recording electrodes that we made by measuring the length of a thread placed over the putative route of afferent pathways between the sensory field and the ganglion studied (from the epicardium, via the cardiopulmonary nerve, ansa, and sympathetic chain, through the muscle, to the ganglion).
2.3. Mechanical stimuli

First, we gently probed various loci on the atrial or ventricular epicardium, using a cotton-tipped applicator that had been soaked in saline. By taking care that any such distortion of cardiac tissues did not also distort the great thoracic vessels or other adjacent mediastinal tissues, we were assured that changes in neuronal activity that occurred after these epicardial loci were distorted occurred because the neurones were directly or indirectly [14] associated with neurites in the epicardium. Thereafter, we occluded the aorta and the superior and inferior vena cavae individually (5–20 s). Neurones whose activity was modified by mechanically induced pressure changes were considered to be associated with mechanosensory neurites. To determine if respiratory dynamics affected the activity generated by these neurones, we altered respiratory rate and then pressure, after which respiration was interrupted for 60 s.

2.4. Chemical stimuli

Purinergic compounds and peptides were then applied for 60–120 s to various loci throughout the heart. The chemicals, obtained from Sigma Chemical Co. (St. Louis, MO, USA), were dissolved in 0.5 ml of normal saline at room temperature and were applied in random order to the epicardium via 1 cm × 1 cm gauze squares. After each had been applied, we flushed the locus being investigated with normal saline (~ 2 ml/s for at least 20 s) to wash all of the applied chemical from the sensory field; the fluid was removed from the thorax by dependent drainage. Depending on the neuronal response elicited, we allowed 5–40 min to elapse between chemical applications to enable the preparation to stabilize. A chemical was reapplied if it elicited a response. Gauze squares soaked with normal saline at room temperature were also applied to the preparation to stabilize. We tested the neurones’ sensitivity to ischaemia after we had applied the mechanical and chemical stimuli, lest their responses to the individual stimuli be altered by changes to the neurones or the myocardium that ischemia produced. If occluding the coronary arteries did not induce ventricular fibrillation, we induced it by applying electrical stimuli (10 V, 1 ms, 100 Hz) to the epicardium in order to determine how ventricular fibrillation affected the activity of afferent neurones studied. Thereafter the ventricles were emptied of blood and squeezed in order to determine if global mechanical distortion influenced neuronal activity.

2.5. Coronary artery occlusion

After completing the interventions described above, we occluded the left anterior descending coronary artery for 1–10 min, then the circumflex coronary artery for 1–10 min, and then both arteries simultaneously for 1 min, waiting at least 10 min between occlusions to allow the preparation to stabilize. We tested the neurones’ sensitivity to ischemia after we had applied the mechanical and chemical stimuli, lest their responses to the individual stimuli be altered by changes to the neurones or the myocardium that ischemia produced. If occluding the coronary arteries did not induce ventricular fibrillation, we induced it by applying electrical stimuli (10 V, 1 ms, 100 Hz) to the epicardium in order to determine how ventricular fibrillation affected the activity of afferent neurones studied. Thereafter the ventricles were emptied of blood and squeezed in order to determine if global mechanical distortion influenced neuronal activity.

2.6. Data acquisition and analysis

Neuronal activity, a lead II electrocardiogram, left atrial and left ventricular chamber pressures, right and left ventricular intramyocardial pressures and tracheal pressure were recorded simultaneously on an 8-channel rectilinear recorder (Astro-Med, Inc., model MT 9500, West Warwick, RI, USA). A videocassette recorder (A.R. Vetter
co., model 820, Rebersburg, PA, USA) stored the data on VHS videotape for later analysis.

Only data from neurones that were associated with sensory terminals in the epicardium and that satisfied the criteria described above, and that continued to function throughout the period of time required for all interventions to be performed (about 6–8 h) were included in this study. Thus, although we found 10–20 neurones that generated spontaneous activity throughout each dorsal root ganglion studied, only the activity generated by 59 neurones in 22 dogs (28 in right T2–T5 ganglia of 10 dogs and 31 in left T2–T5 ganglia of 12 dogs, an average of 3 neurones per dog) was analysed. Data derived from 5 additional dogs were not analysed: in 2, we could not satisfactorily identify neurones associated with epicardial sensory fields; in another 3, we identified such neurones, but they became inactive during or after the first ischaemic episode.

