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Parasympathetic Control of the Heart*

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I. Introduction

THE classical studies of Otto Loewi (209) not only provided proof for the release of an active substance during stimulation of the vagus nerve of the perfused frog heart, and thus laid the ground work for modern concepts of neurohumoral transmission, but gave early indication of the complexities of vagal control of the heart. Loewi's observations could not be immediately corroborated by other investigators because of technical difficulties arising from inadvertent stimulation of sympathetic fibers in the mixed vagosympathetic trunk; in some instances stimulation of this trunk produced cardiac inhibition and at other times acceleration. The intimate anatomical association of adrenergic and parasympathetic fibers innervating the heart and the complex interactions between adrenergic and cholinergic mechanisms have caused the subject of the parasympathetic control of the heart to be clouded by conflicting observations. For many years the parasympathetic system was considered to be of little if any importance in the control of cardiac function. However, an ever increasing number of histological and physiological studies now indicate the existence and physiological importance of the terminal parasympathetic innervation of the myocardium, the specialized conduction fibers, and the coronary vessels. It was therefore considered appropriate to take stock of the situation at this juncture, focusing particular attention upon the more recently elucidated observations of parasympathetic control of the heart.

II. Histoanatomy

A. Histological Evidence for Parasympathetic Innervation of Ventricles

While the existence of a luxuriant parasympathetic innervation of atrial structures is well established, the terminal parasympathetic innervation of the ventricles has been a subject of considerable controversy. Until the past decade, both histological (67, 239, 240) and physiological observations (51, 88, 276, 280, 321) were generally considered to indicate an absence of direct parasympathetic innervation of the mammalian ventricle, other than of the atrioventricular junction (21, 127, 269). Indirect histological evidence for the presence of parasympathetic nerve fibers beyond the atrioventricular node was suggested by studies utilizing stains for cholinesterase (50, 170); differences in the depth of cholinesterase staining indicated both adrenergic (light staining) and cholinergic (dark staining) nerve terminals. Although the presence of high concentrations of cholinesterase was detected in the bundle of His and the proximal bundle branches, (50, 170), none was observed in the ventricular myocardium. The absence of cholinergic terminals in the ventricular myocardium was consistent with earlier findings using silver impregnation staining techniques (182, 289).

On the other hand, some early studies had actually shown the presence, albeit sparse, of parasympathetic innervation of the ventricular myocardium. Nonidez (239), using the silver impregnation method of Cajal, detected extensive plexuses of cholinergic

fibers in the atrial myocardium, as well as a few cholinergic nerve fibers in the ventricular wall of young canine and feline hearts. These few cholinergic fibers followed the coronary arteries into the ventricles, but they were not observed to form plexuses and no parasympathetic ganglia were identified in the ventricular myocardium. Tcheng (293) demonstrated not only preganglionic parasympathetic fibers coursing along the coronary arteries into the ventricular myocardium, but he also showed the presence of ventricular intramural ganglia, presumably parasympathetic in type. Parasympathetic fibers were found to exist both at the base and apex of the ventricle but, in both locations, were far sparser than sympathetic fibers. Although Davies *et al.* (71), in a study of a variety of mammals, observed ganglion cells, suggesting the presence of parasympathetic nerve fibers, in the myocardium of only Cetacea and Artiodactyla, Mitchell and collaborators (226), using combined intravital and supravital staining techniques, observed ventricular ganglion cells in primates and rabbits as well.

In more recent studies, Cooper, Hirsch, Napolitano, and their co-workers (61, 142, 143, 234, 235) characterized the extent of parasympathetic innervation of the canine ventricle. Acetylcholinesterase activity in ventricular tissue was shown to be present at levels approximating one-third of those measured in atrial tissue (61). Moreover, since the association of nerve fibers with ganglia in an organ is characteristic of the parasympathetic segment of the autonomic nervous system, the finding of a system of nerves associated with ganglia in the canine ventricular septum suggested a relatively rich parasympathetic innervation of at least this portion of the ventricles (142). This interpretation was supported by the failure of these fibers to degenerate after total extrinsic surgical denervation of the heart in rabbits (144) and dogs (143). The persistence of these ganglia indicated that they were, in fact, intrinsic ganglia and therefore parasympathetic in type.

Jacobowitz *et al.* (167), utilizing the thiocholine method in order to identify acetylcholinesterase activity in normal and denervated feline hearts, observed a dense parasympathetic innervation of the atria with numerous cholinergic cell bodies in the atrial septum near the atrioventricular node but only a small to moderate number of cholinergic nerve fibers unevenly distributed throughout the ventricles. Light microscopic examination of the hearts of dogs subjected either to total extrinsic cardiac denervation or cervical vagotomy indicated that both parasympathetic and sympathetic nerves contribute to the perimysial plexi within the ventricular myocardium (144). Electron microscopic studies have confirmed the persistence of intrinsic neural elements in the atrial and ventricular myocardium after cardiac denervation (234, 235). These intrinsic nerves represent postganglionic elements; their existence in ventricular tissue after total cardiac denervation and cardiac transplantation (234), which produces complete depletion of cardiac norepinephrine stores (63), provides additional strong evidence for parasympathetic innervation of the ventricles. Furthermore, there is some evidence that acetylcholine (ACH) is synthesized in nerves in the ventricles as well as in the atria (37, 218).

B. Physiological Evidence for Parasympathetic Innervation of Ventricles

The anatomical evidence for parasympathetic innervation of the ventricular myocardium as well as of the coronary vessels penetrating the myocardium summarized above has been confirmed in numerous physiological studies over the past decade, which have clearly demonstrated alteration in ventricular function and in the coronary circulation during stimulation of the vagi (20, 77, 99, 119). Several investigators have observed inhibitory effects of vagal

stimulation on ventricular automaticity (94) and ventricular rate in dogs with complete atrioventricular block (95) and in the adrenalectomized dog after extirpation of the stellate and upper cervical ganglia and section of the bundle of His (119). Similarly, as reviewed in detail below, a number of studies has demonstrated a small but distinct effect of vagal stimulation on ventricular contractility (68, 77). Additional support for the existence of vagal innervation below the atrioventricular junction is derived from experiments in which efferent vagal stimulation exerts a direct vasodilating effect upon the coronary arteries (20, 99).

C. Terminal Distribution of Vagal Nerve Fibers

Parasympathetic nerve fibers traversing the right and left vagus nerves appear to be distributed to different areas of the heart. Early studies indicated that the fibers from the left vagus act primarily upon the atrioventricular node and exert negative dromotropic effects, *i.e.*, they slow atrioventricular conduction, while the right vagus acts primarily on the sinoatrial node, exerting a more intense negative chronotropic effect (58, 106, 114, 322). Hamlin and Smith (130) recently observed that submaximal direct right and left vagal stimulation caused comparable effects on heart rate and atrioventricular conduction, but that supramaximal stimulation of the right vagus nerve produced sinus arrest, while similar stimulation of the left vagus caused complete atrioventricular block. Misu and Kirpekar (225) observed that strong stimulation of the right vagus nerve produced only sinus arrest and had little effect on the atrioventricular node, while left vagal stimulation affected the two nodal structures to similar extents. The right vagus nerve also appears to have a greater influence on the atrial myocardium (241, 252, 326). While some studies indicate equal effects of stimulating the two vagi on the ventricular myocardium (68, 77), others suggest that the right supplies a greater number of cholinergic fibers while the left vagus carries a greater number of adrenergic fibers to the ventricles (241, 252, 257).

Although the vagi supply the preganglionic parasympathetic nerve fibers both to the atria and ventricles, as already indicated, they also carry sympathetic fibers to the heart of the dog (54, 176, 179, 229, 241, 252, 254–257), rabbit (98), and baboon (254), but, apparently not the cat (136). Randall and co-workers (241, 252, 255, 257) have demonstrated that some adrenergic fibers from the stellate ganglion pass through the anterior and posterior ansa subclavia primarily and to a lesser extent by other unidentified pathways to enter the posterior cervical ganglion and course to the heart *via* the vagi. From these studies it also appears that the adrenergic and cholinergic components of the vagal nerves, which might more properly be termed the vagosympathetic trunks, are asymmetrically distributed to the ventricles; stimulation of the cervical trunks produces a net inhibitory effect characteristic of cholinergic stimulation on the left ventricle and a net excitatory effect, which is characteristic of adrenergic stimulation, on the right ventricle. However, *beta*-adrenergic blockade unmasks the cholinergic effect on the right ventricle; *i.e.*, in the presence of propranolol, vagal stimulation depresses right ventricular contractility (252, 255, 257), indicating that some parasympathetic fibers do course to this chamber.

III. Mechanisms of Action of Acetylcholine and of Parasympathetic Stimulation

A. Release of Cardiac Catecholamine Stores

The parasympathetic neurotransmitter, acetylcholine (ACH), and parasympathomimetic agents interact with cardiac adrenergic nerve fibers, with specific myocardial receptor sites, with components of the myocardial cell membrane, and with intracellular enzyme systems. Undoubtedly,

these multiple actions mediate the inotropic and chronotropic effects of parasympathetic stimulation of the heart. A single mechanism which accounts for all of the effects of parasympathetic stimulation of the heart appears unlikely when one considers that whereas vagal stimulation usually causes a negative inotropic effect on both atrial (21, 114, 127, 270, 275, 319) and ventricular (68, 76, 77) tissue, a less obvious positive inotropic effect is evoked under some circumstances (12, 95, 192, 206, 225, 241). Furthermore, ACH usually produces a negative inotropic effect on the atrium (36, 103, 104, 190) but results in both negative (36, 103, 190, 206) and positive (36, 53, 103, 131, 155, 193, 206, 224) inotropic effects on ventricular myocardium. Both ACH (4, 12, 25, 85, 95, 103, 171, 220) and vagal stimulation (56, 64, 85, 95, 192, 223, 225, 241) under some circumstances cause a positive chronotropic effect in addition to or instead of the more characteristic negative inotropic effect. The appearance of positive chronotropic and inotropic effects with vagal nerve stimulation can be explained in part by the mixed cholinergic and adrenergic nature of this trunk; in the presence of atropine only the effect of stimulation of adrenergic fibers is observed; however, this explanation is not relevant to the excitatory effects observed with the administration of ACH in the presence of atropine.

In regard to the first mechanism by which parasympathetic intervention may influence cardiac function, *i.e.*, interaction with adrenergic nerve fibers, abundant evidence has been presented to show that cholinergic stimulation may release norepinephrine from depots in the heart (42, 44, 45, 49, 53, 100, 155, 261). In 1945, Hoffman *et al.* (155) noted an "epinephrine-like" effect of ACH on the atropinized hearts of a variety of species; the positive inotropic and chronotropic effects produced by the parasympathetic neurotransmitter were associated with the appearance of an epinephrine-like substance in the perfusate of the isolated heart. Subsequent investigators confirmed this epinephrine-like effect of ACH on the isolated heart and demonstrated that it may be markedly attenuated by pharmacological (49, 56, 64, 81, 192, 193, 224, 225) or surgical (49, 81, 193) cardiac sympathectomy, by *beta*-adrenergic blocking agents (25, 81, 225), and by drugs which inhibit the release of norepinephrine from postganglionic adrenergic nerve endings (12). Thus, when the cardiac adrenergic neurotransmitter stores are depleted or the cardiac adrenergic receptors are blocked, the cardiac-stimulating effects of parasympathetic agents are essentially abolished, observations which strongly support the contention that cholinergic stimuli may influence cardiac function by releasing norepinephrine from cardiac adrenergic nerve fibers. Furthermore, Richardson and Woods (261) have shown that the positive inotropic effect of ACH observed in the isolated atropinized rabbit heart is accompanied by the release into the coronary effluent of norepinephrine, identified both chemically and by bioassay. Angelakos and Bloomquist (4) have confirmed these observations in the isolated perfused guinea pig heart, and they noted further that the cardiac stores of norepinephrine were significantly higher following perfusion with ACH. Lassberg *et al.* (191) also reported this increase in cardiac norepinephrine concentration after ACH, and these findings (4, 191) suggested that ACH may also stimulate norepinephrine synthesis.

