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# Multi-receptive Sensory Properties of Epicardial Afferent Neurons in Canine Dorsal Root Ganglia

by

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for the degree of Doctor of Philosophy

at

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## Chapter 1 Introduction

Chapter 1 reviews the progresses of our understanding of dorsal root ganglion afferent neuronal mechanisms. These include anatomic and physiological data concerning neuronal somata of dorsal root ganglion neurons, their central and peripheral terminals involved in afferent signal transduction.

### 1.1 Historical overview

William Harvey, the discoverer of the circulation of the blood in the 17th century, was the first to examine whether the heart has sensation. He concluded that it does not when a patient whose heart he touched through an unhealed thoracic fistula did not feel the slightest sensation (Willis, 1847). Heberden was the first to use the concept angina pectoris (Heberden, 1772). In 1928, Keefer and Resnik provided the early detailed description of angina pectoris, a concept originally proposed by Heberden (1772), suggesting that chest pain was related to transient myocardial ischemia (Keefer & Resnik, 1928). The involvement of sympathetic afferent neurons in transmitting angina pain has been proposed since the later 1920s (Leriche & Fontaine, 1927, Sutton & Lueth, 1930; While & Smithwick, 1941), based on the clinical experience that excising the upper thoracic dorsal root ganglia eliminates angina pectoris. The first convincing anatomic evidence that linked the heart with sympathetic afferent neurons was provided by Nettleship (1936), who reported that removing the dorsal root ganglia produced degeneration of axons in the endocardial net at the apex of the ventricles and from the walls of coronary vessels. Brown (1964, 1967) pioneered the electrophysiological recording of neuronal activity

generated by *in situ* cardiac sympathetic afferent axons. During the 1970's and 1980's, Malliani and associates (1982) and Uchida and Murao (1974a, b, c) performed a series of electrophysiological studies which furthered the understanding of cardiac sympathetic afferent neuronal mechanisms. Data derived from the past 20 years support the original hypothesis of Lewis (1932) that certain pain-producing substances, such as bradykinin, released during myocardial ischemia may be involved in the genesis of angina pectoris and related symptoms (Felder & Thames, 1982; Foreman, 1991; Lombardi et al., 1981; Uchida & Murao, 1974c). Recently, Sylvén et al. (1986, 1989) provided convincing clinical evidence that exogenous adenosine, a purine compound that the heart releases during myocardial ischemia, provokes angina-like chest pain in humans. Indirect and direct evidence provided by Thames and associates (Dibner-Dunlap et al., 1993; Thames et al., 1993) and others (Montano et al., 1991) have suggested that adenosine activates cardiac sympathetic afferent axons.

## 1.2 Anatomy and physiology of afferent neurons in mammalian dorsal root ganglia

### 1.2.1 Gross anatomy

Afferent axons associated with cardiac sympathetic sensory endings course in thoracic cardiac nerves to cranial thoracic sympathetic ganglia and then into the ramus communicants to reach corresponding spinal nerves. After a short course within the intervertebral foramina, axons enter the dorsal roots to join their cell bodies, neuronal somata, located in a dorsal root ganglion. Neurons in dorsal root ganglia are pseudounipolar: their axons bifurcate into

peripheral axons connected with afferent sensory endings and central processes synaptically connected with dorsal horn spinal neurons. Primary sensory neurons which receive afferent input from the heart are located bilaterally in the $C_6$-$T_6$ dorsal root ganglia, the greatest population existing in the $T_2$-$T_4$ ganglia (Hopkins & Armour, 1989; Vance & Bowker, 1983).

There are two types of dorsal root ganglion neurons: small and large. A general relation exists between the size of neuron's cell bodies and that of their axons: large somata give rise to large axons, and small somata to small axons (Dogiel, 1908; Ramon y Cajal, 1909). Yoshida and Matsuda (1979), using the horseradish peroxidase cell-filling technique, described a linear relationship between cell body and axon diameters. Using conduction velocity measurements, a method frequently employed to estimate axon size, several studies found a linear relationship between dorsal root ganglion cell body size and conduction velocity (Yoshida & Matsuda, 1979; Cameron et al., 1986). The large axons are myelinated and small ones are unmyelinated. Thus, it has been assumed that large neurons have myelinated axons, and small neurons have unmyelinated axons (Ranson, 1912).

Traditionally, dorsal root ganglion neurons have also been categorized as "light " or "dark", based on the neurohistochemical stains and the organization of their Nissl substance. Lightly staining (relatively organelle free) neurons tend to be large whereas intensely staining (relatively organelle-concentrated) neurons tend to be small (Scharf, 1958; Lieberman, 1976).

### 1.2.2 Neurotransmitter-like substances associated with dorsal root ganglion neurons

Several neurochemicals or neuronal markers including peptides, purines, amino acids, and other enzymatic markers have been associated with dorsal root ganglion neurons. The evidence that some neurochemicals identified in these neurons serve as afferent neurotransmitters is persuasive, albeit incomplete. Five criteria must be met for a proposed afferent neurotransmitter to be associated with dorsal root ganglion neurons (Jensen, 1980): 1) the neurons contain and synthesize the neurochemical, 2) the neurochemical is released from central and/or peripheral terminals of neurons upon appropriate stimuli, 3) following its release, the chemical reacts with postsynaptically located specific receptors on neurons or other cells, 4) local mechanisms must exist that rapidly "turn off" the biological effects of the chemical, and 5) microapplication of the neurochemical to postsynaptic target sites mimics the effects of neuronal stimulation, such effects being blocked by specific antagonists to the neurochemical.

#### 1.2.2.1 Peptides

Immunohistochemical staining has localized 13 peptides in dorsal root ganglion neurons: substance P, somatostatin, cholecystokinin, calcitonin gene-related peptide, bombesin, vasoactive intestinal polypeptide, galanin, vasopressin, dynorphin, enkephalin, α-neo-endorphin, corticotropin-releasing factor and neurokinin-A (Willis & Coggeshall, 1991). Many of these peptides are colocalized in these neurons; for instance, colocalization of substance P and

calcitonin gene-related peptide has been identified immunohistochemically in dorsal root ganglion neurons (Willis & Coggeshall, 1991). By means of *in situ* hybridization, mRNA for substance P has been identified in many dorsal root ganglion neurons (Warden & Young, 1988), indicating that these neurons synthesize substance P. Endogenous release of substance P and calcitonin gene-related peptides in the dorsal horn of the spinal cord (Bergstrom et al., 1983; Bordin et al., 1987; Duggan et al., 1989; Games et al., 1979; Go & Yaksh, 1987; Morton & Hutchison, 1989) and in the peripheral nerve endings of dorsal root ganglion neurons (Butler & Hammond, 1980; Hougland et al., 1986) can be induced by electrical stimulation of unmyelinated afferent fibers, capsaicin administration, or thermonociceptive and mechanociceptive stimuli. Substance P receptors located postsynaptically have also been found in dorsal horn spinal neurons (Massari et al., 1985) which receive afferent input from dorsal root ganglion neurons. When substance P is applied by ionophoresis adjacent to dorsal horn spinal neurons, these neurons display prolonged excitation (Henry et al., 1974; Henry, 1976). Furthermore, substance P-mediated spinal excitatory responses can be blocked by a substance P antagonist (Urban & Randic, 1984). Thus, substance P fulfills the basic criteria to serve as an afferent neurotransmitter.

#### 1.2.2.2 Purines

The enzymatic immunoreactivity of adenosine deaminase (Nagy & Daddona, 1985; Airhart et al., 1990), a key enzyme which catalyzes the irreversible deamination of adenosine to produce inosine (Frederiksen, 1966), and the immunoreactivity of 5'-nucleotide-hydrolysing acid phosphatase, which

hydrolyzes guanosine-, uridine-, and inosine-5'-monophosphate (Dodd et al., 1984; Nagy & Daddona, 1985), have been localized in the cell bodies and axon terminals of mammalian dorsal root ganglion neuron. The co-existence of these enzymes with peptides such as substance P in these neurons has also been reported (Nagy & Daddona, 1985). Endogenous release of purinergic compounds from primary afferent sensory endings supports the concept proposed by Holton (1954, 1959) that purinergic compounds synthesized and regulated in dorsal root ganglion neurons may be released at their central and peripheral terminals upon appropriate stimulation.

Physiological evidence indicates that a distinct population of neurons from lamina II of the dorsal horn in tissue culture are sensitive to ATP (Jahr & Jessel, 1983). Central application of ATP to functionally identified feline dorsal horn spinal neurons *in vivo* can modify both nociceptive and non-nociceptive neurons (Fyffe & Perl, 1984; Salter & Henry, 1985). These data suggest that ATP may act as a neurotransmitter at afferent synapses in the dorsal horn of the spinal cord. However, several important criteria remain to be fulfilled before a transmitter action of ATP can be established. Thus, as yet there is no evidence that ATP is released from the central terminals of dorsal root ganglion neurons, although the ATP release from peripheral terminals of sensory nerves has been demonstrated (Holton & Holton, 1954; Holton, 1959). Also, specific $P_2$ receptors on dorsal horn spinal neurons have yet to be identified.

Adenosine is an important neuromodulator involving noxious sensory inputs (Goodman & Snyder, 1982). Biochemical studies indicate that endogenous adenosine originating from primary afferent terminals is released in dorsal horn of the spinal cord (Sawynok et al., 1989). Both $A_1$ and $A_2$ adenosine receptors are identified in the dorsal horn spinal neurons, which

probably receive primary afferent input (Choca et al., 1987; Goodman & Snyder, 1982; Sawynok et al., 1989). Furthermore, spinal application of adenosine can excite dorsal horn adenosine receptors, resulting in antinociceptive responses (Sawynok et al., 1989). These data suggest that adenosine probably released from the central terminals of dorsal root ganglion neurons exerts neuromodulatory effects on spinal cord neurons. Adenosine has been shown to excite cardiac sympathetic afferent axons associated with thoracic dorsal root ganglion neurons. For instance, activity generated by cardiac sympathetic afferent axons increased moderately following epicardial application of high concentration of adenosine (Montano et al., 1991). Furthermore, intracardiac administration of adenosine increases renal sympathetic efferent nerve activity, presumably by activating cardiac sympathetic afferent neurons (Thames et al., 1993). These data suggest that cardiac sensory terminals of dorsal root ganglion neurons can be activated by adenosine.

#### 1.2.2.3 Amino acids

The excitatory amino acid glutamate is a candidate for a primary afferent neurotransmitter (Willis & Coggeshall, 1991). Glutamate has been associated immunohistochemically with a population of dorsal root ganglion neurons. Cytosolic glutamic oxaloacetic transaminase is localized in dorsal root ganglion neurons and dorsal root ganglion neurons contain glutamine, the precussor of glutamate. Glutaminase, an enzyme which degrades glutamate, is localized in dorsal root ganglion neurons. These findings indicate that a subpopulation of dorsal root ganglion neurons is involved in glutamate storage and metabolism. Glutamate receptors are also found in spinal cord interneurons (Mclennan &

Lodge, 1979). Curtis and associates (1959, 1960) were the first to demonstrate that ionotophoretic administration of glutamate into the vicinity of spinal cord interneurons exerts an excitatory action on these neurons. Thus, it is generally accepted that glutamate, a putative neurotransmitter of dorsal root ganglion neurons, exerts excitatory effects on spinal interneurons (Willis & Coggeshall, 1991)

With respect to the heart, only a few neurotransmitter-like substances have been associated with cardiac primary afferent neurons. Immunoreactivity of substance P and calcitonin gene-related peptide has been associated with cardiac afferent axons that are probably derived from dorsal root ganglion neurons in guinea-pigs (Hougland et al., 1986; Urban & Papka, 1985). However, substance P reportedly exerts no effects on dorsal root ganglion neurons in culture (Nowak & MacDonald, 1982) or on cardiac sympathetic afferent endings *in situ* (Nishi et al., 1977).

### 1.2.3 Synapses in dorsal root ganglia

There is some early anatomical literature on possible synapses in the dorsal root ganglia. This was summarized by Scharf (1958) who published diagrams of the possible arrangements of such synapses. Light microscopic evidence indicated that processes may arise from neurons either within or outside dorsal root ganglia that form synaptic endings on dorsal root ganglion neurons (Scharf, 1958). Lieberman (1976), however, has stated that no electron microscopic evidence exists which supports the contention that synaptic mechanisms exist in dorsal root ganglia. Three recent studies deserve consideration. First, Kayahara et al. (1981) have identified synaptic terminals

that end on the cell bodies of feline cervical and thoracic dorsal root ganglia by electron microscope. Second, they have identified synaptic terminals in dorsal root ganglia that apparently arise from motor neurons of the spinal cord (Kayahara et al., 1984, 1986). These important findings indicate that a population of dorsal root ganglion neurons may be under direct synaptic control from spinal cord neurons.

*In situ* intracellular recording studies demonstrated the prepotentials in feline dorsal root ganglia following electrical stimulation of central or peripheral portion of dorsal root. Such prepotentials are similar to the excitatory postsynaptic potentials generated by other autonomic neurons (Miletic & Lu, 1993; Lu et al., 1993). Furthermore, spontaneous activity has been identified in dorsal root ganglia in which associated central and peripheral dorsal root connections were anesthetized or sectioned (Lu et al., 1993). Based on such morphological and physiological evidence, it has been postulated that some dorsal root ganglion neurons which generate spontaneous activity independent of peripheral input may modulate other dorsal root ganglion afferent neurons (Miletic & Lu, 1993).

### 1.2.4 Spinal organization of visceral afferent input

Neuroanatomical studies have shown that neurons throughout laminae I to VII in the dorsal horn of the thoracic spine receive afferent information from visceral mechanosensitive and chemosensitive nerve endings, the major concentration of these neurons being located in lamina V (Carstens & Trevino, 1978; Giesler et al., 1979; Trevino, 1976; Willis et al., 1979). These findings correlate well with the electrophysiological identification of visceral afferent input

termination in the spinal cord (Ammons et al., 1985; Blair et al., 1981). By means of autoradiographic labeling techniques, various possible postsynaptic chemical receptors have been identified in the dorsal horn. The presence of various neurochemicals in dorsal root ganglion neurons and of corresponding neurochemical specific receptors on dorsal horn spinal neurons may have implications for the colocalization of neurochemicals within neurons which subserve diversified sensory functions. Thus, a hypothesis of the relationships between cytochemical properties and sensory modality transmission of dorsal root ganglion neurons has been proposed (Carr & Nagy, 1993). No physiological evidence exists to support this hypothesis.

Anatomic (Kuo et al., 1984) and electrophysiological (Foreman, 1991) studies indicate that spinal cord neurons which receive cardiopulmonary afferent input project mainly to the contralateral anterior quadrant of the spinal cord to form two major ascending pathways: the spinothalamic tract and spinorecticular tract (Foreman et al., 1984). At the level of the brainstem, interneurons interface with the nucleus ambigus and with vasomotor centers to alter efferent vagal and sympathetic neuronal activity. Some afferent sympathetic fibers do not ascend to higher centers but interact via interneurons with efferent sympathetic neurons at various levels of the spinal cord (Foreman, 1991).

## 1.3 Cardiac sensory endings of dorsal root ganglion neurons

Afferent axons with cardiac sensory endings travel with thoracic sympathetic nerve into ramus communicants and finally form the dorsal roots of upper thoracic dorsal root ganglia. Thus, dorsal root ganglion cardiac afferent axons are also called cardiac sympathetic afferent axons (Malliani, 1982).

Brown (1964, 1967) first recorded and Ueda et al. (1969) carried out the first detailed electrophysiological study of the activity generated by these cardiac sympathetic afferent axons. These axons were found to be responsive to both epicardial mechanical probing and hemodynamic stimuli. Subsequent work by Malliani et al. (1972, 1973) demonstrated that some cardiovascular sympathetic afferent axons display spontaneous activity related to normal hemodynamic events. Thus, it has been proposed that cardiovascular sympathetic afferent neurons are tonically involved in the neural regulation of the cardiovascular system.

### 1.3.1 Atrial mechanosensitive nerve endings

Atrial mechanosensitive afferent nerve endings are excited when a mechanical probe is applied to their sensory fields. Axons arising from these endings have been reported to be myelinated (Malliani et al., 1973; Uchida & Murao, 1974a; Uchida, 1975) or unmyelinated (Uchida & Murao, 1974a; Uchida, 1975). While most atrial sympathetic sensory nerve endings generate spontaneous activity, some are relatively quiescent during physiological states (Ueda et al., 1969). The spontaneous activity generated by these afferent axons generally express a temporal relation to a particular phase of intraatrial pressure waves. For instance, activity generated by atrial afferent axons was found to be phase-related to either atrial systole (Malliani et al., 1973; Uchida & Murao, 1974a) or atrial diastole (Malliani et al., 1972). Such relationships change spontaneously to other patterns at other times (Malliani et al., 1973). The physiological significance of such a temporal relationship is not understood. Activity generated by sympathetic afferent axons with atrial sensory endings can

be modified when cardiodynamics are altered. Thus, activity generated by atrial afferent axons increases when atrial pressure rises (Malliani et al., 1973; Uchida & Murao, 1974a; Kostreva et al., 1975b) but decreases (and can even become abolished) when atrial pressure falls (Malliani et al., 1973).

### 1.3.2 Ventricular mechanosensitive nerve endings

Ventricular mechanosensitive endings are distributed throughout both left and right ventricles (Ueda et al., 1969). It is generally agreed that most of the myelinated sympathetic afferent axons with ventricular epicardial endings generate spontaneous activity which is irregular with respect to cardiac cycle, although a regular discharge pattern synchronized with ventricular systole (Malliani et al., 1973; Nishi et al., 1977) or diastole (Ueda et al., 1969) can be observed infrequently. There is no clear-cut difference in discharge pattern between myelinated and unmyelinated cardiac sympathetic afferent axons. Ventricular sympathetic sensory endings can be excited during increases in ventricular pressure (Ueda et al., 1969; Malliani et al., 1973; Nishi et al., 1977; Uchida, 1975; Nishi et al., 1977), and their activity decreases during reductions in ventricular pressure (Malliani et al., 1973; Uchida, 1975).

### 1.3.3 Chemosensitive cardiac sensory endings

Atrial and ventricular mechanosensitive sensory endings with both myelinated and unmyelinated axons in sympathetic afferent nerves are responsive to epicardial or intracoronary administration of certain chemicals, including bradykinin (Baker et al., 1980; Felder & Thames, 1982; Lombardi et

al., 1981; Uchida & Murao, 1974c), histamine and potassium (Nishi et al., 1977; Uchida & Murao, 1974d). Although adenosine has been proposed to be a stimulant of cardiac sympathetic afferent nerves (Sylvén et al., 1986, 1987; Montano et al., 1991; Dibner-Dunlap et al., 1993), only one study has directly identified a moderate increase in cardiac sympathetic afferent activity following epicardial applications of high concentration of adenosine (Montano et al., 1991). Mechanosensitive ventricular sensory nerve endings also called "polymodal" ventricular sensory nerve terminals which can be modified by chemical stimuli in normal hemodynamic conditions, and chemical substances can enhance those endings' mechanosensitivity (Malliani, 1982).