Cardiac variables were measured for 30 s and their means ± s.e.m. were calculated. Neuronal activity was counted for 60 s immediately before each intervention and for 60 s during the maximal responses the intervention elicited. When two neurones were found to be active at a locus (this only occurred when quiescent neurones became active), the activity generated by individual units was analysed by means of a window discriminator (C.J. Hartley Instrumentation Development Laboratories, Baylor College of Medicine, Houston, TX, USA). We considered activity changes of greater than 10% to constitute a response, including changes that occurred after reperfusion had begun. Student’s t-test was used to analyze the significance of these changes. Contingency tables with corrections for continuity were constructed, so that neuronal responses elicited by each chemical could be compared to those elicited by another chemical and to those induced by mechanical stimuli or coronary artery occlusion. A significance value of $P < 0.01$ was used for these determinations.

### 3. Results

#### 3.1. Overview (Figs. 1–7)

Each afferent neurone from which data were analysed was affected idiosyncratically by the various stimuli that were applied to the epicardial sensory endings that were associated with that neurone. Of the 59 neurones identified with epicardial neurites, 54 displayed spontaneous activity (1.8 ± 0.6 impulses/second [ips]; range, 0.4–16 ips) and 5 (each of which was adjacent to a spontaneously active neurone) were quiescent during control periods. Forty-six (85%) of the spontaneously active neurones were polysensory (i.e., they responded both to altered cardiology and to local chemical stimuli), whereas 5 neurones responded only to mechanical stimuli and 3 responded only to chemical stimuli. The quiescent neurones were activated only by chemical stimuli. Approximately half of the chemosensitive neurones were associated with sensory fields located on more than one chamber.

Electrical stimulation of epicardial sensory fields activated 47 (87%) of the spontaneously active neurones (Fig. 1 inset and Fig. 3F). Action potentials were generated after a fixed latency when the frequency at which the field was stimulated varied between 0.1 and 5 Hz. The conduction velocity that we calculated using the estimated lengths of the axons associated with the neurones ranged from 0.61 to 6.4 m/s (mean, 2.7 ± 0.4 m/s). The activity pattern a neurone generated bore no relationship to whether the axon with which it was associated was type B or type C according to the Erlanger-Gasser classification scheme (the conduction velocity of type B fibres is 2–14 m/s; that of type C fibres is < 2 m/s [15]).

#### 3.2. Coronary artery occlusion

For 37 of the 46 spontaneously active polysensory neurones, we were able to identify associated mechanosensitive fields in the left ventricle. The activity generated by 29 of these neurones was modified when transient left ventricular ischaemia was induced (Fig. 1), increasing (on average) by 263% in 25 and decreasing by 60% in 4 cases (Table 1). The activity of the 5 quiescent neurones increased from 0 to 15.3 ± 5.7 ips (range, 9–38 ips) during ischaemia. The 3 spontaneously active neurones associated with only chemosensitive fields were activated during ischaemia. All ischaemia-sensitive neurones were affected by epicardial application of at least one of the chemicals studied.

The mean latency of visually detectable changes in neuronal activity that occurred after ischaemia had been induced in spontaneously active neurones was 15 s (range, 10–29 s). Neuronal changes lasted up to 45 min after reperfusion began. The activity generated by 8 neurones increased even more during reperfusion than during coronary arterial occlusion, the mean latency for onset of such responses being 58 ± 29 s after reperfusion began.

In general, left ventricular intramyocardial and chamber systolic pressures and left ventricular chamber end-diastolic pressure did not change when ischaemia was induced; occasionally, however, and particularly when two coronary arteries were occluded simultaneously, the systolic pressures decreased or the end-diastolic pressure increased (Fig. 1). In 3 dogs, heart rate and left ventricular systolic pressure increased minimally during reperfusion.

Ventricular fibrillation occurred during coronary artery occlusion or upon reperfusion in 17 of 22 dogs. The activity generated by 13 (35%) of the ischaemia-sensitive neurones that were associated with left ventricular sensory fields increased further as fibrillation persisted, with the peak activity elicited during fibrillation being 205 ips (Fig. 3). Neuronal activity persisted for at least 20 min after
Fig. 1. Activity generated by a neurone that was located in a left T7 dorsal root ganglion and was associated with mechanosensory neurites on the ventral surface of the left ventricle. In control periods, activity occurred early in isovolumetric contraction or at the end of diastole every 2–11 cardiac cycles. During ischaemia (occlusion of the circumflex and ventral descending coronary arteries began at arrow below), activity occurred during most cardiac cycles early in diastole (fast trace on right). Activity increased when left ventricular systolic pressure increased, when the receptive field was touched or when adenosine and bradykinin were studied (not shown). Inset (lower right): activity recorded 69 ms after delivery of an electrical stimulus (artifact on the left) to an associated sensory field in the epicardium of the left ventricle (estimated conduction velocity of the associated axon was 4.2 m/s).

