Chemotransduction properties of nodose ganglion cardiac afferent neurons in guinea pigs

G. W. THOMPSON, M. HORACKOVA, AND J. A. ARMOUR

Department of Physiology and Biophysics, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7

Received 18 November 1999; accepted in final form 6 March 2000

To understand how information arising from the heart is transferred to the central nervous system, the capacity of cardiac afferent neurons to transduce sensory stimuli needs to be characterized as fully as possible (17, 22, 25). Sensory nerve terminals (neurites) located in both ventricles are associated with myelinated and unmyelinated afferent axons that course centrally in cardiopulmonary nerves and the vagosympathetic complexes to unite with afferent cell bodies in nodose ganglia bilaterally (13). These neurites are capable of transducing mechanical and/or chemical stimuli, with many being sensitive to local ischemia too (2, 3, 19, 25).

Whether these neurites respond to a variety of chemical stimuli, some of which may be liberated locally during hypoxia or during sympathetic neuronal excitation, remains unknown. Thus the present experiments were devised to study the response characteristics of ventricular sensory neurites associated with nodose ganglion cardiac afferent neurons in situ to a variety of ion channel-modifying agents and neurochemicals.

Extracellular activity generated by the somata of individual afferent neurons in nodose ganglia can be recorded for relatively prolonged periods of time using tungsten microelectrodes, a procedure aided by the lack of detectable motion of such ganglia when left attached to surrounding tissue in situ (2). This experimental design permits an assessment of the response characteristics of individual cardiac sensory neurites to a variety of chemical stimuli, as responses so elicited can be characterized over several hours. Employing this technique, we were able to determine that ventricular sensory neurites associated with cardiac afferent neurons are capable of transducing more chemical stimuli than appreciated previously. Some of these chemicals include those known to be released from adjacent sympathetic efferent postganglionic nerve terminals.

METHODS

Animal preparation. All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals [DHEW Publication No.(NIH) 85–23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20205] and were approved by the institutional animal care and use committee of Dalhousie University. Forty-five adenine; adrenergic; angiotensin II; adenosine 5′-triphosphate; bradykinin; calcitonin gene-related peptide; histamine; H2O2; ion channel modifiers; nicotine; nitric oxide donor; substance P, vasoactive intestinal peptide.

TO UNDERSTAND HOW INFORMATION ARISING FROM THE HEART IS TRANSFERRED TO THE CENTRAL NERVOUS SYSTEM, THE CAPACI-...
adult guinea pigs of either sex, weighing 500–1,000 g, were anesthetized with ketamine hydrochloride (80 mg/kg im) and xylazine (10 mg/kg im). After endotracheal intubation and initiation of positive-pressure ventilation, a bilateral thoracotomy was made to expose the heart. The ventral pericardium was incised and retracted laterally via sutures. Left ventricular chamber pressure was measured by placing a PE-50 catheter into that chamber via its apex. Aortic pressure was monitored via another PE-50 catheter placed into the left common carotid artery. These catheters were connected to Bentley Trantec model 800 transducers. Each animal was placed on a Baxter Health Care hot water blanket attached to an American Pharamsel (model K-20-C) control unit. Body temperature was monitored throughout each experiment via a subcutaneous thermoprobe connected to a Shiley (Irving, CA) temperature monitor. At the end of each experiment, the animal was administered an overdose of Euthanol intravenously.

Neuronal recording. Neurons in 40 right- and 5 left-sided nodose ganglia were studied. Neurons in one nodose ganglion on either side of the neck were studied in each animal following their exposure via a ventral neck incision. Ganglia on either side of the neck were studied in each animal. Neurons in one nodose ganglion were identified and studied for several hours, a process permitted when the amplitudes of their action potentials were identified by means of a window discriminator (Hartley Instrumentation Development Laboratories, Baylor College of Medicine, Houston, TX). With the use of these techniques and criteria, the microelectrode does not record action potentials generated by axons of passage when placed in the adjacent vagus nerve. Rather, this methodology permits recording action potentials generated by cell bodies and/or dendrites in ganglia (2).