The postganglionic cells within the myocardium were originally considered to be the source of cholinergically releasable norepinephrine, since the positive inotropic and chronotropic effects could be abolished by ganglionic blocking agents (12, 25, 64, 138, 155, 193, 224, 225). However, recent observations casting doubt upon the traditional concept that the action of these agents is limited to the ganglion (45, 221), the failure to observe chromaffin tissue in the myocardium, and the histochemical evidence demonstrating that nearly all of

the norepinephrine in the heart is located in sympathetic nerve fibers (3, 62, 70) suggested the alternative explanation that ACH releases norepinephrine from cardiac postganglionic adrenergic terminals rather than from ganglion cells. This mechanism is consistent with the observations and the hypothesis of Burn and Rand (42, 44, 45), that ACH plays an integral part in the liberation of norepinephrine from adrenergic nerve fibers in a variety of tissues. The finding that sympathetic denervation of the heart markedly attenuates the cardiac sympathomimetic response to ACH (49, 62, 198) also supports this hypothesis. Such denervation depletes catecholamines in postganglionic adrenergic fibers but does not deplete these stores in chromaffin tissue (62). Furthermore, agents which block the release of norepinephrine from adrenergic postganglionic nerve fibers also block the sympathomimetic effects of cholinergic stimuli (160, 186). Finally, electroneurographic studies have documented the activation of postganglionic adrenergic fibers by cholinergic stimuli (49, 129). These lines of evidence strongly support the contention that the norepinephrine released by cholinergic interventions originates from the action of ACH on postganglionic adrenergic nerve fibers.

Several recent studies indicate that some of the norepinephrine released by cholinergic stimuli may be released from extra neural stores as well (56, 64, 302); vagal stimulation still elicited a cardiostimulatory effect after depletion of neural stores of catecholamines by reserpine (300) or sympathectomy (56). In addition, postvagal stimulation tachycardia (302) and tachycardia upon cessation of ACH infusion (53, 157) persist, although to a lesser extent, in the presence of a dose of bretylium sufficient to block adrenergic nerve fibers but inadequate to prevent release of norepinephrine from chromaffin cells in the adrenal medulla (302). Possibly related to the latter observation, Endoh *et al.* (96) have recently described a catecholamine-dependent inotropic response to ACH in the canine papillary muscle preparation. The catecholamine-independent responses occurred with lower doses of ACH than the catecholamine-dependent responses and were abolished by atropine, but they were unaffected by ganglionic-blocking agents and *beta*-adrenergic receptor blocking agents. A catecholamine-independent cardiac-stimulating effect of vagal stimulation was also apparent in a number of earlier studies (23, 53, 200, 206).

B. Activation of Multiple Cardiac Cholinoreceptive Sites

In order to explain the observation that the inotropic effect of ACH persists in ventricular tissues depleted of neural norepinephrine stores, Buccino *et al.* (36) have proposed the existence of two or three specific cholinergic receptors (fig. 1). The first receptor site, type I, responds to small concentrations of ACH, exerts a negative inotropic effect, is blocked by atropine, and may be termed muscarinic. The other, type II, responds to large concentrations of ACH, exerts a positive inotropic effect, and is not blocked by atropine. Since the type II receptor is also not blocked by hexamethonium, it is considered to be distinct from the so-called nicotinic receptor. The latter mediates a positive inotropic response with small concentrations of nicotine, prior to depletion of cardiac catecholamine stores. These investigations suggested that the type I cholinergic receptors are intimately related to vagal nerve endings, whereas the type II receptors are spatially separate from these endings. In this study ACH was found to have a predominantly negative inotropic effect on isolated feline atria and a predominantly positive inotropic effect on feline ventricular papillary muscles. Since, as already pointed out, vagal nerve fibers and, therefore, parasympathetic nerve endings are considerably more abundant in the atria than in the ventricles (61, 67, 167, 239, 240), it was suggested that type I receptor sites

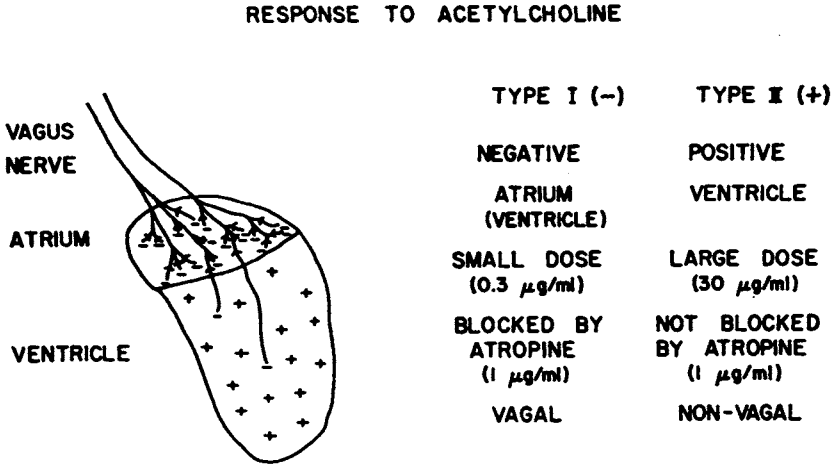

FIG. 1. Schematic representation of two types of cholinergic receptor sites in the heart. (Reproduced from Buccino *et al.* (36) by permission of the American Heart Association.)

have a similar density and distribution, thus accounting for the predominantly negative inotropic effect of ACH on the atria. By the same rationale, type I sites in the ventricle are few, corresponding to the lesser number of vagal fibers and parasympathetic nerve endings there (61, 167). A predominance of type II receptor sites in the ventricle, unrelated to the vagus nerve, would permit the manifestation of a positive inotropic effect at higher, perhaps nonphysiological concentrations of ACH in ventricular muscle. Type II sites appear to be present in addition to, but as already pointed out, are not identical to classically defined nicotinic receptor sites (308), since the positive inotropic effect of high doses of ACH on ventricular tissues could not be blocked by hexamethonium.

Relatively high concentrations of ACH are required to demonstrate the type II sites, and these actions of ACH may represent a nonspecific effect on the cell membrane rather than an action on a discrete receptor. This action of ACH on the proposed type II site occurred only in the presence of low frequencies of contraction and became more prominent with elevated extracellular Na^+ concentration and with lowered extracellular Ca^{++} concentration (103). The dependence of this positive inotropic effect of high concentrations of ACH in ventricular tissue on these conditions, which tend to diminish intracellular Ca^{++} concentration (184, 188, 236), suggested that the phenomenon was due to nonspecific alterations in cellular membrane permeability to Ca^{++}. Furthermore, this positive inotropic action of ACH could not be elicited in the intact feline heart depleted of catecholamines by previous extrinsic denervation or reserpinization and in the innervated heart pretreated with propranolol or d-tubocararine, indicating that the positive inotropic effects in the intact heart are mainly catecholamine dependent (81). Thus, although it seems possible that under special circumstances two mechanisms may exist by which ACH can produce a positive inotropic effect, the significance of the catecholamine-independent mechanism seems to be much less than the catecholamine-dependent mechanism.

C. Alterations in Myocardial Membrane Permeability

ACH has another, more familiar, influence upon the myocardial cell membrane. While it does not appear to shorten the duration of the transmembrane action potential of the ventricular myocardium appreciably (154), its negative inotropic

(38, 39, 104, 126, 315) and chronotropic (38, 150, 154, 163, 328) effects on atrial tissue are associated with a distinct shortening of the action potential of that tissue. This may be considered to be a muscarinic effect of vagal stimulation and ACH infusion, since both the inotropic effect and the shortening of the action potential were potentiated by physostigmine (104) and blocked by atropine (38, 104, 126, 215). Burgen and Terroux (39) proposed that the negative inotropic effect of ACH could be due to the inability of this temporarily shortened action potential to cause the excitation of as many contractile elements as one of normal duration. Since the duration of the action potential is one of the factors which control the cellular influx of Ca^{++} (124–126, 274), shortening of its duration could be the mechanism by which cholinergic interventions decrease the cellular uptake of Ca^{++} (126) and exert a negative inotropic effect. Grossman and Furchgott (126) have also demonstrated that ACH and adenosine, at concentrations which profoundly depress the strength of contraction, significantly reduce the transcellular exchange of that Ca^{++} associated with contraction.

Webb and Hollander (315) demonstrated a correlation between the shortening of the action potential and the depression of contractile state of the murine atrium induced by ACH and carbachol, whereas the positive inotropic effect of epinephrine was associated with a lengthening of the duration of the action potential. However, they pointed out that the shortened action potential may not be the *cause* of the negative inotropic effect but may be the result of an independent action or the result of the reduced inotropic state itself. Furchgott *et al.* (104) made similar observations on paced isolated guinea pig atria. The latter investigators noted also that epinephrine was able to counteract the effect of ACH on the strength of contraction and the duration of the action potential. With specific dosage combinations of ACH and epinephrine it was possible to shorten markedly the action potential without reducing contractile strength. Prolonged exposure of atria to high concentrations of ACH caused a desensitization of the actions of ACH on both contractile strength and on the duration of the action potential. Thus, it appears that although the negative inotropic effect of parasympathomimetic agents is closely associated with an abbreviation of the duration of the action potential (125, 126), a clear-cut cause and effect relationship is not established.

Several studies have shown that ACH and vagal stimulation also alter the myocardial cellular permeability to K^+ and Na^+ (90, 101, 154, 156, 300). The increased permeability of the myocardial cells to K^+ appears to be closely related to the decreased rate of diastolic depolarization of pacemaker cells (300) and the augmented rate of repolarization of non-specialized myocardial cells (101, 154).

D. Regulation of Myocardial Cyclic Adenosine 3′,5′-Monophosphate

ACH appears to have a direct effect on the intracellular enzyme systems which may mediate the effect of adrenergic transmitters on the inotropic state. Several groups of investigators (55, 250, 265, 282) have demonstrated an increase in myocardial cyclic adenosine 3′,5′-monophosphate (cyclic AMP) concentrations closely associated in time with the characteristic inotropic effect of catecholamines. In myocardial cells, as well as in other tissues, the activation of phosphorylase and the increased glycogenolysis produced by catecholamines have been shown to be mediated by activation of adenylate cyclase and the resultant increased levels of cyclic AMP (217, 266). Several investigators (24, 137, 307) have shown that catecholamines increase phosphorylase and glycogenolysis and that ACH and choline esters attenuate this response to catecholamines in both atrial (24, 307) and ventricular (24) tissue. While Blukoallotey *et al.* (24) were not able to

demonstrate an associated attenuation of the inotropic action of catecholamines on ventricular tissue in the presence of ACH, Hollenberg *et al.* (157) have elicited such an antagonism in the intact canine ventricle. Hess *et al.* (137) have observed that vagal stimulation and ACH infusion decrease phosphorylase levels in association with decreased heart rate and force of contraction of the murine heart, but these actions are not considered to be causally connected. In another study (231) ACH and its cogeners reduced the formation of adenosine 3′,5′-monophosphate by preparations of cardiac adenylate cyclase from a number of species, and this effect was blocked by atropine. Recently, carbamylcholine was shown to produce parallel decreases in tension development, adenylate cyclase activity, and cyclic AMP levels in atrial and ventricular tissue, whereas norepinephrine produced parallel increases in these variables (189, 190) (fig. 2). Although the effects of norepinephrine were the same for both atrial and ventricular myocardium, the cholinergic agents were much less effective in ventricular tissue, perhaps related to a relative paucity of cholinergic receptors in ventricular tissue.