EKG = electrocardiogram; LAP = left atrial chamber pressure; Resp = tracheal pressure; LVP = left ventricular chamber pressure; Neuro = afferent neuronal activity. The same abbreviations are used in Figs. 3, 4, 5 and 7. Vertical calibration bar to the left of the neurogram = 0.1 mV.

fibrillation had been induced and was unaffected by the ventricles being emptied of blood.

3.3. Mechanical stimuli

The activity generated by all 46 spontaneously active polysensory neurones was modified by aortic occlusion and that of 45 (98%) was modified by vena cava occlusion. Changes in neuronal activity began as soon as the aorta was occluded (Fig. 5) and subsided soon after this stimulus was removed. Changes that occurred after chemical stimuli were administered developed over time and lasted long (up to 32 min) after the stimulus was removed. Reapplying vascular occlusions induced similar changes in neuronal activity. In control states, mechanosensory neurones frequently generated activity which related to the

Fig. 2. Activity was generated by a quiescent afferent neurone (as presented by window discriminated activity) located adjacent to a spontaneously active neurone in a left T7 dorsal root ganglion following epicardial application of adenosine and ischaemia. (A) Adenosine (10 μM), when applied to the lateral surface of the left ventricle (between arrows), induced activity (maximum of 38 ips). (B) After 20 min had elapsed, this neurone became active about 2 min after circumflex coronary artery occlusion began (onset at arrow).
Fig. 3. Mechanical-, chemical- and ischaemia-sensitive left atrial afferent neurone. (A) Activity generated by an afferent neurone in a left T₅ dorsal root ganglion increased when its ventral left atrial sensory field was touched (T, between arrows). (B) Occlusion of the inferior vena cava (IVC Occ) reduced left atrial pressure and increased neuronal activity (0.1 to 54 Hz). Activity was enhanced when substance P (C), adenosine (D) or ATP (not shown) was applied to its sensory field. (E) Ischaemia induced by left anterior descending coronary artery occlusion (onset at arrow) induced ventricular fibrillation which was followed by increased activity (to 205 Hz). (F) Unipolar electrical stimulation of the sensory field activated the neurone. The conduction velocity of the axon connecting this neurone to its sensory field was estimated to be 3.3 m/s. Vertical calibration beside neuronal activity (Neuro) = 0.5 mV.

Fig. 4. Activity generated by a mechanical- and chemical-sensitive afferent neurone in a right T₂ dorsal root ganglion associated with a right ventricular conus sensory ending. (A) Activity increased from 4 to 22 ips when substance P (10 μM) was applied to its sensory field (arrow) even though cardiac variables were unchanged. (B) Gently touching its sensory field (touch, between arrows) excited this neurone. (C) Application of ATP (10 μM, between arrows) to its epicardial receptive field increased activity. Cardiovascular-related and respiratory-related activity continued to be generated when activity was enhanced by chemicals. This neurone was not affected by adenosine or bradykinin. The estimated conduction velocity of its axon was 2.4 m/s. Vertical calibration beside neuronal activity = 0.2 mV.
cardiac cycle, not necessarily during every cardiac cycle (Fig. 1). The activity generated by 35 of the 46 spontaneously active polysensory neurones increased (from 1.7 ± 0.6 ips to 5.5 ± 2.6 ips; P < 0.01) when their sensory fields were touched, and that of 9 decreased (from 2.1 ± 0.6 ips to 0.6 ± 0.2 ips; P < 0.01). No preferential distribution of epicardial sensory fields was identified with respect to neurones in left as opposed to right dorsal root ganglia. The mechanosensitive fields—approximately 1–4 cm² in area—of 37 of these 44 neurones were located in the ventral, lateral or dorsal surfaces of the left ventricle. Six had sensory fields in the right ventricular conus, 3 had sensory fields in the right ventricular sinus and 9 had sensory fields in the left atrium. Thus, 9 neurones were associated with mechanosensitive fields in two cardiac chambers.