At the end of each experiment, the conduction velocity of individual afferent axons was estimated by delivering electrical stimuli (1–4 V, 1 ms, 0.1–5 Hz) to the associated epicardial sensory field (see Mechanical stimuli) using a unipolar ball electrode, with the indifferent electrode being attached to adjacent mediastinal tissues. The time between the beginning of the stimulus artifact and a consistently generated action potential was then determined. Placing a thread between the epicardial site and the recording site permitted an estimate of the distance between the stimulating and recording electrodes to be made. Estimating this distance, along with measuring the interval of time between an epicardial electrical stimulus and action potential generation, permitted calculation of the conduction velocity of impulses generated along their interconnecting afferent axons.

Mechanical stimuli. Once a spontaneously active neuron had been identified in a nodose ganglion, 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 with a saline-soaked cotton swab. When epicardial loci were touched with a saline-soaked cotton swab, no mechanical disturbance occurred elsewhere, including investigated nodose ganglia, nor were monitored cardiovascular indices altered. Neurons generating activity that was modified by gentle mechanical distortion of ventricular epicardial tissues were considered to be associated with cardiac mechanosensitive sensory neurites.

Epicardial application of chemicals. Multiple chemicals were applied to the sensory field of each investigated afferent neuron. Gauze squares (1 × 1 cm) soaked with chemicals (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 ventricular sinus, the right ventricular conus, as well as the ventral, lateral, and dorsal surfaces of the left ventricle. After each chemical had been applied, the epicardial region investigated was flushed with normal saline for at least 30 s. At least 15 min were allowed to elapse between each chemical application to enable each preparation to stabilize before the next intervention. Gauze squares soaked with room-temperature normal saline were applied to identified epicardial sensory fields to determine whether neuronal responses elicited by chemical application were due to vehicle effects or the mechanical effects elicited by gauze squares.

Chemicals, obtained from Sigma Chemical (St. Louis, MO) and BDH (Toronto, Ontario, CAN), were dissolved in physiologic Tyrode solution. The following ion channel-modifying agents were investigated: 1) the nonspecific potassium-channel blocker barium chloride (BaCl₂, 5 mM), 2) the nonspecific modifier of Ca²⁺-channels and Ca²⁺-activated K⁺-channels, CdCl₂ (200 μM), 3) CaCl₂ (10 mM), 4) the chelating agent EGTA (5 mM), 5) the membrane-depolarizing agent KCl (40 mM), 6) the nonspecific inhibitor of L-type Ca²⁺-channels nickel chloride (200 μM), 7) tetraethylammonium chloride (TEA; 10 mM), a chemical that inhibits voltage-sensitive potassium channels as well as Ca²⁺-activated K⁺-channels, and 8) the specific modifier of Na⁺-selective channels veratridine (7.5 μM). The following peptides were investigated: 1) angiotensin II (10 μM), 2) bradykinin acetate salt (1 μM), 3) calcitonin gene-related peptide (CGRP; 10 nM), 4) substance P (10 μM), and 5) vasoactive intestinal peptide (VIP; 50 μM). The adrenergic agonists tested were 1) the α₁-adrenoceptor agonist phenylephrine hydrochloride (10 μM), 2) the α₁-adrenoceptor agonist clonidine hydrochloride (10 μM), 3) the β₁-adrenoceptor agonist prenateral hydrochloride (10 μg/ml), 4) the β₂-adrenoceptor agonist terbutaline hemisulfate salt (10 μM), and 5) the β₁- and β₂-adrenoceptor agonist norepinephrine (50 μM). Other chemicals tested were 1) the purinergic compound adenosine (10 μM), 2) the nitric oxide donor nitroprusside (50 μM), 3) histamine (10 μM), 4) nicotine (10 μM), 5)
oxygen-derived free radicals (H₂O₂, 100 µM, and 6) an acidic saline solution (hydrochloric acid; pH 6.0).

Dose-response curves were performed in preliminary studies to determine the lowest dose of each agent that would consistently modify the activity generated by identified afferent neurons. Monitored heart rate, left ventricular chamber pressure, and aortic pressure were unaffected when mechanical (Fig. 1) or chemical stimuli were applied to epicardial loci. Thus the confounding variable of altering left ventricular pressure when epicardial stimuli were applied could be minimized, thereby avoiding affecting cardiac afferent sensory neurites indirectly. When smaller doses of these agents were tested, neuronal activity changes were induced, but with less consistency. Larger doses were not studied as that would have increased the likelihood of a chemical entering into the systemic circulation in sufficient doses to affect ventricular dynamics. Multiple applications of each agent were performed to study the consistency of elicited responses. All chemicals were administered in the same doses into carotid arterial blood to test whether their systemic administration would modify afferent neuronal activity.

