Responsiveness of in situ canine nodose ganglion afferent neurones to epicardial mechanical or chemical stimuli

J A Armour, M H Huang, A Pelleg, and C Sylvén

Objective: The aim was to determine the capacity of nodose ganglion afferent neurones with epicardial sensory endings to respond to mechanical and chemical stimuli, in particular to purinergic compounds. **Methods:** Alterations in spontaneous activity generated by epicardial afferent neurones in nodose ganglia in situ of 17 anaesthetised dogs were identified using extracellular recording techniques when mechanical and chemical stimuli were applied to their receptor fields, as well as during brief periods of coronary artery occlusion. **Results:** 92 cardiac afferent neurones were identified. Localised epicardial distortion modified the activity generated by 34 neurones [0.19(SEM 0.02) to 1.2(0.4) impulses·s^{-1}]. Application of bradykinin, substance P, N^6-cyclopentyl-adenosine or β,γ-methylene adenosine 5'-triphosphate to localised epicardial fields altered the activity of 69 neurones. Thus the majority of identified epicardial neurones responded to chemical stimuli alone (63%) as opposed to mechanical stimuli alone (25%), 12% responding to both types of stimuli. Activity was enhanced overall by chemical stimuli from a mean range of 0.1-0.4 to 11.6-13.2 impulses·s^{-1}. Following termination of short lasting chemical as opposed to mechanical stimuli, activity remained increased for up to 45 min. Activity generated by 16 chemosensitive neurones was modified by brief periods of coronary artery occlusion [0.26(0.12)-1.66(0.61) impulses·s^{-1}]; activity increasing further [2.51(0.47) impulses·s^{-1}] during reperfusion periods. **Conclusions:** (1) Chemical stimuli induce an order magnitude greater enhancement of activity generated by nodose ganglion cardiac afferent neurones than do mechanical stimuli, such enhancement persisting long after removal of chemical as opposed to mechanical stimuli. Thus qualitative and quantitative differences exists between central neuronal inputs derived from nodose ganglion epicardial afferent neurones sensitive to chemical as opposed to mechanical stimuli. (2) Adenosine and ATP can activate nodose ganglion cardiac afferent neurones.

Cardiovascular Research 1994;**28:**1218-1225

Myelinated and unmyelinated afferent axons arising from cardiac mechanosensitive or chemosensitive nerve endings course centrally in cardiopulmonary nerves.[1] These join either the vagosympathetic complexes[2-5] to unite with afferent cell bodies in the nodose ganglia bilaterally,[6] or the subclavian ansae and thoracic rami[7-11] to unite with neurones in dorsal root ganglion bilaterally.[6] Activity generated by cardiac mechanosensitive nerve endings varies depending on their location.[1 4 12-15] For example, atrial mechanosensitive nerve endings respond primarily to unidirectional strain of their receptor field.[3 13] Different activity patterns are generated by right and left ventricular mechanosensitive nerve endings in response perturbations.[1 5 10 11 16] The activity of left ventricular mechanosensitive nerve endings may either increase[12 16 17] or decrease[1] in response to increased systemic vascular pressure. The majority of left ventricular endocardial mechanosensitive endings generate increased activity when left ventricular systolic pressure increases.[1] Cardiac afferent nerve endings also can be affected by brief periods of coronary artery occlusion or local application of noxious chemicals.[1 12 18] Ventricular receptors have been reported to display polymodal behaviour manifested by their capacity to be modified by both mechanical and chemical stimuli.[2 10 19]

In order to understand the transfer function of information from cardiac afferent neurones to central neurones, the function of afferent neurones involved in cardiovascular regulation needs to be characterised as fully as possible. Mikhailova *et al*[20] reported that the activity of in situ mammalian nodose ganglion neurones can be altered by ventricular ischaemia or fibrillation. ATP can modify the transmembrane properties of in vitro intrinsic cardiac neurones[21 22] and the activity generated by in situ intrinsic cardiac neurones.[23] In addition, adenosine has been implicated in the genesis of cardiovascular reflexes initiated by cardiac receptors.[24] Whether purine compounds and peptides can modify the activity generated by in situ nodose ganglion cardiac afferent neurones remains unknown. Furthermore, the responses of such cardiac afferent neurones to mechanical as opposed to chemical stimuli remains unknown.

Thus the present experiments were devised to quantify the capacity of cardiac afferent neurones in the mammalian nodose ganglion in situ to respond to localised cardiac mechanical or chemical stimuli, as well as to transient myocardial ischaemia. Extracellular activity generated by the somata of individual neurones can be recorded over long periods of time due to the lack of detectable motion of in situ

Department of Physiology and Biophysics, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, B3H 4H7, Canada: J A Armour, M H Huang; Likoff Cardiovascular Institute, Hahnemann University, Philadelphia, Pennsylvania, USA; A Pelleg; Department of Medicine, Huddinge University Hospital, Huddinge, Sweden: C Sylvén. Correspondence to Dr Armour.

nodose ganglia. This allowed us to determine the capacity of individual nodose ganglion cardiac afferent neurones to respond to various mechanical and chemical stimuli, as well as to compare these effects to those elicited by brief periods of coronary artery occlusion.

Methods

Animal preparation

Seventeen mongrel dogs of either sex, weighing 18-24 kg, were sedated with intravenous sodium thiopentone (12-15 $mg \cdot kg^{-1}$) and anaesthetised with α chloralose (100 $mg \cdot kg^{-1}$ intravenous bolus plus 25 $mg \cdot kg^{-1}$ every hour throughout the experiments or more frequently, as required). Following initiation of positive pressure ventilation, a bilateral thoracotomy was made to expose the heart. A miniature solid state pressure transducer (Konigsberg Instruments, model P190; 5 mm diameter, 1.5 mm thick) was inserted into the midwall region of the left ventricular ventral wall to record regional intramyocardial pressure. Left ventricular chamber pressure was measured using a Bentley Trantec model 800 transducer connected to a Cordis No 7 catheter inserted into that chamber via a femoral artery.