The 37 neurones associated with mechanosensitive nerve endings in the left ventricle generated 5 types of response to similar changes in left ventricular chamber pressure: (I) 14 were 'high pressure active', generating greater activity as left ventricular chamber and intramyocardial systolic pressures rose, less activity as these pressures fell (Fig. 5A), and no activity during systemic vascular hypotension; (II) 9 were 'low pressure active', generating greater activity as left ventricular systolic pressure fell and less activity as it rose (Fig. 5B); (III) 7 were 'high/low pressure active', generating greater activity when left ventricular chamber systolic pressure was either above or below the physiological range of 88–125 mmHg (Fig. 7A) and minimal activity when it was within the physiological range; (IV) 5 were 'high/low pressure inactive', generating reduced (or no) activity when left ventricular chamber systolic pressure deviated from physiological values and maximal activity when it was within the physiological range (Fig. 7B); and (V) two neurones generated activity that was not related to the cardiac or respiratory cycles in any detectable fashion. The activity patterns generated by 15 of these neurones were stable enough during mechanical interventions that we could subject them to graphic descriptions; the activity patterns of the others were less stable because we had difficulty maintaining relatively stable changes in systolic pressure during vascular occlusions for the 10–20 s needed to obtain steady-state activity patterns. Post-stimulus responses—activity changes that persisted after the pressure change ended—lasted 3–15 s (Figs. 3 and 5A).

The 9 neurones that were associated with sensory fields in the right ventricle displayed type I activity patterns when right ventricular pressure changed. Three of these neurones generated activity that was related simultaneously to the dog's respiratory rate and heart rate (Fig. 4); the latter ceased, along with changes in right ventricular intramyocardial systolic pressure oscillations that were respiration-related, when artificial ventilation ceased. These neurones' activity was affected when epicardial, but not respiratory, tissues were distorted (Fig. 4B).

Seven of the 9 neurones that were associated with mechanosensitive endings in the left atrium generated type I activity patterns when left atrial pressure was changed; the other two displayed type II activity patterns (Fig. 3B).
3.4. Chemical stimuli

The activity generated by all 49 chemosensitive spontaneously active neurones (46 of which were polysensory) and by all 5 quiescent neurones was modified by more than one of the 4 chemicals studied. About 80% of chemosensitive afferent neurons responded to more than one chemical. The frequency with which such changes occurred (and their magnitude) did not vary significantly from one chemical to another. When a chemical was applied to a chemosensory field a second or third time, the responses were similar to those that followed the first application; when saline was applied, no alteration in activity was detected.

Cardiodynamics were not changed overall when chemicals were applied to the epicardium. Twelve neurones were associated with sensory fields that were adjacent to the intramyocardial pressure sensor in the left ventricle and two were associated with sensory fields adjacent to the pressure sensor in the right ventricle. In these instances local chemical stimuli did not affect regional intramyocardial pressure (for instance, left ventricular intramyocardial systolic pressure was $108 \pm 11$ mmHg both before and after chemical application), whereas mechanical stimuli

Table 1
Activity generated by 46 polysensory spontaneously active dorsal root ganglion afferent neurones during control states (Control) as well as in response to ischaemia, vascular occlusions and chemical stimuli

<table>
<thead>
<tr>
<th>Interventions</th>
<th>No. of neurones responding</th>
<th>No. of neurones</th>
<th>Increased activity (impulses/second)</th>
<th>Decreased activity (impulses/second)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>Intervention</td>
</tr>
<tr>
<td>Coronary artery occlusion</td>
<td>29/37 (78%)</td>
<td>25</td>
<td>2.2 ± 1.6</td>
<td>8.0 ± 2.9 $^*$ ($+263%$)</td>
</tr>
<tr>
<td>Aortic occlusion</td>
<td>23 (type I and II)</td>
<td>14</td>
<td>1.9 ± 0.6</td>
<td>12.0 ± 4.7 $^*$ ($+531%$)</td>
</tr>
<tr>
<td>IVC or SVC occlusion</td>
<td>45/46 (98%)</td>
<td>19</td>
<td>1.4 ± 0.6</td>
<td>10.6 ± 4.0 $^*$ ($+657%$)</td>
</tr>
<tr>
<td>Epicardial adenosine</td>
<td>33/46 (72%)</td>
<td>30</td>
<td>1.5 ± 0.4</td>
<td>9.1 ± 2.1 $^*$ ($+506%$)</td>
</tr>
<tr>
<td>Epicardial ATP</td>
<td>28/46 (61%)</td>
<td>23</td>
<td>2.4 ± 1.8</td>
<td>9.8 ± 3.2 $^*$ ($+308%$)</td>
</tr>
<tr>
<td>Epicardial substance P</td>
<td>30/46 (65%)</td>
<td>25</td>
<td>1.6 ± 1.1</td>
<td>6.8 ± 2.8 $^*$ ($+325%$)</td>
</tr>
<tr>
<td>Epicardial bradykinin</td>
<td>29/46 (63%)</td>
<td>26</td>
<td>1.8 ± 1.1</td>
<td>7.2 ± 2.6 $^*$ ($+300%$)</td>
</tr>
</tbody>
</table>