Some investigators have proposed that a second type of chemosensitive ventricular sensory ending exists, which is nonresponsive to mechanical stimuli but can be activated by epicardial application of high concentrations of bradykinin (Uchida & Murao, 1974c) or by prolonged asphyxia (Takenaka et al., 1970). This type of cardiac sensory ending has been proposed to represent a "pure" chemosensitive type of ending. Malliani (1982) have challenged this concept (Malliani, 1982), suggesting that the existence of "pure" chemosensitive cardiac nerve endings does not exclude the possibility that sensory endings located deep in the ventricles might not respond to epicardial probing (Uchida & Murao, 1974 a & c). Thus, more vigorous and global mechanical stimuli, such as aortic occlusion, might modify their activity (Lombardi et al., 1981).

## 1.4 Cardiac nociception

### 1.4.1 Cardiac nociceptive sensory endings

So far, no substantial evidence exists to support the thesis that a distinct class of cardiac sympathetic afferent nerve endings, which respond only to "noxious" stimuli (Sherrington, 1906), exists in the heart. Nociceptive sensory nerve endings belong to a class of sensory nerve terminals that normally have no spontaneous activity, being unresponsive to mechanical stimuli and excited by chemical stimuli only (Burgess & Perl, 1973; Häbler et al., 1990; Schaible & Schmidt, 1988). These sensory endings are activated in inflamed joints (Schaible et al., 1988) and in diseased urinary bladders (Häbler et al., 1990). The existence of a small population of nociceptive cardiac sympathetic nerve endings has been claimed (Baker et al., 1980; Coleridge & Coleridge, 1980). However, since such chemosensitive nerve endings displayed background activity, they did not fit the nociceptive criteria described by other investigators (Burgess & Perl, 1973; Häbler et al., 1990; Malliani, 1982; Schaible & Schmidt, 1988). Brown and Malliani (1971) reported that a few "silent" cardiac sympathetic afferent axons were activated during interruption of coronary artery flow in spinal transected animals. Such a recruitment of afferent axonal activity was initially interpreted as suggesting the existence of specific nociceptors excited by myocardial ischemia; however, the same authors pointed out that the lack of background activity of these "silent" axons could have been due to insufficient cardiodynamic stimuli as a result of the low arterial blood pressure which occurs following spinal transection, a fact that might have resulted in a lack of spontaneous activity generated by ventricular mechanosensitive nerve endings (Malliani, 1982; Malliani & Lombardi, 1982).

It is generally agreed that cardiac dorsal root ganglion afferent neurons are essential to the perception of cardiac pain in man (Leriche & Fontaine, 1927; Sutton & Lueth, 1930; Lindgren & Olivecrona, 1947; White, 1957; White & Smithwick, 1941). Cardiac vagal afferent neurons may exert some modulating effects on sympathetic afferent mediated cardiac pain (Foreman, 1991). Two hypotheses have been proposed to explain potential cardiac pain mechanisms: the specificity and intensity theories of pain (Perl, 1971). The specificity theory requires the existence of anatomically and functionally separate afferent neurons that extend from the periphery to the cortex and that specifically signal information associated with tissue damage (Willis & Coggeshall, 1991). Current experimental evidence derived from somatic (skin, muscle, and joints) and visceral (urinary bladder) organs support the specificity theory (Cervero & Jänig, 1992); however, Malliani (1982) has questioned whether this theory can account for cardiac pain. The intensity theory postulates the existence of nonspecific peripheral afferent pathways which may functionally separate in the central nervous system. Thus, cardiac nociception has been thought to result from the intensity of the tonic discharge of nonspecific sympathetic sensory neurons in response to noxious mechanical or chemical stimuli applied to the heart (Lombardi et al., 1981; Malliani & Lombardi, 1982). Since all ventricular sympathetic sensory endings have been presumed to generate activity during normal hemodynamic states, they cannot be considered to function as pure nociceptors. Based on this assumption, Malliani and Lombardi (1982) have suggested that the "intensity" theory can account for the peripheral afferent neural mechanism subserving cardiac nociception.

### 1.4.2 Pain mediators

Although it was proposed decades ago that the myocardium releases chemical substances during ischemia which might be responsible for visceral pain (Lewis, 1932; Gutzman & Lim, 1962), no conclusive evidence exists for or against this hypothesis. According to Lewis (1932), the criteria for a candidate substance acting as a pain messenger during myocardial ischemia should include: 1) that it is released in sufficient quantities during ischemia, 2) that it can provoke angina pectoris-like pain experimentally, and 3) that it can excite cardiac afferent neurons.

Potassium has the capacity to depolarize afferent neurons, acting at high concentrations as a local irritant provoking pain. Epicardial application of 10 mM KCl increased the axonal activity generated by cardiac sympathetic afferent neurons (Uchida & Moura, 1974). During myocardial ischemia in animals (Haddy & Scott, 1971) and humans (Webb et al., 1986) coronary sinus potassium concentrations do not exceed 5 mM. These data imply that endogenously released potassium during transient myocardial ischemia may not occur in sufficient quantities to excite cardiac afferent neurons independently.

Bradykinin is, on a molar basis, one of the most potent afferent neuronal excitatory substances (Felder & Thames, 1982; Lombardi et al., 1981; Uchida & Murao, 1974c). Endogenous release of bradykinin from the heart during myocardial ischemia has been demonstrated in animals (Kimura et al., 1973). However, percutaneous transluminal coronary angioplastic occlusions in humans which elicit signs and symptoms of myocardial ischemia do not induce detectable bradykinin release into coronary sinus blood (Eldar et al., 1992).

Furthermore, it has been reported that intracoronary administration of bradykinin does not induce angina pectoris-like pain in humans (Rafflenbeul et al., 1986).

Substance P, a putative neurotransmitter associated histochemically with 10-30 % dorsal root ganglion neurons, is known to be involved in visceral and somatic nociception (Willis & Coggeshall, 1991), but it reportedly exerts no effects on cardiac sympathetic afferent endings (Nishi et al., 1977). Although, when administered into coronary artery blood, substance P does not provoke chest pain in humans (Crea et al., 1990; Gaspardone et al., 1992), substance P, however, can facilitate the induction of chest pain induced by coronary artery administration of adenosine in humans (Gaspardone et al., 1992).

Adenosine can elicit cardiac pain when administered to healthy humans. Adenosine also provokes chest pain in patients with stable angina (Sylvén et al., 1986, 1987). Such adenosine-mediated pain does not differ qualitatively from the chest pain induced by myocardial ischemia (Sylvén et al., 1986). In addition, the time course of the onset of chest pain induced by intravenous adenosine administration was similar to the onset of the atrioventricular block (Sylvén et al., 1987). Thus, such pain was proposed to originate from a myocardial site. In subsequent studies, administration of adenosine to the coronary artery of patients with stable angina was found to precipitate chest pain with a character, location and radiation similar to those of angina pain (Crea et al., 1990, 1992). Thus, it was concluded that the pain induced by adenosine originated from the heart (Crea et al., 1990, 1992). Pain elicited by both intravenous and coronary artery administration of adenosine is attenuated by aminophylline (Sylvén et al., 1986; Crea et al., 1990), and that produced by intravenous adenosine is potentiated by dipyridamole (which blocks adenosine transport into cells) (Sylvén et al., 1986). The mechanism of the cardiac pain

produced by adenosine was proposed to be due to activation of cell-surface adenosine receptors on sensory nerve terminals located in the myocardium. The role of endogenous adenosine in ischemic pain has also been examined. Aminophylline reduces exercise-induced chest pain (Crea et al., 1990) and delays the onset time of angina during exercise-induced ischemia (Crea et al., 1989). Similarly, theophylline reduces pain resulting from ischemic work in the forearm (Jonzon et al., 1989). In neither case, however, was pain abolished. The reduction of ischemia-induced chest pain by an adenosine-receptor antagonist suggests that endogenous release of adenosine with subsequent activation of the adenosine receptors of cardiac afferent nerves may be a significant factor in the generation of such pain. Montano et al. (1991) showed that epicardial application of adenosine increases the activity generated by cardiac sympathetic afferent axons, but they reported that the excitatory effects on cardiac sympathetic afferents that adenosine elicited were minimal, much less than those induced by bradykinin. Thus, the relative contribution of adenosine and bradykinin to the activation of sympathetic afferent neurons associated with cardiac sensory endings remains to be determined.

Among the pharmacological agents that have been shown experimentally to excite cardiac afferent nerves, only adenosine has been demonstrated to cause angina pectoris-like pain in humans. The fact that adenosine antagonists reduce, but do not abolish ischemia-induced cardiac pain (Crea et al., 1990; Jonzon et al., 1989) indicates that ischemia-induced pain of cardiac origin may not be mediated by a single "pain mediator". Rather, complex interactions between a number of factors may be involved in the genesis of cardiac pain during myocardial ischemia.

## 1.5 Cardiogenic sympatho-sympathetic reflexes

It has become clear that cardiac sympathetic afferent nerves can mediate reflexes involved in cardiovascular regulation (Malliani, 1982; Smith & Thames, 1994). Evidence supporting the existence of a cardiac sympatho-sympathetic reflex came from several types of experiments. Electrical stimulation of afferent cardiac sympathetic fibers elicits reflex increases in heart rate (Malliani et al., 1973), myocardial contractility (Malliani et al., 1972) and arterial pressure (Peterson & Brown, 1971). Mechanical stretch of aortic mechanosensitive nerve endings, without altering arterial pressure, induces increases in heart rate, cardiac contractility, and arterial blood pressure in sinoaortic afferent nerve denervated and adrenalectomized preparations (Lioy et al., 1974). Such cardiovascular augmentation was drastically reduced after β-adrenergic antagonists were administered, indicating that cardiac efferent sympathetic neurons were activated by this reflex. Cardiocardiac sympathetic reflexes can also be induced by chemosensitive cardiac sympathetic afferent neurons. Furthermore, intracoronary administration of veratridine, a chemical which excites cardiac afferent sensory endings, elicits enhanced myocardial contractility in vagotomized animals in which the upper spinal cord was transected (Malliani et al., 1972). Such cardiac augmentation was abolished after the transection of the upper thoracic sympathetic rami. Epicardial application of bradykinin, an endogenous substance known to excite cardiac afferent sympathetic nerve endings, induces cardiac augmentation, presumably by activating cardiac sympathetic afferent sensory endings (Staszewska-Barczak et al., 1976). Excitatory cardiovascular sympatho-sympathetic reflexes also are activated during myocardial ischemia. Using single-axon recording,

Malliani et al (1969a, b) showed that most preganglionic sympathetic efferent axons from the left third thoracic ramus communicants, which contribute to the innervation of the heart (Bronk et al., 1936; Randall et al., 1957), are activated during coronary artery occlusion. Reflex-mediated cardiac sympathetic efferent activation induced by coronary artery occlusion was also demonstrated in acutely (Felder & Thames, 1979) and chronically (Lombardi et al., 1981) sinoaortic nerve denervated preparations. Furthermore, increases in left ventricular pressure associated with increased cardiac sympathetic efferent activity can be elicited during coronary artery occlusion (Lombardi et al., 1981). As reflex-mediated cardiac changes were induced in spinal vagotomized animals, a cardiocardiac spinal sympathetic reflex pathway has been advanced. Cardiocardiac reflexes may not be always functioning during myocardial ischemia in animals with an intact neuraxis since activation of cardiac afferent endings associated with vagal afferent neurons at the same time causes central inhibition of efferent sympathetic neurons (Öberg & Thorén, 1973; Thorén, 1979; Thames et al., 1978; Minisi & Thames, 1991). Thus, the net cardiovascular efferent response induced during coronary occlusion will depend on central integration of these various afferent inputs.

Adenosine has also been shown to elicit cardiac augmentations when it was administered into a coronary artery in humans (Cox et al., 1989; Crea et al., 1992). Thames and associates (Dibner-Dunlap et al., 1993; Thames et al., 1993) showed that sympathetic efferent renal nerve activity increased following intracoronary administration of adenosine, suggesting that adenosine excites cardiac sympathetic afferent receptors, which in turn elicit excitatory sympatho-sympathetic reflexes. Furthermore, myocardial release of adenosine may be

involved in cardio-renal sympathetic reflexes during myocardial ischemia (Thames et al., 1993).

## 1.6 Rationale for the study

Previously characterization of sympathetic cardiac sensory nerve endings has been done by means of recording the activity generated by afferent axons in the ramus communicates (Malliani, 1982; Uchida, 1975) or subclavian ansae (Peters et al., 1980). No recording has been made of activity generated by *in situ* cardiac dorsal root ganglion neurons, the somata of sympathetic afferent axons arising from cardiac sensory terminals. When activity generated by single cardiac afferent axon is recorded, a limited number of interventions can be performed due to the fact these are short-lasting preparations since it is difficult to maintain adequate recordings of axonal action potentials for very long. Recording activity generated by *in situ* dorsal root ganglion neuronal somata provides a stable preparation which allows prolonged (~ 8 hours) periods in which responses of individual neurons to multiple physiological and pathological interventions can be assessed. Thus, the latter method of study was used for the work reported in this thesis.

Although bradykinin, an endogenous algesic substance released from animal heart during myocardial ischemia (Kimura et al., 1973) can excite cardiac sympathetic sensory endings (Lombardi et al., 1981; Uchida & Murao, 1974), little information exists concerning the effects of other endogenous chemicals on these sensory endings. Substance P, a putative neurotransmitter associated with dorsal root ganglion neurons (Hökfelt et al., 1975), has been implicated in visceral nociception (Nicoll et al., 1980; Pearson et al., 1982). Intravenous

administration of substance P facilitates adenosine-induced cardiac pain in humans (Gaspardone et al., 1992). Despite that, afferent nerve recordings so far have failed to demonstrate substance P-mediated excitatory effects on cardiac sympathetic afferent nerves (Nishi et al., 1977). As mentioned above, adenosine has been implicated in the genesis of cardiac pain during myocardial ischemia in humans (Sylvén et al., 1986, 1987; Crea et al., 1990, 1992). Adenosine and adenosine 5'-triphosphate (ATP) release are enhanced during myocardial ischemia (Berne, 1963; Clemens & Forrester, 1981; Forrester & Williams, 1977; Paddle & Burnstock, 1974). Although exogenously applied pharmacological doses of adenosine to the heart has been reported to increase activity generated by cardiac sympathetic afferent axons, it is not known whether physiological or pathophysiological concentrations of adenosine can do so. Furthermore, it is not known whether endogenous adenosine modulates the cardiac sensory nerve endings of dorsal root ganglion neurons.

The hypothesis of this study is that multiple receptors co-exist on cardiac sensory endings of individual dorsal root ganglion neurons and that these are modified simultaneously in physiological and pathophysiological states. The objectives of the present study are to: 1) characterize cardiac dorsal root ganglion afferent neurons respond to cardiovascular hemodynamic changes; 2) to determine whether peptides and purinergic compounds (adenosine and ATP) can modify sensory nerve endings of *in situ* dorsal root ganglion neurons; 3) determine whether substance P- or adenosine-mediated afferent neuronal responses are receptor specific and whether endogenously released substance P and adenosine modify the cardiac sensory endings of dorsal root ganglion neurons; 4) determine whether myocardial ischemia modifies cardiac afferent neurons sensitive to specific chemical stimuli.

## Chapter 2 Materials and Methods

### 2.1 Animal preparations

Thirty-one mongrel dogs of either sex, weighing 9-15 kg, were tranquilized with sodium thiopental (12-15 mg/kg i.v.) and anesthetized with α-chloralose (100 mg/kg i.v., α-chloralose was dissolved in propylene glycol at a concentration of 50 mg/ml). Supplemental doses of α-chloralose (20-35 mg/kg i.v.) were administered during the experiments, as required. Animals were intubated, and respiration was maintained by means of a Bird Mark 7 positive-pressure respirator. Pulmonary pressure was measured via a catheter which was inserted into the tracheal tube and connected to a Bentley Trantec model 800 transducer (Bentley Trantec Inc., Irvine, CA). A bilateral thoracotomy was performed at the fourth intercostal interspace to expose the heart. The ventral pericardium was incised and retracted laterally to expose the epicardial surface of the heart. The origins of the left anterior descending and circumflex coronary arteries were identified, and threads were placed around them so that they could be transiently occluded later in the experiment. One miniature (5 mm diameter, 1.5 mm thick) solid state pressure transducer (Konigsberg Instruments, model P19D, Pasadena, CA) was inserted into the right ventricular conus and another was inserted into the ventrolateral wall of the left ventricle to record right and left ventricular regional intramyocardial pressures. Left ventricular chamber pressure was measured via a Cordis #7 catheters (Cordis Corporation, FL) that was inserted into the chamber via a femoral artery and was connected to a Bentley Trantec model 800 transducer (Bentley Trantec Inc., Irvine, CA). Left atrial chamber pressure was measured via another Cordis #7 catheter inserted into the left atrium via left atrial appendage and connected

to a Bentley Trantec model 800 transducer. Umbilical tape was placed around the descending aorta and superior and inferior vena cavae, so that these structures could be occluded partially later in the experiments.

Thereafter, a dorsal laminectomy was performed to expose dorsal root ganglia at the $T_2$-$T_5$ levels of the spinal cord, leaving the dura of the spinal cord intact. The $T_1$ and $T_6$ vertebral processes were secured with a spinal cord stereotaxic apparatus in order to minimize spinal cord motion. Exposed thoracic dorsal root ganglia were covered with mineral oil throughout the experiments.

## 2.2 Extracellular recording of activity generated by a single neuron

Spontaneous activity generated by 280 neurons in left or right $T_2$-$T_5$ dorsal root ganglia was investigated. A tungsten microelectrode (Frederick Haer Co. #25-10-3, Brunswick, ME) mounted on a Märzhäuser micromanipulator (25033-10, Fine Scientific Tool Inc., North Vancouver, BC) was driven into exposed dorsal root ganglia in micrometer increments (the indifferent electrode was attached to structures adjacent to the ganglion). Signals were differentially amplified by a Princeton Applied Research model 113 amplifier, which had bandpass filters set at 300 Hz to 10 KHz and an amplification range of 100-500X. The output of this device was further amplified (50-200X) and filtered (band width 100 Hz-2k Hz) using an optically isolated amplifier (Applied Microelectronics Institute, Halifax, N.S., Canada) and the output of this amplifier was led to a Nicolet model 207 oscilloscope (Nicolet Instrument Corporation, Madison, Wisconsin) as well as to an audio monitor (Grass AM8B, Grass Instruments, Quincy, Mass) for monitoring neuronal activity. Activity generated by individual neurons was identified by the amplitude

and shape of the recorded action potentials. Generated action potentials with signal-to-noise ratios greater than 3:1 were identified for several hours, a process facilitated by the lack of motion of dorsal root ganglia *in situ*. Action potentials generated by a single neuron were defined using the following criteria: 1) action potentials generated by one neuron displayed the same configuration and amplitude over a number of hours and 2) action potentials maintained the same configuration when the microelectrode was moved micrometers away from the site where maximal activity was recorded even though their amplitude changed.

The conduction velocity of afferent axons connected with identified cardiac afferent neurons in a dorsal root ganglion was estimated by delivering electrical stimuli (1-4 V, 1 ms, 0.1 Hz) to the identified epicardial receptor field at the end of the experiments by means of a unipolar ball electrode (the indifferent electrode being attached to the thoracic wall). The latency of activation of dorsal root ganglion neurons was determined, and the distance between the stimulating and recording electrodes was estimated.

