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The Parasympathetic Neuroeffector Junction of the Heart*

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I. Introduction

THE concept of chemical neuroeffector transmission has evolved greatly since the turn of the century (reviews refs. 159, 201). Since then research on transmitter substances has focused on *general* aspects of synaptic transmission which now allows one to recognize more precisely organ-specific differences. It appears that the cardiac parasympathetic neuroeffector junction exhibits properties that are extremely different from those of the neuromuscular junction of skeletal muscle, although both belong to the family of cholinergic junctions. These differences are readily appreciated from the distinct roles of these cholinergic junctions in affecting the function of the heart and the skeletal muscle, respectively.

A major goal of this article is to substantiate the physiological "reasons" for, and the mechanisms of, the organ-specific characteristics of cardiac neuroeffector transmission. It is impressive how well tissue structure and the release, removal, and postsynaptic actions of acetylcholine (ACh) are organized to produce the typical parasympathetic influence on the heart beat.

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II. Anatomy of the Cardiac Parasympathetic Innervation

A. Vagal Innervation

The *preganglionic nerves* of the right and left cardiac parasympathetic fibers originate ipsilaterally in the me-

dulla oblongata which serves as the major center for mediating cardiovascular reflexes. For investigating the precise location of the cardiac vagal preganglionic somata, the horseradish peroxidase technique (53, 158) was used in combination with electrical stimulation of the medullary nuclei (88). In the bird, the dorsal motor nucleus constitutes the principal location (review, ref. 39). In the cat, the nucleus ambiguus contains the bulk of somata (72%) while successively fewer cell bodies were located in the dorsal motor nucleus (19%) and the intermediate zone (9%). It has been suggested that these differences might reflect a phylogenetical ventral migration of the anlagen of the cardiac neurons (241; review, ref. 39).

Recently the problem was considered as to whether the localizations of the somata of the cardiac vagal neurons in essentially two medullary nuclei are of functional significance. Although the problem is not yet settled, there has been a report (88) showing a differential cardiac response to electrical stimulation of the two nuclei. Stimulation of the dorsal motor nucleus resulted in a reduced force of contraction with unchanged heart rate, while stimulation of the nucleus ambiguus mediated the converse reaction. Consistent with this report is the observation that in the cardiac vagal branches of the dog, chronotropic fibers are not intermingled with inotropic fibers to a large extent (266). Geis et al. (88) suggested that vagal inotropic neurons of the dorsal motor nucleus may be controlled by one set of reflexes while vagal chronotropic neurons of the nucleus ambiguus may be affected by a different set. However, evidence for this kind of differential reflex pathway is still lacking. No systemic account is yet available for inputs to the preganglionic cardiovagal cells, whereas the multiple inputs to the sympathetic counterpart have been partially identified (198, 284).

The *cervical and thoracic vagi* give off several major cardiac branches which vary considerably among vertebrates. In man, the fine cardiac nerves can be separated into cardio-inhibitory and cardio-acceleratory nerves, whereas, in other mammals interconnections between the two systems were frequently observed (266). In the dog, even the cervical vagus carries sympathetic efferent fibers (260) originating, at least in part, in the brain stem itself (334).

According to fiber diameter and conduction velocities, the cervical vagus is composed of B- and C-fibers. Conduction velocities of the preganglionic parasympathetic fibers projecting to the heart were all in the range of myelinated B-fibers (2.8 to 15.5 m/s) (review, ref. 212; 236).

The vagus nerves innervate the *intracardiac parasympathetic ganglia*. Much of the present knowledge on the transmission through these ganglia has been obtained on the isolated interatrial septum of the frog heart (52, 217) which is so thin (5 to 100 μm) that the ganglia can be studied within the muscular tissue by light and electron microscopy in combination with intracellular recording of the excitatory postsynaptic potential (EPSP). Dennis et al. (52) found in this preparation that, on each ganglion cell, 10 to 20 synaptic boutons are usually derived from a single preganglionic vagal fiber and that these fibers branch extensively to innervate several septal ganglion cells. Fibers derived from the right and left vagus intermingled at the entry of the septum (48, 52), such that even 40% of all ganglion cells studied received inputs from both right and left nerves (275). Similarly, a significant bilateral innervation of the cardiac ganglia was found in the chicken heart by measuring output of ACh in response to stimulation of the right and/or left vagus nerves (193). One of the bilateral inputs may be subthreshold with respect to postganglionic activation. Bilateral vagal innervation of intrinsic cardiac neurons would also explain why unilateral vagal denervation of the dog heart, in contrast to complete surgical denervation of the heart (including bilateral preganglionic denervation), failed to cause supersensitivity to the negative inotropic effect of nicotine (261, 262) which is evoked by stimulation of the intrinsic parasympathetic neurons. Similarly, in rat (206) and cat (31, 32) hearts, bilateral preganglionic parasympathectomy markedly reduced the ACh content, whereas unilateral vagotomy was nearly or totally ineffective. It seems that cardiac effects of unilateral vagotomy and decentralization are small even at a time when sprouting and re-innervation processes may not yet be effective. Partial bilateral innervation of the intrinsic parasympathetic neurons could explain the small responses to unilateral vagotomy.