$^*$ $P < 0.01$. 

Data obtained from 5 spontaneously active neurones responsive to mechanical stimuli only, 3 spontaneously active neurones responsive to chemical stimuli only and 5 quiescent neurones are not included. Due to opposing responses induced by types III and IV neurones, aortic occlusion data were derived only from high pressure (type I, $n = 14$) and low pressure (type II, $n = 9$) active afferent neurones ($n = 23$).
did. If the fields associated with polysensory neurones were touched when neuronal activity was enhanced by epicardial application of a chemical, the activity increased even more. Cardiodynamics also affected neuronal activity when the activity was enhanced by chemical stimuli (Fig. 4A,C).

### 3.4.1. Purinergic compounds

Purinergic compounds increased the activity generated by most, but not all, spontaneously active polysensory neurones (Table 1). Purinergic compounds activated 3 of the quiescent neurones. Adenosine (average 9.1 ± 2.1 ips; peak 61 ips) and ATP (average 9.8 ± 3.2 ips; peak 49 ips) induced similar enhancement of activity. Adenosine altered the activity of each ischemia-sensitive spontaneously active neurone that was associated with a sensory field in the left ventricle. The mean latency of onset of the neuronal responses elicited after the epicardial application of adenosine and ATP was 8 ± 3 and 18 ± 5 s, respectively. The responses lasted 2.5–8 min after we removed the chemicals. Most polysensory neurones were activated by both purinergic agents; 3 generated opposing responses to the two purinergic agents. Epicardial application of β,γ-mATP increased the activity of the 10 neurones so studied (from 1.8 ± 0.6 to 8.6 ± 2.4 ips; P < 0.01). When the purinergic agents were administered systemically, a few previously inactive neurones that had not been affected by any of the interventions described above were activated. As we could not be sure whether these responses were induced directly or indirectly, however, we did not analyze them.

### 3.4.2. Peptides

Epicardial application of substance P or bradykinin modified the activity generated by 65 and 63% of the

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**Fig. 7.** Type III (above) and IV (below) left ventricular mechanosensory afferent neuronal responses. (A) High and low pressure-active cardiac afferent neurone response (Type III) generated by an afferent neurone in a right T3 dorsal root ganglion with neurites located in the left ventricular ventral wall. Maximum activity occurred when left ventricular chamber systolic pressure decreased during occlusion of inferior vena cava (between arrows on left) or increased when the descending aorta was partially occluded (between arrows on right). (B) A Type IV response (high and low pressure-inactive) generated by an afferent neurone in a right T2 dorsal root ganglion associated with neurites in the left ventricular ventral epicardium. Activity decreased when left ventricular chamber systolic pressure decreased after the inferior vena cava was occluded (between arrows, first panel) or when that pressure increased during aorta occlusion (between arrows on the right of the second panel). It also decreased when left ventricular systolic pressure was reduced spontaneously (asterisk, second panel). The neurone in the upper, but not lower, panel was sensitive to chemicals.
polysensory neurones, respectively, increasing the activity in most (Figs. 3 and 4) but not all instances (Table 1). These chemicals activated 4 of the 5 quiescent neurones. The peak activity achieved after the epicardial application of bradykinin and substance P was 38 and 61 ips, respectively. The mean latency of neuronal response onset induced by substance P (18 ± 6 s) was less than that induced by bradykinin (33 ± 6 s). Responses induced by peptides lasted 4–28 min after the chemical was removed. Application of the substance P receptor antagonist, spantide, to sensory fields associated with 10 neurones that were sensitive to substance P reduced their activity (from 0.9 ± 0.3 to 0.4 ± 0.2 ips; P < 0.01). Reapplying substance P to the sensory fields of these neurones in the presence of spantide failed to excite the neurones (activity remained at 0.5 ± 0.3 ips), but applying bradykinin and mechanical stimuli did. The simultaneous application of mechanical and chemical stimuli induced activity changes in multiple frequencies simultaneously (Fig. 4), the frequencies generated thereby usually occurring at 0.2, 1.1, 4.0, 19, and 40 Hz.