## 2.3 Identification of epicardial afferent neurons

Once spontaneously active neurons were identified in a dorsal root ganglion site, 10 minutes of baseline activity was recorded. Thereafter, various atrial or ventricular loci were gently probed by means of a saline-soaked Q-tip applicator. In addition, loci in the pulmonary tissues, the thoracic aorta, and the superior and inferior vena cava were probed. Neurons whose activity was modified following epicardial probing and/or other cardiac mechanical stimuli were considered to be connected to epicardial sensory nerve endings.

## 2.4 Interventions

### 2.4.1 Mechanical stimuli elicited by vascular occlusion

The aorta, superior vena cava, and inferior vena cava were individually occluded (5-20 sec) by means of umbilical tape previously placed around each of these vessels. In this way, epicardial mechanosensitive endings which may not have been affected by gentle epicardial probing were identified, and those identified by touch were modified.

### 2.4.2 Mechanical stimuli elicited by altering pulmonary dynamics

The respiratory rate was altered (rate and pressure), followed by cessation of respiration for 60 seconds, in order to determine if altered respiratory dynamics which may result in alterations of cardiac mechanics and/or coronary artery blood gas affected activity generated by identified epicardial afferent neurons.

### 2.4.3 Sensory-field application of chemicals

#### 2.4.3.1 Bradykinin, substance P, and a substance P antagonist

Peptides were applied for 60-120 seconds to mechanically identified epicardial sensory fields. Neurochemicals (0.5 ml), applied via 1 cm x 1 cm gauze squares, were obtained from Sigma Chemical Co. (St. Louis, MO). The peptides investigated were bradykinin acetate salt and substance P. Four concentrations of bradykinin (1, 10, 100, and 750 μM) were tested on identified epicardial sensory fields of six dorsal root afferent neurons. Bradykinin, when

studied at these four concentrations, elicited neuronal responses; however, 750 μM bradykinin elicited maximal afferent neuronal responses. This concentration was used in the remaining experiments. Similarly, four concentrations of substance P (1, 10, 100, and 300 μM) were tested on the epicardial sensory fields of eight dorsal root afferent neurons. Substance P elicited neuronal responses at all four concentrations but the 300 μM concentration elicited the maximal responses, so that concentration was used for the remaining experiments. The effects of the substance P antagonist [D-Arg$^{1}$, D-Trp$^{7,9}$, Leu$^{11}$]-substance P (spantide) (10 μM, Sigma Chemical Co.) was also studied, to determine whether endogenously released substance P modifies epicardial afferent neurons. This was done by applying an antagonist (0.5 ml) soaked-gauze pledglet to a previously identified substance P sensory field for 1- 2 minutes and then applying substance P (10 μM) to the same pledglet, so that it was tested in the presence of spantide. To determine whether bradykinin effects were due to the release of substance P from sensory nerve endings (Geppetti, 1993), bradykinin (100 μM) was applied in the presence of the substance P antagonist (10 μM) to the epicardial sensory fields of four afferent neurons that had previously responded to bradykinin and substance P in a similar fashion.

After each chemical had been applied, the epicardial region investigated was flushed with normal saline (~ 2 ml/sec) for at least 20 seconds to wash all the applied chemical from the sensory field. Depending on the neuronal response elicited, 5-20 minutes was allowed to elapse between the application of each neurochemical, to enable preparation stabilization. A chemical was reapplied if it elicited a response. In addition, gauze squares soaked with room-temperature normal saline were applied to identified epicardial sensory fields in

order to determine whether neuronal responses elicited by chemical application was due to chemical or vehicle effects.

2.4.3.2 Adenosine and adenosine antagonists

Three concentrations of adenosine (0.75, 10, and 100 μM) were tested on epicardial sensory fields associated with ten dorsal root ganglion cardiac afferent neurons. As 0.75 μM adenosine elicited maximum neuronal responses and did not elicit bradycardia and systemic hypotension, that dose was used for all subsequent studies. In addition, adenosine (3-10 ml, 0.75 μM) was administered into the left atrium via the left atrial catheter in order to determine whether administering adenosine into the systemic circulation can elicit afferent neuronal responses similar to those induced by applying adenosine to the epicardial sensory field.

Adenosine $A_1$ and $A_2$ receptor-specific antagonists (Fredholm et al., 1994) were individually applied at the ventricular epicardial sensory fields of fifteen dorsal root ganglion neurons which were activated by the epicardial application of adenosine and/or substance P. This was done by applying 0.5 ml of the selective adenosine $A_1$ receptor antagonist DPCPX (1,3-dipropyl-8-cyclopentylxanthine, 2 μM) or $A_2$ selective adenosine-receptor antagonist KF 17387 (1,3-dipropyl-8-(3,4-dimethoxystyryl)-7-methylxanthine, 2 μM) via a 1 cm x 1 cm gauze square on identified epicardial sensory fields which had been sensitive to adenosine. After a 1-2 minute application of the $A_1$ or $A_2$ antagonist, adenosine (0.5 ml, via a syringe) was applied to the same gauze. Thereafter, the effects of a sensory-field application of the nonselective ($A_1$+$A_2$)

adenosine antagonist CGS-21680 hydrochloride (CGS, 2 μM) on these afferent neurons was studied.

#### 2.4.3.3 Epicardial application of ATP and an ATP analog

Three concentrations of ATP (0.75, 10, and 100 μM) were tested on epicardial sensory fields associated with ten dorsal root ganglion cardiac afferent neurons. As 0.75 μM ATP elicited maximum neuronal responses, this dose was used for all subsequent studies. In five neurons in which an epicardial application of ATP elicited afferent neuronal responses, the ATP analog β, γ -methyleneadenosine 5'-triphosphate (β, γ -ATP, 75 μM) was also applied to identified ATP-sensitive epicardial sensory fields.

### 2.4.4 Myocardial ischemia

After the interventions described above were completed, the left anterior descending coronary artery was occluded for 1- 5 minutes. Thereafter, the circumflex coronary artery was occluded for 1-5 minutes. At least 10 minutes separated these coronary arterial occlusions in order to allow preparation stabilization. In addition, substance P antagonist spantide (10 μM) and nonselective adenosine antagonist CGS (2 μM) which had modified epicardial afferent activity were reapplied individually to the epicardial sensory fields during myocardial ischemia of seven afferent neurons whose activity was enhanced by myocardial ischemia.

### 2.4.5 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 Astro-Med, Inc., model MT 9500 eight-channel rectilinear recorder (Astro-Med, Inc., West Warwick, R.I.) and were stored on VHS video tape (T120 Scotch, 3M, Canada Inc., London, Canada) using a VCR recorder (A.R. Vetter, Co. Model 820, Rebersburg, PA) for later analysis. Heart rate, left atrial pressure, as well as left ventricular intramyocardial and chamber systolic pressures, were measured for 20 consecutive beats and their means ± s.e.m. were calculated. Neuronal activity was counted for 60-second periods in order to establish average activity. This was done immediately before and during the maximal responses elicited during each intervention. If more than one cardiac afferent neuron was active at one locus, the activity generated by individual neurons was analyzed by means of a two-channel window discriminator (Hartley Instrumentation Development Laboratories, Baylor College of Medicine, Houston, TX). A change in neuronal activity was defined as occurring when activity differed by more than 20% from baseline values. One-way ANOVA and paired t-test with Bonferroni correction for multiple tests were used for statistical analysis. In addition, to determine whether the magnitude of neuronal excitation elicited by each intervention differs, contingency tables were constructed with corrections for continuity to compare responses elicited by each neurochemical and to compare responses elicited by neurochemicals with those induced by mechanical stimuli or coronary artery occlusion. A significance value of $p < 0.05$ was used for these determinations.

## Chapter 3 Results

### 3.1 Overview

Fifty-six afferent neurons identified in dorsal root ganglia (31 right and 25 left) associated with epicardial sensory endings were fully characterized with mechanical (atrial and ventricular epicardial touch and vascular occlusion) and multiple chemical stimuli. Of these 56 neurons, five generated no activity during control states or when mechanical stimuli were applied to the heart. They were subsequently identified since they generated activity when chemical stimuli were applied to their sensory fields shared by other spontaneously active cardiac afferent neurons. The mean baseline activity generated by the 51 spontaneously active neurons before any intervention was $1.5 \pm 0.3$ impulses/sec (ips) (range 0-17 ips). Forty-three (84%) of these spontaneously active neurons responded to both mechanical and chemical stimuli (Table 1). Of 31 neurons that were tested with coronary artery occlusion, 22 (71%) responded to that intervention (Table 1). Mechanical stimuli initiated responses immediately after they were applied, and the responses subsided relatively rapidly after stimulus was removed. Chemical stimuli initiated responses which lasted long after removal of the chemicals (up to 32 minutes). Axons connecting epicardial sensory endings to dorsal root afferent neurons had an average conduction velocity of 2.7 m/s (range 0.61-6.4 m/s).

## Responses Elicited By Epicardial Polymodal Afferent Neurons to Different Stimuli

| Stimulus | Responding vs total # neurons tested | Increased activity (impulses/sec) | | | Decreased activity (impulses/sec) | | |
|---|---|---|---|---|---|---|---|
| | | # of neurons | Control | Intervention | # of neurons | Control | Intervention |
| **Epicardial touch** | 26 / 43 (60%) | 21 | 1.7±0.6 | 5.5±2.6** | 5 | 2.1±0.6 | 0.6±0.2 |
| **Aortic occlusion** | 39 / 43 (91%) | 20 | 2.0±0.6 | 12.0±4.7** | 19 | 1.4±0.3 | 0.3±0.1** |
| **IVC or SVC occlusion** | 39 / 43 (91%) | 19 | 1.4±0.6 | 10.6±6.0** | 20 | 1.3±0.3 | 0.4±0.1** |
| **Epicardial bradykinin** | 28 / 43 (65%) | 25 | 1.8±1.1 | 7.2±2.6** | 3 | 16.1±5.2 | 10.1±5.0 |
| **Epicardial substance P** | 29 / 43 (67%) | 24 | 1.6±1.1 | 6.8±2.8** | 5 | 1.1±0.3 | 0.1±0.1 |
| **Epicardial adenosine** | 31 / 43 (72%) | 28 | 1.5±0.4 | 6.0±2.1** | 3 | 1.1±0.4 | 0.1± 0.2 |
| **Epicardial ATP** | 26 / 43 (60%) | 21 | 2.6±1.8 | 9.8±4.2** | 5 | 1.9±0.9 | 0.4±0.2 |
| **Coronary artery occlusion** | 22 / 31 (71%) | 19 | 2.2±1.6 | 8.0±2.9** | 3 | 1.0±0.5 | 0.4±0.2 |

Table 1. Responses of 43 identified dorsal root epicardial afferent neurons elicited by cardiac mechanical, chemical and ischemic stimuli. Data obtained from chemosensitive only neurons and quiescent neurons was not included in the table. ** denotes $p < 0.01$.

## 3.2 Cardiopulmonary mechanical stimuli

### 3.2.1 Cardiovascular stimuli

The sensory fields of individual neurons responding to epicardial touch (maximum activity of 15 ips) were approximately 1 cm x 1 cm. Two or three separate sensory fields were associated with 60% of identified neurons. No preferential distribution of epicardial sensory fields was identified with respect to neurons in left as opposed to right dorsal root ganglia. Sensory fields were identified on the ventral, lateral, or dorsal surfaces of the left ventricle and on the right ventricle. Thirty-eight percent of neurons asociated with ventricular mechanosensitive fields also had their epicardial sensory fields in atria.

Four types of neuronal responses were elicited from 39 of 43 afferent neurons with sensory endings on the left ventricular epicardium when left ventricular pressure changes were induced: fourteen (32%) displayed a type I (high-pressure-active) response, which consisted of increased activity being generated when left ventricular chamber systolic pressure increased and reduced activity when ventricular chamber systolic pressure decreased (Figs. 1 & 2); nine (22%) displayed a type II (low-pressure-active) response, in which neuronal activity increased when left ventricular chamber systolic pressure decreased and activity decreased when chamber systolic pressure increased (Figs. 2 & 3); six (14%) displayed a type III (high-low pressure active) response, in which activity increased regardless of whether the left ventricular chamber systolic pressure increased or decreased from control values, and was lowest when left ventricular systolic chamber pressure was in the physiological range (90-130 mmHg) (Figs. 2 & 4); and ten (24%) displayed a type IV (high-low pressure inactive) response, in which neuronal activity decreased regardless of

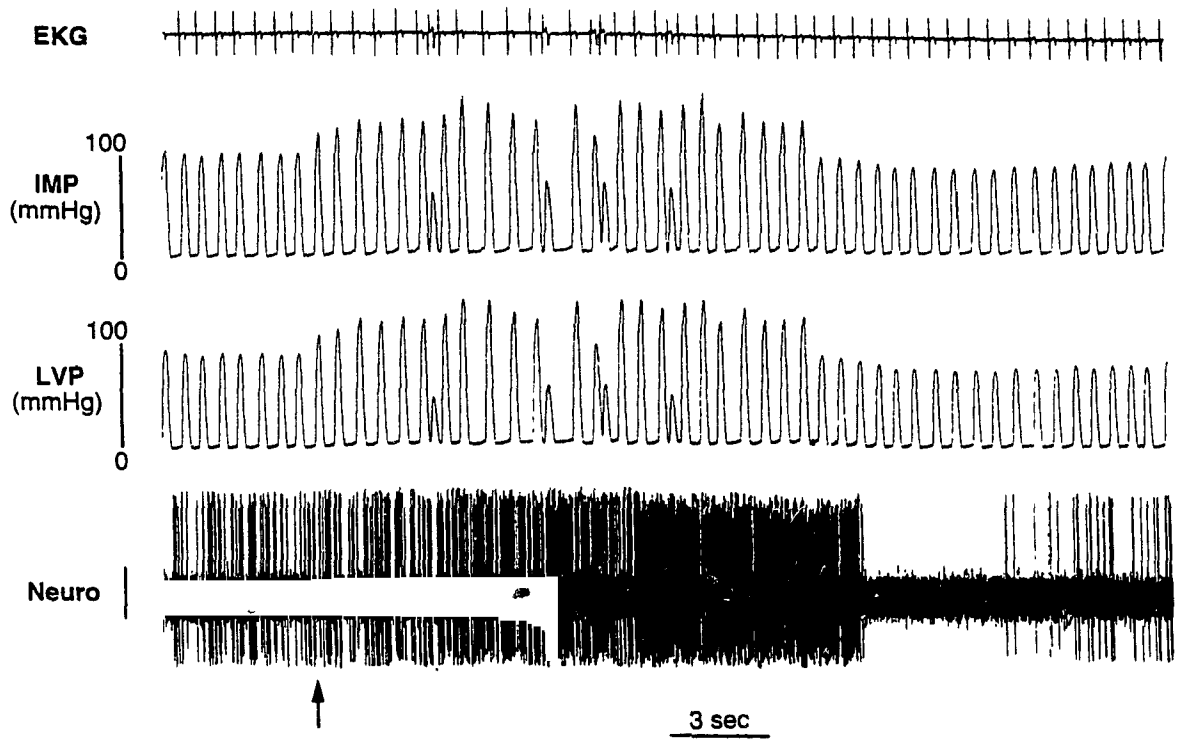


Figure 1. Activity generated by a left ventricular epicardial afferent neuron in a right $T_2$ dorsal root ganglia increased when the left ventricular chamber systolic pressure increased following aortic occlusion (arrows). Occlusion lasted 15 seconds. Vertical calibration beside neuronal activity is 0.1 mV. EKG = electrocardiogram; LAP: left atrial chamber pressure. IMP = left ventricular ventral wall intramyocardial pressure; LVP = left ventricular chamber pressure; Neuro = neuronal activity; Resp = respiration. The same abbreviations are used in all figures.

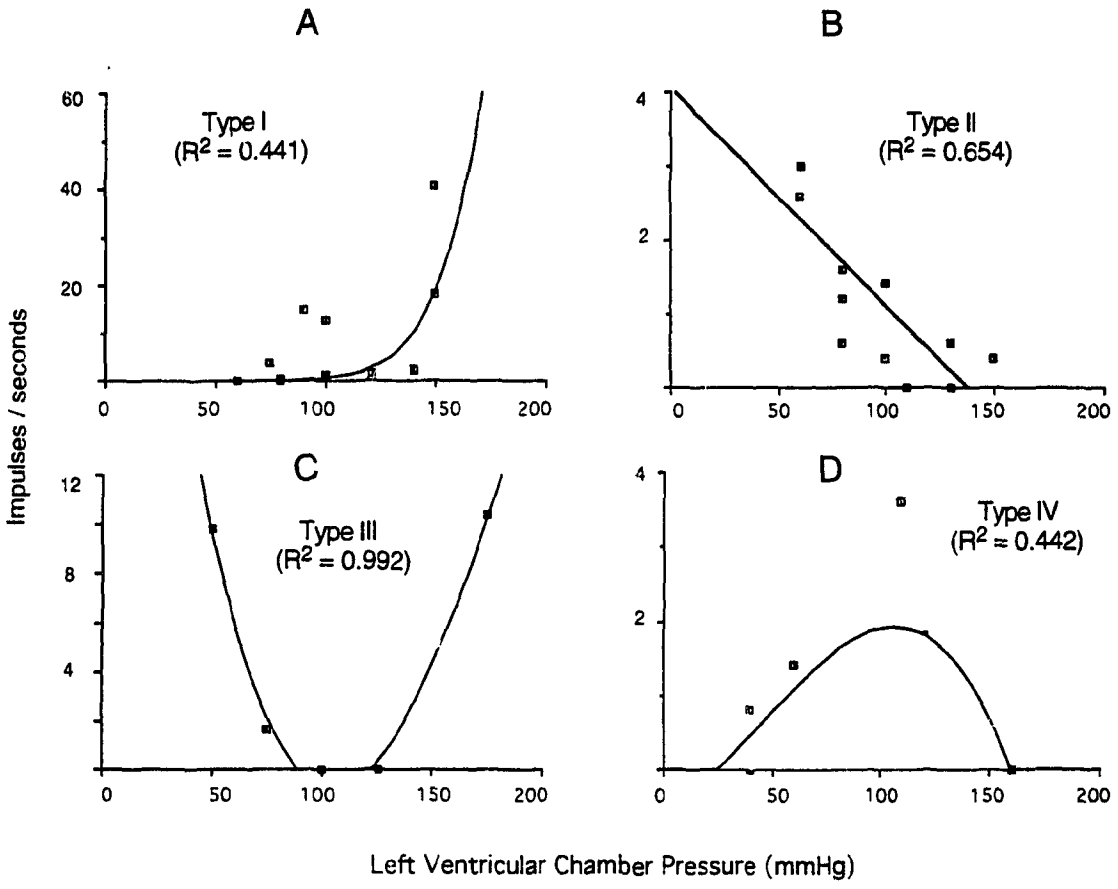


Figure 2. Four different types of cardiac afferent neuronal responses elicited by mechanosensitive neurons when left ventricular chamber systolic pressure was changed. Plot of type I afferent neuronal responses demonstrates a logarithmic relationship between left ventricular chamber systolic pressure and neuronal activity. This curve was derived from data obtained from four afferent neurons. Plot of type II afferent neuronal responses which displayed an inverse relationship between left ventricular chamber systolic pressure and neuronal activity. This curve was derived from four afferent neurons. Plots of type III and IV afferent neuronal responses displaying polynomial and inverse polynomial relationships, respectively, between left ventricular systolic chamber pressure and neuronal activity. Data for types III and IV afferent neurons were derived from one and two afferent neurons, respectively. $R^2$ denotes the square of the correlation coefficient.