Data analysis. Heart rate (using a 2-lead electrocardiogram), left ventricular chamber systolic pressure, and aortic pressures were measured and averaged over 20 consecutive cardiac cycles, and their means ± SE were calculated. Individual action potentials were counted for 60 s immediately before and during maximal responses to determine changes in the average number of impulses per minute generated by individual neurons. Activity changes were ascribed when neuronal activity changed by more than 20% from baseline values. Data are expressed as means ± se. One-way ANOVA and paired t-test with Bonferroni correction for multiple tests were used for statistical analysis. A value of P < 0.05 was considered to represent significant activity differences from control values.

RESULTS

One to three spontaneously active units, as determined by the amplitude of individual action potentials, that were associated with ventricular epicardial sensory fields were identified in a locus of each nodose ganglion investigated. 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. Baseline activity generated by most of the 102 neurons associated with identified epicardial sensory fields was sporadic in nature and thus not correlated to cardiac mechanical events. During control states, the activity generated by identified afferent neurons ranged from 7 ± 3 to 104 ± 37 impulses/min (Table 1). None of the neurons subsequently found to respond to epicardial stimuli were completely inactive during periods between interventions. The level of activity generated by individual identified afferent neurons did change over time, frequently remaining relatively depressed or excited when so affected by epicardial application of a chemical, for instance, after excitation of many neurons by epicardial application of a chemical activity, although returning toward pre-stimulation levels, never achieving that state. The average conduction velocity of the axons associated with identified afferent neurons was estimated to be 1.4 ± 0.5 m/s (range 0.7–2.1 m/s). The varied conduction velocities associated with identified neurons did not relate to whether sensory neurons proved to be responsive to mechanical or chemical stimuli or, for that matter, to specific chemicals. Heart rate, left ventricular systolic pressure, and aortic pressure remained stable throughout the period of study. Monitored cardiovascular indices remained unaffected when local mechanical or chemical stimuli were applied to epicardial loci. Similar activity response characteristics were displayed by right- or left-sided neurons to mechanical stimuli and individual chemicals.

Effects of mechanical stimuli. Application of a gentle mechanical stimulus to an epicardial locus did not result in any detectable motion in the nodose ganglia from which activity recordings were being made. The activity generated by 66% of the identified nodose ganglion afferent neurons associated with ventricular sensory neurites responded to mechanical stimuli. They did so by increasing their activity from 1.2 ± 0.4 to 47.1 ± 9.8 impulses/min (P < 0.01). These activity changes were initiated promptly after application of mechanical stimuli (Fig. 1). Such activity changes ceased immediately after the removal of the mechanical stimulus, displaying no adaptation on repeat application of mechanical stimuli. The epicardial regions that contained the sensory fields associated with identified nodose ganglion afferent neurons were ~1 cm in diameter, being located in the conus or sinus of the right ventricle and less often on the ventral and lateral epicardial surfaces of the left ventricle. As the activity generated by mechanosensory afferent neurons was sporadic in nature, it was not related to cardiodynamic events. No difference was detected comparing the activity generated by afferent neurons that proved to be responsive to mechanical stimuli or not.
Table 1. Changes in afferent neuronal activity induced by the various chemicals studied