B. Regional Distribution of Pre- and Postganglionic Neurons

Assuming that the parasympathetic postganglionic neurons give rise to short axons, information concerning the localization of these cell bodies would provide accurate assessment of the specific cardiac regions influenced by postganglionic neurons. The predominant location of the *cardiac ganglia* is the posterior aspect of the atria within the subepicardial connective tissue. The ganglia are considerably more prominent on the right than on the left atrium (review, ref. 39). Further preferential locations of cardiac ganglia are the interatrial septum (217, 275), the entrances of the venae cavae, near the roots of the aorta and pulmonary artery, and along the coronary arteries (39). The most complete picture of the development of vagal innervation of the heart has been obtained in the chick where the parasympathetic ganglia are mainly situated in and around the coronary sulcus (270). An elegant description of the migration and maturation of neural crest cells that give rise to parasympathetic cardiac ganglia has been given by Kirby and Stewart (173). Bilateral extirpation of neural crest from somites 1 to 3 appeared to be optimal for interrupting vagal innervation of embryonic chick heart as assessed

by reduction of ^{3}H-choline uptake and acetylcholinesterase (AChE, EC 3.1.1.7) staining.

The presence of ventricular ganglion cells is a more constant feature of the *avian heart* than of the mammalian one (294). Many ganglia of the avian heart were observed in association with the coronary and epicardial plexuses of the ventricular myocardium (294). In contrast to the atria, the ventricles exhibit a more prominent innervation of the ventral than dorsal surfaces (270). The incidence of ventricular ganglia correlates with the ACh content of the ventricles. This is obvious from comparison between avian and mammalian hearts (172, 294) and between ventricles and atria. In the avian heart, the total number of ganglia (270) and the total ACh content (54) of the ventricles are higher than those of the atria, though both properties, when expressed per gram of tissue, are markedly lower in the ventricles than in the atria (see below). Finally, the physiological significance of the parasympathetic fibers occurring in the ventricles is easier to demonstrate in the avian than in the mammalian heart, since transmural stimulation of isolated ventricular strips as well as application of exogenous ACh evoked a marked atropine-sensitive reduction in force of contraction in avian but not in mammalian preparations (11, 12, 21, 22).

The observation that atria of avian and mammalian hearts receive a much greater overall cholinergic innervation than the ventricles has never been a matter of controversy (review, ref. 266). Consistent with this is the finding that the amount of ACh as well as the activities of its synthesizing and hydrolyzing enzymes, i.e., *choline acetyltransferase* (ChAT, EC 2.3.1.6.) (see section III C) and cholinesterases (see section IV), respectively, are higher in atria than in ventricles. For example, in the rat heart, the activity of ChAT was highest in the sinoatrial node (1775 nmol of ACh synthesized $g^{-1}h^{-1}$) and decreased in the order: interatrial septum (781)>rest of right atrium (712)>left atrium (416)>basal part of right ventricle (366)>apical part of right ventricle (250)>interventricular septum (239)>basal and apical part of left ventricle (208 and 205) (ref. 293). Likewise, the ACh content of chicken and rabbit ventricles (per g of wet weight) was 41% and 19% of the atrial content, respectively (172). In various regions of the cat (31) and rat heart (300) the ACh content decreased in the order: right atrium > left atrium > right ventricle > left ventricle.

Although the parasympathetic innervation of the mammalian *ventricular myocardium* is sparse (170), there is now a broad consensus that the ventricles of mammals including man receive a parasympathetic innervation. The contents of ACh and ChAT in the ventricles, as presented above, indicate a density of cholinergic innervation that is about one-fifth of that of the atria. According to light microscopic analysis, however, the innervation density seems to be even lower than one fifth. Cholinergic innervation of the ventricular myocardium as identified by AChE staining was limited to perivascular terminals, axonal trunks and sparse branches that originate from the larger trunks in both canine and human hearts (170). Possibly, AChE staining may be inadequate to identify all the *ventricular* cholinergic neurons; a comparative study of AChE-containing and ChAT-immunoreactive axons in mammalian ventricles would be intriguing. At 17 days after complete denervation of the canine heart, intrinsic cardiac nerves were still observed in the ventricles by using electron microscopy (231); these axons presumably originate from the ventricular ganglion cells. The existence of a cholinergic innervation of the ventricular myocardium is supported by the presence of muscarinic receptors in the ventricle (section V) and by ventricular responses to vagal activity and to muscarinic agonists (section VI).

In contrast to the scant innervation of the ventricular myocardium, the atrium and also parts of the ventricular *conducting system* of the vertebrate heart, including that of humans, are richly innervated as identified by specific histochemical stains for AChE (19, 170). Consistent with the high incidence of ganglia at the posterior aspect of the right atrium is the fact that the density of the postganglionic varicose fibers in the heart is highest in the sinoatrial node (237, 312). In this region, cholinergic nerves run in close apposition with adrenergic nerves. As shown by histochemical stains for AChE, the cholinergic innervation is more prominent in the sinoatrial node, Purkinje fibers, and atrioventricular node than in either atrial or ventricular myocardium (18, 19, 152, 304). In human hearts, cholinergic terminal fibers run parallel with the Purkinje fibers in the left bundle of His (170). It has been proposed that these fibers tend to raise the ventricular fibrillation threshold (170; see section VI A and B), and that resting vagal tone exerts a significant effect on human ventricular refractoriness (263, 320).