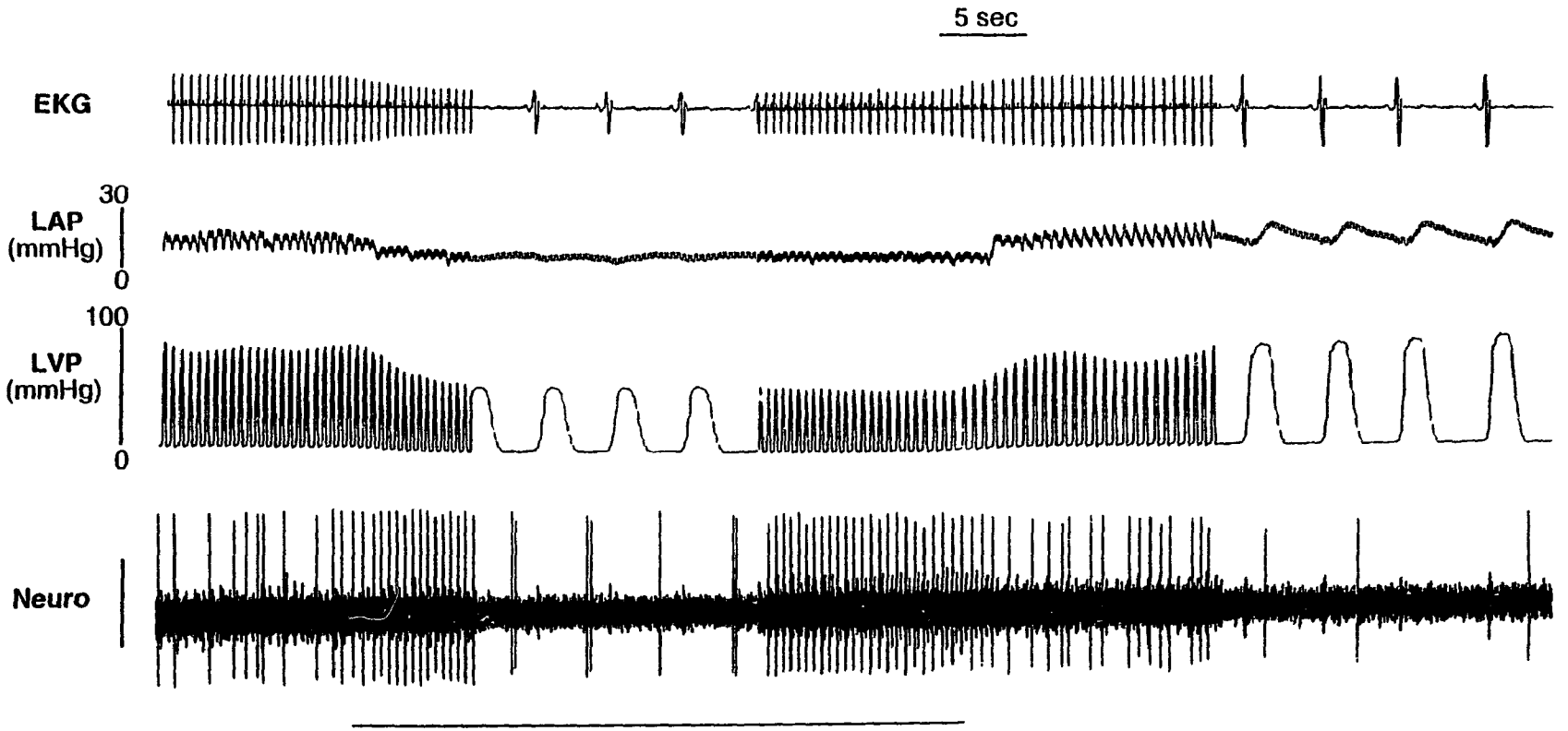


Figure 3. Activity generated by a left ventricular epicardial afferent neuron in a right $T_3$ dorsal root ganglia increased when the left ventricular chamber systolic pressure decreased during superior vena cava occlusion. Note that activity generated by this neuron was entrained with each cardiac cycle (~2 per systole) during the hypotension. Horizontal bar at bottom indicates the duration of the occlusion. Vertical calibration bar beside neuronal activity = 1 mV.

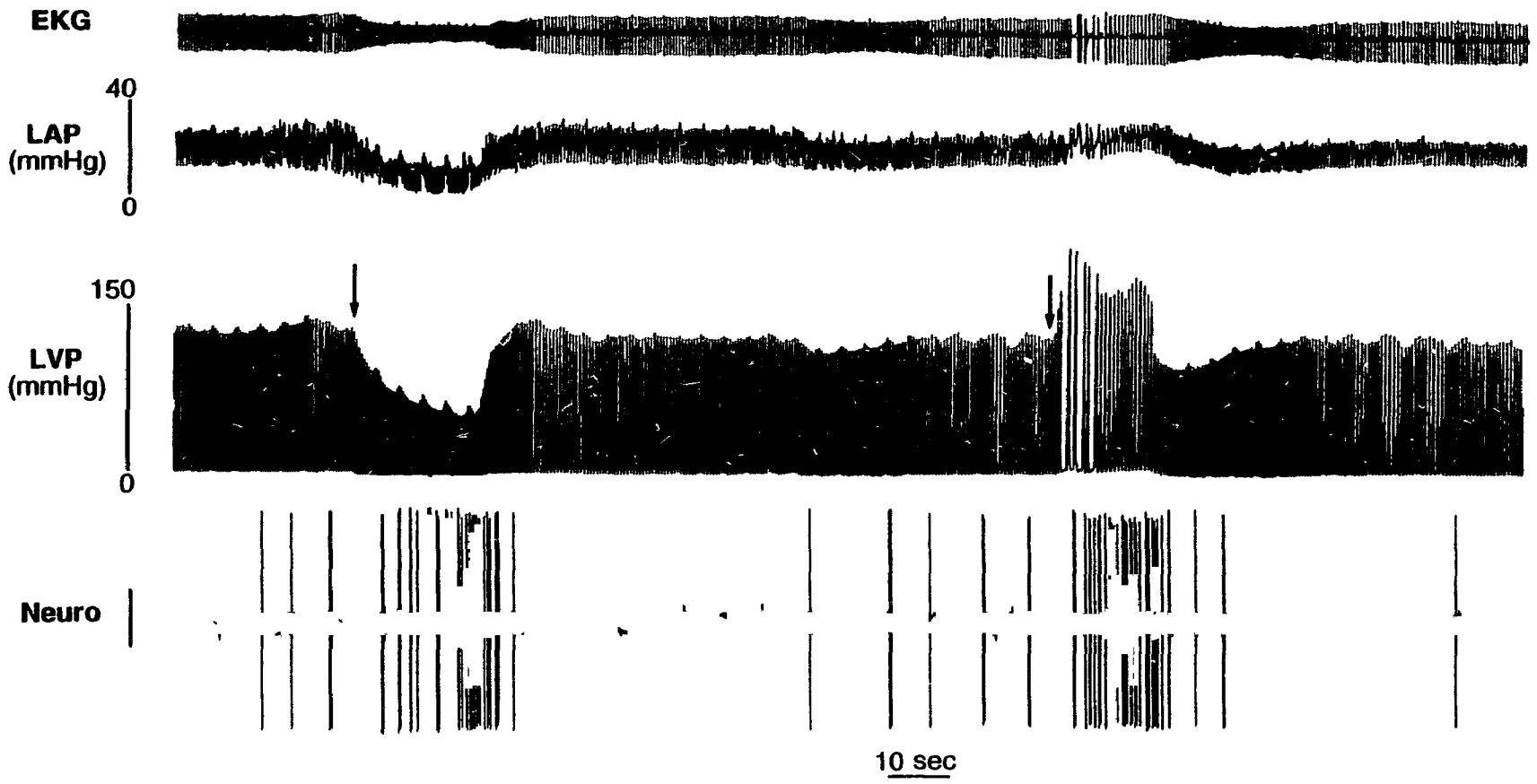


Figure 4. Activity generated by a left ventricular epicardial afferent neurone in a right $T_3$ dorsal root ganglion. Neuronal activity increased when left ventricular chamber systolic pressure was either decreased during occlusion of inferior vena cava (onset at first arrow) or increased when the descending aorta was partially occluded (onset at second arrow). Vertical calibration beside neuronal activity = 1 mV.

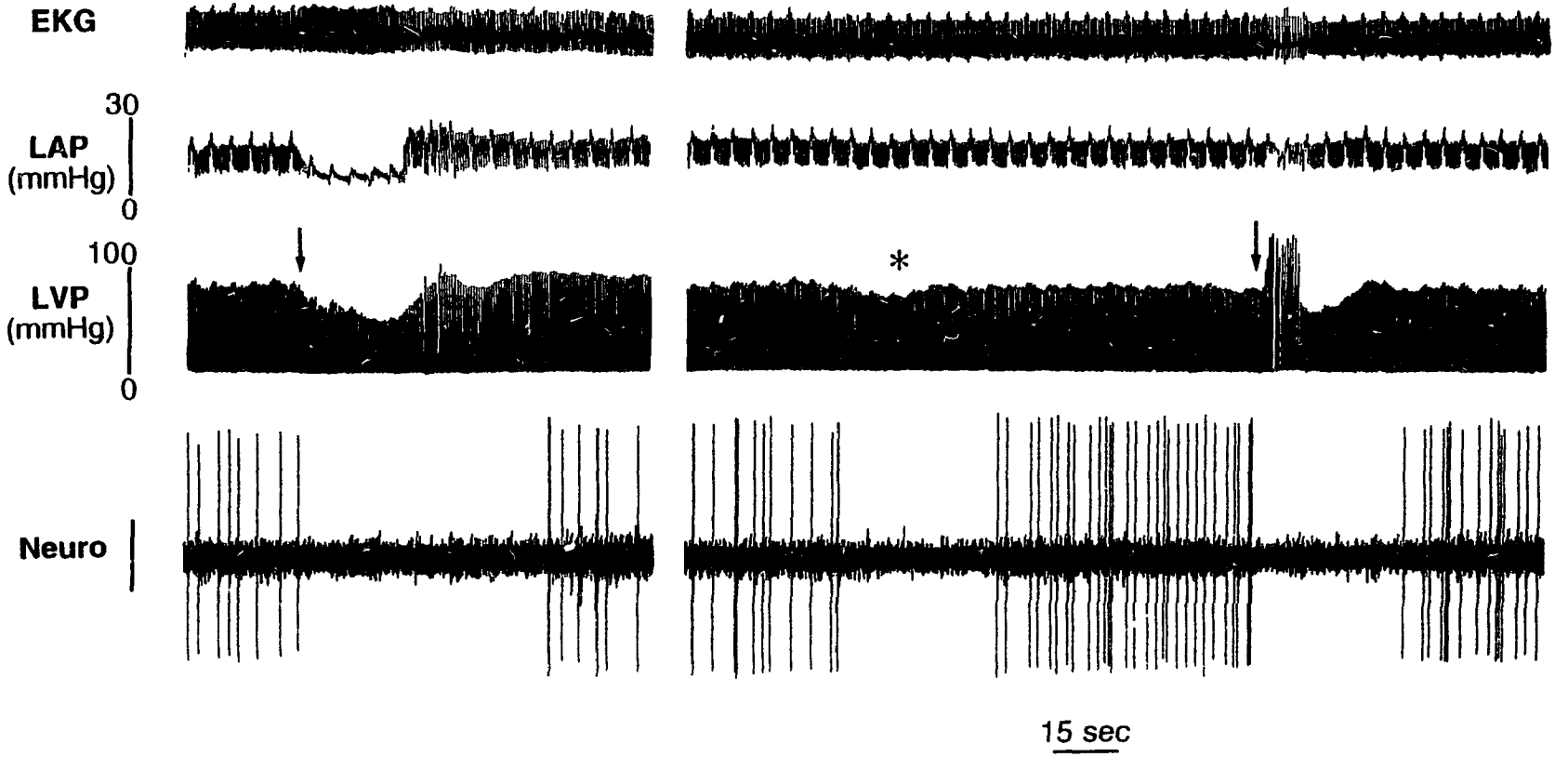


Figure 5. Activity generated by a left ventricular epicardial afferent neuron in a right $T_2$ dorsal root ganglion. Activity decreased when left ventricular chamber systolic pressure was reduced following occlusion of inferior vena cava (first arrow) or increased following partial occlusion of the descending aorta (second arrow). * denotes a spontaneous reduction in left ventricular systolic pressure which was accompanied by reduced activity. Vertical calibration beside neuronal activity = 0.5 mV.

whether the left ventricular chamber systolic pressure increased or decreased from physiological values (Figs. 2 & 5), and was maximal when the pressure was in the physiological range. Post-stimulus responses, the afterdischarge phenomenon, occurred in 70% of epicardial afferent neurons studied and lasted up to 15 seconds.

### 3.2.2 Respiratory stimuli

#### 3.2.2.1 Epicardial afferent activity

Respiratory-related activity was generated by seven neurons with an identified ventricular epicardial sensory field (Figs. 6, 10). Respiratory-related activity was not generated by these neurons when artificial ventilation was terminated (Fig. 10), but it persisted when afferent activity was increased by mechanical or chemical stimuli (Figs. 6 &10). Respiratory-related changes in the systolic intramyocardial pressure of the right ventricle were detected in one of these instances (Fig. 6).

#### 3.2.2.2 Pulmonary afferent activity

Four other afferent neurons were identified which generated activity that was altered when the respiratory rate was altered or when the pulmonary parenchyma was touched gently. These neurons were not modified by an epicardial application of mechanical or chemical stimuli. Four other neurons, which were associated with mechanosensitive fields in intercostal muscles, generated quasi-respiratory-related activity, activity that persisted after artificial ventilation was stopped.

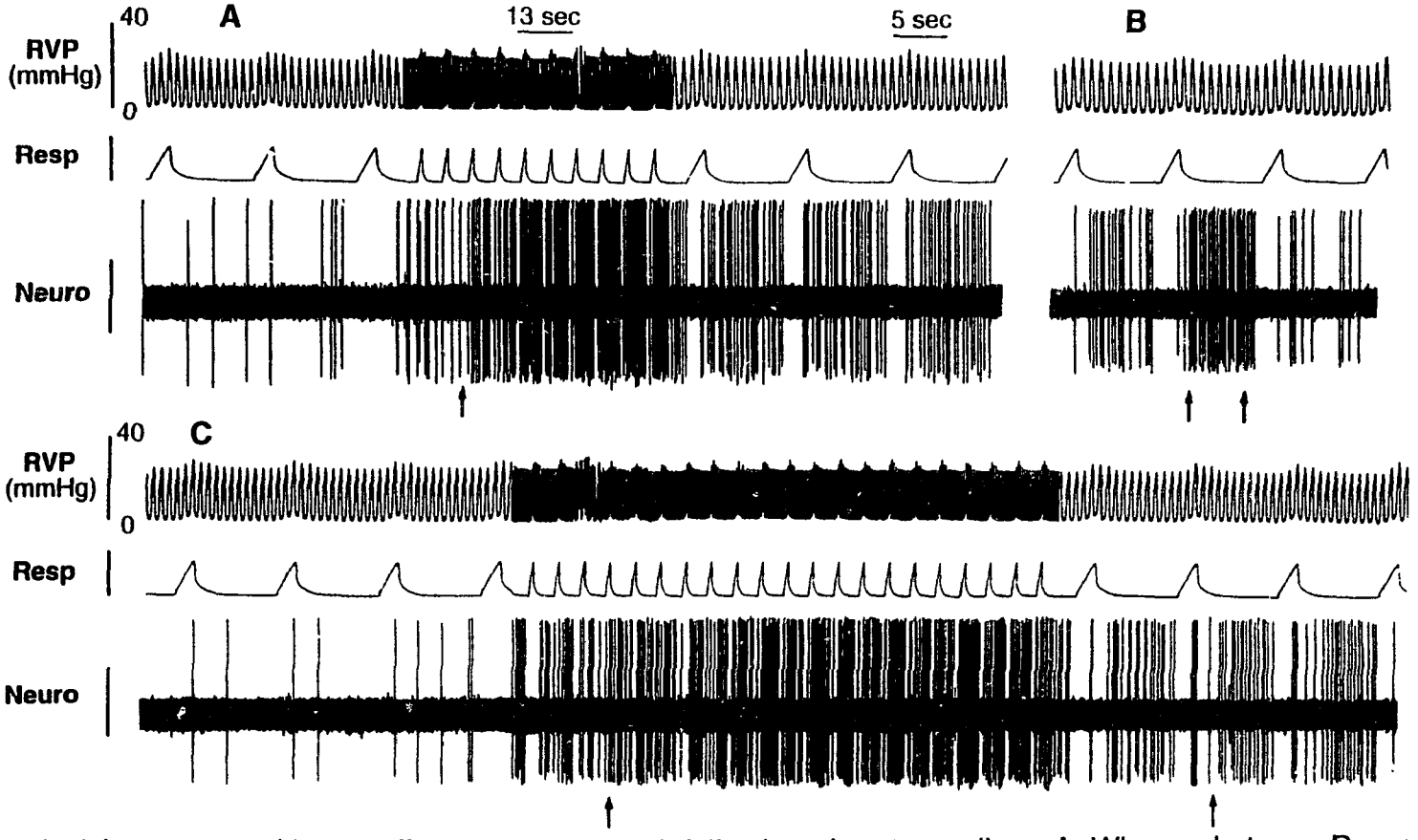


Figure 6. Activity generated by an afferent neuron in a left $T_3$ dorsal root ganglion. A. When substance P was applied (arrow) to its right ventricular epicardial field for 2 seconds (onset at arrow) afferent activity was enhanced. B. Touching the right ventricular epicardial sensory field (between arrows) augmented activity. C. Application of ATP (between arrows) to its sensory field enhanced activity. This activity was entrained to respiratory dynamics, increasing from 4 to 22 impulses / respiratory cycle particular during expiration. Calibration beside neuronal activity = 0.5 mV.

## 3.3 Chemical stimuli: overview

The activity generated by 84% (43 of 51) of identified dorsal root ganglion neurons that had epicardial sensory fields and were sensitive to mechanical stimuli was modified by epicardial application of neurochemicals (Table 1). An additional five afferent neurons were identified that were quiescent during control periods and when mechanical stimuli were applied to their epicardial sensory fields. These were only activated by an epicardial application of a neurochemical. Three neurons whose activity was not modified by mechanical stimuli were activated by an epicardial application of a neurochemical. When saline was applied to an epicardial sensory field, neuronal activity was unaffected.