Neuronal recording

Neurones in eight right and nine left sided nodose ganglia were studied in situ. The nodose ganglion, exposed on one side of the neck, was left bound by adjacent connective tissue to the base of the skull to minimise ganglionic motion. A tungsten microelectrode, mounted on a micromanipulator, was placed over the exposed nodose ganglion so that it could be slowly advanced into the ganglion to search for activity generated by spontaneously active neurones. The indifferent electrode was attached to adjacent tissue. Signals were amplified by an amplifier which had bandpass filters set at 300 Hz to 10 KHz and an amplification range of 100-500×. The output of this device was further amplified (50-200×) and filtered (bandwidth 100 Hz-2 kHz) using an optically isolated amplifier. All data, including an electrocardiogram, were recorded on an eight channel rectilinear recorder. Signals from the conditioner unit were stored on disks using a Bernouille box and were later analysed on the optical recorder or via a memory oscilloscope. The frequency response of the entire recording system was linear to ~1500 Hz.

Action potentials recorded in a given locus with the same configuration and amplitude were considered to be generated by a single unit, as has been described previously.[23 25] Neurones associated with epicardial receptors were investigated to permit application of mechanical and chemical stimuli to their receptor fields, thereby avoiding generalised cardiac effects secondary to altered peripheral vascular resistance which occurs when chemicals are given systemically. Action potentials with signal to noise ratios greater than 2.5:1 were studied, one to three different action potentials being identified at active loci. In order to rule out the possibility that recorded action potentials were generated by axons in the nodose ganglion, the recording electrode was also inserted into the ipsilateral vago-sympathetic complex adjacent to the nodose ganglion in an attempt to record spontaneous activity propagated by axons in that complex. In order to estimate the conduction velocity of afferent axons connected with identified nodose ganglion neurones, electrical stimuli (1-4 V, 1 ms, 0.1 Hz) were delivered at the termination of the experiment to an ispilateral intrathoracic cardiopulmonary nerve via a unipolar electrode, the other electrode being attached to the thoracic wall. The latency of activation of nodose ganglion neurones was determined and an estimate of the distance between the stimulating and recording electrodes obtained by measuring the length of a thread which had been placed between the stimulation site and the nodose ganglion studied.

Mechanical distortion

Once a spontaneously active neurone had been identified in a nodose ganglion, loci in the following tissues were gently probed by means of a saline soaked cotton tipped applicator. First, epicardial loci on the ventral, lateral, and dorsal surfaces of the atria were touched. Then epicardial loci on the ventral, lateral, and dorsal walls of the left ventricle and the conus and sinus of the right ventricle were touched gently. Then loci on the vena cava, aorta, and lungs were touched, as were loci on the skin of the upper limbs, neck, thorax, and abdominal wall. Pressure was applied to the abdominal contents in order to identify neurones with mechanosensitive receptors within the abdomen. Then the thoracic aorta and the superior and inferior venae cavae were occluded individually for brief periods of time (5-10 s) by means of umbilical tape which had previously been placed around them. When tissues were gently distorted or vessels occluded, no mechanical disturbance was detected in the nodose ganglia investigated. Neurones which generated activity modified by gentle mechanical distortion of atrial or ventricular epicardial tissues were considered to be associated with cardiac mechanosensitive receptors.

Epicardial application of chemical agents

Gauze squares (1 cm × 1 cm) soaked with the agent under investigation (0.5 ml) or normal saline were individually applied for brief periods of time (60-100 s) to discrete epicardial loci on the ventral and lateral surfaces of the right and left atria, the ventral and lateral surfaces of the right ventricular sinus, all over the right ventricular conus, as well as to the ventral, lateral and dorsal surfaces of the left ventricle. After each agent had been applied, the epicardial region investigated was flushed with normal saline at a rate of ~3 $ml \cdot s^{-1}$ for at least 30 s. At least 5 min were allowed to elapse after the effect of each agent had terminated to enable the preparation to stabilise before the next intervention. Four pharmacological agents, obtained from Sigma, were applied to focal epicardial fields. Two peptides were investigated: bradykinin acetate salt (20, 50, 100, 200, and 300 μM) and substance P (20, 50, 100, 200, and 300 μM). Two purine compounds were investigated: N^6-cyclopentyladenosine (CPA; 75 μM, 0.2 mM, and 0.4 mM) and β,γ-methylene adenosine 5'-triphosphate (β,γ-mATP; 75 μM, 0.2 mM, and 0.4 mM).

Coronary artery occlusions

After the interventions described above were completed the left anterior descending, circumflex, and right coronary arteries were occluded individually for 1 min by means of snares previously placed around the origins of these vessels. Then the left anterior descending and circumflex coronary arteries were occluded simultaneously for 1 min. At least 5 min separated consecutive arterial occlusions to allow recovery to baseline conditions. At the termination of each experiment all cardiac regions were distorted manually in order to determine if mechanoreceptor fields associated with each investigated neurone were located in regions of the heart other than those identified by epicardial touch.

Data analysis

Heart rate as well as left ventricular intramyocardial and chamber systolic pressures were measured for 20 consecutive beats and their means(SEM) calculated. Individual action potentials were counted for at least 60 s immediately prior to and during maximum responses in order to determine changes in the average number of $impulses \cdot s^{-1}$ generated by individual neurones. Activity changes were ascribed when neuronal activity changed by more than 20% from baseline values. Neuronal activity was further analysed by comparing data obtained before and after each intervention with data obtained during other interventions using the multiple paired t test. To determine whether activity responses induced by various interventions were elicited with different frequencies and magnitudes, contingency tables were constructed with corrections for continuity to compare responses induced by different agents as well as to compare responses elicited by chemical agents with those induced by mechanical stimuli or brief periods of coronary artery occlusion. A p value <0.05 was considered to be significant for these determinations.