C. Ultrastructure of the Neuroeffector Junction

The terminal cholinergic innervation is composed of long chains of varicose fibers similar to the adrenergic innervation. Varicosities of the cholinergic fibers exhibit dense accumulations of vesicles and are assumed to release the transmitter *en passage*, i.e., in response to an action potential running from one varicosity to the next. McMahan and Kuffler (217) could trace individual cholinesterase-stained varicose axons back to ganglion cells in the interatrial septum of the frog heart. Moreover, after surgical denervation of the heart (bilateral sympathectomy and preganglionic parasympathectomy), the remaining varicosities contained only agranular vesicles indicating that these varicose axons had their cell bodies in the septum ("intrinsic cardiac nerves") (231) and were cholinergic. The average density of varicosities (adrenergic and cholinergic) in the frog heart was 1.9 varicosities/100 μm^2 in the sinus venosus and 0.7 varicosities/100 μm^2 in the ventricle (113). The cholinergic terminal

fibers coursing between the myocardial cells seem to form varicosities unrelated to their distance to the nearest muscle cell (113). Thus the distance from a varicosity to the nearest muscle cell varies from 200 Ångstrom units (Å) to many micrometers (101, 113, 217). In areas of high innervation density, as in the sinoatrial node (237, 312), the atrioventricular node (304), the atrial and ventricular conducting system (19), "close neuroeffector contacts" (< 600 Å) were observed more frequently and may even be predominant. For example, Purkinje fibers are often accompanied by one or more varicose nerves that course in a tortuous fashion, the neuroeffector distance being about 600 Å (18, 170). Further morphometric analyses are necessary to quantify the incidence of close contacts in relation to the cardiac region. Evidence for a rich cholinergic innervation of the cardiac specialized tissue has been summarized above (section II B).

Even when "close contacts" are observed (e.g., 18, 237), the postsynaptic cardiac membrane lacks obvious structural specializations as seen in the central nervous system, in ganglia, and in the motor end-plate. In the heart, muscarinic receptors are randomly distributed over the entire cellular surface as indicated by ionophoretic application of ACh and mapping of ACh sensitivity (113) and by binding of [^{3}H]quinuclidynlbenzilate (^{3}H-QNB) and autoradiography (113, 291). It is concluded that the parasympathetic neuroeffector junction of the heart does not display synaptic specializations, a conclusion that had been drawn as early as 1958 by Fawcett and Selby (71).

III. Neuronal Mechanisms

A. Resting Release of Acetylcholine

Release of transmitters occurring in the absence of neuronal action potentials is difficult to assess in vivo and to distinguish from the release due to "tonic" activity of the nerves. Assuming that tonic activity of the intrinsic parasympathetic neurons of the heart is absent after isolation of the organ, release of ACh from the isolated unstimulated heart must be "resting release." Unfortunately, we cannot be sure that this release is not, at least partially, an artifact due to the isolation and perfusion procedures. Addition of cholinesterase inhibitors to isolated atria of dogs evoked effects, such as decrease in the action potential duration and hyperpolarization of atrial cells, that were blocked by atropine (313). After inhibition of the cholinesterase activity, isolated rabbit atria released 5.4 pmol/min of ACh (about 7 pmol $g^{-1}min^{-1}$) (100) and isolated perfused chicken hearts liberated as much as 64 pmol $g^{-1}min^{-1}$ (195). Assuming these preparations had ACh contents of 6.7 and 4.5 nmol/g, respectively (172), the amounts of ACh released per min and expressed as percent of the ACh content were equal to 0.1% in rabbit atria and 1.4% in chicken hearts. Corresponding values determined in isolated ileum, diaphragm, and superior cervical ganglion are in the same range.

The question remains unanswered whether this ACh originates from neuronal or extraneuronal sources (section III C). The answer will not be found easily because of the presence in the heart of extraneuronal carnitine acetyltransferase (EC 2.3.1.7) (211, 293, 339), a second enzyme that is capable of acetylating choline. Thus although ChAT activity was found to be associated exclusively with neuronal and not extraneuronal structures (see section III C), ACh could be synthesized in, and released from, extraneuronal stores. About 30 years ago several studies were published which emphasized the possibility of a resting release of ACh from myocardial cells (23, 279) and envisaged ACh as a "local hormone" involved in cardiac automaticity (38; see also refs. 45 and 293).

The opinions on subcellular events involved in resting and evoked release of neuronal ACh are controversial (review, ref. 43; 343). In the heart, resting release in contrast to evoked release of ACh was not altered by low Ca^{2+} and by atropine (200). Therefore, it appears that resting release is Ca^{2+}-independent and is not modulated by the presynaptic muscarinic feedback mechanism (see section III B). In analogy to the resting release of ACh from motor nerves (168, 325) a substantial fraction of cardiac resting release may be due to "leakage" through the axolemma rather than due to quantal release. Unfortunately, the membrane potential of the heart cell does not show changes equivalent to the mEPPs observed at the neuromuscular junction, i.e. changes that would allow a selective study of quantal release. The experimental identification of extraneuronal resting release of ACh in the heart is further complicated by two circumstances, namely the intrinsic position of the postganglionic parasympathetic neuron and parasympathetic neuroeffector junctions that are not restricted to specialized synaptic areas. The problem remains unsettled whether the cardiac resting release of ACh is based on one, two, or more mechanisms and originates from one or more sources.

By analogy the results obtained on various tissue preparations, it can be assumed that digitalis glycosides, which inhibit the Na,K-ATPase, facilitate *resting* release of ACh also in the heart, though a direct proof is still lacking (92, 259). A brief look at ouabain and the release of norepinephrine shows that the drug facilitates resting release of axoplasmic norepinephrine through the intact membrane (non-exocytotic release) (182, 190) presumably by an effect on the Na-dependent amine carrier system (97, 282). Further details are discussed in a recent review article (259). Finally it is emphasized that effects of digitalis glycosides on ACh resting release are observed at high concentrations (> 10^{-5}mol/of ouabain) and are not expected to play a significant role in the parasympathetically mediated effects of these drugs observed in the heart under clinical conditions (see next section).