IV. Parasympathetic-Sympathetic Interactions

A. Modulation of Parasympathetic Influences by Background Sympathetic Tone

Although an antagonism between the two divisions of the autonomic nervous system is a well known phenomenon, the interaction between the parasympathetic and sympathetic limbs appears to be far more complex than a simple antagonism. Under certain circumstances simultaneous activation of sympathetic nerve fibers may exaggerate the cardiovascular response to the parasympathetic intervention, whereas under others it may be markedly blunted.

In a series of experiments, Gellhorn and co-workers (109–113) produced an enhancement of the responses to cholinergic inter-

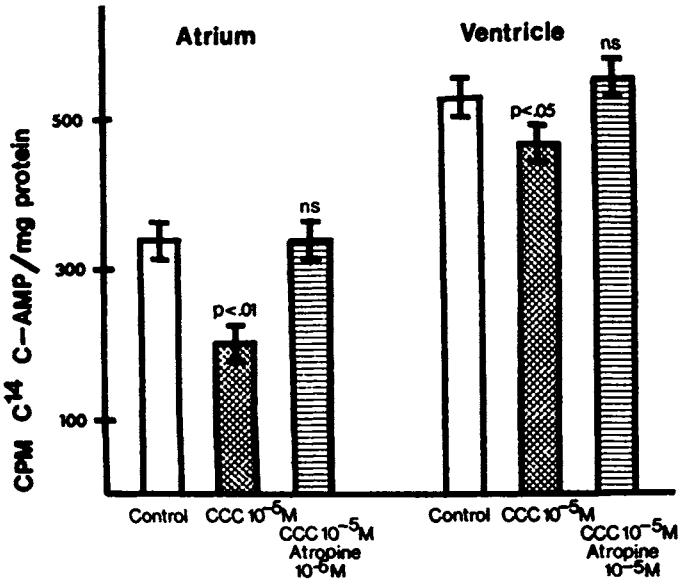

FIG. 2. Effect of carbamylcholine chloride (CCC) on the accumulation of cyclic [^{14}C]AMP (C^{14} C-AMP) by slices of rabbit atrial and ventricular myocardium.

In the atrium, carbamylcholine chloride reduced cyclic [^{14}C]AMP significantly and this effect was blocked by atropine. Carbamylcholine chloride also had a slight but significant effect on ventricular cyclic [^{14}C]AMP, and this effect was also blocked by atropine. (Reproduced from La Raia and Sonnenblick (190) by permission of the American Heart Association.)

ventions by activation of hypothalamic centers. Either direct or reflex stimulation of centers in the anterior hypothalamus induced cardiovascular responses characteristic of parasympathetic stimuli. On the other hand, direct or reflex activation of centers in the posterior hypothalamus resulted in the enhancement of responses to sympathetic interventions and attenuation in those to parasympathetic interventions. Gellhorn termed this phenomenon "autonomic tuning" and "reciprocal inhibition"; states of pre-existing elevated sympathetic tone were accompanied by enhanced sympathetic and decreased parasympathetic reactivity, whereas the reverse changes occurred in states of preexisting increased parasympathetic activity. Similar findings have been reported recently by Gebber and coworkers (107, 108, 183) who noted considerable modulation of baroreceptor responsiveness by suprabulbar structures and established that it took place within the central nervous system. In these studies, electrical stimulation of selected areas of the lateral and posterior hypothalamus, which increased heart rate and blood pressure in the intact cat, blocked the bradycardia evoked by carotid sinus nerve stimulation in the high spinal preparation (108); in other words, sympathetic nervous activation by the hypothalamus was associated with simultaneous inhibition of the efferent component of the baroreceptor reflexes mediated by the vagi. On the other hand, stimulation of forebrain sites within the area pre-optica, the area septalis, and the anterior hypothalamus greatly facilitated the parasympathetic components of the baroreceptor reflexes (183). A number of other investigators (141, 146, 283) have also recognized this parasympathetic-sympathetic interaction in the central nervous system.

Recent studies have suggested that this central autonomic interaction may be responsible for attenuation of parasympathetically mediated bradycardia of the baroreceptor reflex in states of elevated sympathetic tone, as occurs during exercise (31, 92, 248) and heart failure (91, 140). Furthermore, this interaction may be substantially altered by the state of arousal (284, 303) and by general anesthesia (32, 246, 251, 303).

The parasympathetic-sympathetic interaction at the effector organ, the heart, has been descriptively termed "accentuated antagonism" by Levy (194). The chronotropic responses to activation of both divisions of the autonomic nervous system are not simply algebraically additive, but, rather, the absolute reduction in heart rate produced by cholinergic interventions is accentuated in the presence of high tonic sympathetic stimulation of the heart (204, 267, 273, 286, 314). In addition, Grodner *et al.* (123) have recently demonstrated a similar interaction in regard to the chronotropic effects of the two autonomic neurotransmitters acting directly on receptor sites; when various combinations of norepinephrine and ACH were added to isolated atrial preparations, the bradycardiac influence of ACH predominated over the tachycardiac effects of norepinephrine (fig. 3). Furthermore, during intense cholinergic activation induced by asphyxia in the anesthetized rabbit, bradycardia persisted when large doses of isoproterenol were administered (fig. 4). The negative inotropic effects of cholinergic interventions appear to be substantially exaggerated in the presence of high cardiac sympathetic tone (81, 157, 200, 203, 216, 286). In this regard, Hollenberg *et al.* (157) have indicated that the negative inotropic effect observed when ACH was infused into the canine coronary artery was greater when the base line level of myocardial contractile force had been elevated by sympathomimetic drugs or by stellate ganglion stimulation. Levy and coworkers (200, 203, 216) demonstrated that accentuated antagonism occurs also in the setting of autonomic nerve stimulation. They noted that vagal stimulation produced a more intense negative inotropic effect when pre-existing sympathetic tone was

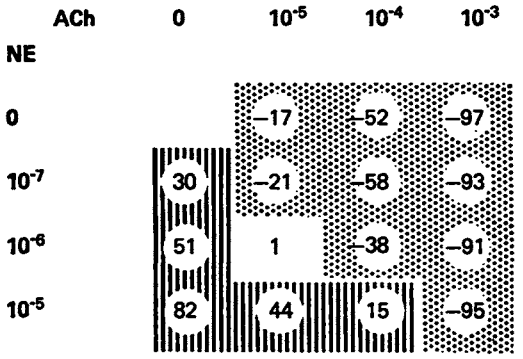

FIG. 3. Diagramatic representation of the change of atrial contraction frequency following the addition of the agents used, expressed as percentage of change from control frequency.

Lined area indicates an increase in frequency, stippled area indicates a decrease in frequency, and the clear area indicates essentially no change. Mean control rate 176 ± 2 beats per min. NE, norepinephrine; ACH, acetylcholine. (Reproduced from Grodner *et al.* (123) by permission of the American Heart Association.)

elevated by either stellate ganglion stimulation or carotid sinus hypotension. These results may be interpreted as indicating that vagal effects on the heart are mediated, at least in part, by antagonizing the positive inotropic influence of the prevailing sympathetic nervous activity. The findings of Dempsey and Cooper (81), indicating an attenuation of the inotropic response to norepinephrine in the presence of ACH, lend support to this possibility.

The concept which arises from the experiments cited above is that vagal stimulation and the addition of ACH are capable of suppressing the chronotropic (123, 204, 267, 273, 314) and inotropic effects (81, 157, 200) of sympathetic stimulation or of norepinephrine.

B. Interaction of Autonomic Neurotransmitters

Grodner *et al.* (123) related the predominance of the cholinergic influences

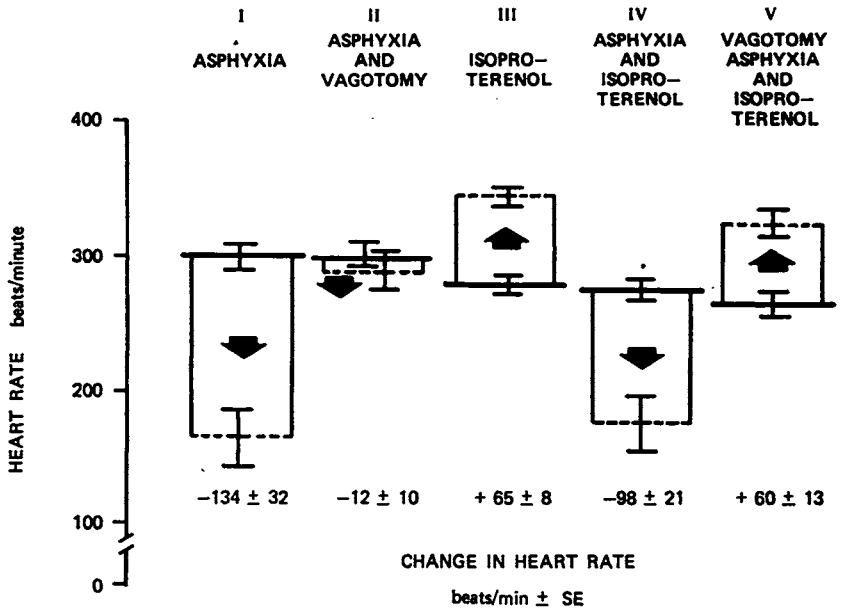

FIG. 4. The change in heart rate of rabbits in response to asphyxia and isoproterenol.

Note that the decrease in heart rate observed during asphyxia is abolished after vagotomy. While isoproterenol alone produced a substantial increase in heart rate, when administered during asphyxia it did not prevent the vagally mediated bradycardia. The direction of the arrows indicates the direction of the change from the control heart rate (heavy line) to the resultant rate (broken line). The mean changes in beats per min produced by each intervention are also indicated. (Reproduced from Grodner *et al.* (123) by permission of the American Heart Association.)

to the interaction of the neurotransmitters at the receptor site; equipotent concentrations of ACH and norepinephrine always caused a negative chronotropic effect on the isolated murine right atrium. The precise mechanism responsible for the predominance of the cholinergic neurotransmitter has not been established but may be the result of any one or a combination of the previously discussed antagonistic actions of ACH and catecholamines on the myocardial cell. ACH counteracts the effect of catecholamines on the mycardial action potential (104), and inhibits the catecholamine-induced increase in cyclic AMP (231).

The effects of sympathetic stimulation on intracellular potassium levels could also be responsible for the sympathetic potentiation of parasympathetic influences; cardiac sympathetic stimulation produces a sudden brief burst of uptake of potassium by the heart (228) and the responsiveness of the cardiac pacemaker to vagal stimulation has been shown to be modified by the level of extracellular potassium (148).

In addition to the predominance of the cholinergic neurotransmitter, the interaction of the two limbs of the autonomic nervous system may also be related to a cholinergically induced reduction in the quantity of norepinephrine released in response to a given level of sympathetic activity. Two groups of investigators (129, 208, 210) have provided clear evidence for a muscarinic inhibition of norepinephrine released from sympathetic nerve fibers to the heart; small concentrations of ACH were found capable of reducing by 80% the norepinephrine released by sympathetic nerve stimulation (210). In contrast, studies of end-plate potentials in skeletal muscle suggest that catecholamines increase the quantity of ACH released per nerve impulse in motor nerve terminals (187). However, the existence of this mechanism in the heart has yet to be established.

In addition to the parasympathetic-adrenergic interactions reviewed above, there also is an apparent simultaneous activation of adrenergic mechanisms during stimulation of the vagi or to be more precise, as indicated above, of the vagosympathetic trunks. A large number of studies has demonstrated a cardiostimulatory response at the cessation of vagal stimulation (53, 85, 192, 200, 225) and during vagal stimulation of the atropinized heart (12, 25, 81, 85, 95, 138, 155, 192, 193, 220, 223, 225, 241, 302). These cardiostimulatory effects appear to be due primarily to release of norepinephrine from depots in the heart (4, 12, 25, 49, 56, 64, 81, 191, 192, 223, 225) and excitation of postganglionic adrenergic nerve fibers (49, 129) by cholinergic interventions, as discussed above in "Mechanisms of Action of Acetylcholine and of Parasympathetic Stimulation."