Results

On average, 16 tracts were explored with the microelectrode in each of the 17 nodose ganglia investigated. One to three spontaneously active units, as determined by amplitudes and shapes of individual action potentials, were identified in each of two to five active loci per ganglion. As few spontaneously active neurones associated with epicardial receptors were identified in the investigated ganglionic tracts, it is presumed that data derived from the present experiments reflect the fact that a subpopulation of cardiac afferent neurones was studied. When the microelectrode tip was placed into the vagus nerve caudal to the nodose ganglia either no action potentials were detected or action potentials with signal to noise ratios less than 2:1 were recorded. This is in accord with the fact that this method of recording does not detect action potentials generated by axons of passage.[25] When limited regions of the skin of the ipsilateral neck or forelimb were distorted mechanically, the activity generated by eight non-cardiovascular related neurones increased (table). Four units were activated when pulmonary tissues were touched. The spontaneous activity generated by these neurones was sporadic and of low frequency. One unit was identified which was activated when pressure was applied to the abdominal contents.

Changes induced in neuronal activity, grouped according to the various interventions studied. Values are means(SEM) with [range]. Numbers of afferent neurones which responded to gentle mechanical distortion of various tissues, as well as during augmentation of systemic vascular pressure by aortic occlusion, are listed on top. Neuronal responses elicited by epicardial application of peptides and purines are tabulated below. The effects induced by occlusion of one coronary artery and the subsequent reperfusion on afferent neuronal activity are provided at the bottom. Average activity generated before (control) and during (intervention) each intervention are listed.

Interventions	*No of responsive neurones*	*Control activity (impulses·s^{-1})*	*Intervention activity (impulses·s^{-1})*
Gentle mechanical distortion	52		
Skin	8+	0.08(0.02) [0.001-0.3]	0.81(0.21)* [0.2-1.2]
Pulmonary tissue	4+	0.06(0.01) [0.001-0.2]	0.95(0.33)* [0.1-1.5]
Superior vena cava	2+	0.03	0.16
Coronary sinus	1+	0.01	0.26
Heart	32+/2–	0.19(0.02) [0.002-0.41]	1.2(0.4)* [0.2-7.1]
Aorta	17+	0.24(0.09) [0.005-0.3]	1.68(0.76)* [0.8-4.1]
Aortic occlusion	20 (19+/1–)	0.23(0.16) [0.01-2.1]	1.56(0.65)* [0.8-2.6]
Epicardial chemical application	69		
Bradykinin	40 (37+/3–)	0.10(0.02) [0.005-0.4]	13.19(1.01)* [0.3-45]
Substance P	43 (41+/2–)	0.19(0.05) [0.001-0.8]	11.66(1.76)* [0.2-35]
CPA	37 (36+/1–)	0.4(0.18) [0.01-2.5]	13.16(1.89)* [0.2-32]
βγATP	23 (22+/1–)	0.16(0.07) [0.01-0.3]	12.91(1.64)* [0.4-48]
Coronary artery occlusion	16 (14+/2–)	0.26(0.12) [0.01-2.0]	1.66(0.61)* [0.05-7.4]
Reperfusion			2.51(0.47)*† [0.8-27]

+ = increase in activity; – = decrease in activity.
*$p < 0.001$ comparing data obtained before and during each intervention; †$p < 0.001$ comparing reperfusion data to data elicited during coronary artery occlusion.

Effects of epicardial mechanical stimulation

Ninety two neurones were identified which generated activity that was changed when mechanical or chemical stimuli were applied to the epicardium. The conduction velocity of axons arising from their cardiac receptors was estimated to be 1.5(SEM 0.3) (range 0.7-2.6) m·s^{-1}. Of the 92 neurones in which activity was modulated when stimuli were applied to the epicardium, 23(25%) responded to mechanical stimuli alone, 58(63%) to chemical stimuli alone, and 11(12%) responded to mechanical and chemical stimuli (table). Activity generated by 34 neurones with mechanosensitive epicardial fields was, on average, 0.19(0.02) Hz (range 0.002 to 0.41 Hz) during control conditions. The majority of these neurones generated activity that was sporadic. Eight afferent neurones generated activity during control conditions which was phase related to the cardiac cycle for short periods of time, usually more than one action potential being generated per cardiac cycle on these occasions (figs 1 and 5). When cardiovascular related activity was generated, it changed during the minor fluctuations in systemic vascular pressure which occur in open chest anaesthetised preparations (fig 1). Gentle mechanical distortion of epicardial sensory fields enhanced the activity of mechanosensitive neurones overall to 1.2(0.4) impulses·s^{-1} (maximum 7.1), the activity of two neurones becoming reduced during sensory field distortion. As activity generated by these neurones varied considerably when

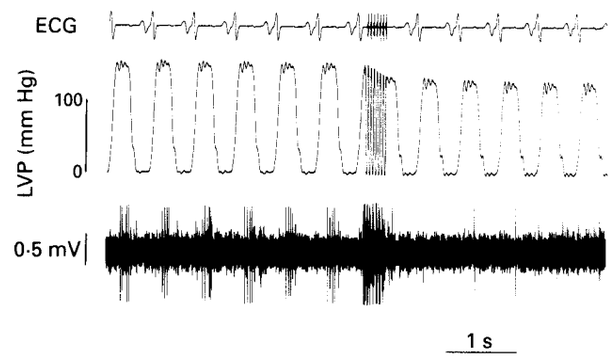

Figure 1 *Cardiovascular related activity generated by neurones in an in situ left sided nodose ganglion. This afferent neurone generated 4-5 action potentials per cardiac cycle when left ventricular systolic pressure (LVP) was 140 mm Hg (left side of figure) and ~1 action potential per cardiac cycle when systolic pressure was 115 mm Hg (right side of figure). The receptor field of this afferent neurone was identified in the epicardium of the left ventricular lateral wall. This neurone did not respond to epicardial chemical application.*

comparing one neurone to another, activity levels could not be correlated to receptor field distortion or systolic pressure. The size of identified epicardial mechanoreceptor fields varied from ~5 mm × 5 mm to ~2 cm × 2 cm. Activity changes were initiated promptly after application of mechanical stimuli, such changes ceasing immediately following removal of the probe or release of the aortic occlusion. Repeat application of mechanical stimuli to a receptive field induced similar neuronal responses. The activity generated by all neurones associated with left ventricular epicardial mechanosensitive nerve endings was altered when central aortic pressure increased during partial aortic occlusion.