B. Release of ACh Evoked by Nerve Activity

Between periods of increased reflex activity, vagal motor neurons may be still active. This "tonic activity" explains the importance of the vagus even in reflex tachycardia. Tonic activity of the vagus is largely produced by input from the carotid sinus and aortic baroreceptor afferent nerves (reviews, refs. 44, 56). The delay with which the effect of a single impulse applied to the carotid nerve appears in the cervical vagus ranges between 30 and 150 ms (148, 165, 215). After the latent period, excitation of vagal motor neurons evoked by the brief intracarotid pressure pulse (50 to 200 ms) increased rapidly, but declined slowly and outlasted the pressure pulse by 1 to 3 s (258). This prolonged and gradual decay is mainly due to a central modulation of the reflex activity.

In man baroreceptors are experimentally stimulated by administration of hypertensive drugs (295), such as phenylephrine or angiotensin, or by the "neck suction"-technique (69). These non-invasive techniques were critically reviewed by Eckberg (57).

When the blood pressure falls or baroreceptor afferents are eliminated by nerve section, the activity of cardiac vagus fibers may decrease from, for example, 20 to 2 spikes/s or may even cease (180). As the mean pressure is raised *gradually*, the vagal efferent fibers discharge phasically with the cardiac cycle until, at elevated pressure and pulse frequencies, the discharge tends to become continuous attaining maximum rates (165, 179, 180, 242). Some problems in the parasympathetic control of heart functions arising from the phasic character of vagal activity are discussed in section VII. The importance of the parasympathetic tone for the regulation of the heart rate at rest or after activation of the baroreceptor reflex is obvious also from the effects of atropine occurring even under resting conditions (58, 126, 242, 254). Another indication of the importance of parasympathetic nerves has been obtained from a critical examination of the Bezold-Jarisch reflex in the pathophysiology of certain cardiovascular disorders. The anatomical features and operation of parasympathetic nerves as mediators of cardiac depressor reflexes in several clinical states (inferoposterior ischemia and infarction, aortic stenosis, chronic heart failure, etc.) have been comprehensively reviewed (210).

The predominant role of the parasympathetic nerves in baroreceptor reflex which has been shown for conscious human subjects, other mammals (126), and birds (review, ref. 39), may disappear under certain conditions. General anesthesia by barbiturates (2, 104) or the steroid anesthetic, althesin (177), depressed the number of vagal impulses and consequently increased heart rate. This centrally mediated depression of the cardiac vagal reflex observed after injection of general anesthetics is supplemented by a concomitant peripheral inhibition of ACh release at the level of the postganglionic parasympathetic neuron (192).

Much of our knowledge concerning the *intracardiac parasympathetic ganglion* was obtained from studies on the interatrial septum of the frog heart (52, 217, 275). Most of the ganglion cells (60%) received inputs from only one vagal fiber (see section II A), which, on the other hand, can exite several ganglion cells. Stimulation of a single vagal axon resulted in an all-or-none postsynaptic impulse, i.e. an EPSP, which triggered one and sometimes two action potentials (52). The parasympathetic ganglion of the frog heart serves as a low-pass amplifier (52). However, this ganglion does not appear to have an important integrative function (52), a conclusion which is supported by a study on perfused chicken hearts by using a different experimental approach (192). The release of ACh from the chicken heart into the perfusate evoked by preganglionic vagal stimulation at various frequencies (0.3 to 30 Hz) was not significantly different from that found after transmural (pre- and postganglionic) stimulation.

The release of ACh at the *neuroeffector junction* of the heart displays certain features of stimulus-secretion coupling (e.g., ref. 197), although the opinions on the subcellular events leading to release differ considerably (43, 344). Briefly, the release evoked by electrical stimulation or by high potassium was dependent on extracellular Ca (55, 94, 191, 200, 323) and was inhibited by Mg, Co, and Mn ions (94, 308). Inhibition by these cations is caused by interference with the slow inward current in the neuron (review, ref. 7). Manganese ions not only inhibited, but also facilitated ACh release possibly by a reduction of the intracellular Ca binding sites (10).

The Ca dependence explains why drugs known to decrease or increase neuronal Ca inward current, like *barbiturates* or *aminopyridines*, respectively, have been reported to change the release of ACh in the heart. At a concentration of 1.7×10^{-4} M, pentobarbital caused a half-maximal inhibition (IC50) of the postganglionic release of ACh in the perfused chicken heart (192). The parasympathetic terminal fibers were more sensitive to pentobarbital than other excitable cells of the preparation. In order to reduce the release of noradrenaline, or the force of myocardial contraction or the conduction in vagal B- or C-fibers, one had to infuse pentobarbital in concentrations well above the IC50 for inhibition of postganglionic ACh release. As discussed above, it is possible that the clinically known "vagolytic" effect of general anesthetics is due to both a central and a peripheral site of action.

Aminopyridines are used as tools to facilitate transmitter release by promoting voltage-dependent Ca influx (reviews, refs. 204, 305). Hence these drugs (e.g. 4-aminopyridine and 3,4-diaminopyridine) not only increased the postganglionic release of ACh (204, 333), but also antagonized the inhibitory effect of pentobarbital on the

release of ACh in cardiac preparations of chickens and cats (333). These effects of barbiturates and aminopyridines demonstrate that the mechanism of ACh release follows general principles of stimulus-secretion coupling even though we are still far from knowing the chain of events involved in transmitter release.