Relevant to the discussion of parasympathetic-adrenergic interactions is a recent finding that both the vasoconstrictor and inotropic actions of a norepinephrine precursor, dopamine, are augmented substantially by cholinergic blockade with atropine (306). The parasympathetic augmentation of dopamine's pressor and inotropic actions was not observed when the animals were studied after anesthesia was induced with sodium pentobarbital (306), suggesting that the interaction was related to the conscious dogs' relatively high levels of basal vagal tone.

V. Parasympathetic Influences on Myocardial Contractility

A. Atrium

1. Effects of vagal stimulation. Since the classical studies of Gesell (114) and Wiggers (319), there has been general agreement that vagal stimulation depresses atrial contractility (21, 127, 270). A number of investigators has corroborated these studies and has estimated the extent of the myocardial depression (134, 241, 252, 253, 255, 257, 275, 326). Sarnoff *et al.* (275) were among the first to reevaluate this concept, and they elicited a consistent and distinct depression of the strength of left atrial

contraction in the paced and unpaced isolated supported canine left heart preparation. The curve describing the relation between mean left atrial pressure and left ventricular stroke work was depressed, whereas that relating left ventricular end-diastolic pressure to stroke work remained unchanged; these findings were interpreted to indicate that although vagal stimulation depressed atrial contractility, it does not exert any direct effect on the left ventricle. However, vagal stimulation raised the late diastolic atrial pressure, lowered the left ventricular end-diastolic pressure, and reduced the elevation of left ventricular end-diastolic pressure and segment length produced by atrial contraction. Thus, by exerting a negative inotropic effect on the left atrium, vagal stimulation was considered to interfere with the atrial contribution to left ventricular filling. Williams *et al.* (326) recorded a decline in right atrial force, which averaged 41%, and a decline in left atrial force, which averaged 19%, measured by atrial strain gauge arches, during stimulation of the right vagus nerve in intact, paced canine hearts. Stimulation of the left vagus nerves and reflexly induced vagal activation resulted in decreases of lesser magnitude in the force of contraction of both atria. In both of the studies described above (275, 326), the negative inotropic effects of vagal stimulation were abolished by atropine, substantiating these as muscarinic actions of vagal stimulation. Harmon and Reeves (134) also measured atrial force with strain gauge arches during vagal stimulation and observed 90% and 71% decreases in left and right atrial force, respectively; left ventricular contractile force decreased by only 5%, thus indicating the greater extent of vagal control of atrial compared to ventricular function. Randall, Priola, Pace and co-workers observed a consistent depression in atrial contractile force during vagal stimulation in the intact canine heart (241, 257) and in a four-chambered isovolumically contracting heart preparation (252, 253, 255). As in the studies of Williams *et al.* (326) cited above, the right vagus nerve exerted a more profound effect than the left on atrial contraction. In most of the studies enumerated above, a distinct positive inotropic effect on the atrial myocardium occurred upon cessation of vagal stimulation (134, 241, 252, 253, 255, 257). After atropine, vagal stimulation produced both positive chronotropic and inotropic effects on the atrium (56, 225, 255), which were abolished by propranolol (255), pronethalol (225), and reserpine (225).

Vagal stimulation may depress cardiac function by two additional effects on the atrium. First, by slowing atrioventricular conduction, vagal stimulation can alter the synchronicity between atrial and ventricular contractions, impair ventricular filling, and interfere with closure of the atrioventricular valves (29, 207, 326). Secondly, as has been pointed out above, cholinergic interventions have been shown specifically to antagonize adrenergic influences on the heart; thus, vagal activation would depress atrial contractility by interfering with preexisting adrenergic tone (123, 194, 200, 204, 266, 273, 314).

2. Effects of ACH infusion. ACH exerts effects on the atrium which are similar to those of vagal stimulation; infusion of ACH in the heart-lung preparations (47) or isolated atrium of several species (12, 104, 118, 126, 315) produced a predominantly negative inotropic effect. ACH decreased the force of contraction in paced (12, 118, 315) and unpaced (104, 126) guinea pig atria, whereas after atropine ACH exerted a positive inotropic effect on this tissue (12, 104). Other choline esters have effects on the atrium similar to those of ACH (315). Atrial strips from cats demonstrated a marked decrease in tension development in the presence of carbamylcholine; this depression of isometric tension was associated with parallel decreases in cyclic AMP, and both effects were blocked by atropine (190). ACH reduced tension development in paced, isolated, cat left atria (36, 103); this negative inotropic effect became more

prominent as the frequency of contraction was increased and was inhibited by atropine (103). While high doses of ACH induced a positive inotropic effect on ventricular tissue, a similar reversal of effect of high doses on atrial tissue was not observed (36, 103).

B. Ventricle

1. Effects of vagal stimulation. The subject of parasympathetic control of ventricular contractility has provoked considerable controversy for many years; some investigators have contended that vagal stimulation has a negligible or no influence on ventricular contractility (14, 51, 82, 88, 135, 268, 269, 271, 297), whereas others concluded that it does depress left ventricular contractility (95, 159, 214, 222, 245, 247, 260, 290, 310, 323). Despite these conflicting results, the prevailing view resulting from these early investigations and the one generally promulgated until recent years was that vagal nerve stimulation exerts no significant inotropic effects on the mammalian ventricle (12, 127, 270). A number of these early studies utilized very indirect indicators of ventricular contractility (51, 82, 268), and did not control pre-load, after-load, or heart rate during vagal stimulation (14, 51, 82, 88, 135, 268, 269, 271, 297). In more recent investigations, Schreiner *et al.* (281) did not elicit a reduction in cardiac output or of the left ventricular function curve during vagal stimulation at an intensity sufficient to lower heart rate by 50% in the unpaced heart; in this study heart rate and resistance to left ventricular ejection were maintained constant. Likewise, Sarnoff *et al.* (275), utilizing a similar preparation to maintain heart rate and cardiac output but not after-load constant, did not observe a vagal-induced elevation of left ventricular end-diastolic pressure or depression of the left ventricular function curve. The use of mean rather than mean systolic aortic pressure to calculate left ventricular work and submaximal stimulation of the vagi may have precluded the elicitation of a negative inotropic effect. Reeves *et al.* (258) using strain gauge arches on the left ventricle, recorded a prominent reduction of maximal isometric tension and rate of tension development along with an increase in the duration of systole during stimulation of the right vagus nerve at various submaximal frequencies. However, these negative inotropic effects were not evident when heart rate was maintained constant during vagal stimulation.

The interpretation of the early studies, which implied that the vagus nerve does exert a negative inotropic effect on the mammalian ventricle, are limited by the same considerations just enumerated (159, 214, 222, 245, 247, 260, 290, 310, 323). More recently, Eliakim *et al.* (95) elicited a decrease in the force of ventricular contraction in 14 of 22 experiments in dogs with surgically produced complete heart block. A distinct negative inotropic effect upon the isovolumic canine left ventricle was also clearly demonstrated by DeGeest and co-workers (76, 77). In the latter studies, stimulation of either vagus nerve produced a reduction in left ventricular systolic pressure when heart rate, coronary perfusion pressure, and end-diastolic volume were kept constant and the left ventricle contracted isovolumically; this reduction ranged from 7% to 34%, depending upon the frequency of stimulation. No significant difference was noted between the negative inotropic effects of right or left vagal stimulation in the paced heart. At all frequencies of nerve stimulation, the relative decreases in frequency of the unpaced heart were greater than the depression of contractility in the paced heart, indicating the greater influence of vagal stimulation on the sinoatrial node than on the ventricular myocardium. This negative inotropic effect on the ventricle may be considered to be a muscarinic action of vagal stimulation, since after atropine such stimulation produced a slight increase in left ventricular systolic pressure. As further documentation of the negative inotropic effect of vagal

stimulation, these investigators demonstrated an appreciable elevation of left ventricular end-diastolic pressure and reduction of left ventricular systolic pressure in the pumping heart preparation with a constant venous return, indicating a depression of the left ventricular function curve (77). These findings were confirmed in the pumping left ventricle with the left atrium and mitral valve excluded, thus obviating the possible effects of vagal stimulation on atrial transport, mitral valve competency, and the sequence of atrioventricular activation (29, 207, 326).

At an intensity of vagal stimulation which was sufficient to reduce heart rate to the level existing prior to bilateral vagotomy, a definite negative inotropic effect was observed, suggesting that a level of vagal tone exists in the intact animal sufficient to participate in the neural control of left ventricular contactility (77). Daggett *et al.* (68) also demonstrated a negative inotropic effect on the left ventricular myocardium by vagal stimulation in a right heart bypass preparation, in which aortic pressure, cardiac output, and heart rate were held constant. Vagal stimulation during synchronized atrioventricular pacing caused a reduction in left ventricular pressure and in the maximal rate of rise of left ventricular pressure, right ventricular contractile force, and duration of systole. Alterations in atrial contractility, ventricular activation, coronary blood flow, or effectiveness of mitral valve closure were shown not to be factors which contributed to the observed decrease in left ventricular contractility, whereas a positive inotropic effect was induced by vagal stimulation after cholinergic blockade.

The negative inotropic effect of vagal stimulation on the ventricular myocardium has been confirmed in the dog by several groups of investigators (134, 200, 241, 252, 253, 255, 257, 286, 325) and has recently been observed in the baboon (254). However, the negative inotropic effect elicited in these studies was usually considerably smaller in amplitude than that observed by De Geest, Levy, and co-workers (77, 200, 205). For example, as already indicated, Harmon and Reeves (134) observed a 71% average reduction in right atrial contractile force during supramaximal vagal stimulation in the paced, canine, right heart bypass preparation, but only a 5% average reduction in left ventricular contractile force and only a 1.3 mm Hg rise in left ventricular end-diastolic pressure. In fact, in 16 of their 57 experiments, left ventricular contractile force was unchanged or increased slightly. Randall, Priola, Pace, Stanton, and their co-workers (241, 252, 253) elicited a small negative inotropic effect on the canine right ventricle as well as on the left ventricle. Priola and Fulton (252), using a unique four-chamber isovolumic preparation, demonstrated that the greatest decrease in isovolumic systolic pressure during vagal stimulation occurred in the right atrium (>30%), followed by the left atrium (12%), right ventricle (4 to 12%), and the left ventricle (1 to 10%). After atropine, all chambers responded with an increase in systolic pressure, which in turn was abolished by propranolol; the greatest increase occurred in the right ventricle (30%), whereas the other chambers increased by 10 to 20%. In this study it was frequently observed that vagal stimulation produced negative inotropic effects in both atria and in one of the two ventricles; the other ventricle exhibited a positive inotropic response. In all instances, any positive inotropic effect could be either abolished or markedly attenuated by pentolinium, propranolol, or surgical sympathectomy. Pace *et al.* (241) observed divergent alterations in the response of the two ventricles during stimulation of either vagus nerve; in the majority of instances, left ventricular systolic pressure and dP/dt fell while right ventricular pressure and dP/dt rose. After propranolol, vagal stimulation consistently reduced right as well as left ventricular systolic pressure.

Randall and co-workers (257) measured contractile force at the base and apical

regions of both ventricles of the dog with strain gauge arches and demonstrated clearly that cervical vagal stimulation, due to the intermingling of adrenergic nerve fibers from large connections with the caudal cervical ganglion, exerts both direct inhibitory and excitatory influences on the ventricle and that the adrenergic and cholinergic fibers, and hence the excitatory and inhibitory influences, were distributed unevenly to each ventricle and to different portions of each ventricle. Excitatory responses were most intense at the bases of both ventricles, particularly in the area of the pulmonary conus of the right ventricle. Furthermore, the response varied depending upon the portion of the vagosympathetic nerve trunk stimulated; stimulation of the craniovagal branch, which arises from the vagosympathetic trunk, at the level of the ansa subclavia, usually produced positive inotropic effects in both ventricles, whereas stimulation of the caudovagal branch, which arises just below the ansa subclavia, produced positive and negative inotropic effects on the right and left ventricles, respectively, and stimulation of the thoracic vagus uniformly produced negative inotropic effects.