Of the 34 neurones associated with epicardial mechanosensitive nerve endings, 14 were located in right and 20 in left nodose ganglia. When right atrial loci were touched the activity of 17 units increased and two decreased; activity generated by nine neurones increased when left atrial loci were touched. When loci on the right ventricular conus (two neurones) or sinus (four neurones) were gently distorted six neurones were activated. When loci on the ventral (four neurones), lateral (six neurones), or dorsal (five neurones) surfaces of the left ventricle were distorted, the activity of 12 units increased whereas that generated by another one decreased. Distortion of two to three separate ventricular epicardial regions altered the activity of three neurones. The activity generated by seven neurones was modified when an atrial and a ventricular locus were gently touched. One unit was modified when the coronary sinus was touched ~1 cm from its ostium. Of the six neurones associated with right ventricular mechanosensitive nerve endings, four were located in left and two in right nodose ganglia. Of the 12 neurones associated with left ventricular receptor fields, seven were located in right and five in left nodose ganglia. In other words the anatomical location of a neurone did not reflect its function. Since at the end of each experiment mechanical distortion of cardiac regions not previously distorted failed to modify activity generated by investigated afferent neurones, it appears that ventricular mechanoreceptor fields associated with investigated neurones were confined to the epicardial fields identified.

The activity of two neurones increased when loci on the ventral surface of the superior vena cava adjacent to the right

atrium were gently touched, and that of 17 increased when loci on the intrathoracic aortic adventitia were gently distorted. When the descending thoracic aorta was partially occluded, the activity generated by 19 units increased and one decreased concomitant with increased ascending aortic pressure. Contingency table analysis determined that distortion of epicardial mechanoreceptor fields elicited afferent responses more frequently from identified cardiac related neurones than did partial occlusion of the aorta or local distortion of the aorta. Epicardial touch and aortic occlusion elicited similar degrees of enhancement of activity generated by neurones with left ventricular mechanosensitive nerve endings. The activity generated by the majority of these neurones before and during aortic occlusion was not related in a temporal fashion to the cardiac cycle. The activity of one neurone was altered when the left ventricular epicardium and the skin of the ipsilateral upper limb were touched independently, and that of two others was modified when the left ventricular ventral epicardium and the surface of the aorta were touched independently.

Effects of chemical stimulation

The activity generated by identified neurones was not affected when saline soaked gauze was applied epicardially. Activity generated by 69 neurones identified in right (38 neurones) and left (31 neurones) nodose ganglia was modified for prolonged periods of time by epicardial application of bradykinin (fig 2), substance P (fig 3), or purinergic agents (fig 4). Responses were elicited soon after chemical application, for instance occurring 22(8) s (range 8-54 s) following the beginning of bradykinin application. Response lasted 19(6) min (maximum 38 min) after removal of bradykinin. Effects elicited by substance P were of similar duration [average 23(9) min, maximum 45 min], being longer than those elicited by CPA [average 7.1(1.2) min, maximum 12 min] or β,γ-ATP [average 3.8(1.6) min, maximum 8 min]. Frequently more than one neurone was modified at a given locus (figs 1-6). When activity was modified by a peptide it was unaffected by the 20 μm doses. As 200 and 300 μM doses induced similar responses the former was used for data analysis. Similar responses were elicited when 200 or 400 μM doses of purinergic compounds were used, lesser responses being elicited when lesser doses were used. Thus data were analysed when 200 μM doses of purinergic agents were employed. Monitored cardiovascular variables were unaffected by these interventions overall, short duration systemic vascular hypotension being induced in five and two animals respectively immediately following the initial epicardial application of substance P or bradykinin (fig 5). Hypotention was rarely induced when these agents were applied a second or third time. Bradykinin, substance P, and CPA affected similar numbers of neurones, β,γ-ATP modifying a lesser number ($p < 0.01$). When maximum activities elicited by each agent were compared, similar enhancement of activity was elicited by all four pharmacological agents tested. When comparisons were made between maximum neuronal responses elicited by mechanical versus chemical stimuli, bradykinin and β,γ-ATP elicited greater neuronal enhancement than did mechanical inputs ($p < 0.05$).

The activity of seven neurones was modified by both substance P and bradykinin and that of four neurones by both CPA and β,γ-mATP. Two neurones were activated by both

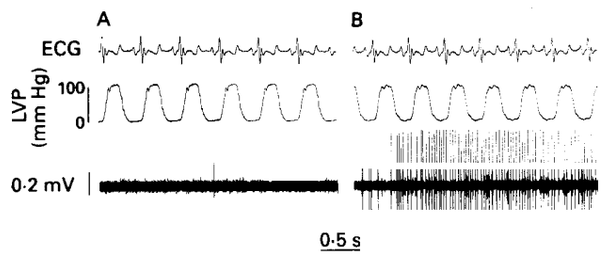

Figure 3 *Application of substance P (applied to the ventral left ventricular epicardium via a substance P soaked 1 cm × 1 cm pledglet, between panels A & B) induced increased activity generated by a neurone in a right nodose ganglion as well as initiating activity by another neurone which generated small signal to noise ratio activity. Slight tachycardia was induced during this intervention. Panel B was obtained 7 min after the epicardial application of substance P had been terminated and the receptor field had been flushed with saline. This neurone did not respond to altered cardiodynamics or mechanical distortion of the receptor field. LVP = left ventricular systolic pressure.*

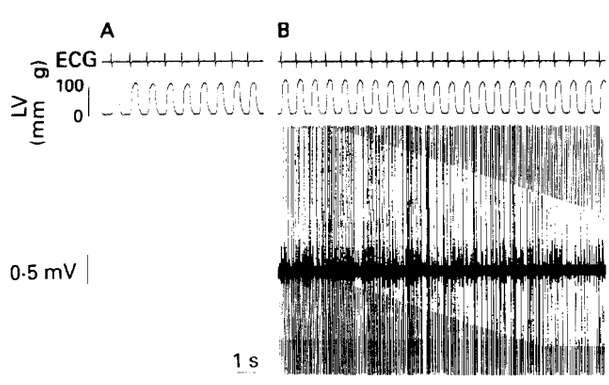

Figure 2 *Bradykinin (applied briefly to the cranial lateral epicardium of the left ventricle via a bradykinin soaked 1 cm × 1 cm pledglet, between panels A & B) induced long lasting increased activity generated by neurones in a right nodose ganglion without alteration in monitored cardiac variables. Panel B was obtained 20 min after bradykinin removal and flushing of the receptor field with saline. This neurone did not respond to altered cardiodynamics or mechanical distortion of the receptor field. LVP = left ventricular systolic pressure.*