Clinical observations support the experimentally achieved concept that efferent vagal influence on the heart is enhanced by *digitalis glycosides* (92, 110). The effects of ouabain (15 μg/kg, i.v.) and vagal stimulation on cardiac sinus function (pacemaker cycle length and sinoatrial interval) in conscious dogs were blocked by atropine and were so similar to each other that most effects of ouabain on sinus node activity seem to be mediated vagally (110). Experiments on isolated heart preparations have not yet provided evidence that ouabain or other digitalis glycosides increase the resting release (see section III A) and evoked release of ACh at subtoxic concentrations ($<5 \times 10^{-7}$ mol/l) (92, 259). On the other hand, several investigators (92, 216, 307) observed that ouabain at low concentrations augmented the negative chronotropic response to vagal stimulation. Moreover, ouabain in the same low concentration range enhanced the cardiac effects of exogenous ACh in various species (92), indicating a postjunctional myocardial site of action. Under therapeutic conditions, however, the vagally mediated effects of digitalis glycosides may act entirely through a central mechanism.

In general, *presynaptic modulation* of ACh release can be classified into three aspects. (1) *Feedback modulation*, i.e. modulation of ACh release through stimulation of presynaptic cholinoceptors. Negative feedback modulation of ACh release has been shown in perfused chicken hearts (171, 200) and rat atria (338). Muscarinic agonists depressed and atropine enhanced ACh release evoked by vagal and field stimulation (171, 200) or by high K^+ (338), and cholinesterase inhibitors were found to lower the release of ACh (195, 338). The results indicate a postganglionic localization of the presynaptic muscarinic inhibition (200), which seems to operate under physiological conditions in both avian and mammalian heart. (2) *Axo-axonal modulation*, i.e. modulation by transmitters released from adjacent noncholinergic neurons. This type of modulation is prominent in organs that are equipped with various kinds of neurons involved in intrinsic reflex modulation or automaticity control, in organs like the central nervous system or the intestinal tract. In the heart, axo-axonal contacts may be restricted to a reciprocal interaction between adrenergic (sympathetic) and cholinergic (parasympathetic) postganglionic neurons. However, other kinds of axo-axonal interactions involving non-cholinergic, non-adrenergic neurons are possible (49, 236). Morphological evidence for close contacts between adrenergic and cholinergic postganglionic neurons has been obtained with electron microscopy in mammalian (59, 306) and amphibian cardiac tissue (217). There is also strong evidence that ACh (185, 202) and vagal stimulation (203, 230) inhibit the release of noradrenaline (reviews, refs. 228, 229). The existence of presynaptic inhibitory adrenoceptors at the cholinergic nerve terminals may be expected by analogy with other organs that have dual cholinergic and adrenergic innervation (review, ref. 324). In the chicken heart, however, adrenoceptor agonists such as methoxamine, clonidine, and isoproterenol failed to modify the release of ACh in response to vagal stimulation at 5 Hz (200), whereas cholinoceptors at sympathetic nerve terminals have been clearly shown to exist in the same preparation (67). Only recently it was found, by using incubated rat atria (338), that the ACh release evoked by high K^+ and by field stimulation (5 Hz) was markedly inhibited by norepinephrine and epinephrine via stimulation of prejunctional α-adrenoceptors. The possibility that the observed species-differences in the prejunctional adrenoceptors follow a phylogenetical pattern is well worth studying. (3) *Transsynaptic (retrograde) modulation*, i.e. modulation of transmitter release by substances released postsynaptically from the innervated target cell. It has been shown that ACh enhances the release of arachidonic acid from membrane phospholipids of various organs (section VI C 2). Thus, in isolated rabbit hearts, vagal stimulation (5 Hz, 10 min) as well as exogenous ACh caused an atropine-sensitive increase in the efflux of prostaglandins (157). Prostaglandins may inhibit cardiac neuroeffector transmission according to some indirect evidence obtained in the same organ, i.e., the isolated rabbit heart (335), a result that contrasts with the prostaglandin-evoked facilitation of cholinergic transmission in smooth muscle (review, ref. 102). However, a role of prostaglandins in cholinergic transmission in general is questionable and appears unlikely in the parasympathetic neuroeffector transmission of the heart. Experiments should be done to evaluate the possibility that the substances modulating synthesis and release of prostaglandins, like acetylsalicylic acid, indomethacin, and also bradykinin affect transmission in this synapse. It should be mentioned that bradykinin was found to slow heart rate by an effect on the intracardiac parasympathetic pathway (288).

Adenosine, purine nucleotides, phosphodiesterase inhibitors, and phospholipid metabolites represent further candidates for modulation of ACh release (reviews, refs. 78, 102, 103). As to the heart, evidence for transsynaptic modulation is still lacking. Certain kinds of modulation may occur selectively in the ischemic myocardium.