<table>
<thead>
<tr>
<th>Chemicals Tested</th>
<th>Number of Responsive Neurons</th>
<th>% Responding</th>
<th>Activity Increased, Impulses/min</th>
<th>Activity Decreased, Impulses/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ion channel agents</td>
<td></td>
<td></td>
<td>Control</td>
<td>Intervention</td>
</tr>
<tr>
<td>BaCl₂ (5 mM)</td>
<td>20 of 20</td>
<td>100%</td>
<td>17</td>
<td>19 ± 10</td>
</tr>
<tr>
<td>CdCl₂ (200 μM)</td>
<td>12 of 13</td>
<td>92%</td>
<td>7</td>
<td>18 ± 7</td>
</tr>
<tr>
<td>CaCl₂ (10 mM)</td>
<td>7 of 12</td>
<td>58%</td>
<td>6</td>
<td>20 ± 7</td>
</tr>
<tr>
<td>EGTA (5 mM)</td>
<td>12 of 15</td>
<td>80%</td>
<td>7</td>
<td>12 ± 8</td>
</tr>
<tr>
<td>KCl (40 mM)</td>
<td>33 of 35</td>
<td>94%</td>
<td>18</td>
<td>12 ± 6</td>
</tr>
<tr>
<td>NiCl₂ (200 μM)</td>
<td>6 of 12</td>
<td>50%</td>
<td>5</td>
<td>18 ± 6</td>
</tr>
<tr>
<td>TEA (10 mM)</td>
<td>11 of 16</td>
<td>69%</td>
<td>9</td>
<td>13 ± 5</td>
</tr>
<tr>
<td>Veratridine (7.5 μM)</td>
<td>33 of 36</td>
<td>92%</td>
<td>30</td>
<td>18 ± 7</td>
</tr>
<tr>
<td>Neurochemicals</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenosine (10 μM)</td>
<td>31 of 33</td>
<td>94%</td>
<td>20</td>
<td>10 ± 3</td>
</tr>
<tr>
<td>Angiotensin II (100 μM)</td>
<td>14 of 18</td>
<td>78%</td>
<td>12</td>
<td>11 ± 4</td>
</tr>
<tr>
<td>Bradykinin (10 μM)</td>
<td>38 of 39</td>
<td>97%</td>
<td>29</td>
<td>11 ± 5</td>
</tr>
<tr>
<td>CGRP (100 nM)</td>
<td>11 of 14</td>
<td>79%</td>
<td>5</td>
<td>37 ± 19</td>
</tr>
<tr>
<td>Clonidine (100 μM)</td>
<td>8 of 10</td>
<td>80%</td>
<td>3</td>
<td>7 ± 3</td>
</tr>
<tr>
<td>Histamine (100 μM)</td>
<td>10 of 16</td>
<td>63%</td>
<td>5</td>
<td>57 ± 14</td>
</tr>
<tr>
<td>Acetic acid (pH 6.0)</td>
<td>7 of 10</td>
<td>70%</td>
<td>7</td>
<td>7 ± 4</td>
</tr>
<tr>
<td>Hydrogen peroxide (100 μM)</td>
<td>19 of 26</td>
<td>73%</td>
<td>13</td>
<td>20 ± 7</td>
</tr>
<tr>
<td>Nicotine (100 μM)</td>
<td>12 of 16</td>
<td>75%</td>
<td>10</td>
<td>11 ± 4</td>
</tr>
<tr>
<td>Nitroprusside (100 μM)</td>
<td>12 of 23</td>
<td>52%</td>
<td>11</td>
<td>104 ± 37</td>
</tr>
<tr>
<td>Norepinephrine (50 μM)</td>
<td>13 of 18</td>
<td>72%</td>
<td>6</td>
<td>63 ± 39</td>
</tr>
<tr>
<td>Phenylephrine (100 μM)</td>
<td>10 of 18</td>
<td>56%</td>
<td>8</td>
<td>18 ± 6</td>
</tr>
<tr>
<td>Prenaterol (10 μg/ml)</td>
<td>12 of 24</td>
<td>50%</td>
<td>12</td>
<td>14 ± 10</td>
</tr>
<tr>
<td>Substance P (10 μM)</td>
<td>27 of 39</td>
<td>69%</td>
<td>19</td>
<td>9 ± 3</td>
</tr>
<tr>
<td>Terbutaline (100 μM)</td>
<td>7 of 12</td>
<td>58%</td>
<td>7</td>
<td>67 ± 24</td>
</tr>
<tr>
<td>VIP (50 μM)</td>
<td>7 of 14</td>
<td>50%</td>
<td>6</td>
<td>59 ± 20</td>
</tr>
</tbody>
</table>

Values are means ± SE. Chemicals are grouped according to whether activity was either increased or decreased. Nos. of neurons tested (n) and percentage of responding neurons elicited by each chemical are listed. TEA, tetraethyl ammonium; CGRP, calcitonin gene-related peptide; VIP, vasoactive intestinal peptide. *P < 0.01.