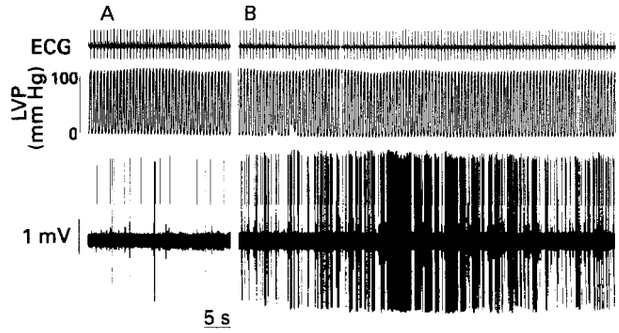

Figure 4 *Epicardial application of N^6-cyclopentyl adenosine (CPA) (applied to the ventral left ventricular epicardium via a CPA soaked 1 cm × 1 cm pledglet, between panels A & B) resulted in activity generated by the neurone with the greatest signal to noise ratio increasing as well as the activation of at least one other neurone. Monitored cardiac variables were not altered. Note that activity generated by the neurones was irregular with respect to time. Panel B was obtained 12 min after termination of CPA application. The activity generated by this neurone was not altered when cardiodynamic changes were induced nor when the receptor field was distorted. LVP = left ventricular systolic pressure.*

a purine compound and a peptide. The activity generated by 11 neurones responsive to epicardial distortion was modified by a chemical agent, three mechanosensitive neurones being modified by a neuropeptide and a purine compound. Chemoreceptive fields were located on both atria, as well as the epicardium of the right ventricular sinus or conus and the ventral, lateral, or dorsal surfaces of the left ventricle. Chemical application to either ventricle modified neurones in right and left nodose ganglia. For instance, left ventricular application of CPA modified three right sided and four left sided neurones. CPA, when applied to the right ventricle, modified six right and eight left nodose ganglion neurones. The maximum size of identified chemoreceptor fields was ~2 cm × 2 cm, as determined by the epicardial region from which neuronal activity responses were initiated following local application of chemical agents. Most chemosensitive receptor fields were confined to one chamber of the heart. For instance, substance P activated only seven of 43 responsive afferent neurones when applied to epicardial receptors in more than one region of the heart.

Chemical stimuli elicited similar neuronal responses, whether neurones were responsive to chemical stimuli alone or to both chemical and mechanical stimuli. Repeat application of each agent to an epicardial receptor induced similar activity changes in individual nodose ganglion afferent neurones. Lower doses induced lesser and shorter duration responses, presumably because receptor fields were subepicardial in location.

Effects of coronary artery occlusion

The activity generated by 16 neurones (nine right sided and seven left sided) was modified when a coronary artery was occluded briefly. The activity of 14 neurones increased (fig 5) and of two others decreased during transient coronary artery occlusion. When the left anterior descending, circumflex, or right coronary arteries were occluded individually, activity generated by six, eight, and four neurones was modified, respectively. When the left anterior descending and circumflex coronary arteries were occluded simultaneously, 11 neurones were modified. Of the eight neurones modified by circumflex coronary artery occlusion, five were modified when the left anterior descending coronary artery was occluded and none when the right coronary artery was occluded. The receptor fields of the five neurones affected when the two left sided coronary arteries were occluded individually were identified on the ventral surface of the left ventricle, a region which was perfused by branches arising from both arteries. Similarly, receptor fields of the four neurones modified by right coronary artery occlusion were identified on the right ventricle. During the initial reperfusion periods, neuronal activity was enhanced further in 11 of the 14 instances in which activity was augmented (table). In one instance activity increased suddenly about 45 s after the reperfusion period began, this change immediately preceding the onset of ventricular fibrillation (fig 6). Cardiovascular variables were not affected overall when the right coronary artery was occluded. When the left anterior descending or circumflex coronary arteries were occluded individually, left ventricular systolic chamber pressure was reduced minimally in two and five animals, respectively. Left ventricular chamber systolic pressure [123(16)-96(11) mm Hg; $p < 0.01$] fell in nine animals when the left anterior descending and circumflex coronary arteries were occluded simultaneously (fig 5). In three of those instances left ventricular end diastolic pressure increased minimally. Left ventricular ventral wall intramyocardial

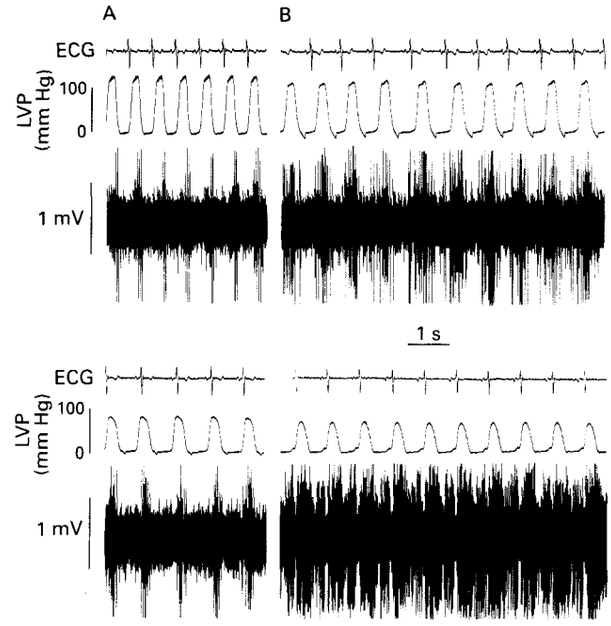

Figure 5 *Effects of bradykinin (upper panels) and coronary artery occlusion (lower panels) on activity generated by a few left sided nodose ganglion afferent neurones. The neurone which generated activity with the greatest signal to noise ratio was associated with a receptor field on the ventral left ventricular epicardium. Upper panel: Despite a reduction in left ventricular systolic pressure, neuronal activity increased compared to control states (A) following application of bradykinin (between panels) (B). Lower panel: Activity generated by neurones increased after a 15 s occlusion of the left anterior descending and circumflex coronary arteries (between panels). Activity generated by this afferent neurone increased when the receptor field was gently distorted (not shown). LVP = left ventricular systolic pressure.*