C. Synthesis of ACh

In general, ACh synthesis depends on the enzymatic transfer of the acetyl moiety from acetyl coenzyme A to choline (reviews, refs. 315, 317): choline + acetyl-CoA $\rightleftharpoons$ ACh + CoA. In the cholinergic neurons of the heart, ACh synthesis is catalyzed by ChAT which seems to be absent in non-cholinergic cells and, therefore, has been

used as a marker for cholinergic neurons (277). It was found that myocardial cells in culture lacked ChAT but contained carnitine acetyltransferase (276), which also is able to acetylate choline, though at a much slower rate than ChAT (339). The K_m of carnitine acetyltransferase for choline (8.7 mM; 339) is about 80 times higher than the K_m of ChAT for choline (discussion, ref. 293). ChAT is highest in the right atrium (285, 293, 314) which shows the highest density of cholinergic neurons (section II B). Moreover, administration of the ganglion blocking drug, chlorisondamine, to rats (61) or total denervation of rat hearts by transplantation (including preganglionic parasympathectomy) produced a selective decrease of ChAT (205), but not of the mitochondrial enzyme carnitine acetyltransferase (discussion, ref. 211) thereby indicating the latter's presence in myocardial cells (211, 293). Similarly to the heart, skeletal muscle cells also contain enzymes able to acetylate choline (222, 316, 318), and it has been claimed that a small amount (<10%) of the total ACh present in the rat extensor digitorum longus muscle is formed and stored in the muscle itself (255). It is emphasized that the presence of myocardial carnitine acetyltransferase and its ability to acetylate choline in the heart homogenate do not necessarily demonstrate the existence of extraneuronal ACh in the intact heart (section III A).

Experiments to identify the source of acetyl groups, with acetate or pyruvate as the most likely candidates (reviews, refs. 315, 317), have not been carried out in the heart. Our knowledge of the second precursor, choline, is more complete. Choline utilized for ACh synthesis in the heart is taken up from the interstitial space by a Na^+-dependent neuronal uptake mechanism which is sensitive to hemicholinium-3 and is activated by stimulation of the vagus nerves (195). The extracellular free choline comes from three sources: (1) free choline in the plasma, which is present at a concentration of about 10 μmol/l in mammals (including humans) (16, 55, 343); (2) choline bound to phospholipids which are present ubiquitously in the heart and constantly release choline (47); and (3) free choline formed from ACh that is released into the interstitial space and is subsequently hydrolyzed (194).

There is evidence for a high capacity of the first two sources, the circulating free choline and the choline phosphoglycerides. The plasma level of free choline, which is rapidly incorported into cardiac ACh (119), is well above the apparent K_m of the high-affinity choline uptake which is generally in the range of 0.5 to 2.0 μmol/l (review, ref. 155). In the heart, the K_m was 0.2 μmol/l (337). Thus, under physiological conditions, the supply of choline by the blood seems sufficient to maintain synthesis of ACh in peripheral organs such as the heart. Choline phosphoglycerides are also capable of providing sufficient choline to permit complete resynthesis of ACh even during high frequency nerve stimulation of the chicken heart that released approximately the entire ACh store within about 20 min (201; review, ref. 199).

One has to keep in mind that the two choline sources, the free choline of the plasma and the tissue-bound choline, are interdependent choline compartments. Incorporation of choline into tissue phospholipids via the cytidine diphosphocholine pathway, oxidation of choline to betaine, and hydrolysis of choline phosphoglycerides contribute to the net formation of free choline in organs and in plasma. Thus, inhibition of choline oxidation in peripheral tissues increases the level of plasma choline (118). In conclusion, both plasma choline and tissue-bound choline supply cholinergic neurons with choline at a rate that is partially determined by the metabolism of choline and choline phosphoglycerides. Therefore, ACh synthesis in the neuroeffector junction of the heart seems less dependent on the third source of choline, namely choline formed from ACh cleavage, as compared, for example, with autonomic ganglia (199).

Changes in turnover of ACh are related to neuronal activity and to ACh release in a way that tends to keep the contents of ACh in peripheral organs at a constant level (reviewed in ref. 209). This holds true also for drug-induced modulations of ACh release (section III B). In anesthetized rats, turnover of cardiac ACh (144 pmol/g·min, about 2.4% of ACh content/min, ref. 33) was slightly higher than the resting release of ACh from various isolated hearts (0.1 to 1.4%/min; section III A).

Drugs that interfere with ACh synthesis directly will, of course, alter the tissue content of ACh and, subsequently, its release. This has been shown in the perfused chicken heart for *hemicholinium-3* (201), an inhibitor of neuronal choline uptake, and this is expected also for *4-(1-naphthylvinyl) pyridine*, a drug that inhibits ChAT activity in heart homogenates leaving carnitine acetyltransferase activity unchanged (339). A similar effect has been described for *bromoacetylcholine* in heart tissue (293). An important problem is the clarification of the mechanisms responsible for the effects of *exogenous choline* on ACh content and release in the heart and in other tissues. These effects have gained physiological (154) and clinical interest (160, 340, 343). Injection of choline chloride, 1.2 mmol/kg s.c. into rats (178) and 0.4 mmol/kg i.v. into guinea pigs (120) increased the cardiac content of ACh. Moreover, infusion of 10^{-5} M choline chloride into isolated chicken and cat hearts caused a two- to three-fold increase in the release of ACh evoked by field stimulation (55). These data are difficult to reconcile with other observations obtained on the same preparation. (1) Resynthesis of ACh in the chicken heart after release of large amounts of ACh met the synaptic demands to maintain a certain level of ACh tissue content (201). (2) The amount of choline taken up in response to a 1 min-period of vagal stimulation was stoichiometrically related to the preceding release of ACh in the absence of exogenous choline and in the presence of

physostigmine (195). (3) Despite large interindividual variations in the resting rate of choline overflow (1.3 to 4.0 nmol $g^{-1}min^{-1}$) from the chicken heart, a significant correlation with the amount of the ACh release evoked by nerve stimulation was absent (195). In conclusion, these data suggest that the extracellular concentration of endogenous choline in the isolated heart is not rate-limiting for neuronal ACh synthesis.