Effects of chemical stimuli. Each identified neuron responded to multiple chemicals. Many chemicals modified the activity generated by each afferent neuron studied, but not all of the chemicals affected every neuron (Table 1). The activity generated by afferent neurons was not affected when saline-soaked gauze was applied to their epicardial sensory fields (29 ± 10–32 ± 9 impulses/min; not significant). Responses were initiated gradually after chemical application. For instance, neuronal activity responses took 16 ± 5 s (range 4–30 s) to reach maximum levels after adenosine application. When lesser doses of an agent than those depicted in the table were tested, induced responses varied presumably because tested receptor fields were located at differing depths within the subepicardium. As greater doses of a neurochemical induced similar responses as the doses listed in Table 1, they were not used for data analysis in case they entered the systemic circulation in sufficient quantities to affect distant tissues. Heart rate, left ventricular chamber pressure, and aortic pressure were unaffected by epicardial application of each chemical in the dosage listed in Table 1. Repeat application of each active chemical to an epicardial locus after various sequences of chemical application had been performed induced similar changes in neuronal activity over time. In some instances, one chemical initiated an excitatory response while another initiated a suppressor response.

Ion channel-modifying agents. The majority of nodose ganglion afferent neurons associated with epicardial sensory neurites examined were sensitive to most of the ion channel-modifying agents tested (Fig. 2A). All afferent neurons studied were sensitive to barium chloride (Table 1). With the exception of potassium chloride, ion channel-modifying agents initiated inhibitory responses with much lesser frequency than excitatory ones.

Neurochemicals. The peptides angiotensin II, bradykinin, and CGRP (Fig. 2C) affected >75% of identified neurons, whereas the peptides substance P and VIP modified fewer neurons. Of the other neurochemicals tested, adenosine modified identified nodose ganglion afferent neurons with the greatest consistency (Table 1). Norepinephrine influenced lesser numbers of afferent neurons, as did α₁ (phenylephrine)- and α₂ (clonidine)-adrenoceptor agonists as well as β₂ (pre-naterol)- and β₂ (terbutaline)-adrenoceptor agonists. Sensory neurites of identified afferent neurons also proved to be responsive to histamine (Fig. 2D), hydrogen peroxide, nicotine, and the nitric oxide donor nitroprusside (Fig. 2B). Nitroprusside generated the greatest level of afferent neuronal activity encountered (Table 1). A number of the identified afferent neurons proved to be sensitive to local application of a mildly acidic solution (pH 6.0) as well.

DISCUSSION

The major finding of the present investigation was that the ventricular sensory nerve endings (neurites)
associated with nodose ganglion afferent neurons transduce multiple chemical stimuli in situ. The sensory neurites associated with two-thirds of identified chemosensory afferent neurons proved to be capable of transducing local mechanical stimuli, being a greater percentage of identified neurons than mechanosensitive ventricular afferent neurons in canine nodose ganglia (2). Identified ventricular sensory neurites proved to be sensitive to not only a host of neurochemicals, but to various ion channel-modifying agents (Table 1).

Previous reports have demonstrated that many cardiac sensory neurites associated with afferent axons in the vagi (3, 25) or nodose ganglion afferent neurons (2) are sensitive to peptides such as bradykinin and substance P. The ventricular sensory terminals associated with neurons identified in this study were capable of sensing the peptides angiotensin II, CGRP (Fig. 1C), and VIP in addition to bradykinin and substance P (Table 1). Excitatory neuronal responses elicited by the peptide bradykinin (+68%) were of greater magnitude than those induced by substance P (+42%), with even greater activity resulting from sensory neurite exposure to the peptide CGRP (+73%). Angiotensin also influenced the behavior of most afferent neurons, something that may have to be taken in account when considering the therapeutic effects of pharmacological agents that modify angiotensin II receptors. All of the chemicals tested, with the exception of the acidic solution and β-adrenoceptor agonists, activated or suppressed afferent neuronal activity depending on the neuron tested (Table 1). Excitatory or suppressor responses appeared to depend, in part, on the level of activity that individual afferent neurons generated in control states. For instance, suppressor responses were elicited most frequently by those afferent neurons that generated relatively high ongoing activity. On the other hand, excitatory responses were generated most frequently by neurons that displayed, on average, lower levels of activity (Table 1). This was particularly evident when angiotensin II, histamine, and norepinephrine were tested. These data suggest that the activity state of individual nodose ganglion cardiac afferent neurons may determine, in part, their functional response characteristics to a chemical stimulus.