Thus, it appears that only the thoracic portion of the vagosympathetic trunk carries predominantly cholinergic nerve fibers, whereas the proximal portion contains adrenergic nerve fibers which enter the vagosympathetic trunk from the stellate ganglion by way of the ansa subclavia and which are distributed to the heart by the craniovagal and caudovagal branches of this trunk.

In further studies from Randall's laboratory simultaneous depression of contractile force and the rate of rise of contractile force during vagal nerve stimulation was recorded by strain gauge arches on the epicardial surface and on the anterior and posterior papillary muscles of the left ventricle (66). This observation documented the direct action of parasympathetic nerve fibers on the papillary muscles. Thus, studies of Randall, Priola, Pace, and co-workers (241, 252, 253, 255, 257) indicate that vagal nerve stimulation does cause a negative inotropic influence on the canine ventricle but that this effect is small and inconsistent compared to its effect on the atrium (134); these observations appear to be due to the fact that cervical vagal stimulation undoubtedly excites both adrenergic and cholinergic pathways simultaneously and that the terminal distribution of the latter varies markedly in different regions of the heart. It is likely that natural physiological stimuli through the vagi are selective and involve reciprocal intensities of impulse traffic in the cholinergic and adrenergic fibers of the trunk; under these circumstances the parasympathetic control of ventricular contractility might be quite significant. In this regard, a number of studies has indicated that not only direct stimulation but also reflex activation of parasympathetic fibers can induce depression of ventricular contractility through cholinergic mechanisms (78, 79, 165, 176, 194, 199); these are presented in greater detail below.

As discussed in "Parasympathetic-Sympathetic Interactions," the effects of vagal stimulation are conditioned by the level of adrenergic stimulation of the heart. In this connection, Levy and co-workers (200, 216, 324) have shown that the magnitude of the reduction of left ventricular systolic pressure generated by stimulation of the isovolumic canine left ventricle during vagal stimulation was more profound when adrenergic tone was increased either directly, by electrical stimulation of the stellate ganglion, or reflexly, by lowering carotid sinus pressure. Several other groups of investigators (81, 286) have also noted accentuation of the negative inotropic effects of cholinergic interventions during increased adrenergic tone. These findings have been interpreted to indicate that the cardiac effects of vagal stimulation are mediated partly by antagonizing the inotropic level established by background adrenergic tone. Under the influence of

positive inotropic stimuli other than adrenergic, such as sustained post-extrasystolic potentiation and digitalis glycosides, the negative inotropic effect of vagal stimulation is not accentuated but actually is reduced (205).

In addition to the negative inotropic effect on the ventricle produced by vagal stimulation, a number of studies has indicated a positive inotropic effect upon cessation of vagal stimulation and during vagal stimulation in the presence of atropine (68, 76, 77, 95, 134, 200, 206, 241, 253, 255, 257). It appears that the rebound positive inotropic effect upon cessation of stimulation, like the negative inotropic effect during stimulation, is less obvious in the ventricles than the atria (134). As discussed above, in "Mechanism of Action of Acetylcholine and of Parasympathetic Stimulation," this positive inotropic effect may be due to stimulation of adrenergic fibers in the mixed vagosympathetic trunk, the release of norepinephrine by ACH from depots in the ventricular myocardium, *i.e.*, the Burn and Rand hypothesis, or to a combination of these factors. It is not possible to distinguish clearly between these mechanisms, but it is of interest to consider that if the time constant for the disappearance of norepinephrine in the ventricles exceeds that of ACH as some studies have indicated (279, 314), then the rebound positive inotropic effect following cessation of stimulation may be caused, at least in part, by electrical stimulation of a nerve which contains both adrenergic and cholinergic fibers with some quantity of the adrenergic neurotransmitter persisting after the ACH has been inactivated. A positive inotropic effect upon cessation of vagal stimulation which appears to be independent of adrenergic mechanisms can also be elicited. Blinks (23), using field stimulation to stimulate vagal nerves in isolated heart muscle preparations, observed a definite rebound in contractile force after cessation of stimulation, which was unaffected by high concentrations of propranolol but was abolished by atropine. Other investigators have also observed a nonadrenergic component of the poststimulation excitatory response (157, 194, 200, 206, 225).

2. Effects of acetylcholine infusion. In addition to the negative inotropic effects on the ventricles resulting from the stimulation of cholinergic fibers, the administration of ACH has been demonstrated repeatedly to exert negative inotropic effects on the ventricles of isolated feline and canine hearts (81, 155), feline papillary muscle preparations (36, 96, 103, 190, 224), canine isovolumic heart preparations (206), heart-lung preparations (47, 155), the ventricles of intact anesthetized dogs with (95) and without (25, 53, 82, 281) complete atrioventricular block. La Raia and Sonnenblick (190) observed that the negative inotropic effect of ACH on ventricular tissue is considerably less than that observed on atrial tissue. As is the case with vagal stimulation, a positive inotropic effect is observed on cessation of ACH infusion (157) or during ACH infusion in the presence of atropine (25, 52, 81, 82, 95, 96, 155, 206, 224). This postinfusion excitatory response is more prominent than that observed after vagal stimulation (157, 206); the positive inotropic effects of ACH appear to be due to a combination of adrenergic mechanisms, *i.e.*, the release of catecholamines from depots in the myocardium, and nonadrenergic mechanisms such as its effect on the type II cholinoreceptive sites, nonspecific effect on membrane permeability to Ca^{++}, and other effects (36, 81, 96, 103) similar to those discussed above for vagal nerve stimulation.

In comparing the effects of ACH administration and vagal nerve stimulation, it is of interest to consider the studies of Blumenthal *et al.* (25) who demonstrated that ACH infused into the canine coronary circulation produced only coronary dilatation at a dose of 0.01 to 0.1 μg; a negative inotropic effect was observed at a 10-fold greater dose and a negative chronotropic effect at a 100-fold greater dose. After

neostigmine, the dose of ACH which caused the negative inotropic and chronotropic effects was reduced to or toward the threshold dose for coronary dilatation. Hence, the wide range of threshold doses of the three effects can be ascribed to differences in cholinesterase activity adjacent to receptor sites. Levy and Zieske (206) demonstrated a similar order of responsiveness of cardiac structures to ACH but a reverse order of responsiveness to vagal nerve stimulation. Thus, in addition to dependence on regional variation of cholinesterase activity, the order of responsiveness undoubtedly depends also upon the distribution of cholinergic nerve endings and receptor density in various portions of the heart.

3. Diastolic distensibility. Since possible changes in ventricular diastolic distensibility can complicate evaluation of the effects of vagal stimulation and of ACH infusion on ventricular performance, a number of investigations has focused upon this question in recent years. The preliminary reports of Tsakaris and co-workers (295, 296), showing a decrease in diastolic distensibility of the intact canine heart after vagotomy, have raised the possibility that, apart from possible changes in the inotropic state, the parasympathetic system may cause an alteration in the left ventricular end-diastolic pressure-volume relationship. Earlier studies had indicated no alteration in diastolic distensibility during cholinergic interventions (13, 212, 213, 227, 285, 297). Mitchell and co-workers (227), employing simultaneous measurements of left ventricular end-diastolic pressure and the length of a segment of ventricular myocardium, observed no change in the left ventricular diastolic pressure-length relationship during vagal stimulation; however, they also failed to observe a negative inotropic effect during stimulation. Likewise, Ullrich, Bauereisen, Sonnenblick and their co-workers (13, 285, 297) did not demonstrate an alteration in diastolic distensibility during vagal stimulation, but these groups also elicited no or only a very slight decrease in left ventricular contractility. Similarly, Lundin (213) and Luisada and Weiss (212) found that ACH did not change the length-tension relationship of strips of ventricular muscle studied *in vitro*.

In more recent studies, promoted by the difference in the studies of Tsakaris *et al.* and earlier observations, a number of investigators has noted a distinct depression of ventricular contractility, unaccompanied by an alteration in diastolic distensibility during vagal stimulation (22, 134, 175, 324, 325). Wildenthal and co-workers reported that inotropic depression of the ventricle during vagal stimulation occurred independently of changes in distensibility (325). In a subsequent study these workers (324) demonstrated that during vagal stimulation no significant change occurred in the relation of end-diastolic pressure to the volume and dimensions of the canine left ventricle, as assessed by biplane cinefluorographic visualization of small lead beads placed near the endocardium in order to outline the left ventricular cavity. Bianco *et al.* (22) demonstrated that the elevations of left ventricular end-diastolic pressure in the canine right heart bypass preparation induced by vagal stimulation were always paralleled by increases in end-diastolic circumference and base-to-apex segment length. They also showed, in the isovolumically contracting left ventricle, that, while efferent vagal stimulation induced a significant lowering of peak, left ventricular systolic pressure and its first derivative, left ventricular end-diastolic pressure, remained constant. Prior *beta*-adrenergic blockade did not unmask an effect of vagal stimulation on diastolic distensibility. Thus, the bulk of available information strongly supports the view that vagal stimulation does not affect left ventricular distensibility. These findings are consistent with previous observations that negative inotropic agents in general do not alter the diastolic length-tension relations of cardiac muscle (27, 175).

4. Physiological significance: vagal tone. The physiological significance of parasympathetic activity in the control of ventricular function remains to be fully assessed under normal conditions and in states of stress. A number of studies has indicated that elicitation of reflexes in anesthetized animals, utilizing powerful stimuli under controlled conditions in the laboratory, can mediate negative inotropic effects on the left ventricle (78, 79, 165, 176, 194, 199), but they do not indicate whether or not parasympathetic influences are involved in the normal control of ventricular contractility. However, DeGeest et al. (77) inferred from their data that the vagi do exert a distinct tonic inhibitory effect on ventricular contractility; electrical stimulation of the vagi at an intensity sufficient to reduce heart rate to the level existing prior to bilateral vagotomy exerted a definite negative inotropic effect. Similarly, Stanton and Vick (286) noted an increase in contractile force and its first derivative in the paced canine right ventricle after bilateral cervical vagotomy and also after cholinergic blockade with atropine, suggesting the release of a degree of tonic negative inotropic influence exerted by the parasympathetic nervous system.

VI. Parasympathetic Influence on Heart Rate, Conduction and Rhythm

A. Heart Rate

1. Paradoxical tachycardia. Although it is well known that the restraining influences of the parasympathetic nerves operate in concert with the excitatory influences of the adrenergic nerves to determine the level of heart rate at any instant, studies in recent years have indicated that this interaction is quite complex. The influence of cholinergic interventions on cardiac frequency is qualitatively similar to its effect on myocardial contractility, in that vagal stimulation and ACH administration in the presence of atropine or upon the cessation of stimulation or infusion cause tachycardia rather than the usual bradycardia (4, 12, 25, 42, 44, 45, 49, 53, 56, 64, 81, 100, 132, 133, 138, 155, 191, 224, 225, 261). This phenomenon is discussed in "Mechanisms of Action of Acetylcholine and of Parasympathetic Stimulation." However, James and co-workers (169, 171–173) did not detect a specific positive chronotropic effect when ACH was infused directly into the canine sinus node through its nutrient artery or in the immediate postperfusion period after local atropine administration to the sinoatrial node. Thus, since adrenergic nerve terminals are present in abundance in the canine sinus node (168), these findings dictate against local release of catecholamines by ACH in this structure. Although these investigators demonstrated a positive chronotropic effect during vagal stimulation after local atropine administration to the sinus node (171), they suggested that stimulation of adrenergic fibers in the cervical vagus might be responsible for the tachycardia rather than the local release of catecholamines by a direct action of ACH on postganglionic sympathetic nerve fibers. An alternative view is that the failure to elicit evidence for the release of catecholamines from adrenergic fibers in the sinoatrial node by ACH may be due to the relatively high cholinesterase activity in this area (170, 263), since the concentration of ACH required to reproduce adrenergic effects, by releasing catecholamines, exceeds that required to elicit cholinergic effects (263).