It is difficult to explain how administration of choline can affect ACh content and release in various organs in vivo and in vitro. No convincing argument is available that could explain the discrepancies in the results obtained in many investigations on the potency of choline to enhance tissue ACh (reviews, refs. 154, 317).

IV. Inactivation of Released ACh

Transmission in cholinergic neuroeffector junctions of skeletal muscle, viscera, autonomic ganglia or heart are different with respect to the time-limits of ACh inactivation which vary between microseconds in the motor end-plate and several seconds in the heart (194). In skeletal muscle, contraction and relaxation are dependent on a very rapid inactivation of ACh and postsynaptic repolarization within milliseconds.

In principle, interstitial clearance of ACh is controlled by hydrolysis and diffusion (98, 99). To reconstruct mathematically the ACh-evoked hyperpolarization of atrial myocardium, rate constants for the irreversible enzymatic hydrolysis K_H of 1 to 5 s^{-1} and a diffusion constant D of 8×10^{-6} cm^2 s^{-1} were used (246, 257). The effect of cholinesterase inhibitors on the vagal cardiac response is impressively seen in isolated heart preparations as well as in vivo. In atrial tissue, peak amplitude and the declining phase of the hyperpolarization evoked by vagal stimulation are drastically increased or prolonged, respectively, by cholinesterase inhibitors (95, 246). The cardiac symptomatology during organophosphorus poisoning is due to inhibition of cardiac and also of extracardiac cholinesterase activity, since the plasma level of ACh was elevated to concentrations of 10^{-8} mol/l or above (244).

The rate of junctional clearance of ACh may determine also the "phase response curves" (151, 184), i.e. the curves describing the dependency of the efficacy of a vagal input on the time within the cardiac cycle (P-P interval) at which the vagal input arrives at the cardiac tissue (section VII). At an early phase of the P-P interval, the vagal input was considerably less effective than later in the cycle so that the chronotropic efficacy was about halved within less than 1 s (151, 184).

The enzymatic activities are not equally distributed throughout the heart either on a regional or on a cellular level. The overall activities of both acetylcholinesterase and butyrylcholinesterase (EC 3.1.1.8) are higher in the atria than in the ventricles of mammals (93; review, ref. 175). However, these facts are of limited value, because hydrolysis of released ACh is determined by only those esterase molecules that are located at the cellular surface ("surface", "external", or "functional" cholinesterase). Acetylcholinesterase activity was observed mainly in cholinergic nerves and was less concentrated in adrenergic neurons and in myocardial cells. Thus, enzymatic staining is used for histochemical mapping of cholinergic nerves (section II B).

Cholinesterase has been localized within mammalian (including human) myocardial cells, where the enzyme was associated with longitudinal and perinuclear elements of the sarcoplasmic reticulum and the A band (152, 161). It has been pointed out by Karnovsky (161) that the "internal" cholinesterase may be important especially in relation to the hypothetical myocardial (non-neuronal) ACh, a problem which is discussed in section III A and C.

How important is diffusion of ACh followed by extracellular washout as the second factor of tissue clearance? Theoretically, the importance of hydrolysis and diffusion are inversely related to each other. In the motor end-plate, for example, hydrolysis is the only important factor, because the apparent rate of diffusion is markedly reduced by receptor binding, and the activity of the surface cholinesterase is extremely high (167). On the other hand, the rate of ACh hydrolysis in the extracellular space of the heart is so low, that a large part of the released ACh diffuses over long distances and escapes into the vascular lumen (section VII C). This has been shown for amphibian, avian, and mammalian hearts (review, ref. 199). Washout of ACh from the extracellular space of the heart is caused not only by diffusion but also by bulk movements of fluid due to the "squeezing" effect of the contracting myocardium (342). After a brief period of vagal stimulation (5 s), the mean extracellular concentration of ACh in the perfused chicken heart declined in a multiexponential fashion and reached the 50% level after 2.5 s due to both factors, hydrolysis and washout (194). In contrast, the decline of the extracellular concentration of both ACh plus choline (formed from ACh) was of first order and was characterized by a rate constant of 0.069 s^{-1} and a half-time of 10 s. The data clearly indicate the importance of both diffusion followed by extracellular washout and hydrolysis as significant factors for the inactivation of released ACh.

V. The Myocardial Muscarinic Receptor

A. Regional Distribution

The availability of radioactively labeled ligands that bind to muscarinic receptors with high affinity has greatly aided the examination of muscarinic receptor distribution in the heart. Tritium-labeled muscarinic receptor antagonists, l-[^{3}H]-QNB and l-[^{3}H]-N-methylscopolamine (NMS), have also been widely used to explore the physicochemical properties of muscarinic receptors (section V B). In an early description of the interaction between ^{3}H-QNB and cardiac muscarinic

receptors (72), the specific binding of ^{3}H-QNB to various regions of the rabbit heart was reported as (fmole/mg of protein): left atrium (302.4), right atrium (200.0), ventricular septum (58.1), right ventricle (52.7), and left ventricle (37.4). These data indicate that the density of ventricular muscarinic receptors expressed as percent of that of atria approximates 20% or less. The regional distribution of muscarinic receptors has also been compared in hearts of rabbits, rats, guinea pigs, and dogs (332); the results obtained were essentially the same as those of Fields et al. (72). In contrast, the corresponding densities, when compared to atria, were 46% in frog ventricle (113), 109% in embryonic chick ventricle (291), and 82% in hatched chick ventricle (297). It is noteworthy that these hearts exhibit a higher density of ventricular parasympathetic innervation as compared with mammalian hearts.