### 3.3.1 Peptidergic stimuli

#### 3.3.1.1 Effects of sensory-field application of bradykinin and substance P

Bradykinin and substance P modified (either increased or decreased) the activity generated by 65% (28 of 43) and 67% (29 of 43) respectively, of cardiac afferent neurons in dorsal root ganglia studied (Table 1; Figs. 6a & 7c). Chemosensitive fields were located on the right and left atria and ventricles. With respect to the left ventricle, they were identified on the ventral, lateral and dorsal surfaces of that chamber. The activity of 60% of neurons responsive to substance P was modified in a similar fashion by bradykinin. The activity of two neurons insensitive to mechanical stimuli was increased following an epicardial application of substance P. The mean latency of responses elicited by an

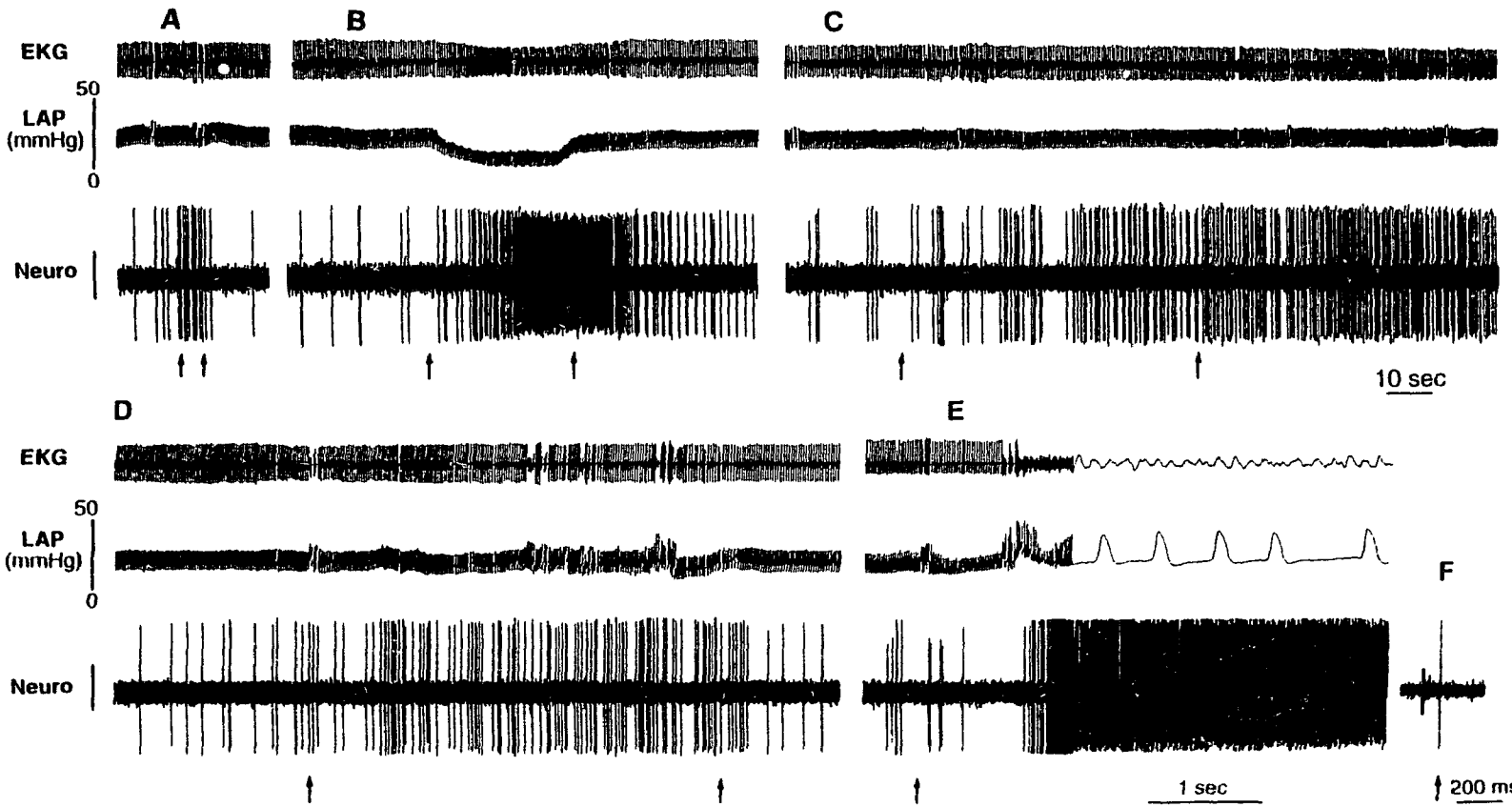


Figure 7. Activity generated by an afferent neuron in a left $T_5$ dorsal root ganglion. A. touching th left atrial epicardium. B. Occlusion of the inferior vena cava resulted in activity increasing (0.1 to 60 Hz). When substance P (C) or ATP (D) were applied to the left atrial sensory field, activity was enhanced. E. Soon after the left anterior descending coronary artery was occluded (arrow) ventricular fibrillation occurred, during which time neuronal activity increased up to 200 Hz. F. Electrical stimulation of the epicardial sensory field induced an action potential, the conduction velocity of the axon connecting this neuron to its sensory ending being 3.3 m/s. Calibration beside neuronal activity = 0.5 mV.

epicardial application of bradykinin was 33 ± 6 seconds, this being longer than that of substance P (18 ± 6 seconds, $p < 0.05$). Maximum activity generated by application of bradykinin or substance P reached 37 and 41 ips, respectively.

#### 3.3.1.2 Effects of sensory-field application of a substance P antagonist

Applying the substance P antagonist spantide (10 μM) on the epicardial sensory fields of twelve neurons which were sensitive to substance P induced decreased activity (from 0.8 ± 0.2 to 0.2 ± 0.1 ips, $p < 0.01$, Fig. 8). Furthermore, reapplying substance P failed to elicit neuronal excitation (0.5 ± 0.3 - 0.5±0.3 ips) in the presence of the spantide. An epicardial application of bradykinin in the presence of spantide excited (0.7 ± 0.2 to 1.8 ± 0.3) three of four afferent neurons tested. Epicardial application of adenosine in the presence of spantide excited two afferent neurons tested (Fig. 8).

### 3.3.2 Purinergic stimuli

#### 3.3.2.1 Effects of sensory-field application of adenosine

Seventy-two percent (31 of 43) of identified neurons responded to epicardial (Figs. 8 & 9) and intracardiac (Fig. 10) applications of adenosine. Afferent neuronal activity usually increased after the application of adenosine (Table 1). The mean latency of onset of the neuronal responses elicited following the application of adenosine was 8 ± 3 seconds. The peak firing frequency elicited by adenosine was 61 ips. The activity generated by one

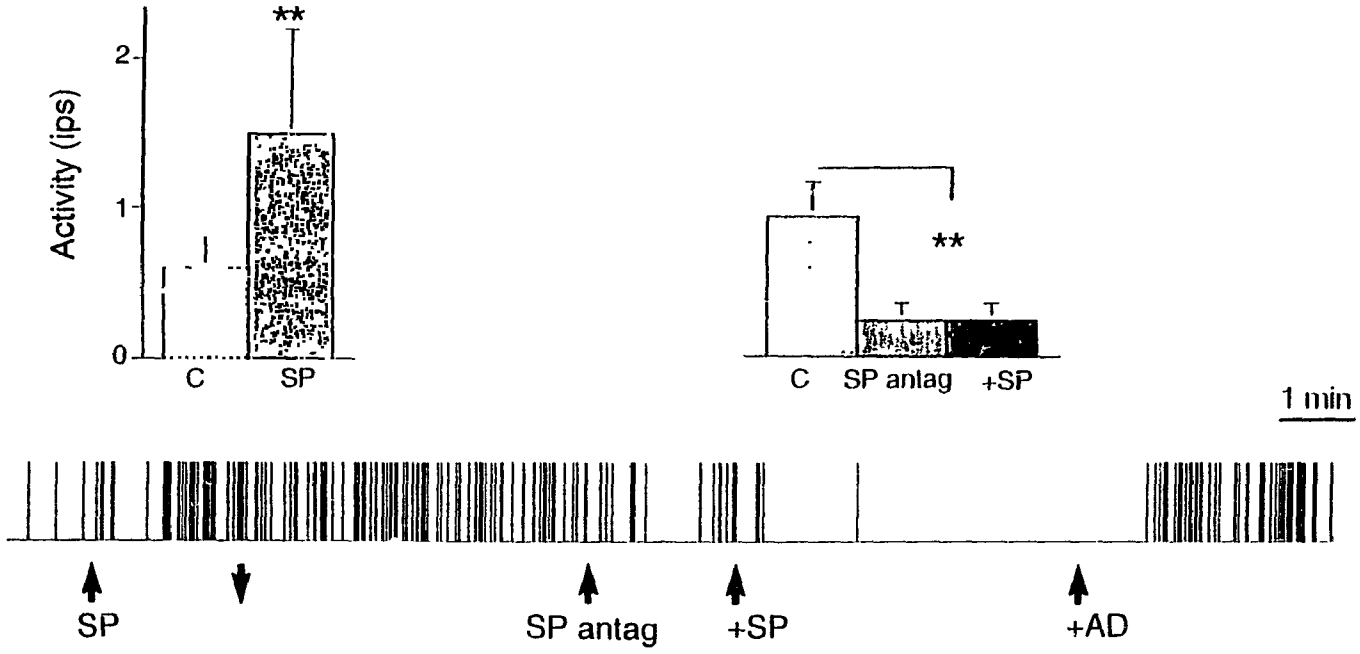


Figure 8. Upper left bar graph: epicardial application of substance P (SP, 20 μM) increased activity (0.6±0.2 to 1.6±0.5 ips) generated by 12 afferent neurons. Upper right bar graph: the substance P antagonist (SP antag) spantide (10 μM) when applied to epicardial sensory fields decreased neuronal activity (0.8±0.2 to 0.2±0.1 ips, $p<0.01$) in 10 of 12 neurons studied. Application of substance P in the presence of spantide failed to elicit neuronal responses. C = control; ** denotes $p<0.01$. Lower panel: effects of epicardial application of SP as well as substance SP in the presence of the SP antagonist is illustrated, as is the effect of adenosine (+AD) in the presence of the SP antagonist. Note that adenosine increased neuronal activity in the presence of substance P receptor blockade.

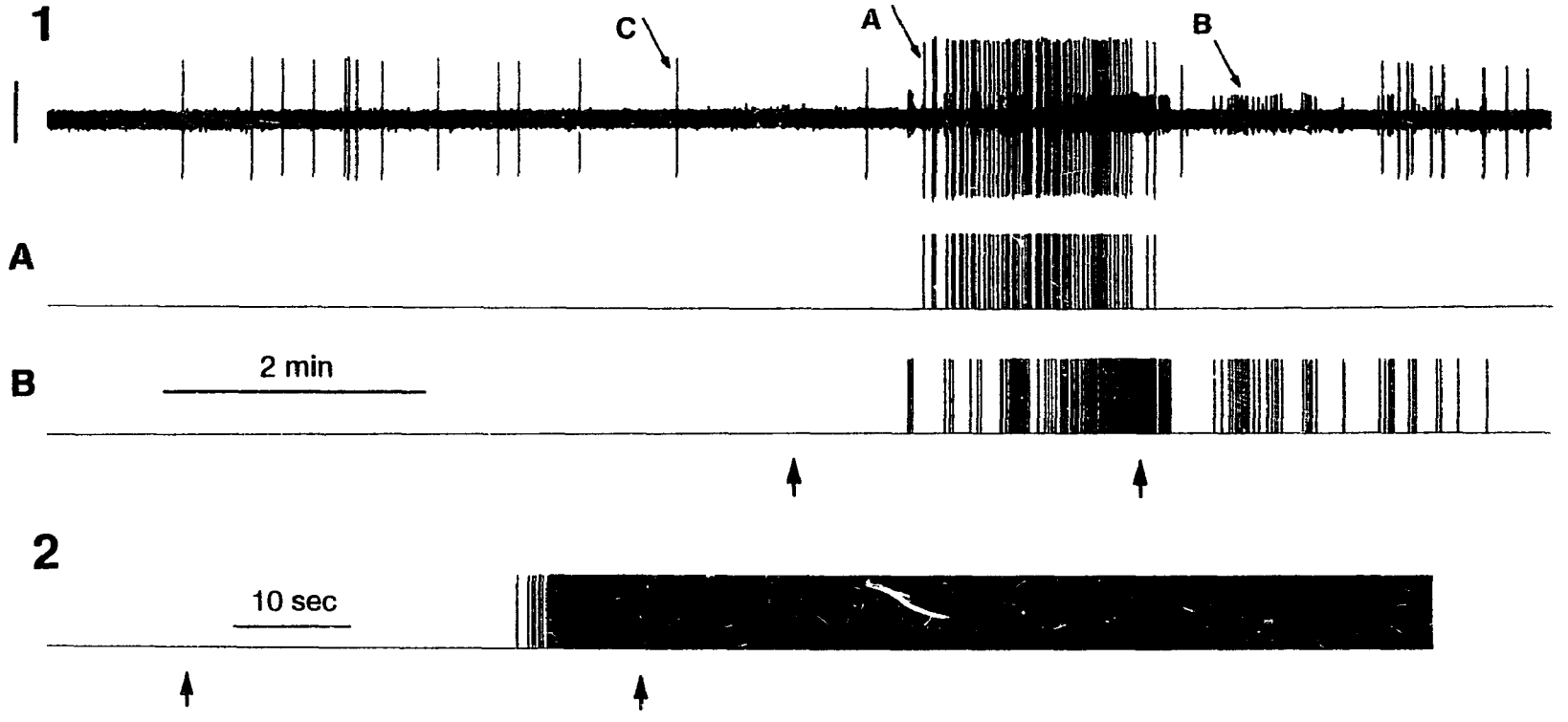


Figure 9, 1. Two adjacent quiescent neurons identified in a right $T_3$ dorsal root ganglion were activated by epicardial application of substance P on the right ventricular conus. Top panel: original neurogram demonstrating activity generated by 3 different neurons. A. Window discriminated activity generated by a previously quiescent neuron (A, top trace) demonstrated activity being initiated following epicardial application of substance P. Its discharge rate reached 2.6 ips. B. Window discriminated activity generated by another previously quiescent neuron (B, top trace) following epicardial application of substance P. Its discharge rate reached 2.8 ips. 2. A quiescent neuron in a left $T_4$ dorsal root ganglion which was activated by epicardial application of adenosine on the right atrium (between arrows). Neuronal activity increased up to 56 ips, activity lasting for 4 min after removal of adenosine. Calibration (top trace) = 0.5 mV.

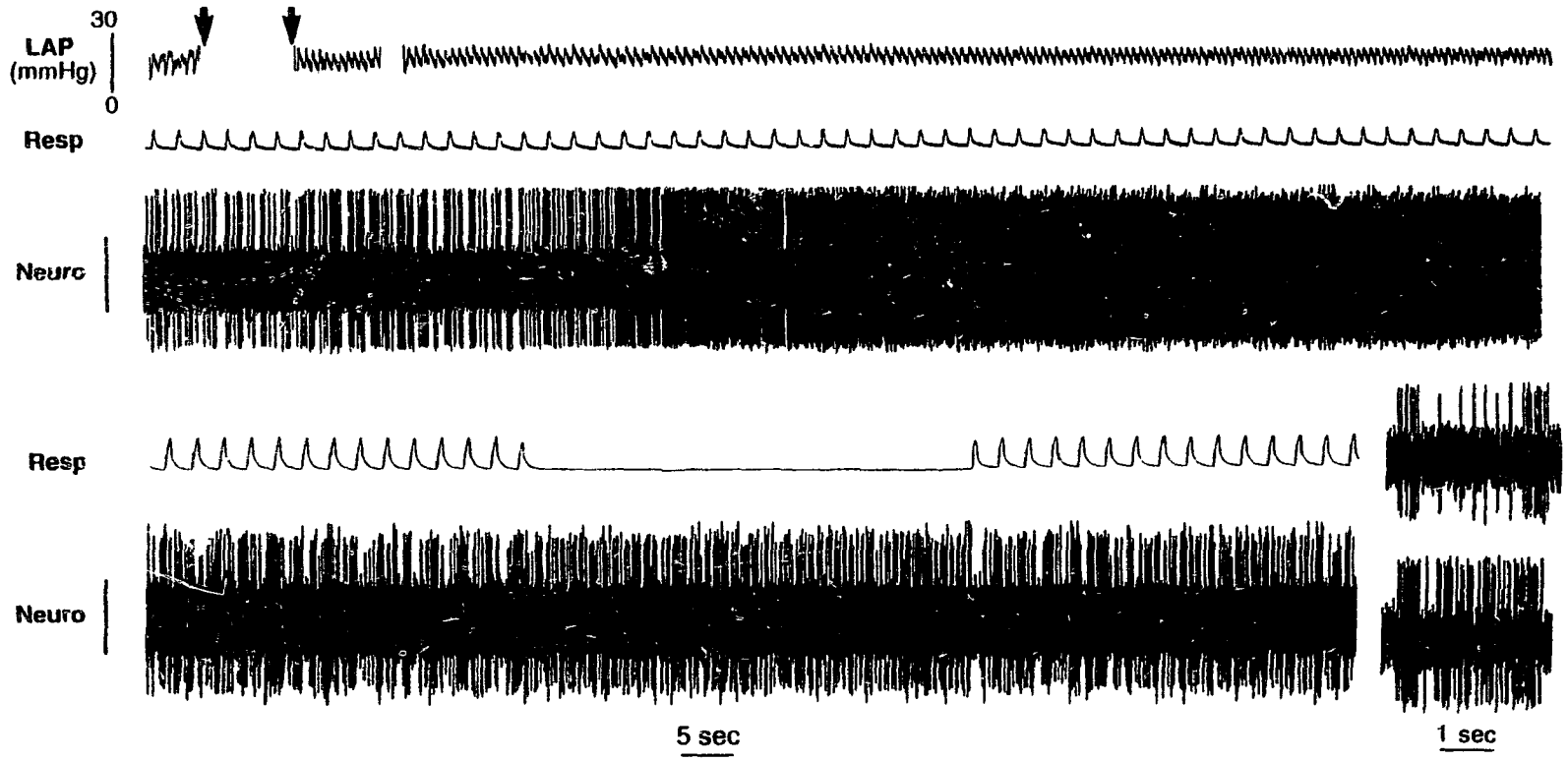


Figure 10. Activity generated by an afferent neuron in a right $T_2$ dorsal root ganglion increased following injection of adenosine (3 ml, 0.75 μM) into the left atrium. Activity increased from 5 to 60 ips and heart rate increased from 140 to 190 beats /min. Note that neuronal activity was entrained with respiration dynamics. Respiratory-related activity was eliminated when respiration was ceased (second neurogram). Calibration beside neuronal activity = 1 mV. Inset, right side, shows baseline respiratory-related activity (top) and the activity increase elicited when the ventral wall of the left ventricle was touched (bottom).

neuron that was unaffected by mechanical stimuli increased after an epicardial application of adenosine. Five neurons that responded with increased activity to an epicardial application of adenosine had similar increased activity after adenosine was administered in the left atrial chamber. Afferent neuronal responses lasted up to 32 minutes after 60 - 120 second exposure to adenosine.