The amount of adenosine liberated by the myocardium increases during myocardial ischemia (20). Consistent with previous reports (2, 16), adenosine either enhanced or suppressed the activity generated by most responsive neurons (Table 1). Ventricular sensory neurites proved to be sensitive to selective α- and/or β-adrenoceptor agonists too and thus to local norepinephrine application (Table 1). These data are consistent with the fact that some somatic sensory neurons express α2-adrenoceptors (6). Thus it is speculated that a population of nodose ganglion cardiac afferent neurons is sensitive to adrenergic agents liberated by adjacent sympathetic efferent postganglionic nerve terminals, forming a substrate for positive-feedback control within the cardiac nervous system. In other words, enhanced liberation of catecholamines from sympathetic efferent postganglionic nerve terminals within the myocardium during activation of cardiac sympathetic nerves could lead to the further excitation of some cardiac afferent neurons. Presumably, this would occur concomitant with the direct effects that locally liberated catecholamines exert on local myocardial contractility, thereby further influencing local sensory neurites associated with many afferent neurons.

The activity generated by 73% of identified afferent neurons was modified when their ventricular sensory neurites were exposed to an acidic solution (pH 6.0). This may have relevance with respect to cardiac afferent neuronal responses to alterations in the local ventricular tissue acid-base balance. As a matter of fact, local application of an acidic solution enhanced afferent neuronal activity almost as much as did the peptides bradykinin or substance P (Table 1). The ventric-
ular sensory neurites associated with 11 of 23 tested afferent neurons were also responsive to locally applied \( \text{H}_2\text{O}_2 \) (Table 1). The latter agent has been reported to modify sensory neurites associated with axons coursing in intrathoracic sympathetic nerves (15). That these afferent neurons share this feature with intrinsic cardiac neurites (24) may have a bearing on the capacity of the hierarchy of cardiac neurons to respond to locally released oxygen-derived free radicals during myocardial ischemia (30). The ventricular sensory neurites of many identified nodose ganglion afferent neurons displayed their greatest sensitivity to the nitric oxide donor nitroprusside (Fig. 1B; Table 1). That a nitric oxide donor modifies the activity generated by cardiac afferent neurons may have implications with respect to the neurocardiological effects of nitrate therapy.

Mammalian autonomic neurons possess a variety of ion channels (1). Data obtained from the present experiments indicate that ventricular sensory neurites associated with afferent neurons in mammalian nodose ganglia are sensitive to agents known to alter neuronal ion channels in vitro. Multiple ion channels have been associated with the capacity of the somata of nodose ganglion afferent neurites to generate action potentials in vitro (10). As a matter of fact, the ventricular sensory neurites associated with identified afferent neurons displayed ionic transduction processes consistent with those of somata derived from nodose ganglia studied in vitro (10).

We employed the neurotoxin veratridine, the effect of which can be blocked by TTX in vitro, to investigate the functional significance of membrane Na\(^+\) channels associated with the sensory terminals of nodose ganglion afferent neurites in situ (Fig. 2A). This alkaloid has been shown to specifically affect the fast sodium channels of neurons (18, 26) and cardiomyocytes (14). Veratridine selectively binds to Na\(^+\) channels in the open conformation, thereby delaying their inactivation (18). This effect results in prolongation of sodium influx, causing membrane afterdepolarization and, as a consequence, the generation of spontaneous action potentials (9, 26). Consistent with such findings, veratridine increased the activity generated by most cardiac afferent neurites when applied to their sensory neurites in situ (Table 1).