4. Discussion

Each neurone in dorsal root ganglia studied that was affected by myocardial ischaemia generated unique activity responses to similar mechanical and chemical stimuli. Although the great majority of such neurones that we identified generated spontaneous activity, a few remained inactive in physiological states. On average, the activity of spontaneously active neurones increased 2.6-fold when ischaemia was induced (Table 1); that of quiescent neurones increased from 0 to 15.3 ips. In accordance with previous reports [16–19], when the neurones we studied had associated neurites in the ventricles they were located in the cranial third of the ventricles.

A population of neurones in the dorsal root ganglia may not be connected to epicardial sensory neurites directly [14]. It seems unlikely, however, that this was the case with the neurones we studied, as fixed latencies were elicited when the sensory fields associated with the neurones were stimulated electrically at different frequencies. Had multiple synapses been interposed between the sensory fields and the neurones, this would not have been the case.

Increases in the activity generated by axons in sympathetic rami during coronary artery occlusion have been ascribed to regional mechanical bulging (i.e., local stretch of sensory terminals) and metabolic changes [2,4]. The increased activity that the polysensory neurones we studied generated in response to ischaemia did not appear to be primarily due to changes in the mechanical milieu of their sensory fields as left ventricular intramyocardial systolic pressure became (if anything) depressed, particularly when two arteries were occluded (Fig. 1). Left ventricular chamber pressure became (if anything) depressed, particularly when two arteries were occluded (Fig. 1).
ganglion neurones, however, the peak activity levels generated in response to the application to the epicardium of maximal mechanical and chemical stimuli were equivalent (Table 1). Moreover, the activity these neurones generated in response to myocardial ischaemia was an order of magnitude greater than that which nodose ganglion neurones that were sensitive to myocardial ischaemia generated when ischaemia was induced [13].

Adenosine, when applied in high doses (0.5–5 mM) to the feline epicardium, induces no change [10] or a brief, minor increase (1.4 ± 0.4 to 3.6 ± 1.3 ips) in the activity of sympathetic afferent axons [9]. The maximum activity that adenosine induced in feline cardiac sympathetic afferent axons was in the range of that found in control states in canine dorsal root ganglion neurones associated with epicardial neurites. In the present study each spontaneously active dorsal root ganglion neurone associated with left ventricular neurites and sensitive to myocardial ischaemia was affected when 10 μM adenosine, a concentration similar to that found in canine coronary venous blood during ischaemia [22], was applied to the epicardium. Most quiescent neurones were activated by adenosine as well. Epicardial application of ATP also affected dorsal root ganglion neurones (Table 1). It is unlikely that responses elicited by ATP were due to the breakdown of ATP to adenosine in all cases as only 48% of afferent neurones sensitive to ATP responded to adenosine, some responding in an opposite direction. The fact that the stable analog β,γ-mATP which does not readily degrade to adenosine activated afferent neurones supports this contention.