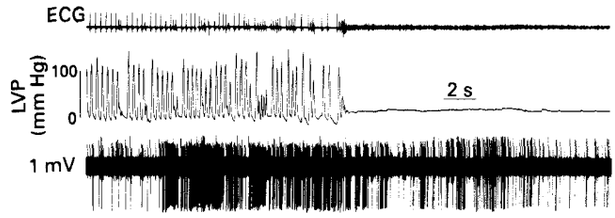

Figure 6 *Activity generated by a few neurones in a right nodose ganglion after termination of a left anterior descending coronary artery occlusion. Activity generated by one neurone (that with the greatest signal to noise ratio) during control periods was, on average, 0.02 Hz. Its activity increased to 0.3 Hz during coronary arterial occlusion. About 20 s after reperfusion began (onset of trace) activity increased further (maximum of 48 Hz). Activity remained increased even following the spontaneous onset of ventricular fibrillation. Neuronal activity persisted despite the fact that ventricular pressure was ~10 mm Hg. Activity also persisted when ventricular chambers were subsequently emptied of blood, left ventricular chamber pressure then being 0 mm Hg (not shown). Previously this neurone had responded to epicardial field (ventral surface of the left ventricle) application of N^6-cyclopentyl adenosine. Activity generated by these neurones was not modified by receptor field probing or epicardial application of β,γ-mATP, bradykinin, and substance P. The fact that neurones responded to chemical as opposed to mechanical stimuli presumably accounted for the fact that they remained active during ventricular fibrillation even when blood was removed from the cardiac chambers.*

systolic pressure was unaffected overall by 1 min occlusion of these two vessels [118(11)-104(12) mm Hg].

Eleven of 16 nodose ganglion neurones which responded to brief periods of coronary artery occlusion were modified by chemical stimuli (fig 5). No statistical differences were found comparing the magnitudes of neuronal changes induced by brief periods of coronary artery occlusion and chemical agents. Of these, eight were modified by both CPA and β,γ-mATP, one by CPA alone, and one by β,γ-mATP alone. Seven ischaemia sensitive neurones were modified by both bradykinin and substance P, another by bradykinin alone, and another by substance P alone. The activity of nine ischaemic sensitive neurones was modified by a purine compound and a peptide. Five ischaemia sensitive neurones responded to mechanical stimuli.

Discussion

The major findings of the present investigation were: (1) the activity generated by in situ nodose ganglion afferent neurones associated with epicardial sensory fields in anaesthetised dogs can be modified by mechanical and/or chemical stimuli; (2) transient myocardial ischaemia and reflow can modulate their activity; (3) the activity generated by some of these neurones can be modified by one type of perturbation while that of others by multiple types of perturbations; (4) the anatomical location of a neurone within a right or left nodose ganglion did not reflect the location or function of its sensory nerve ending. These data are in accord with previous reports that cardiac receptors with afferent axons in the vagi generate activity which can be modified by alterations in cardiodynamics or the chemical *milieu* of the heart.[2 4 5 14 15 19 26] Furthermore, they are in accord with a previous report which indicated that the activity generated by nodose ganglionic neurones can be modified by myocardial ischaemia.[20]

Atrial mechanosensitive nerve endings usually generate multiple action potentials per cardiac cycle.[1 3 13 14] The activity generated by ventricular epicardial mechanosensitive nerve endings is reportedly irregular (~1 to 1.5 impulses per systole) during physiological conditions.[10 12 14–16 19] In the present study, the majority of nodose ganglion neurones associated with epicardial mechanosensitive nerve endings generated less than one impulse per cardiac cycle [0.19(0.02) impulses·s^{-1}; table], activity patterns in the majority of instances not relating to the cardiac cycle. Thus their activity patterns did not relate in a simple fashion to regional intramyocardial dynamics such as have been reported to occur with respect to vagal afferent axonal activity arising from feline ventricular mechanosensitive nerve endings.[2 5 19] Unlike rabbit or cat physiological data,[27] but similar to canine anatomical studies,[6] neurones associated with epicardial and aortic receptors were located throughout the nodose ganglia bilaterally. In contrast to data indicating that left ventricular mechanosensitive nerve endings with vagal afferent axons lie primarily in the dorsal wall of the left ventricle,[28] left ventricular epicardial sensory fields associated with canine nodose ganglion afferent neurones were distributed relatively equally throughout the ventral, lateral, and dorsal surfaces of the left ventricle, as well as between ventricular chambers. Some afferent neurones received mechanical or chemical inputs from two sensory fields, as has been found with individual intrathoracic cardiac afferent axons.[1 12]

The activity generated by a small population of these afferent neurones was temporally related to the cardiac cycle, particularly during systole (figs 1 and 5). Such activity was abolished when ventricular systolic pressure fell by as little as 10-15 mm Hg (fig 1), indicating that some nodose ganglion afferent neurones are influenced by minor changes in ventricular systolic events, not diastolic events. In accord with data concerning activity generated by left ventricular mechanosensitive nerve endings,[4 5 12 16] the activity generated by 19 afferent neurones associated with left ventricular mechanosensitive nerve endings increased when systemic vascular pressure increased and decreased when systemic vascular pressure was reduced. In contrast to vagal afferent axonal activity data,[5] activity generated by one left ventricular mechanosensitive neurone decreased when systemic vascular pressure increased (table). Thus, although the activity generated by many nodose ganglion neurones connected with ventricular epicardial mechanosensitive nerve endings did not relate in a linear fashion to changes in ventricular systolic or diastolic pressure or, for that matter, to rate of ventricular pressure change, that generated by the majority increased when left ventricular systolic pressure increased.