A paradoxical increase in heart rate can be induced by vagal stimuli delivered at specific frequencies and at specific times during the cardiac cycle. In 1934, Brown and Eccles (35) were the first to demonstrate that a single vagal stimulus may produce a variable effect on pacemaker activity, depending on its timing during the cardiac cycle. A number of later studies have verified the existence of this phenomenon (86, 185, 196–198, 259, 290). The paradoxical increase in heart rate with vagal impulses

delivered at specific times in the cardiac cycle is not dependent on adrenergic mechanisms, since it persists after *beta*-adrenergic blockade and decentralization of the stellate ganglia (185). Levy and co-workers (196, 197) showed that with single or repetitive stimuli delivered at a frequency exactly or approximately equal to that of the prevailing heart rate, the precise timing of the stimulus within the cardiac cycle determined whether an increase or decrease in sinoatrial rate ensued. The paradoxical effect was the increase in heart rate with increasing frequency of stimulation, but the heart rate at any frequency of vagal stimulation was less than that in the absence of vagal stimulation. In these experiments, using single stimuli, the cardiac pacemaker became synchronized with the activity in the vagus nerves over a narrow range of frequencies; *i.e.*, cardiac frequency followed the frequency of vagal stimulation precisely. When bursts of repetitive stimuli were employed, the band of frequencies at which synchronization occurred was considerably wider (196). Thus, as the frequency of vagal nerve stimulation was raised over a narrow band, heart rate rose paradoxically. By relating the P-P interval to the interval between the preceding P wave and the vagal stimulus (P-St interval) "pacemaker response curves" were constructed (fig. 5). When the P-St interval was incrementally varied, a sinusoidal-like relationship was elicited between the P-P and P-St intervals in which the average maximum P-P interval occurred at a P-St interval of 135 msec and a minimum at 349 msec. Thus, a relative increase o decrease in pacemaker frequency was produced depending on the timing of vagal stimulation (fig. 5). The range of P-P intervals represented by the "pacemaker response curve" in each animal indicated the band of frequencies over which the sinus rate became synchronized with vagal stimuli; within this band, raising the frequency of vagal stimulation produced paradoxical increases in sinus rate. A similar sinusoidal relationship was observed between the P-R and P-St intervals. A corollary of these observations was the finding that when the frequency of vagal nerve impulse traffic was close to but not equal to the prevailing cardiac frequency, the arrival of the vagal impulse at different times in the cardiac cycle produced oscillations in the P-P interval due to temporal variations in the responsiveness of the sinoatrial node as a function of the timing of the vagal stimulus. The frequency of oscillations of the P-P interval was equal to the number of complete cardiac cycle sweeps each minute and the latter was equal to the difference between the frequencies of vagal stimuli and of cardiac contraction. In the band of frequencies at which synchronization of vagal stimuli and heart rate occurred, the frequencies at the upper limit of this band fell at a point in the cardiac cycle at which the responsiveness of the sinoatrial node was minimal. Vagal impulses preceding the next P wave by less than 200 msec had no effect on the time of occurrence of the next P wave, *i.e.*, they were ineffective in altering

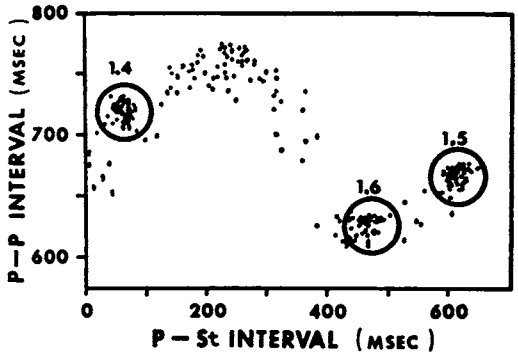

FIG. 5. The P-P intervals are plotted as a function of the P-ST interval during step changes in frequency of stimulation of both cervical vagus nerves.

Each point represents the relationship for one heart beat. The points obtained at stimulation frequencies of 1.4, 1.5, and 1.6 pulses per sec are enclosed in the corresponding circles. All other points were inscribed when the vagus nerves were stimulated at 1.3 pulses per sec. (Reproduced from Levy *et al.* (197) by permission of the American Heart Association.)

heart rate (198) because this period of time (200 msec) appeared to be insufficient for vagal impulse transmission and ACH release and diffusion.

Reid (259) described the same phenomenon of paradoxical increases in the rate of feline and murine hearts with increasing frequencies of vagal stimulation over specific narrow bands of stimulation frequencies. He described this phenomenon as "locking" the pacemaker to the input frequency so that it discharges in fixed ratio to the frequency of vagal stimulation and noted that the stimulus predictably fell during ventricular repolarization when the ratio of sinoatrial and vagal frequencies was fixed. Although heart rate could be increased by certain frequencies of vagal stimulation, the maximum rate to which a pacemaker could be driven in the stable or locking zones of vagal impulse frequencies was always below the spontaneous rate of the pacemaker, *i.e.*, that existing prior to the initiation of vagal stimulation. In other words, as the frequency of vagal stimulation was increased, heart rate reached a minimum but then tended to return to that existing without stimulation. Levy *et al.* (196) and Dong *et al.* (86) also noted that with repetitive volleys of vagal activity the cardiac pacemaker tended to become synchronized in some fixed ratio of vagal stimuli to P waves, and this tendency became greater and the band of frequencies at which synchronization occurred became broader as the number of stimuli per volley increased. Within any range of synchronization, a paradoxical effect occurred; increasing the frequency of the volleys of stimuli while the frequency of the individual stimuli of which the volley were composed were held constant caused increases rather than the expected decreases in cardiac frequency. On the other hand, when the volley of stimuli was delivered at precisely the same P-St interval, increasing the number of stimuli per volley produced a greater negative chronotropic effect.

2. Timing of vagal activity during cardiac cycle. A number of additional studies in recent years has confirmed the importance of the timing of the arrival of vagal impulses at the sinoatrial node in the neural regulation of heart rate (180, 198). This phenomenon may have functional significance since efferent cardiac vagal activity does not occur in a random fashion throughout the cardiac cycle but rather there is a definite tendency toward grouping of impulses in each cardiac cycle (176, 180). Such periodic-grouped impulses are probably initiated by stimulation of the arterial baroreceptors by each pressure pulse, since it has been demonstrated that a single stimulus applied to the baroreceptor nerve is followed by a specific volley of impulses in vagal fibers innervating the heart (164, 202).

Besides providing an explanation for some types of paradoxical increases in cardiac frequency observed with vagal stimulation, these experiments (196–198, 259) indicate the manner by which the restraining influence of vagal activity is finely tuned. Not only does a greater number of impulses per volley of vagal activity produce a greater slowing of heart rate, but the relation of this volley to the preceding P wave also regulates the degree of slowing produced. Thus, vagal restraint of heart rate appears to be achieved by modulations in both the frequency and timing of vagal impulses and apparently is not a simple linear function of the total release of ACH and its concentration near the sinoatrial node, as had been previously believed (313, 314).

3. Summation of simultaneous adrenergic and cholinergic interventions. Since it is likely that the heart rate at any instant is influenced importantly by the interaction of the sympathetic and parasympathetic influences on the sinoatrial node, the nature of this interaction is of considerable importance in the understanding of the parasympathetic control of heart rate. Most investigations dealing with this topic have indicated that simultaneous stimula-

tion of the vagus and of adrenergic nerves resulted in definite slowing of heart rate (204, 267, 271, 273, 314). The study of Samaan (273) was the first to elicit definite evidence that the cholinergic nervous system predominates over the adrenergic in the control of heart rate, and it demonstrated that moderate vagal stimulation can mask the effect of strong sympathetic stimulation. Warner and Russell (314) and Levy and Zieske (204) have devised a mathematical model to describe the predominance of parasympathetic influences during simultaneous stimulation of the two divisions of the autonomic nervous system in the dog.

As already noted above, two recent studies (52, 123) have indicated that the parasympathetic neurotransmitter, ACH, is capable of suppressing the response of the sinoatrial node to the adrenergic transmitter, norepinephrine. These investigations (52, 123) indicated that when equipotent (123) or equimolar (52) doses of ACH and norepinephrine were added to the fluid bathing the spontaneously contracting atrium, the inhibitory influences of ACH clearly predominated at each dose (fig. 3). The action of ACH was abolished by atropine, indicating that this interaction was not due to a blockade of the *beta*-adrenergic receptor by the parasympathetic transmitter. Grodner *et al.* (123) also demonstrated that the predominance of the cholinergic influences on heart rate exists in the intact animal; in rabbits with intense parasympathetic activation induced by asphyxia, large doses of isoproterenol were not capable of increasing heart rate (fig. 4).

B. Conduction System

1. *Sinoatrial node and atrial pacemaker cells.* There has been general agreement for more than a decade that cholinergic influences regulate automaticity and conduction in the sinoatrial node, atrioventricular node, and atrial tissue (53, 65, 89, 149, 152–154, 162, 163, 243, 294, 300, 317, 318). ACH and vagal stimulation produces three changes in the action potential of pacemaker cells of the sinoatrial node recorded by intracellular microelectrodes: 1) a reduction in the slope of spontaneous diastolic depolarization; 2) an elevation of the maximum diastolic potential; 3) a reduction in the duration of the action potential; and 4) a shift of the pacemaker to another site in the node (149, 154, 309). With high concentrations of ACH, spontaneous diastolic depolarization may be abolished completely and conduction within the node may be markedly impaired (149, 154). The effects of cholinergic interventions on automatic cells located outside the sinoatrial node are similar to those described for the sinoatrial cells, but they are usually less intense. An exception to this rule is the effect of cholinergic influences on the duration of the action potential; shortening of the duration of the action potential and acceleration of repolarization is less marked in sinoatrial cells than in automatic cells of the crista terminalis and is most marked in nonspecialized atrial muscle fibers (149, 153, 154, 318). Hoffman and Suckling (154) demonstrated in the *in situ* and isolated canine heart that, although vagal stimulation and ACH decreased the duration of the action potential and increased the rate of repolarization of atrial tissue, they had no effect on the resting or action potential of ventricular cells. In this preparation, strong vagal stimulation slightly increased the atrial diastolic conduction velocity, markedly decreased the duration of the absolute refractory period of the atrium, and increased its vulnerability to fibrillation (153). Again, no effect was observed on ventricular excitability or conduction velocity.

2. *Atrioventricular node.* The cholinergic influences on the atrioventricular node were delineated by the investigations of Cranefield, Hoffman, Paes de Carvalho, and their co-workers (65, 150, 242–244). ACH was found to inhibit transmission in the atrioventricular node and, in sufficient concentrations, to cause partial or complete atrioventricular dissociation by an effect on

cells at the atrial margin of the node (65, 150). Furthermore, it was shown to promote decremental conduction by reducing the rate of rise of the action potential and its amplitude, as measured in cells of the upper atrioventricular node by intracellular electrodes (65, 244).