3.3.2.2 Effects of sensory-field application of adenosine antagonists

Fourteen dorsal root ganglion neurons with identified ventricular sensory endings that were sensitive to adenosine were studied with adenosine antagonists. Activity generated by these neurons increased by 164%±46% after application of adenosine (1 μM) to their sensory fields (Fig. 11). Sensory-field application of the adenosine $A_1$ (DPCPX, 2 μM) or $A_2$ (KF 17387, 2 μM) receptor antagonists suppressed the activity generated by ten of these neurons. Reapplication of adenosine in the presence of DPCPX or KF increased neuronal activity. Application of the nonselective adenosine-receptor antagonist CGS (2 μM) decreased the activity generated by these ten neurons. Reapplication of adenosine in the presence of CGS failed to elicit responses. These data indicate that these neurons' sensory endings possessed both adenosine $A_1$ and $A_2$ receptors. The neuronal excitation elicited by $A_1$ and $A_2$ receptor activation was not significantly different from that elicited by the activation of either $A_1$ or $A_2$ receptors alone (Fig. 11). The activity generated by two other neurons was suppressed when the $A_1$, but not the $A_2$, receptor antagonist was applied, and the fact that reapplication of adenosine in the presence of the $A_1$, but not the $A_2$,

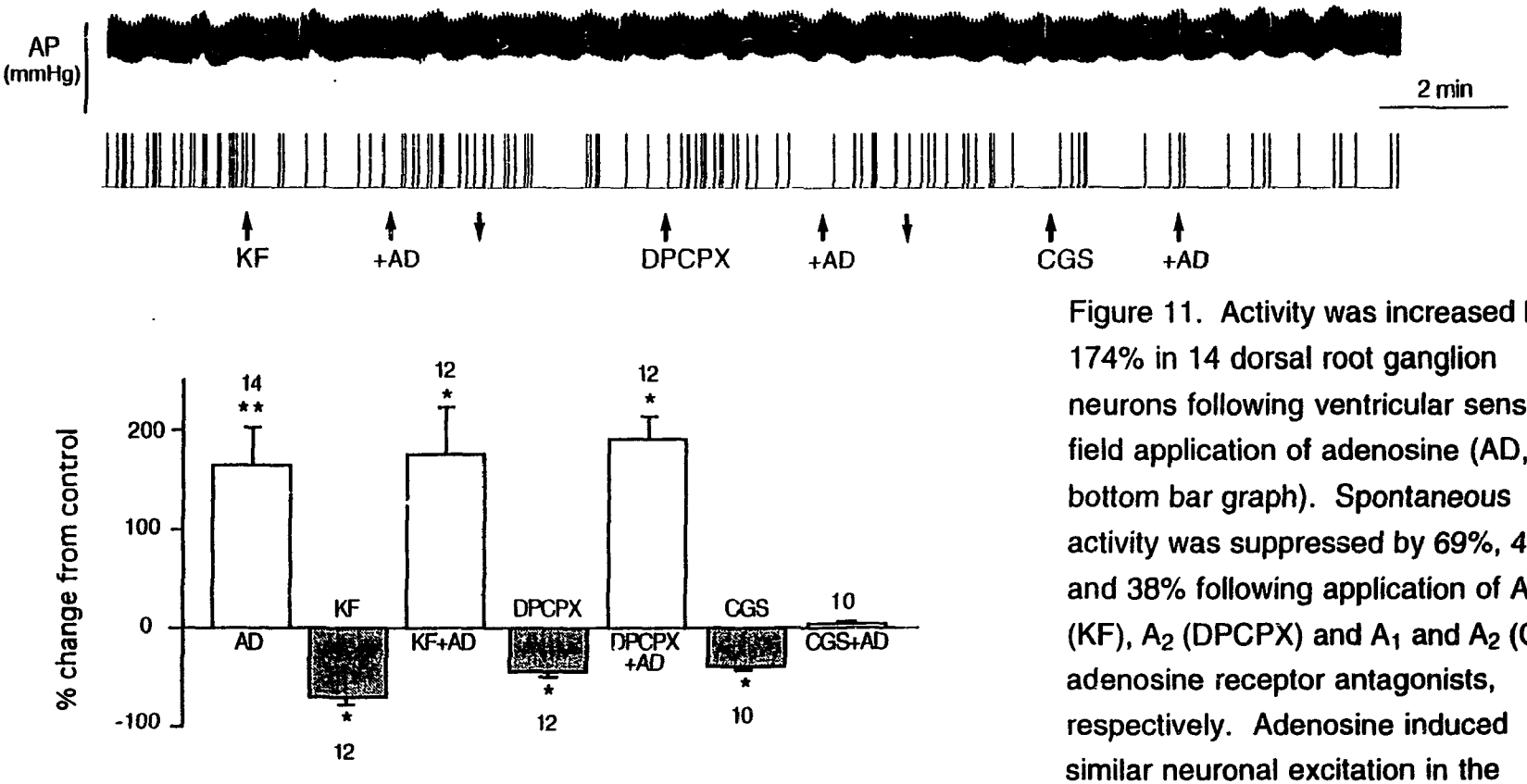


Figure 11. Activity was increased by 174% in 14 dorsal root ganglion neurons following ventricular sensory field application of adenosine (AD, bottom bar graph). Spontaneous activity was suppressed by 69%, 43% and 38% following application of $A_1$ (KF), $A_2$ (DPCPX) and $A_1$ and $A_2$ (CGS) adenosine receptor antagonists, respectively. Adenosine induced similar neuronal excitation in the presence of KF (164%) or DPCPX (190%). Application of CGS not only decreased neuronal activity but also eliminated effects elicited by local application of adenosine. Baseline activity did not differ before each antagonist was applied. One-way ANOVA and paired t-test with Bonferroni correction for multiple tests were used for statistical analysis (* and ** = $p<0.05$ and $p<0.01$, respectively; # above bar = # of neurons). Top panel shows aortic pressure (AP) and activity generated by one neuron possessing both $A_1$ and $A_2$ receptors. Activity was suppressed when KF, DPCPX or CGS were applied individually. Adenosine failed to elicit a response in the presence of CGS, but did after KF or DPCPX. Down arrow denotes removal of chemicals from sensory fields and washing with saline.

antagonist failed to elicit responses in these neurons suggests that the sensory endings of these neurons possessed only $A_1$ receptors. In two other neurons, spontaneous activity was suppressed by the $A_2$, but not the $A_1$, antagonist, and reapplication of adenosine in the presence of the $A_2$, not the $A_1$ antagonist, failed to elicit responses in both neurons (Fig. 12), implying that their sensory endings possessed only $A_2$ receptors. Biphasic responses, characterized by initial excitation (10 ~ 100 sec) followed by a suppression of activity (Fig. 12), were observed in 55%, 56% and 44% of afferent neurons following application of $A_1$, $A_2$ as well as $A_1$, and $A_2$ adenosine antagonists, respectively.

### 3.3.2.3 Effects of sensory-field application of ATP and an ATP analog

Sixty percent of tested neurons were modified by the epicardial application of ATP (Figs. 6c & 7d), with afferent neuronal activity either increasing or decreasing, depending on the neuron studied (Table 1). The mean latency of onset of the neuronal responses elicited following the application of ATP was $18 \pm 5$ seconds. The peak firing frequency elicited by ATP was 49 ips. Only 32 % of neurons tested with both adenosine and ATP responded to both compounds, and five of these neurons generated opposite responses. Five neurons which responded with increased activity following epicardial application of ATP were activated following an epicardial application of $\beta, \gamma$-ATP ($1.8 \pm 1.4$ to $8.6 \pm 3.2$, $p<0.05$).

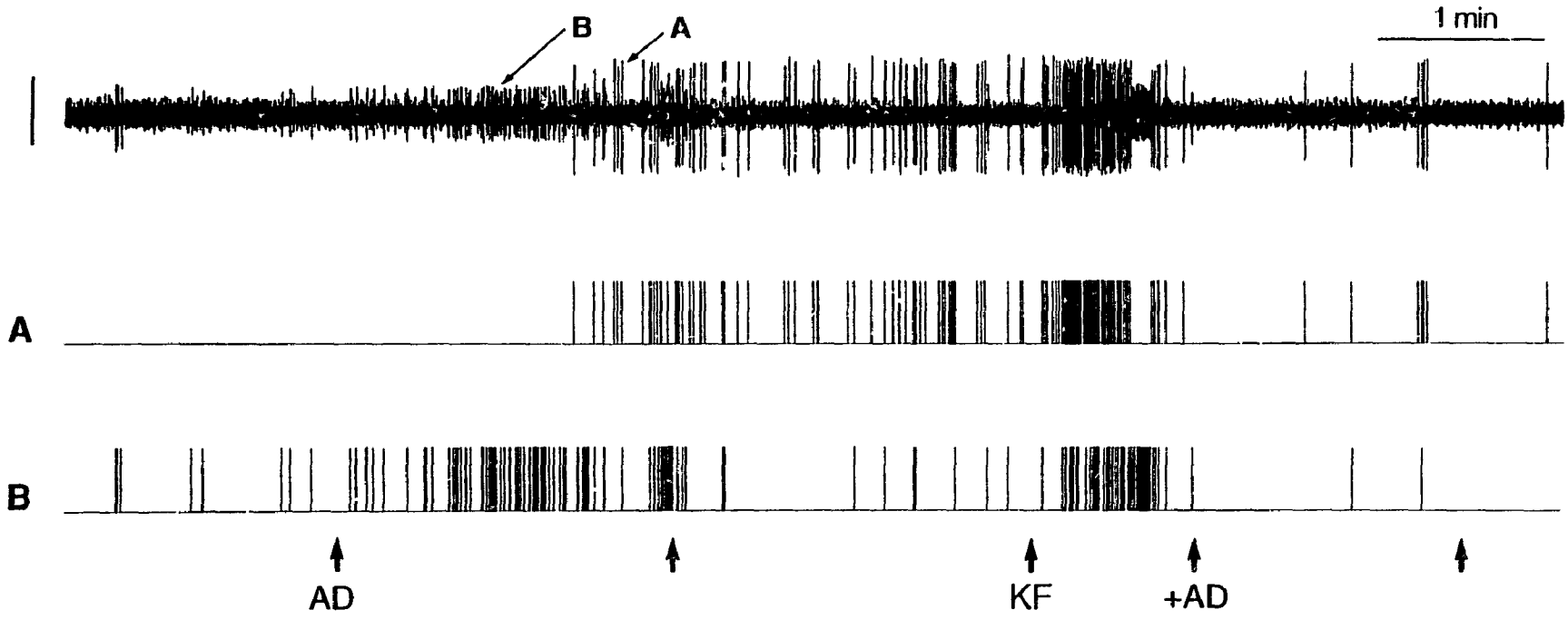


Figure 12. Activity generated by 2 adjacent neurons (A and B) in a right $T_3$ dorsal root ganglion increased when adenosine (AD, between first two arrows) was applied to a left ventricular anterior epicardial region. Application of KF briefly enhanced activity generated by both neurons followed by inhibition of activity. Reapplication of adenosine (+AD) in the presence of KF failed to activate these neurons (chemicals were removed at unlabeld arrow). Neurons were activated by adenosine 20 minutes after removal of KF (not shown). Top trace: original neurogram displaying activity generated by both neurons (calibration bar = 0.5 mV). Tracings A and B discriminated unit activity generated by neurons A and B identified in top tracing.

### 3.3.3 Effects of chemical stimuli on "silent" afferent neurons

Five separate afferent neurons which had been quiescent during control periods and during mechanical stimulation were activated by epicardial application of neurochemicals. No activity had been generated by these neurons for two or more hours before chemical application, including when mechanical stimuli were applied to their sensory fields. The mean left ventricular systolic chamber pressure while these neurons were inactive was 115 ± 6 mmHg. Aortic occlusion, a mechanical stimulus which raised left ventricular chamber systolic pressure to 200 mmHg, did not activate these quiescent neurons.

Four of these quiescent neurons became activated following the epicardial application of peptides: two had right and left atrial sensory endings sensitive to bradykinin, and the other two had left ventricular sensory endings sensitive to substance P (Fig. 9). The mean activity generated by these previously quiescent neurons following peptide application was 4.4 ± 1.8 ips, with an average latency of 30 ± 8 seconds occurring after their sensory fields were exposed to the peptides. The activity generated by these neurons lasted up to 2 minutes after removal of the peptide and washing of the sensory fields with saline.

Three of these quiescent neurons were activated following epicardial application of adenosine (1 animal) or ATP (2 animals). Two were modified by individual applications of ATP and bradykinin. The mean discharge rate of these previously quiescent neurons reached 29 ips (range, 1.2-56 ips) following purine application, with an average latency of activation of 31 seconds occurring

before activity became initiated. Activity generated by these neurons lasted up to 4 minutes after adenosine was removed (Fig. 9).

### 3.4 Coronary artery occlusion

Of thirty-one dorsal root ganglion afferent neurons sensitive to cardiac mechanical or chemical stimuli, 71% were modified by transient coronary artery occlusion (Table 1). Nineteen neurons responded to brief periods of coronary artery occlusion by increasing their activity and three neurons by decreasing their activity. One neuron which was quiescent until activated by bradykinin and ATP was also activated by coronary artery occlusion (Fig. 13). The mean latency of onset of the neuronal responses generated by spontaneously active neurons following coronary artery occlusion was 15 seconds (range 10-30 sec). Afferent neuronal responses lasted up to 1.5 hours following 2 minutes of coronary artery occlusion. The activity generated by four neurons increased more after the coronary artery occlusion than during it. The mean latency for such change was $58 \pm 29$ seconds after reperfusion began. Ventricular fibrillation occurred spontaneously during coronary artery occlusion or reperfusion in 17 animals, and the activity generated by 35% of the 17 neurons being recorded in these instances increased further. The increase of afferent activity lasted up to 20 minutes following the induction of ventricular fibrillation. The peak activity recorded during ventricular fibrillation was 200 ips (Fig. 7e). Activity development during ventricular fibrillation was not affected by emptying the ventricles of blood.

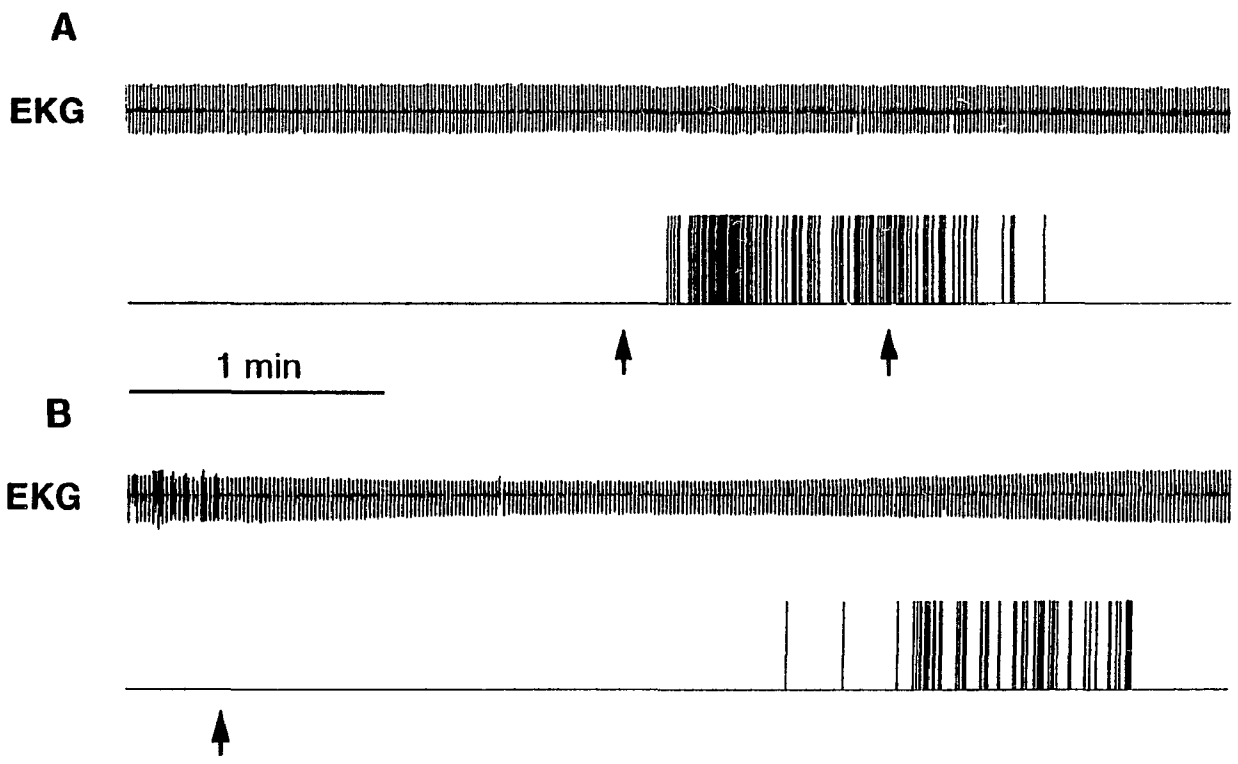


Figure 13. A. A previously quiescent neuron in left $T_2$ dorsal root ganglion was activated following application of bradykinin to the left ventricular ventral epicardium (between arrows). B. This afferent neuron was also activated by occlusion of the anterior descending coronary artery (onset at arrow in B) as well as epicardial administration of ATP (not shown).

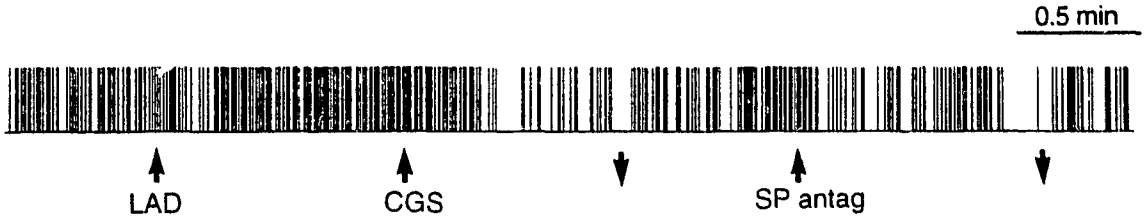


Figure 14. Application of the nonselective adenosine receptor antagonist CGS and later the substance P receptor antagonist suppressed activity generated by a right $T_2$ dorsal root ganglion neuron which had been increased by occluding the left anterior descending coronary artery continuously (onset at arrow, LAD). Down arrows denote when chemicals were removed and sensory field washed with normal saline.

Adenosine $A_1$+$A_2$ receptor antagonist CGS was applied during myocardial ischemia to the sensory fields of seven neurons which did not progress to ventricular fibrillation. CGS attenuated ($3.3 \pm 0.9$ to $0.5 \pm 0.2$ ips, $p < 0.05$) neuronal excitation generated by five of these neurons in the presence of myocardial ischemia (Fig. 14).

3.5 Responses of individual epicardial afferent neurons to multiple stimuli

Nearly 80% of the chemosensitive dorsal root ganglion neurons examined were modified by more than one neurochemical: eighteen were modified by two peptides and two purinergic compounds, twelve were modified by one peptide and one purinergic compound, four were modified by two peptides, and six were modified by two purinergic compounds. The activity generated by 50% of the chemosensitive neurons was modified when chemicals

were applied to two or more epicardial sensory fields. In a number of instances, different sensory fields of the same neuron responded to different neurochemical stimuli. The frequency of occurrence and the intensity of afferent neuronal responses elicited following application of each of the four neurochemicals tested did not differ significantly.