TEA differs in its mode of action on different populations of potassium channels, depending on the neuron type and species studied. TEA inhibits the transient outward \( K^+ \) current (\( I_{\text{KA}} \)). Such an effect would tend to increase the firing frequency of neurons (5); this mechanism may account for the fact that most afferent neurites affected in the present study were excited by locally applied TEA. TEA (1–10 mM) also partially inactivates the delayed rectifying \( K^+ \) current (\( I_{\text{Kr}} \)) of rat sympathetic (4) and parasympathetic (29) neurones in vitro. This effect of TEA slows action potential repolarization and thus decreases the spontaneous activity generated by such neurons, perhaps accounting for the population of neurons in which activity was suppressed by this chemical (Table 1). In addition to its effects on \( I_{\text{Kr}} \), TEA (>5 mM) also inhibits the Ca\(^{2+}\)-dependent \( K^+ \) current (\( I_{\text{KC}} \)), a current that also tends to decrease neuronal excitability when neurons undergo repetitive firing (12, 21), thus possibly contributing to the increased neuronal activity observed in the present study.

The activity generated by most afferent neurites increased when their associated sensory neurites were exposed to the nonselective \( K^+ \)-channel inhibitor BaCl\(_2\). BaCl\(_2\) modifies \( I_{\text{Kr}} \) and muscarinic sensitive \( K^+ \) channels (\( I_{\text{M}} \)) in rat autonomic neurones (7, 28) as well as inhibiting \( I_{\text{KC}} \) in rat (4) and guinea pig (8) sympathetic neurones. Both \( I_{\text{KC}} \) and \( I_{\text{M}} \) can function concomitantly to reduce electrical excitability. Thus agents that block either of these two currents could lead to increased neuronal activity (5). Consistent with these data derived in vitro, local administration of BaCl\(_2\) to epicardial sensory fields increased the activity generated by their associated afferent neurones (Table 1). As with BaCl\(_2\), CdCl\(_2\), an agent that inhibits Ca\(^{2+}\)-channels nonselectively (23) as well as neuronal Ca\(^{2+}\)-activated \( K^+ \) channels (27), modified the activity generated by most sensory neurones investigated. Although blockade of voltage-activated Ca\(^{2+}\)-channels would decrease sensory neurite excitability, blockade of calcium-activated \( K^+ \) channels would act to increase neuronal activity (i.e., CdCl\(_2\) and BaCl\(_2\)). In accordance with the latter, cadmium increased the spontaneous activity generated by most identified nodose ganglion afferent neurones in situ in a fashion that was similar to that induced by BaCl\(_2\) (Table 1). These parallel findings provide further support for the concept that blockade of calcium-activated potassium currents associated with the sensory nerve endings of cardiac afferent neurones leads to increased neuronal excitability and thus enhanced action potential generation.

It does not seem likely that the afferent neuronal responses elicited by chemical stimuli were secondary to altered regional ventricular mechanics, as local epicardial application of mechanical or chemical stimuli did not alter monitored cardiovascular indices. These data are in accord with those obtained in the canine model in which local ventricular sensory field distortion can be directly assessed (11). Activity generated by identified afferent neurones was not modified when saline was applied to their associated receptor fields. Thus afferent neuronal activity changes induced after epicardial chemical application did not appear to be related to the effects of the vehicle or local tissue mechanical distortion.

**Perspectives**

That the ventricular sensory neurites associated with individual afferent neurones are capable of transducing multiple chemical stimuli, including alterations in tissue pH, locally produced hydrogen peroxide, and locally released adenosine, peptides, and histamine, has implications with respect to the variety of chemicals that influence the feedback reflex mechanisms within the cardiac nervous system. That locally re-
leased nitric oxide donors also modify cardiac sensory neuronal processing may help to explain why administration of nitrates can affect the neurocardiological status of a patient. In addition, catecholamines released from sympathetic efferent postganglionic nerve terminals influence the transduction capabilities of some adjacent cardiac sensory neurites. The latter observation necessitates an appraisal of yet another feedback mechanism within the cardiac nervous system, one that could act to amplify local sympathetic neuronal efficacy if required.

The authors gratefully acknowledge the technical assistance of Richard Livingston.

This work was supported by the Nova Scotia Heart and Stroke Foundation (J. A. Armour), the New Brunswick Heart and Stroke Foundation (M. Horackova), and the Medical Research Council of Canada (Grants MT-10122 and MT-4128).

REFERENCES