Most identified epicardial neurones were modified by chemical stimuli, 12% responding to both mechanical and chemical stimuli (table). Bradykinin, a substance which activates cardiac sensory endings with axons in sympathetic nerves,[7 10 29] modified 58% of identified nodose ganglion chemosensitive neurones. A similar population of neurones was modifed by substance P. These data are in accord with the fact that cardiovascular reflexes can be induced by epicardial application of bradykinin[28] or substance P.[30] The present experiments show, for the first time, that nodose ganglion epicardial afferent neurones can be modified for relatively long periods of time following brief exposure of their receptor fields to analogues of adenosine and ATP. The persistence of afferent neuronal responses after removal of chemical stimuli may have been due to continued alteration in the chemical milieu of the receptor field, something that may account for the persistence of effects elicited following termination of brief periods of myocardial ischaemia. The fact that the ATP analogue modified a number of afferent neurones which were not sensitive to the adenosine analogue suggests that a population of nodose ganglion cardiac afferent neurones exists which are sensitive to ATP as opposed to adenosine. That the adenosine analogue modified neurones insensitive to the ATP analogue implies the reverse as well. Many purinergic sensitive neurones were insensitive to substance P or bradykinin. The reverse held true as well. Neuronal activity was not altered when the majority of chemosensitive receptor fields were mechanically distorted. Furthermore, as application of a chemical to an epicardial receptor field directly over the intramyocardial pressure sensor failed to alter local muscle dynamics, it does not seem likely that chemically induced neuronal activity changes were due to direct modification of regional mechanics. In accord with that, activity generated by afferent neurones was not modified when saline was applied to their receptor fields, indicating that when activity changes were induced following epicardial application of chemical agents soaked gauze they were not related to this vehicle or local mechanical disturbance induced by the gauze. Thus some nodose ganglion neurones associated with epicardial sensor endings sense alterations in the chemical milieu of the atria as well as the ventricles.

Activity generated by some atrial[26] and ventricular[1 9 31 32] receptors increases during coronary artery occlusion, presumably reflecting regional mechanical (that is, receptor

field elongation) or metabolic alterations.[10 19] Nodose ganglion neurones associated with cardiorespiratory afferent sensory endings can be modified by myocardial ischaemia as well.[20] In the present study, brief (1 min) periods of coronary artery occlusion increased activity generated by 14 afferent neurones with epicardial sensory endings. Activity increased gradually after initiation of coronary artery occlusion, not promptly as has been reported to occur with respect to feline cardiac vagal afferent axons.[31] It is unlikely that such activity changes were due to increased deformation of ventricular mechanoreceptor fields since left ventricular wall and chamber systolic pressures were unchanged or fell during coronary artery occlusions. As left ventricular chamber diastolic pressure was unchanged in the majority of these instances, particularly when single vessels were occluded, it is unlikely that neuronal responses were due to altered diastolic mechanical events. Neurones which proved to be modified by coronary artery occlusion also were not affected by aortic occlusion, the latter inducing noticeable left ventricular distention and increased left ventricular end diastolic pressure. As the majority of neurones responsive to transient coronary artery occlusion were modified by chemical (fig 5) as opposed to mechanical stimuli, it appears that local chemical changes accounted to a large extent for neuronal responses elicited during transient ischaemia. Activity generated by cardiac vagal afferent axons increases after flow is re-established, but reportedly not to levels generated during the early phase of occlusion.[31] In the present experiments greater activity was achieved during reperfusion than during occlusion (fig 6; table). Perhaps this was due to the fact that local metabolites and ions which had accumulated during the occlusion were released upon reperfusion, thereby modifying sensory endings.

The present experiments indicate that the majority of nodose ganglion epicardial afferent neurones are sensitive to chemical stimuli, lesser numbers being sensitive to mechanical stimuli and few being sensitive to both modes of stimulation. Furthermore, even though activity generated by most nodose ganglion afferent neurones associated with ventricular epicardial mechanosensory endings was not directly related to changes in ventricular systolic or diastolic pressure, activity generated by the majority of these neurones increased as ventricular chamber systolic pressure increased. The present data also indicate for the first time that, in addition to peptides, purinergic compounds enhance activity generated by nodose ganglion cardiac afferent neurones. As epicardial nodose ganglion neuronal activity increases elicited by chemical as opposed to mechanical stimuli were much greater (10-fold) and of longer duration, alterations in the chemical milieu of the heart appear to induce greater afferent input to medullar neurones than alterations in the mechanial milieu of the heart. That activity enhancement induced during brief periods of coronary artery occlusion and, in particular, reperfusion occurred in purinergic and peptidergic sensitive neurones is in accord with the fact that these chemicals are known to be released by the ischaemic myocardium. It is concluded that most canine nodose ganglion epicardial afferent neurones are sensitive to either mechanical or chemical stimuli, the duration and type of neuronal response elicited depending on the modality of challenge and the neurone type.

The authors gratefully acknowledge the technical assistance of Richard Livingston. This work was supported by the Nova Scotia Heart and Stroke Foundation and the Medical Research Council of Canada (MRC-MT 10122). MHH is a recipient of an MRC studentship award and support from Totts Gap Medical Research Laboratories, Inc, Bangor, PA. AP is supported by NIH grant HL43006. CS is supported by the Swedish Medical Association.

Key terms: adenosine; ATP; bradykinin; ischaemia; substance P.

Received 31 January 1994; accepted 25 March 1994. Time for primary review 42 days.