Although substance P has been reported to exert no effect on sensory endings of cardiac spinal afferent axons [3] or dorsal root ganglion neurones in culture [12], more than half of the dorsal root ganglion neurones studied responded to epicardial application of substance P (Table 1). In accord with that, substance P immunoreactivity has been associated with thoracic dorsal root ganglion neurones and cardiac afferent nerve terminals [30]. In every case but one cardiac variables were unaffected by substance P; in one case left ventricular systolic pressure increased minimally. Thus afferent neuronal responses induced by substance P were not secondary to systemic vascular hypotension resulting from substance P entering the circulation in sufficient quantities to directly affect peripheral vessels. That some neurites of afferent neurones possessed substance P receptors was supported by the finding that neuronal activity was suppressed following receptive field application of the selective substance P receptor antagonist, spantide, and that substance P failed to modify neurones in the presence of this antagonist. Bradykinin excites cardiac sympathetic afferent sensory endings [1]. Epicardial application of bradykinin modified the activity generated by a population of afferent neurones after 33 s, a longer latency than occurred when the other chemicals were tested or is induced by bradykinin in cats [10]. Perhaps the neurites of these neurones respond differentially to these chemicals or perhaps bradykinin-sensitive ones were located at a greater depth below the epicardial surface than substance P or purine-sensitive ones. If bradykinin induced the release of substance P from sensory nerve endings to generate such responses [31], this was not the case with respect to all neurones studied since 41% of bradykinin-sensitive neurones were not modified by substance P. This finding is supported by the fact that bradykinin activated the sensory endings of neurones in the presence of spantide. Some substance-P-sensitive afferent neurones were not influenced by bradykinin as well. Epicardial mechanical stimuli influenced neuronal activity which had been enhanced by chemical stimuli. In agreement with that, normal mechanical perturbations continued to influence neuronal activity which was increased by chemical stimuli (Fig. 4A,C). Simultaneous application of mechanical and chemical stimuli to the receptive fields of polysensory neurones synergistically enhanced activity occurring at fixed frequencies. Thus the profile of the activity patterns so generated depended on the response characteristics of the studied neurone to such stimuli as well as the type and intensity of the stimuli applied (Fig. 4).

Although it has been reported that quiescent afferent neurones in dorsal root ganglion are not associated with the heart [1,2], 5 neurones which proved to be sensitive to chemical stimuli were inactive during control states and when mechanical stimuli were applied (Fig. 2). The lack of activity generated by these neurones for over an hour prior to and after application of chemical stimuli presumably was not due to the fact that insufficient mechanical stimuli were applied to their sensory endings since they were not activated when their sensory fields were distorted locally or when, for instance, left ventricular systolic pressure rose above 180 mmHg.

One limitation of the present study is the fact that only neurones with epicardial sensory fields were studied. This was done so that stimuli could be applied to neurone sensory fields while minimizing indirect effects consequent to altering cardiac dynamics such as occurs when chemical stimuli are administered systemically. The sensory fields of some ventricular afferent neurones are located deep within the myocardium [16]. Perhaps some of the neurones studied had sensory fields located deeper within the myocardium than the epicardium. The extent of such receptive field anatomy was not studied. Pathophysiological doses of chemicals were studied. This was done to ensure that if neuronal sensory endings lay deep in the epicardium, sufficient doses of chemicals reached them to induce maximum responses. Since all neurones were not modified by all chemicals, it appears that the doses of chemicals used did not induce nonspecific responses. Not all dorsal root ganglion neurones associated with left ventricular neurites were modified when two major coronary arteries were occluded. This may have been due to the fact that their receptive fields lay in tissues perfused by arterial branches arising central to the sites of coronary arterial
occlusion. The origins of the major coronary arteries were not chosen for sites of occlusion as dissecting tissues adjacent to them might have disrupted sensory afferent axons which accompany large coronary arteries. The modulating effects of efferent autonomic neurones upon afferent neuronal sensory fields, including their modulation of local myocyte contractility, was not studied. Presumably this might have affected afferent neuronal responses to ischaemia and vascular occlusions. In order to minimize this effect, local epicardial stimuli were studied. The effects of ischaemia on the capacity of afferent neurones to sense mechanical or chemical stimuli was not studied.

It is concluded that most canine dorsal root ganglion neurones that have epicardial sensory neurites respond to mechanical and chemical stimuli. There are, however, a few neurones in these ganglia that respond only to mechanical or chemical stimuli. The polysensory responses that most dorsal root ganglion neurones associated with epicardial neurites display can be explained (1) by their sensory neurites being polysensory, (2) by convergence of other primary afferent neurones onto the sensory neurites of these neurones or (3) by interactions among individual mechanosensory and chemosensory neurones in dorsal root ganglia. Even though the latter possibility seems unlikely given the fact that the latency of activity of neurones following electrical stimuli being delivered to their neurites at different frequencies did not vary, it could not be excluded. Such would have happened if synapses were interposed between the neurites and somata studied. During myocardial ischaemia each of these neurones displays unique activity patterns which depend upon their varied sensory characteristics as well as the type and magnitude of the various stimuli they received at any time.

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