### 3.6 Cardiodynamic effects elicited following chemical activation of afferent neurons

Cardiodynamic changes were induced in 12% of the cases when the epicardial application of bradykinin modified afferent neuronal activity. Heart rate increased from 160 to 200 beats/min in one instance, left ventricular intramyocardial systolic pressure increased from 94 ± 8 to 118 ± 11 mmHg in five instances and left ventricular chamber systolic pressure increased from 125 to 145 mmHg in two instances. The epicardial application of substance P augmented the left ventricular intramyocardial (90-100 mmHg) and chamber (120-140 mmHg) systolic pressure in one animal after afferent neuronal activity had been enhanced. Epicardial or atrial chamber administration of adenosine elicited increased cardiac rate or pressure in 17 % of those instances in which afferent activity was modified (Figs. 10 & 15). Heart rate increased (133 ± 13 - 157 ±21 beats/min) in four instances, left ventricular intramyocardial pressure increased (88±10 - 103±11 mmHg) in 6 instances and left ventricular chamber systolic pressure increased (102 ± 11 - 118 ± 13 mmHg) in five instances. The epicardial application of ATP elicited increased ventricular contractility in one instance (left ventricular intramyocardial and chamber systolic pressures increased from 100 to 140 mmHg). When adenosine (3 ml, 0.75 μM) or ATP (3

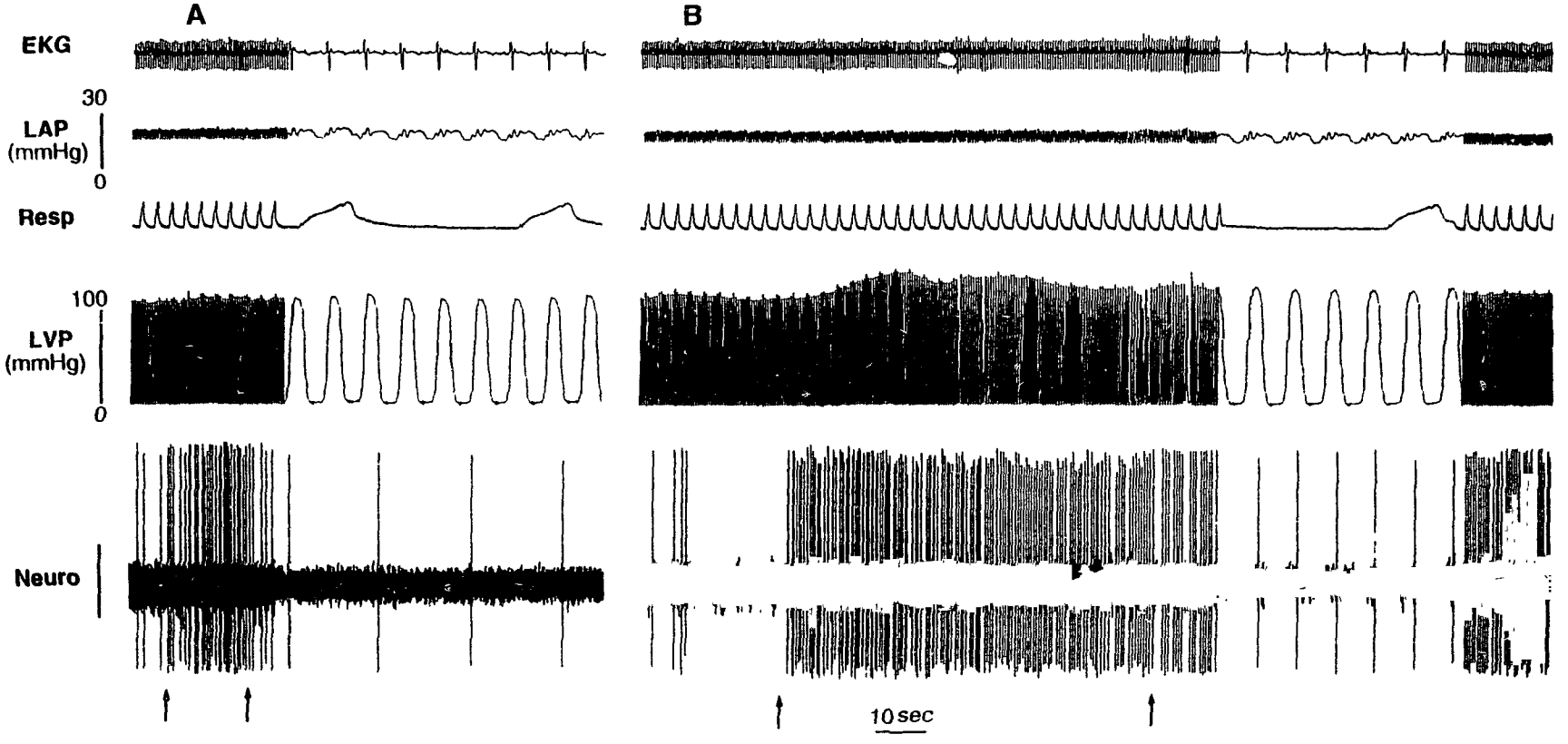


Figure 15. Activity generated by a left ventricular epicardial afferent neuron in a $T_2$ right dorsal root ganglion. A. Activity increased when its left ventricular epicardial sensory field was touched (between arrows at bottom). B. Activity increased when adenosine was applied to this sensory field (between arrows at bottom). Left ventricular systolic chamber pressure increased when activity was enhanced following adenosine application, activity becoming entrained to each cardiac cycle. Vertical calibration bar beside neuronal activity = 0.5 mV.

ml, 0.75 μM) was administered into the left atrial chamber, no significant hypotension was elicited. When 0.1 mM adenosine or ATP were administered into the left atrium, bradycardia and hypotension were induced. Data obtained in the latter group were not used, since it was not possible to differentiate direct purinergic effects from secondary cardiovascular effects induced by these agents.

Ventricular chamber and intramyocardial systolic pressures decreased overall and no significant change was detected in left ventricular diastolic pressure overall during coronary artery occlusion. During coronary reperfusion in two instances heart rate increased from 116 to 124 beats/min, left ventricular chamber systolic pressure increased from 100 to 120 mmHg and left ventricular intramyocardial systolic pressure increased from 90 to 108 mmHg compared to preocclusion values.

# Chapter 4 Discussion

## 4.1 Overview

This is the first effort to demonstrate that multiple stimuli excite the activity generated by individual dorsal root ganglion cardiac afferent neurons. It is also the first time to demonstrate that primary cardiac sensory neurons are activated tonically by endogenously released substance P and adenosine. Cardiac afferent neurons were identified in canine $T_2$-$T_5$ dorsal root ganglia bilaterally, confirming previous anatomical (Hopkins & Armour, 1989; Kuo et al., 1981; Vance & Bowker, 1983) and functional (White, 1957) data. Dorsal root ganglion epicardial afferent neurons displayed four types of responses to similar hemodynamic changes, three of which (types II, III and IV) have not been reported before. In addition, the majority of identified neurons sensing mechanical perturbations were also modified by peptides and/or purinergic agents. Furthermore, 71% of identified neurons sensitive to chemical stimuli were modified by brief periods of ischemia.

## 4.2 Mechanical stimuli

### 4.2.1 Four types of cardiac mechanosensitive afferent neurons

The spontaneous activity generated by sympathetic afferent axons arising from atrial or ventricular mechanosensitive endings has been reported to increase or decrease when their chamber systolic pressure increases or decreases, respectively (Peters et al., 1980; Malliani et al., 1973; Uchida, 1975). Thirty-two percent of afferent neurons associated with ventricular epicardial sensory endings identified in the present study displayed similar responses. We

change in activity occurs when left ventricular pressure deviated from basal physiological states. According to a recently proposed neuronal network model of canine intrathoracic cardiac ganglion neurons, a sudden increase or decrease in neuronal activity provides the central nervous system with equal and maximal information content (Wong & Armour, 1992). It is speculated that types I and II mecha osensitive afferent neurons provide the central nervous system with quantitative information of the states of ventricular pressure. On the other hand neuronal responses generated by types III and IV afferent neurons may signify that cardiodynamic disturbance (ventricular hypotension or hypertension) has occurred. These data indicate that a varied spectrum of afferent information is transmitted to spinal cord neurons by these different types of dorsal root ganglion neurons in response to similar ventricular systolic pressure alterations. Varied afferent inputs may be required to ensure accurate assessment of a single type of cardiovascular perturbation by the central nervous system neurons. In this way the central nervous system neurons may intervene more effectively in response to a wide range of cardiodynamic states. These data may explain, in part, why cardiac sympathetic efferent preganglionic nerve activity can be either enhanced or suppressed when ventricular pressure increases (Pagani et al., 1974).

The afterdischarge phenomenon were generated by many epicardial mechanosensitive afferent neurons after removal of mechanical stimuli (Fig. 7b). Afterdischarge of mechanosensitive nerve endings has been described in non-cardiac sympathetic afferent endings associated with, for instance, esophageal mechanoreceptors (Sengupta et al., 1990). The mechanisms responsible for the afterdischarge phenomenon are not known. It has been proposed that this phenomenon may be due to the release of neurochemicals such as substance

change in activity occurs when left ventricular pressure deviated from basal physiological states. According to a recently proposed neuronal network model of canine intrathoracic cardiac ganglion neurons, a sudden increase or decrease in neuronal activity provides the central nervous system with equal and maximal information content (Wong & Armour, 1992). It is speculated that types I and II mechanosensitive afferent neurons provide the central nervous system with quantitative information of the states of ventricular pressure. On the other hand neuronal responses generated by types III and IV afferent neurons may signify that cardiodynamic disturbance (ventricular hypotension or hypertension) has occurred. These data indicate that a varied spectrum of afferent information is transmitted to spinal cord neurons by these different types of dorsal root ganglion neurons in response to similar ventricular systolic pressure alterations. Varied afferent inputs may be required to ensure accurate assessment of a single type of cardiovascular perturbation by the central nervous system neurons. In this way the central nervous system neurons may intervene more effectively in response to a wide range of cardiac states. These data may explain, in part, why cardiac sympathetic nerve activity can be either enhanced or reduced when pressure increases (Pagani et al., 197

The afterdischarge phenomenon was observed in many epicardial mechanosensitive afferent neurons to mechanical stimuli (Fig. 7b). Afterdischarge of mechanosensitive neurons has been described in non-cardiac sympathetic afferent endings associated with, for instance, esophageal mechanoreceptors (Sengupta et al., 1990). The mechanisms responsible for the afterdischarge phenomenon are not known. It has been proposed that this phenomenon may be due to the release of neurochemicals such as substance

P from sensory nerve endings during mechanical perturbation, thereby resulting in further excitation of sensory terminals (De Groat, 1986). In agreement with the fact that neurochemicals may be responsible for this phenomenon, most of the mechanosensitive epicardial afferent neurons displaying afterdischarge responses were excited by sensory-field application of peptides or purines.

### 4.2.2 Simultaneous transmission of heart rate- and respiratory-related activity

Respiratory-related activity generated by ventricular sympathetic afferent neurons has not been reported previously. Such activity was generated by 12% of ventricular epicardial afferent neurons and was modified when pulmonary dynamics changed (Figs. 6 & 10) but not when pulmonary tissues were distorted. In contrast, neurons associated with pulmonary mechanosensitive endings identified in this study and pulmonary afferent axons in sympathetic nerves identified previously (Kostreva et al., 1975a) are modified when pulmonary tissues are touched. As a consequence of changes in ventricular intramyocardial systolic pressure that were induced by respiratory mechanical changes, some epicardial mechanosensitive endings may generate respiratory-related activity (Fig. 6). The fact that five ventricular epicardial polymodal afferent neurons generated such activity without detectable respiratory-related ventricular dynamic changes suggests that coronary arterial blood gas tension variation, which is known to occur during each respiratory cycle (Purves, 1966), may be responsible for the periodic changes in such activity. This is in accord with the fact that respiratory-related activity can be generated by feline carotid body chemosensitive nerve endings (Biscoe & Purves, 1967). These data

indicated that respiratory-related activity generated by dorsal root ganglion ventricular epicardial afferent neurons may be due to respiratory related changes in ventricular dynamics and/or the chemical milieu of the heart. That individual epicardial afferent neurons can generate respiratory- and heart-rate-related activity simultaneously indicates that afferent input arising from epicardial sensory neurons simultaneously encodes information on a beat-to-beat and breath-to-breath basis related to cardiopulmonary dynamics as well as changes in the chemical milieu of the heart.

## 4.3 Chemical stimuli: overview

Eight-two percent of identified mechanosensitive afferent neurons responded to the epicardial application of bradykinin, substance P, adenosine, or ATP and most of these were modified by more than one neurochemical. In agreement with previous reports (Baker et a., 1980), sensory fields of 50% of chemosensitive afferent neurons were located in more than one epicardial region. Three chemosensitive afferent neurons were not modified by mechanical stimuli, which supports the notion that a small population of dorsal root cardiac afferent neurons are associated with chemosensitive rather than mechanosensitive nerve endings (Nishi et al., 1977; Takenaka et al., 1970). On the other hand, the fact that 10% of identified mechanosensitive afferent neurons did not respond to epicardial chemical stimuli could have been due to the fact that their sensory endings were located far enough below the epicardium to be unaffected by epicardially applied neurochemicals or to the fact that they did not sense chemical stimuli.

### 4.3.1 Peptidergic sensitive afferent neurons

The activity generated by ~ 50% of identified afferent neurons was modified by bradykinin or substance P. Bradykinin, an algesic substance which is released during myocardial ischemia (Kimura et al., 1973), is known to increase the activity of cardiac sympathetic afferent fibers (Felder & Thames, 1982; Lombardi et al., 1981; Uchida & Murao, 1974c), and bradykinin may release substance P from sensory nerve terminals to modulate visceral sensory nerve endings (Hougland et al., 1986; Geppetti, 1988). Substance P, a putative neurotransmitter in a subpopulation of primary afferent neurons, is synthesized in these neurons' soma and transported to central and peripheral afferent terminals (Harmar & Keen, 1982; Sweet, 1980). Substance P immunoreactivity has been associated with thoracic dorsal root ganglion neurons (Hougland et al., 1986) and cardiac nerve terminals (Lundberg et al., 1985; Urban & Paka, 1985). Substance P is released from cardiac afferent nerve terminals (Geppetti et al., 1988; Manzini et al., 1989). Although it has been proposed that substance P released from the central terminals of dorsal root ganglion neurons mediates excitatory cardiac afferent responses (Staszewska-Woolley et al., 1986), intravenous administration of substance P (30 μg/kg) does not modify the activity of cardiac sympathetic afferent fibers (Nishi et al., 1977). Furthermore, substance P reportedly exerts no effects on dorsal root ganglion neurons in culture (Nowak & MacDonald, 1982). Despite that, substance P has been reported to facilitate adenosine-induced cardiac pain in humans (Gaspardone et al., 1992).

In the present study, more than half of the afferent neurons tested responded to the epicardial application of substance P. Neuronal responses

were not due to cardiac dynamic alterations as a consequence of substance P entering the circulation and thereby inducing hypotension, which in turn modifies cardiac mechanics. Intramyocardial and ventricular chamber pressures were usually unaffected when afferent activity was enhanced following the application of substance P to a small epicardial region (Figs. 6a & 7c). As the activity generated by afferent neurons sensitive to substance P was suppressed following the application of a substance P specific antagonist (Fig. 8), it appears that substance P is endogenously released to tonically activate substance P receptors on epicardial sensory endings. Endogenously released substance P is degraded by neutral endopeptidase-24.11(Petersson et al., 1993), an enzyme synthesized by vascular endothelial cells (Graf et al., 1992). As substance P is possibly released from cardiac sensory endings of dorsal root ganglion and is degraded by neutral endopeptidase-24.11 located on cardiac endothelial cells, based on the evidence that exogenously applied and endogenously released substance P can modify cardiac sensory nerve endings, it appears that substance P may act as a neurotransmitter on the peripheral endings of cardiac dorsal root ganglion neurons via an autoreceptor mechanism.

Fifty-nine percent of afferent neurons sensitive to substance P were also sensitive to bradykinin. Since 41% of neurons sensitive to bradykinin were not modified by substance P, it is unlikely that bradykinin's effects on cardiac afferent neurons was primarily due to its release of substance P from sensory nerve endings (Hougland et al., 1986; Geppetti et al., 1988; Geppetti, 1993). Such a contention was further supported by the fact that bradykinin elicited neuronal responses in the presence of a substance P antagonist. As bradykinin receptors have been identified autoradiographically in the somata as well as in the afferent axons of dorsal root ganglion neurons (Steranka et al., 1988), it

appears that some bradykinin-induced neuronal responses were due to direct modification of the bradykinin-sensitive receptors of afferent nerve terminals rather than being secondary effects due to sensory terminal release of substance P.

### 4.3.2 Adenosine receptor and subtypes

The epicardial application of adenosine affected 72% of sympathetic afferent neurons. Montano et al. (1991) reported that the epicardial application of a high concentration of adenosine (10 mM) increased the activity generated by feline cardiac sympathetic afferent fibers from 1.1 ips to 3.6 ips. Dibner-Dunlap et al. (1993) reported that adenosine (3.7 mM), when administered into a coronary artery, excited cardiac sympathetic afferent nerves only in the presence of systemic dipyridamole, which prevents cellular adenosine uptake. Intracardiac adenosine formation originates from two sources, an intracellular pool and the extracellular dephosphorylation of ATP, both being dependent on the intracellular oxygen supply/demand ratio (Bardenheuer & Schrader, 1986). During myocardial ischemia, these two pathways are equally important (Mathias et al., 1991). That adenosine plays a role in the pathogenesis of angina pain, as proposed by Sylvén et al. (1986, 1987), has been supported recently (Crea et al., 1990, 1992). Adenosine release into coronary sinus blood is greatly enhanced during myocardial ischemia in humans (Haneda et al., 1989; Feldman et al., 1992). Arterial blood concentrations of adenosine in man normally are about 0.25 μM (Edlund et al., 1985; Feldman et al., 1992). This concentration increases two fold in patients with coronary arterial diseases during atrial-pacing-induced myocardial ischemia (Feldman et al., 1992). The concentration

of adenosine employed in the present study is in the same range as that found in human coronary arteries during myocardial ischemia. Adenosine-elicited long-lasting excitatory effects on cardiac sensory endings (Fig. 10) cannot be ascribed solely to adenosine's binding to the afferent receptors, as adenosine is rapidly eliminated from interstitial spaces due to cellular uptake (Olsson & Pearson, 1990). Moreover, the half-life of adenosine in the bloodstream is less than 10 seconds due to its rapid elimination by adenosine deaminase and erythrocyte uptake (Olsson & Pearson, 1990). The long-lasting afferent neuronal excitation elicited by adenosine is similar to that "long-term potentiation" (persistent neuronal excitation lasting for minutes or hours after brief periods of sensory nerve stimulation) initially described in hippocampal neurons (Voronin, 1983). The mechanism for long-term potentiation remains unclear. It has been proposed in hippocampal neurons that long-term potentiation is due to a postsynaptic influx of calcium via *N*-methyl-D-aspartate receptor-linked channels (Lynch et al., 1990). These data are in accord with a resent publication that long-term potentiation is associated with synaptic transmission between dorsal root ganglion neurons and dorsal horn spinal neurons (Randic et al., 1993). Long-term excitation elicited by adenosine sensitive dorsal root ganglion cardiac afferent neurons may account, in part, for the fact that neuronal excitation lasted for long periods of time after terminating short duration coronary artery occlusions. This is in accord with the clinical finding that angina pectoris can persist after termination of coronary artery occlusion (Parodi et al, 1976).