3. Purkinje system and ventricular pacemaker cells. The actions of cholinergic stimuli on the His-Purkinje system and ventricular pacemaker sites are more controversial. Early investigations indicated that cells of the specialized conduction system below the atrioventricular junction are unaffected by ACH (41, 149, 150, 153, 154, 242). However, Bailey *et al.* (10) recently presented evidence that the specialized conducting cells below the atrioventricular junction, when rendered automatic by anatomical isolation *in situ*, were significantly responsive to the negative chronotropic effects of ACH. Using intracellular recording techniques from the bundle of His and right bundle branch of the canine heart, they demonstrated that the slope of diastolic depolarization (phase 4 of the action potential) was markedly depressed by the addition of ACH; as automaticity was depressed, conduction in these fibers was augmented. Recent studies (16, 95, 101, 120, 301) have also suggested that cholinergic intervention may also influence the electrical activity of latent ventricular pacemaker cells. Fisch and co-workers (101) have shown that ACH consistently increased T wave amplitude, independent of changes in extracellular K^+ concentrations; it was presumed from this observation that ACH increased the rate of repolarization of ventricular cells. Greenspan *et al.* (120) showed that during vagal stimulation there was an increase in the speed of ventricular repolarization and amplitude of the T wave of the electrocardiogram; however, in that study heart rate was not controlled. Other investigations have indicated that ACH and vagal stimulation can lower the idioventricular rate (16, 95, 301).

It has been suggested that cholinergic influences alter automaticity and conduction by increasing membrane permeability to K^+ (101, 154, 161, 294). Since diastolic depolarization appears to be a consequence of decreased potassium conductance during electrical diastole (74, 219, 299), the increased potassium conductance produced by ACH (101, 154, 161, 294) permits a more stable diastolic potential and delays diastolic depolarization.

C. Participation in Genesis of Arrhythmias

It is likely that parasympathetic influences are intimately involved in the induction and facilitation of a number of arrhythmias (1, 40, 46, 47, 69, 145, 211, 232, 233, 238, 309). Stimulation of specific sites in the central nervous system has resulted in arrhythmias which suggest enhanced parasympathetic discharge, *i.e.*, sinus bradycardia, atrial asystole, atrial flutter, and atrial fibrillation (145). However, most centrally induced arrhythmias probably represent the net effect of adrenergic and parasympathetic discharges (309). In concert with simultaneous adrenergic stimulation, parasympathetic influences promote instability of the predominant pacemaker site (1, 69, 309). Parasympathetic activity may induce arrhythmias by depressing the activity of the sinus pacemaker cells, by blocking or slowing atrioventricular conduction, and by promoting nonuniformity of repolarization of adjacent regions of the myocardium (1, 309). In this regard, cholinergic stimulation has been known for a number of years to be associated with the induction and prolongation of atrial fibrillation (1, 40, 46, 47, 69, 170, 171, 211, 232, 233, 238, 279). Nahum and Hoff (233) produced atrial fibrillation in dogs by applying methacholine directly to the right atrial epicardium, and atrial fibrillation persisted for the duration of action of this agent. Subsequent studies have indicated that atrial fibrillation may be induced by both vagal stimulation (66, 211) and perfusion of ACH and parasympathomimetic agents into the

artery of the sinus node (169, 171, 232). This appears to be a direct muscarinic effect of ACH, since it can be abolished by atropine (169, 171). Thus, cholinergically induced atrial fibrillation has been attributed to a shortening of the refractory period of atrial tissues, an increase in diastolic conduction velocity, an acceleration of membrane repolarization, a variable degree of homogeneity in the excitability, and a greater disparity in the duration of the refractory period of atrial cells which occur during cholinergic stimulation (1, 40, 46, 279). Alessi *et al.* (1) demonstrated that direct and reflex vagal stimulation increased the nonuniformity of the duration of the refractory period of various atrial sites of the canine heart, an action which favors the development of atrial fibrillation and other arrhythmias (150, 151).

Parasympathetic activation has been used in therapy of supraventricular tachycardia (30) as well as being implicated in the activation and facilitation of arrhythmias. It is most likely by this mechanism, *i.e.*, increased vagal activity, that direct electrical stimulation of carotid sinus nerves, has been reported to be beneficial in the therapy of refractory supraventricular tachycardia (30).

VII. Parasympathetic Influences on the Coronary Circulation

A. Indirect Effects on Coronary Vascular Resistance

The results of numerous investigations of the effects of vagal stimulation on coronary hemodynamics have been conflicting; although some studies have demonstrated an apparent effect on the coronary circulation, vasoconstriction in a few (5, 147, 312, 321), and vasodilatation in others (20, 68, 99, 105, 181, 206), the majority have failed to elicit a distinct and consistent effect (34, 82, 93, 281, 291, 327). This controversy appears to be attributable to the alteration of a number of factors during vagal stimulation, each of which affects coronary vascular resistance to some extent, and the incomplete control of these critical variables. As discussed in previous sections, vagal stimulation may increase or decrease heart rate, decrease arterial perfusion pressure, raise or lower extravascular myocardial compression of coronary vessels, and stimulate or depress ventricular contractility. Since each of these factors can indirectly alter coronary vascular resistance (19, 28), their precise control is necessary for the accurate interpretation of the direct effects of vagal stimulation on the coronary vascular bed. As discussed earlier, the cervical vagus nerve in the dog is a mixed cholinergic-adrenergic nerve and hence blockade of adrenergic receptors is an additional requisite. Finally, the possibility must be considered that in some studies in which no effects of vagal stimulation on coronary resistance were detected (82, 93, 281, 327) inadvertent destruction of nerve fibers by the transection of the coronary artery required for the measurement of coronary blood flow had taken place, further complicating the experimental interpretation.

In those studies demonstrating coronary vasoconstriction with vagal stimulation there were associated decreases in cardiac work and hence myocardial oxygen consumption, due to a fall in heart rate (5, 147, 321), arterial pressure (5, 147, 310, 321), or cardiac output (310). For example, Wang *et al.* (310) noted decreases in coronary blood flow associated with reductions in cardiac work during three-fourths of the instances of vagal stimulation of paced canine hearts and no change in coronary flow, associated with a stable cardiac output in one-fourth, suggesting that the coronary vasoconstriction in response to vagal stimulation in their study was an indirect effect resulting from attendant decreases in myocardial contractile activity. Schreiner *et al.* (281) maintained coronary perfusion pressure and heart rate constant during vagal stimulation but did not detect any change in the left ventricular function

curve, myocardial oxygen requirements, or coronary vascular resistance. Since these investigations measured only *mean* coronary inflow, small changes in instantaneous flow might have occurred but not have been detected. Furthermore, the absence of any effect of vagal stimulation in this study, in which a Gregg cannula was tied into the transected coronary artery, might have been a consequence of destruction of pericoronary nerve fibers.

B. Direct Vascular Effect

The earliest study which clearly indicated a vagally mediated coronary vasodilatation was that of Katz and Jochim (181), who controlled perfusion pressure, heart rate, and extravascular compression by utilizing fibrillating hearts with an isolated perfused coronary circulation. In this preparation, vagal stimulation caused an increase in coronary outflow in 10 of 35 experiments, and this vasodilatation was abolished by atropine. Berne *et al.* (20) detected a small but consistent increase in coronary inflow and decrease in coronary resistance during cervical vagal stimulation in paced and nonpaced beating hearts as well as in fibrillating hearts with a constant coronary perfusion pressure. In the fibrillating heart the increase in coronary blood flow during vagal stimulation was associated with an increase in coronary sinus PO_2, substantiating that the vasodilatation was a direct vascular effect of vagal stimulation rather than an indirect effect due to an increase in myocardial oxygen requirements. In the beating, paced heart the increase in mean flow was due to a major extent to an increase in systolic coronary flow, suggesting that a decrease in extravascular myocardial compression resulting from the negative inotropic effect contributes to the vagally induced coronary vasdilatation. In each of the preparations studied, the coronary vasodilatation was greater during stimulation of the left than of the right vagus nerve. Likewise, Daggett *et al.* (68), utilizing a canine right heart bypass preparation in which aortic pressure, cardiac input, and heart rate were held constant, demonstrated an increase in coronary blood flow and no significant change in myocardial oxygen consumption during vagal stimulation. *Beta*-adrenergic blockade did not influence this increase of coronary blood flow. More recently, Feigl (99) has presented clear evidence for vagally mediated coronary vasodilatation in a study in which the dilatation was shown to be independent of alterations in aortic pressure, heart rate, myocardial systolic compression, myocardial oxygen requirements, and reflex or direct adrenergic activation. The increase in coronary blood flow and decrease in coronary resistance during vagal stimulation were abolished by cholinergic blockade.

Although it appears from the foregoing that coronary vasodilatation can be elicited by vagal stimulation under specific experimental conditions when its indirect effects on the coronary circulation are precisely controlled, the extent of reflex regulation of the coronary circulation remains to be determined. Hackett *et al.* (128) have recently presented data indicating that cholinergic fibers participate in the efferent limb of both the baroreceptor and chemoreceptor reflexes to produce coronary vasodilatation. However, from numerous studies on the coronary circulation, it is evident that local factors within the heart are of primary importance in controlling the coronary blood flow and neural factors play a less significant role (19).

There is general agrement that ACH, when infused directly into the coronary arteries, is a potent direct vasodilator (18, 19, 82, 93, 206, 281). Schreiner *et al.* (281) showed a substantial increase in coronary blood flow after intracoronary injection of ACH when heart rate, coronary perfusion pressure, and aortic pressure were maintained constant. Blumenthal *et al.* (25) and Levy and Zieske (206) have shown that ACH produces coronary vasodilatation after intracoronary injection of doses too

small to elicit inotropic or chronotropic effects.

VIII. Cholinergic Participation in Cardiovascular Reflexes

A. Baroreceptor Reflex

Parasympathetic nerve fibers in the vagosympathetic trunk form a portion of the efferent limb of a number of cardiovascular reflexes. Depending upon the type of stimulus and the afferent receptor stimulated, vagal inhibitory influences on heart rate or contractility, or both, may be activated, whereas under opposite circumstances tonic vagal restraint may be withdrawn. Several investigators have demonstrated the importance of efferent parasympathetic activation of the baroreceptor reflex (75, 116, 194, 199, 248, 264, 278). Controversy has existed concerning the relative participation of the adrenergic and parasympathetic limbs of the autonomic nervous system in the negative chronotropic effects provoked by baroreceptor hypertension and direct electrical stimulation of the carotid sinus nerves (17, 83, 91, 92, 112, 116, 139, 248, 264, 278, 292, 298, 303, 304, 316). However, it is now clear that in conscious human subjects (91, 92, 116, 248, 264) and dogs (116, 139, 278, 292, 303, 304) at rest, the predominant mechanism is through parasympathetic activation. In the presence of general anesthesia in the dog, withdrawal of tonic sympathetic activity assumes greater importance in mediating reflex bradycardia (17, 83, 112, 292, 303, 316). This finding is consonant with the observations that barbiturate anesthesia depresses vagal activity and vagally mediated reflexes (7, 26, 32, 246, 251, 280). Robinson *et al.* (264) demonstrated in human subjects that increases in sympathetic tone induced by exercise allowed the reflex withdrawal of sympathetic tone to assume greater importance in mediating the bradycardia occurring as a consequence of stimulation of baroreceptors.

Recently, a new technique has been devised by Bristow, Smyth, and co-workers (33, 122) for evaluating the sensitivity of the baroreceptor reflex to pharmacologically induced acute elevations in arterial pressure. Since the reflex bradycardia observed with activation of this reflex has been shown to be unaffected by prior *beta*-adrenergic receptor blockade but abolished by atropine (248), this technique appears also to be a sensitive test for evaluating the parasympathetic control of the heart under a variety of conditions and disease states. These investigators have noted that the reflex bradycardia is diminished in elderly patients (122) and patients with essential hypertension (33, 122) and almost totally abolished during exercise in the upright position (248). Eckberg *et al.* (92) have demonstrated, in a group of conscious, unsedated patients with chronically implanted carotid sinus nerve stimulators, that the reflex bradycardia induced by direct stimulation of this nerve is not attenuated by propranolol but is abolished by atropine administration. These investigators observed that the average prolongation of the R-R interval during stimulation with the patients in the supine position was 269 msec prior to the administration of any autonomic receptor blocking agents, not shortened after propranolol and only 44 msec after atropine, thus substantiating the importance of the parasympathetic efferent limb in the reflex slowing of heart rate. In order to evaluate the influence of background autonomic activity on this reflex, it was reevaluated with the patients standing and during moderate treadmill exercise. While the erect position did not alter the response, the prolongation of the R-R interval observed during stimulation of the carotid sinus nerves during treadmill exercise was reduced to 40 msec in subjects who had not received atropine.