1 Armour JA. Thoracic and cardiovascular afferent nerves. In: WC Randall, ed. *Neural regulation of the heart.* New York: Oxford University Press, 1977:13–42.
2 Baker DG, Coleridge HM, Coleridge JCG. Vagal afferent C fibres from the ventricle. In: Hainsworth R, Kidd C, Linden RJ, eds. *Cardiac receptors.* Cambridge: Cambridge University Press, 1979:117–37.
3 Chapman KM, Pankhurst JH. Strain sensitivity and directionality in cat atrial mechanosensitivity nerve endings *in vitro. J Physiol (Lond)* 1976;**259:**405–26.
4 Coleridge HM, Coleridge JCG, Kidd C. Cardiac receptors in the dog, with particular reference to two types of afferent endings in the ventricular wall. *J Physiol (Lond)* 1964;**174:**323–39.
5 Thorén P. Characteristics of left ventricular receptors with nonmedullated vagal afferents in cats. *Circ Res* 1977;**40:**415–21.
6 Hopkins DA, Armour JA. Ganglionic distribution of afferent neurons innervating the canine heart and physiologically identified cardiopulmonary nerves. *J Auton Nerv Syst* 1989;**26:**213–22.
7 Baker DG, Coleridge HM, Coleridge JCG, Nerdrum T. Search for a cardiac nocireceptor: stimulation by bradykinin of sympathetic afferent nerve endings in the heart of the cat. *J Physiol (Lond)* 1980;**306:**519–36.
8 Bosnjak ZJ, Zuperku EJ, Coon RL, Kampine JP. Acute coronary artery occlusion and cardiac sympathetic afferent nerve activity. *Proc Soc Exp Biol Med* 1979;**161:**142–8.
9 Casati R, Lombardi F, Malliani A. Afferent sympathetic unmyelinated fibers with left ventricular endings in cats. *J Physiol (Lond)* 1979;**292:**135–48.
10 Malliani A. Cardiovascular sympathetic afferent fibers. *Rev Physiol Biochem Pharmacol* 1982;**94:**11–74.
11 Uchida Y, Murao S. Excitation of afferent cardiac sympathetic nerve fibers during coronary occlusion. *Am J Physiol* 1977;**226:** 1094–9.
12 Armour JA. Physiological behavior of thoracic cardiovascular receptors. *Am J Physiol* 1972;**225:**177–85.
13 Arndt JO, Brambring P, Hindorf K, Röhnelt R. The afferent discharge pattern of atrial mechanoreceptors in the cat during sinusoidal stretch of atrial strip *in situ. J Physiol (Lond)* 1974; **240:**33–52.
14 Coleridge JCG, Hemingway A, Holmes RL, Linden R. The location of atrial receptors in the dog: a physiological and histological study. *J Physiol (Lond)* 1957;**136:**174–97.
15 Sleight P, Widdicombe JG. Action potentials in fibres from receptors in the epicardium and myocardium of the dog's left ventricle. *J Physiol (Lind)* 1965;**181:**235–58.
16 Peters SR, Kostreva DR, Armour JA, *et al.* Cardiac, aortic, pericardial and pulmonary vascular receptors in the dog. *Cardiology* 1980;**65:**85–100.
17 Malliani A, Recordati G, Schwartz PJ. Nervous activity of afferent cardiac sympathetic fibres with atrial and ventricular endings. *J Physiol (Lond)* 1973;**229:**457–69.
18 Nishi K, Sakanashi M, Takenaka F. Activation of afferent cardiac sympathetic nerve fibers of the cat by pain producing substances and noxious heart. *Pflügers Arch* 1977;**372:**53–61.
19 Thorén P. Role of cardiac vagal C-fibers in cardiovascular control. *Rev Physiol Biochem Pharmacol* 1979;**86:**1–94.
20 Mikhailova SD, Semushkina TM, Kositsky GI. Changes in electrical activity of ganglion nodose neurons in ventricular ischemia and fibrillation. *Biull Eksp Biol Med* 1986;**10:**274–6.
21 Allen TGJ, Burnstock G. The actions of adenosine 5'-triphosphate on guinea-pig intracardiac neurons in culture. *Br J Pharmacol* 1990;**100:**269–76.
22 Fieber LA, Adams DJ. Adenosine triphosphate-evoked currents in cultured neurones dissociated from rat parasympathetic cardiac ganglia. *J Physiol (Lond)* 1991;**434:**239–56.
23 Huang MH, Sylvén C, Pelleg A, Smith FM, Armour JA. Modulation of canine *in situ* intrinsic cardiac neurons by local application of adenosine and ATP. *Am J Physiol* 1993;**265:** R914–22.
24 Cox D, Vita JA, Treasure CB, Fish RD, Selwyn AP, Ganz P. Reflex increase in blood pressure during intracoronary administration of adenosine in man. *J Clin Invest* 1989;**84:** 592–6.
25 Armour JA. Activity of *in situ* middle cervical ganglion neurons in dogs using extracellular recording techniques. *Can J Physiol Pharmacol* 1985;**63:**704–16.

26 Recordati G, Schwartz PJ, Pagani M, Malliani A, Brown AM. Activation of cardiac vagal receptors during myocardial ischemia. *Experientia* 1971;**27:**1423–4.
27 Donaghue S, Garcia M, Jordan D, Spyer KM. Identification of brain-stem projections of aortic baroreceptor afferent neurones in nodose ganglia of cats and rabbits. *J Physiol (Lond)* 1982;**322:** 337–52.
28 Walker JL, Thames MD, Abboud FM, Mark AL, Klopfenstein HS. Preferential distribution of inhibitory cardiac receptors in the left ventricle of the dog. *Am J Physiol* 1978;**235:**H118–92.
29 Lombardi F, Bella PD, Casti R, Malliani A. Effects of intracoronary administration of bradykinin on the impulse activity of afferent sympathetic unmyelinated fibers with left ventricular endings in the cat. *Circ Res* 1981;**48:**69–75.
30 Rioux F, Bachelard H, Barabé J, St-Pierre S. The cardiovascular response to epicardial application of neurotensin in guinea pigs. *Peptides* 1986;**7:**1087–94.
31 Brown AM. Excitation of afferent cardiac sympathetic nerve fibers during myocardial ischemia. *J Physiol (Lond)* 1967;**190:** 35–53.
32 Thorén P. Activation of left ventricular receptors with nonmedullated vagal afferent fibers during occlusion of a coronary artery of a cat. *Am J Cardiol* 1976;**37:**1046–51.