That spontaneous activity generated by dorsal root ganglion neurons was suppressed by the sensory-field application of low concentrations of the nonselective ($A_1$+$A_2$) adenosine-receptor antagonist CGS indicates that these

receptors can be tonically influenced by endogenously released adenosine. These data are in accord with the clinical finding that the adenosine antagonist aminophylline can reduce cardiac pain induced during myocardial ischemia (Crea et al., 1990). A previous study proposed that cardiac sympathetic afferent endings possess only $A_1$ adenosine receptors since adenosine-mediated cardio-renal reflexes were elicited by $A_1$ but not by $A_2$ selective agonists (Dibner-Dunlap et al., 1993). In the present study, however, although two afferent neurons were identified that possessed only $A_1$ receptors, $A_2$ as opposed to $A_1$ receptors were associated with the sensory nerve endings of two other afferent neurons (Fig. 12) and sensory endings of the majority (71%) of tested dorsal root ganglion afferent neurons possessed both $A_1$ and $A_2$ receptors (Fig. 11). Neuronal excitation elicited by either $A_1$ or $A_2$ receptor stimulation was similar to that elicited following simultaneous activation of both $A_1$ and $A_2$ receptors, implying that neuronal effects elicited by sensory terminal $A_1$ or $A_2$ adenosine receptors are not additive when both are activated simultaneously.

The cellular mechanisms underlying the effects of adenosine on the sensory nerve endings of dorsal root ganglion neurons are not known. Evidence derived from the somata of cultured dorsal root ganglion neurons demonstrates that adenosine analogs suppress a nonspecified type of inward calcium current (Scott & Dolphin, 1986; MacDonald et al., 1986). Furthermore, adenosine is known to suppress the calcium-activated potassium current ($I_{K(Ca^{2+})}$) in the somata of rat dorsal root ganglion neurons in culture (Scott & Dolphin, 1986). High pharmacological doses of the adenosine-receptor antagonist caffeine increase intracellular $Ca^{2+}$ in dorsal root ganglion neurons by initiating the release of $Ca^{2+}$ from intracellular sites, which can further increase somata $I_{K(Ca^{2+})}$ (Neering & McBurney, 1984; Thayer et al., 1988).

Increased $I_{K(Ca^{2+})}$ would induce membrane hyperpolarization and thus decrease neuronal excitability. Thus, it is assumed that the inhibition of dorsal root ganglion neuronal activity that follows the ventricular sensory-field application of adenosine-receptor antagonists might have been due to enhanced $I_{K(Ca^{2+})}$ in the neurons' sensory nerve terminals.

Intracellular $Ca^{2+}$ is also regulated by $Na^{+}$-$Ca^{2+}$ exchange (Baker et al., 1969; Blaustein et al., 1991). The excitatory phase elicited in a number of dorsal root ganglion neurons following the sensory-field application of adenosine- receptor antagonists (Fig. 12) might have been due to the activation of the $Na^{+}$-$Ca^{2+}$ exchange. Increased intracellular $Ca^{2+}$ resulting from intracellular calcium mobilization as a result of adenosine-antagonist application would have activated the $Na^{+}$-$Ca^{2+}$ exchange thereby increasing $Na^{+}$ influx, which, in turn, would lead to membrane depolarization and thus to increased neuronal excitability. If the intracellular $Ca^{2+}$ concentration increased further, $I_{K(Ca^{2+})}$ might be enhanced, resulting in hyperpolarization and thereby inhibiting neuronal excitability. Thus, the intensity of the neuronal inhibition induced by an adenosine antagonist might depend on the respective contributions of calcium activated-potassium conductance and $Na^{+}$-$Ca^{2+}$ exchange. Hypothetical mechanisms of adenosine-receptor-mediated neuronal responses are illustrated diagrammatically in Figure 16.

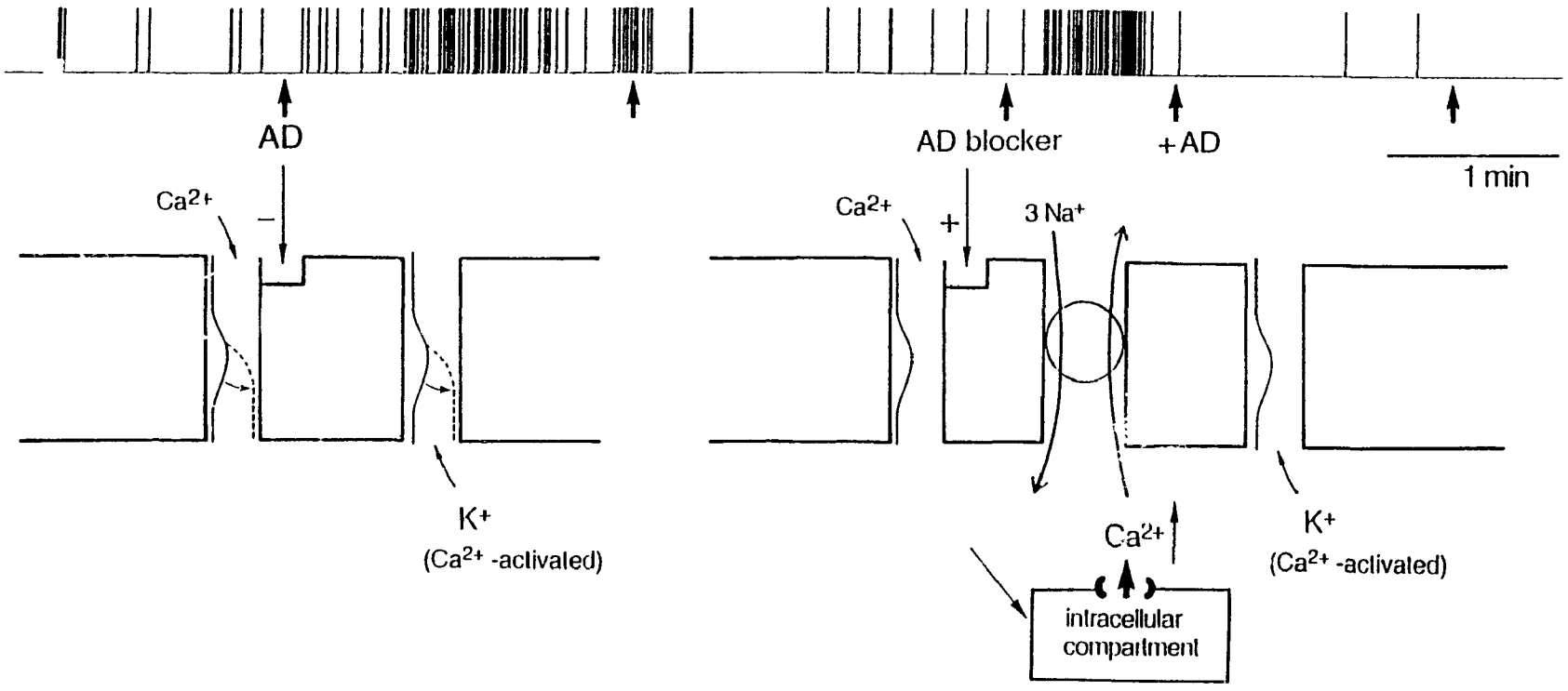


Figure 16. Adenosine (AD) suppresses (-) the inward $Ca^{2+}$ current which leads to decreased intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$). Low $[Ca^{2+}]_i$ suppresses the calcium-activated $K^+$ current which leads to membrane depolarization and thus increased neuronal excitability (top tracing, between first 2 arrows). Adenosine receptor blocker (AD blocker) not only antagonizes (+) adenosine-mediated $Ca^{2+}$ current suppression but also increases $[Ca^{2+}]_i$ by initiating $Ca^{2+}$ release from intracellular compartment. These result in enhanced calcium-activated $K^+$ current which leads to membrane hyperpolarization thereby decreased neuronal excitability (top tracing, between last 2 arrows). High $[Ca^{2+}]_i$ induced by AD blocker may activate $Na^+$-$Ca^{2+}$ exchange thereby increasing $Na^+$ influx which, in turn, leads to membrane depolarization and thus increased neuronal excitability, responses being elicited frequently by AD blocker.

### 4.3.3 ATP sensitive receptors of epicardial afferent neurons

There is no data available concerning the effects of ATP on cardiac sympathetic afferent nerve endings. It is unlikely that the afferent responses elicited by ATP in the present investigation were due to the breakdown of ATP to adenosine (Headrick & Willis, 1989), as only 48% of afferent neurons sensitive to adenosine responded to ATP, with some responding in an opposing fashion. The fact that the ATP agonist β, γ-ATP, which presumably does not affect adenosine receptors activated afferent neurons further supports the contention that some epicardial sensory nerve endings possess ATP-sensitive receptors. Enzymes which regulate ATP and adenosine metabolism such as 5'-nucleotidase and adenosine deaminase, have been detected in mammalian dorsal root ganglion neurons (Nagy & Daddona, 1985). ATP and other purine compounds are released from the peripheral terminals of some sensory neurons (Burnstock, 1989; Holton, 1959). Furthermore, myocardial ATP release increases up to eight fold during myocardial ischemia (Paddle & Burnstock, 1974). It appears that endogenous ATP may activate cardiac sympathetic afferent nerve endings that possess ATP-sensitive receptors. Taken together, these data indicate that the epicardial sensory endings of some dorsal root ganglion neurons possess both adenosine and ATP sensitive receptors.

### 4.3.4 Quiescent epicardial afferent neurons

Sympathetic sensory endings that are insensitive to mechanical stimuli have been associated with other viscera (Cervero & Jänig, 1992; Häbler et al., 1989). For instance, a small population of silent unmyelinated afferent axons

with sensory endings in the feline urinary bladder do not respond to mechanical contraction or distention of the urinary bladder but do respond to acute inflammation of the urinary bladder (Häbler et al., 1989). With respect to the heart, Malliani et al. (1982) did not identify any silent cardiac afferent sympathetic axons in cats, but others have (Uchida & Murao, 1974a; b). However, the existence of such silent cardiac nociceptive afferent neurons has been questioned because the lack of activity has been presumed to be due to insufficient cardiomechanical stimuli (Malliani & Lombardi, 1982; Malliani, 1982, Malliani et al., 1989).

In the present study five afferent neurons which subsequently responded to peptidergic, purinergic, or ischemic stimuli remained quiescent for several hours during control periods and when various sufficient cardiac mechanical stimuli were applied (Fig. 9). The lack of background activity of these neurons cannot be ascribed to insufficient cardiodynamic stimulation, since mechanical stimuli such as those elicited when left ventricular systolic pressure was raised above 150 mmHg did not activate them. The fact that adenosine induced the highest intensity and longest duration responses from quiescent (Fig. 9) or spontaneously active (Fig. 10) epicardial afferent neurons supports the contention that adenosine can mediate cardiac pain based on either the intensity (Malliani & Lombardi, 1982; Malliani et al., 1989) or specificity (Cervero & Jänig, 1992; Perl, 1971) theories of visceral-pain generation.

### 4.4 Ischemia-mediated afferent responses

Seventy-one percent of dorsal root ganglion afferent neurons tested with myocardial ischemia were modified by brief periods of coronary artery occlusion.

That this coronary artery occlusion increased the activity generated by dorsal root afferent neurons is in accord with results obtained by other investigators studying cardiac sympathetic afferent axonal activity (Brown, 1967; Uchida & Murao, 1974c). The increased activity generated by sympathetic afferent axons during coronary artery occlusion has been ascribed to regional mechanical bulging (i.e. receptor- field stretch) and, to a lesser extent, metabolic changes (Malliani, 1982). It is unlikely that the increased afferent activity elicited during coronary artery occlusion in the present study was primarily due to a regional mechanical stretch of cardiac sensory endings. This conclusion is supported by five lines of evidence:

- there was no change in ventricular end-diastolic pressure during brief periods of myocardial ischemia.

- aortic occlusion, an intervention which dilates the heart, failed to excite some of these neurons.

- neurons with epicardial sensory endings that were inhibited during hypotension induced by vena cava occlusion (type I neurons) were excited by ischemia even though regional intramyocardial systolic pressure and ventricular chamber systolic pressure was reduced in the latter.

- in many instances the neuronal-activity increases that were elicited during the ischemia were not reversed upon reperfusion but increased even more despite the fact that regional ventricular dynamics (i.e. intramyocardial systolic pressure) returned to control states.

- the sensory-field application of substance P or adenosine-receptor antagonists reduced ischemia-induced afferent activation. That activity generated by all afferent neurons which were sensitive to transient

myocardial ischemia increased following the epicardial application of one or more neurochemicals, supports the contention that alterations to the chemical milieu accounted for much of the increase in activity displayed during brief periods of myocardial ischemia.

Bradykinin, adenosine, and ATP are released during myocardial ischemia (Berne, 1963; Clemens & Forrester, 1981; Forrester & Williams, 1977; Hougland et al., 1986; Kimura et al., 1973; Paddle & Burnstock, 1974). The fact that ischemia-induced afferent neuronal excitation was suppressed by adenosine or substance P antagonists applied to the cardiac sensory fields of dorsal root ganglion neurons (Fig. 14) indicates that myocardial ischemia may activate cardiac afferent neurons, in part, by modifying the adenosine receptors of sensory nerve terminals by endogenously released chemicals. That a further increase of activity was elicited during reperfusion is in accord with the fact that the release of purinergic compounds increases further during coronary reperfusion (Bernauer, 1991; Kuz'min et al., 1990).

Thirty-five percent of epicardial afferent neurons generated more activity during ventricular fibrillation (Fig. 7e) than during either coronary artery occlusion or control states, even though the ventricles had been emptied of blood to prevent ventricular distention. These data indicate that the activation of such afferent neurons during ventricular fibrillation was probably due to alterations of the heart's chemical milieu rather than of its mechanical milieu.

## 4.5 Cardiogenic reflexes

Increases in heart rate and in cardiac-chamber and intramyocardial pressures were observed in 30% of instances following the epicardial or

intracardiac administration of adenosine (Figs. 10 & 15). Chronotropic and inotropic augmentation was always preceded by increased afferent activity. Dibner-Dunlap et al. (1993) reported that the intracoronary administration of adenosine (1 mg/ml, or 3.7 mM) in the presence of dipyridamole elicited an increased renal sympathetic efferent nerve activity which was assumed to be mediated via adenosine-sensitive cardiac sympathetic sensory endings. Since applying physiological concentrations of adenosine epicardially or intracardially in the present study did not induce systemic hypotension, the cardiac augmentation that occurred after the activation of afferent neurons by adenosine could not have been secondary to the hypotensive effects which can excite type II cardiac mechanosensitive sympathetic afferent neurons (Figs. 3 & 7b). That cardiac augmentation was induced following the activation of epicardial sympathetic afferent neurons by adenosine is in accord with clinical findings that cardiac augmentation can be elicited after the onset of chest pain following an intracoronary or intraatrial infusion of adenosine at doses that do not produce systemic hypotension (Cox et al., 1989; Crea et al., 1990, 1992). Moreover, adenosine induced cardiac augmentation presumably was due in part to the activation of cardiac sympathetic efferent neurons secondary to the excitation of adenosine-sensitive cardiac sympathetic afferent neurons.

Cardiac augmentation that presumably was mediated by sympatho-sympathetic reflex was elicited in less than 30% of neurons that were excited by the epicardial application of chemicals. The lack of such reflexly mediated effects presumably occurred because centrally mediated vagal suppression of sympathetic efferent neuronal activity was elicited (Minisi & Thames, 1991). Bradykinin, substance P, adenosine, or ATP can excite cardiac vagal afferent nerve terminals (Armour et al., 1994). Epicardial vagal afferent receptor

activation may occur simultaneously with epicardial sympathetic afferent neuronal excitation following the epicardial application of a chemical.

## Chapter 5 Conclusion: Implications of multi-receptive properties of epicardial dorsal root ganglion afferent neurons

The major finding of the present study was that most dorsal root ganglion afferent neurons with epicardial sensory nerve endings could be modified by mechanical and multiple neurochemical stimuli (Table 1; Figs. 7, 8, &14). With respect to mechanical stimuli, some were simultaneously modified by cardiac and respiratory dynamics. These data indicate that each dorsal root ganglion cardiac afferent neuron is capable of encoding information from multiple cardiac stimuli simultaneously to spinal cord neurons. Furthermore, the existence of four different types of mechanosensitive afferent neurons may provide spinal cord neurons with more accurate information over a wide range of cardiodynamic changes than would occur if only one type (namely, type I) existed. Histochemical evidence demonstrates that neuropeptide-like or purine-like chemicals localize in afferent or efferent nerve terminals in the heart (Burnstock, 1980 & 1989; Lundberg et al., 1985; Urban & Papka, 1985). It has been proposed that substance P and purinergic chemicals are released from cardiac nerve terminals (Burnstock, 1980, 1989; Geppetti, 1993; Manzini et al., 1989). The present study demonstrates for the first time the co-existence of peptidergic and purinergic receptors in individual cardiac sensory endings of dorsal root ganglion afferent neurons. These receptors appear to be tonically influenced by endogenously released substance P and by adenosine. The neuronal excitation mediated by substance P receptors can occur independently of that mediated by adenosine receptors. This contention is supported by the fact that neuronal inhibition induced by adenosine antagonists differed from that induced by the substance P antagonist, in that the response elicited by the latter

lacked an excitatory phase. Furthermore, adenosine elicited neuronal excitation in the presence of substance P- receptor blockade (Fig. 8). It is not known whether cardiac afferent input initiated by a specific endogenous chemical signifies a specific type of information to the spinal cord neurons. However, it is important to note that, although adenosine may serve as a messenger between myocardial ischemia and the sensory neuronal activation which may lead to angina pain, a number of other factors may be involved synergistically in the sympathetic afferent neuronal excitation elicited by myocardial ischemia, such as endogenously released substance P and bradykinin. The complex interactions that may occur between a number of variables in sympathetic cardiac sensory nerve endings presumably induce a varied spectrum of cardiac afferent neuronal responses, which may account for the variety of symptoms and reflexes encountered during myocardial ischemia.

Limitation of the study: That afferent terminals (cardiac and pulmonary) of sympathetic afferent neurons respond to multiple stimuli had been reported previously (Coleridge et al, 1975). In addition, the present study indicate that respiratory- and heart rate-related afferent activity can be generated by a single dorsal root ganglion afferent neuron associated with ventricular sensory endings. Although mechanical probing of exposed pulmonary tissue did not modify the activity generated by these neurons, the possibility that respiratory-related activity originated from pulmonary endings which were relatively inaccessible could not be totally excluded. This could be due to the fact that their nerve endings were located deep in the lungs making it difficult to distort them mechanically. It should be pointed out, however, that all these neurons did have identified cardiac sensory fields.

Mechanisms responsible for of mechanosensitive responses need to be elucidated. For instance, it is not known whether mechanosensitive nerve endings generate activity by stretch receptors independent of chemical receptors influence or whether certain chemicals are released from nerve terminals by mechanical stimulation to enhance mechanosensitive activity. This could be studied by applying mechanical stimuli to sensory fields in the presence of pharmacological blockage of specific chemical receptors or following depletion of endogenous chemicals known to tonically influence sensory nerve endings.

Capsaican can enhance the release of substance P from sensory nerve terminals. Thus, the effects of endogenous substance P could also have been determined in the presence of capscian on sensory nerve endings. This was not done due to the fact that capscian may modify other chemicals than substance P in neurons. Further characterization of cardiac sensory neurons is warranted in order to understand the complex nature of their function *in situ*.

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