Recent studies have indicated a profound blunting of this vagally mediated component of the baroreceptor reflex in conscious human subjects with a variety

of heart diseases (91) and in conscious dogs with cardiac hypertrophy or failure, or both (140). The slope of the regression line relating the prolongation of the R-R interval to this rise in systolic arterial pressure during the transient elevation of arterial pressure induced by an intravenous injection of 1-phenylephrine was several times greater in normal human subjects and dogs than in those with cardiac disease (figs. 6 and 7). The difference between the normal response and the response in heart failure was not altered by propranolol, whereas reflex slowing was abolished by atropine (91). The extent of this dysfunction of the baroreceptor reflex arc appeared to be a function of the severity of the cardiac abnormality, since the dogs with hypertrophy but without overt heart failure had less reduction of sensitivity than the group with overt heart failure (fig. 7). Furthermore, the increases in heart rate induced by the administration of atropine after propranolol administration were found to be substantially less in patients with heart disease than in normal individuals. This was interpreted as showing a reduced degree of parasympathetic restraint on the sinoatrial node in these patients (91). Similarly, atropine produced much less tachycardia in conscious dogs with experimental heart failure than in normal healthy dogs (305). Another study noted an absence of tachycardia after atropine administration and less reflex bradycardia during acutely induced hypertension in patients with chronic

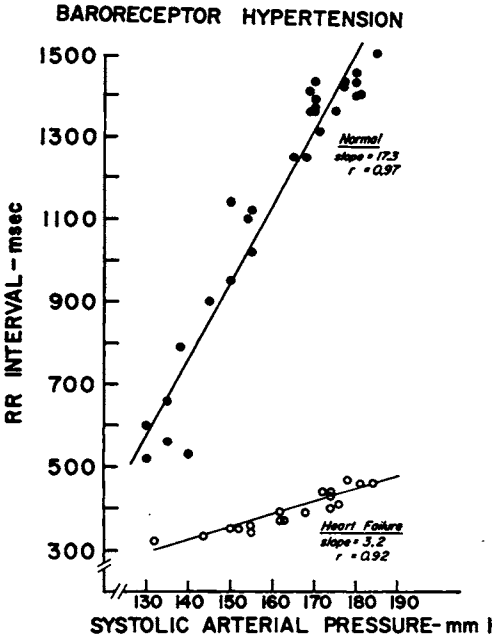

FIG. 6. Each systolic arterial pressure is plotted against the following R-R interval during the transient rise in arterial pressure induced by a bolus intravenous injection of 1-phenylephrine in the same dog in the normal state (closed circles) and in the heart failure state (open circles).

There is a marked depression in the slope of the regression line in the heart failure state compared to the slope existing in the normal state. (Reproduced from Higgins *et al.* (140) by permission of the American Society for Clinical Investigation, Inc.)

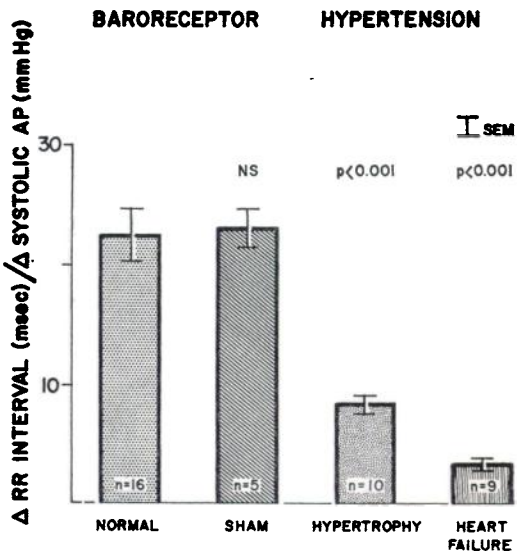

FIG. 7. The mean values and S.E. for the slopes relating the prolongation of the R-R interval (milliseconds) as a function of the rise in systolic arterial pressure (mm Hg) produced by intravenous injection of 1-phenylephrine in normal and sham-operated conscious dogs are compared with those in conscious dogs with cardiac hypertrophy and failure.

While the mean slopes were almost identical in normal and sham-operated animals, the slopes were markedly depressed both in hypertrophy and heart failure. Note also that the hypertrophy group with presumably less severe cardiac overload has less reduction in baroreceptor reflex sensitivity than the group with overt heart failure.

Chagas' disease of the heart compared to patients with a variety of other cardiomyopathies (2). The results of these studies then point to a prominent defect in cardiac control by the parasympathetic system in patients with heart disease.

The mechanisms responsible for this defect have not been clearly established. However, as detailed in "Parasympathetic-Sympathetic Interactions," the baroreceptor reflex can be either facilitated or inhibited by activation of higher centers in the central nervous system (107–113, 183), and the degree of inhibition is increased in situations of circulatory stress (109–112). Indeed, considerable evidence exists that in states of heightened adrenergic tone, parasympathetic reflexes are attenuated (109–112). It is of interest that upright exercise and the heart failure state are both associated with increased adrenergic activity (57, 97) and attenuation of the vagally mediated responses to baroreceptor hypertension.

The reflex tachycardia evoked by carotid sinus hypotension in conscious dogs with experimental heart failure (140) and systemic hypotension in patients with moderate to severe heart failure (15) has also been shown to be profoundly attenuated. This reflex tachycardia in response to baroreceptor hypotension is generally considered to involve a greater participation of adrenergic stimulation than withdrawal of vagal tone (17, 83, 116, 166, 249, 311). However, recent observations in human subjects (248) and in conscious dogs (292, 305) indicate that withdrawal of resting vagal tone may be more important than previously believed. In fact, in normal conscious dogs the vagal contribution to the tachycardia appears to be more important, whereas in conscious dogs with experimental heart failure the reverse tends to hold true (305).

Although earlier studies (75, 80, 115, 276) indicated that the alterations in ventricular contractility in response to activation of the baroreceptor reflex were mediated solely by adrenergic mechanisms, recent studies (199, 201) have demonstrated that cholinergic mechanisms are involved as well. Levy *et al.* (199) demonstrated that sudden elevations of pressure in the isolated carotid sinuses elicited a mild depression in peak systolic pressure in the innervated isovolumically contracting canine left ventricle; after adrenergic blockade with bretylium, the depression was reduced by about one-fourth from that observed prior to blockade, but it was completely abolished by the addition of atropine or by cooling of the cervical vagi.

B. Other Reflexes

The vagi also mediate the reflex alterations in heart rate which occur in response to increases in pressure in the right (8, 11) and left (9) sides of the heart. These alterations in heart rate appear to be variable and apparently dependent on the level of the control heart rate; bradycardia is observed in those instances in which the control heart rate is high (59, 177), and tachycardia when the control rate is low. The reflex response to distension of the atria, whether tachycardia or bradycardia, is markedly attenuated by vagotomy (9, 11, 158) or atropine (6), but it is unaffected by division of the adrenergic accelerator nerve (11) or propranolol (158).

Recently, Horwitz and Bishop (158) observed in the conscious dog that heart rate always rose when left atrial pressure was raised by rapidly infusing saline and there was a consistent relationship between mean left atrial pressure and heart rate. Since parasympathetic blockade prevented this response while propranolol had little effect, reflex inhibition of parasympathetic tone appeared to be the major autonomic mechanism responsible for this response.

When the interfering effects of hyperpnea and its attendant tachycardia are avoided, the negative chronotropic effects produced by stimulation of carotid chemoreceptors also appear to be mediated predominantly by the vagi (60, 72, 73, 79, 87, 272, 287). In addition, the negative inotropic effects

on the atrium (87) and ventricles (79, 87) in response to stimulation of the carotid body chemoreceptors are mediated, at least in part, by parasympathetic nerve fibers. Specifically in paced, innervated, isovolumic canine left ventricular preparations in which the lungs were deflated, hypoxia of the isolated carotid sinus region usually elicited a negative inotropic effect on left ventricular performance, but after vagotomy a slight increase in peak left ventricular pressure was observed (79). However, stimulation of aortic chemoreceptors produces a positive inotropic effect which is mediated by adrenergic mechanisms (287). Electroneurographic studies indicate that rhythmic variations in the frequency of impulse traffic in both divisons of the autonomic nervous system occur at a frequency similar to the respiratory frequency (165, 176). Not surprisingly the variations in heart rate and myocardial contractility occurring at the frequency of the respiratory cycle have also been shown to be mediated in part by the vagi (78, 117, 195).

IX. Summary

In the last decade, evidence has been obtained which indicates that the terminal innervation of the ventricles as well as the atria includes parasympathetic nerve fibers. The demonstration of ganglia within the ventricular myocardium suggested the presence of parasympathetic nerve fibers and the vagal nature of these ganglia was supported by their persistence after total surgical cardiac denervation and cardiac transplantation. Although it is now clear that parasympathetic fibers are present throughout the ventricles, their density is considerably sparser in these chambers than in the atria. Furthermore, the vagal trunks receive abundant adrenergic fibers from the stellate ganglia *via* the ansa subclavia and actually are mixed parasympathetic-adrenergic trunks. The adrenergic and parasympathetic components of the trunks are unevenly distributed to the ventricles; it appears that greater numbers of adrenergic fibers terminate in the right ventricle and greater numbers of cholinergic fibers in the left ventricle.

Cholinergic interventions produce their action upon the heart by a variety of mechanisms. The inhibitory actions appear to be closely related to their ability to decrease the duration of the action potential and subsequent cellular influx of ionic calcium. In addition ACH and choline esters decrease adenylate cyclase activity and cyclic adenosine 3′,5′-monophosphate accumulation of broken cell preparations from mammalian atria and ventricles. On the other hand, under special conditions, *i.e.*, after atropine administration, cholinergic interventions may cause stimulatory effects through interactions with adrenergic and nonadrenergic mechanisms. There is now abundant evidence to indicate that ACH releases norepinephrine from depots in the heart; these depots are located predominantly in postganglionic adrenergic nerve fibers. In addition, particularly in the presence of conditions tending to lower intracellular ionic calcium, large doses of ACH produce positive inotropic effects on ventricular myocardium, in spite of prior depletion of cardiac catecholamine stores.

Cholinergic and adrenergic influences interact synergistically and antagonistically in their actions upon the heart. It appears that under conditions involving a high level of sympathetic tone, the vagal center in the medulla is inhibited by higher centers in the hypothalamus. In addition, cholinergic and adrenergic mechanisms interact at the receptor level, the inhibitory actions of ACH on automaticity and contractility on atrial tissue predominating over the excitatory actions of catecholamines.

Although an inhibitory effect of the vagi upon ventricular contractility has been questioned for many years, recent investigations have clearly indicated that stimulation of the vagosympathetic trunk produces a negative inotropic effect in the canine ventricle. In addition, there is now convincing evidence that stimulation of

parasympathetic nerve fibers produces distinct vasodilatation in the coronary vascular bed, independent of its indirect effects on the determinants of myocardial oxygen requirements and myocardial extravascular compression.

Parasympathetic nerve fibers serve as a component of the efferent limb of the baroreceptor, chemoreceptor, and of other cardiovascular and respiratory reflexes involved in the regulation of cardiac automaticity and contractility. Recent studies using a sensitive technique to evaluate the responsiveness of the baroreceptor reflex indicate that reflexly induced parasympathetic stimulation of the heart is attenuated by general anesthesia, during exercise, in hypertensive patients, and in normotensive, elderly patients. Observations in man and in experimental animals indicate that parasympathetic tone and parasympathetically mediated reflexes are profoundly depressed in heart failure and in various forms of heart disease.

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