The regional distribution of parasympathetic innervation is discussed in section II B and the regional variation of myocardial responses to vagal activity and muscarinic agonists in section VI A.

B. Receptor Properties

It has been proposed that the muscarinic receptor is a highly conserved protein because its molecular weight (about 80,000 daltons) and physicochemical properties are remarkably similar in a variety of tissues (14, 319). When solubilized in digitonin/cholate, the atrial muscarinic receptor from pig heart binds very specifically to lectin affinity resins (123). Approximately 50% of the binding of solubilized muscarinic receptor to wheat germ agglutinin was prevented by treatment with neuraminidase indicating that the receptor is a sialoglycoprotein (123). Of particular interest is the observation that the binding of ^{3}H-QNB to the solubilized muscarinic receptor (apparent molecular weight, 75,000 ± 5,000 daltons) was increased by a nondialyzable, protease-sensitive factor that could be a cytoskeleton fragment (124). The cytoskeleton has been implicated in the desensitization-induced loss of muscarinic receptor by virtue of its role in endocytosis (see below).

The question of the existence of different subtypes and of different states of the muscarinic receptor has been addressed. The antagonist, pirenzepine, but not atropine, has been reported to bind selectively to a subtype (M_1) of muscarinic receptor in brain (106). Because pirenzepine has a much lower affinity for cardiac, as compared to brain, muscarinic receptors, the predominant cardiac muscarinic receptor has been classified as belonging to the M_2 subtype (106). Limited proteolysis yielded essentially the same peptide fragments from muscarinic receptors in a variety of tissues including heart; these results are not consistent with multiple oligomeric forms of the muscarinic receptor (319). By contrast, experiments done with the muscarinic antagonist, N-methyl-4-piperidyl *p*-azidobenzilate (3), provided evidence for multiple forms of the muscarinic receptor in brain and heart tissues. Oligomeric forms of the muscarinic receptor, with molecular weights of 40,000, 80,000, and 160,000 daltons, were identified; the 80,000 and 160,000 dalton polypeptides have been tentatively related to the high and low agonist affinity states of the muscarinic receptor (3). This conclusion is doubtful. When purified to homogeneity (100,000-fold), the detergent-solubilized muscarinic receptor from porcine atrium had a molecular weight of ~ 78,000 daltons and had ligand-binding properties very similar to those of membrane-bound receptors (252). Oligomeric forms of the cardiac muscarinic receptor were not detected in this highly purified preparation which exhibited high and low affinity states for carbachol (252). A comprehensive review of the concepts of subtypes and of forms of the muscarinic receptor has recently been presented (131).

The muscarinic receptor can exist in several states of agonist affinity and guanine nucleotides and cations regulate the transitions among these states in embryonic (80) and adult hearts (reviews, refs. 60, 141, 248, 329). In the rat heart (141) and the chick atrium (298), carbachol displaces ^{3}H-NMS or ^{3}H-QNB from the muscarinic receptor existing in superhigh (SH), high (H), and low (L) affinity states. In the absence of exogenous guanine nucleotides, the rat heart and chick atrium display three receptor states for carbachol with similar K_D values. Addition of guanine nucleotides eliminates the occupation of the SH receptor state by carbachol and yields a preponderance of the L state. Occupation of muscarinic receptors in chick heart by ACh has also been reported to be consistent with the presence of three interconvertible affinity states (140). In these experiments, the proportions of muscarinic receptors occupied by agonist in the SH and H state were reversibly increased by Mg^{2+} while the respective K_D's were not changed. By contrast, Mg^{2+} decreased muscarinic receptor affinity for the antagonist [^{3}H]-QNB (138) and it has become apparent that Mg^{2+} and also monovalent cations, in addition to guanine nucleotides, can serve as important modulators of muscarinic receptor properties (218; review, ref. 15).

Recent experiments with ^{3}H-oxotremorine (^{3}H-OXO), a potent muscarinic agonist, have extended our knowledge of muscarinic receptor properties. In rat heart membranes, ^{3}H-OXO bound to a single class of receptors with high affinity (K_D = 0.3 nM and 2 nM, respectively) according to Harden et al. (108) and Waelbroeck et al. (326). The muscarinic agonist occupied approximately 48% (326) and 75% (108) of the receptor sites that bound ^{3}H-QNB. Because of the rapid dissociation of ^{3}H-OXO, the presence of a low affinity binding site (up to 20% of total) could not be excluded. Whereas both groups reported that guanine nucleotides reduced the binding of ^{3}H-OXO to muscarinic receptors, Harden et al. (108) concluded that there was no change in the K_D for muscarinic agonist while Waelbroeck et al. (326) reported

agonist affinity to be significantly affected. This discrepancy remains unsolved.

It has been suggested that the L state is more likely associated with certain pharmacological actions of agonist in embryonic chick atria [negative chronotropic effect (105)], in ileal smooth muscle [contraction (13)], and in human atria [inhibition of adenylate cyclase (51)]. By contrast, the active state of the muscarinic receptor in embryonic chick heart could not be identified in pharmacological experiments which demonstrated a loss of ^{3}H-QNB binding sites and a decreased affinity of receptor for agonist during chronic exposure (15 min) to carbachol (80). In a subsequent report, the results of experiments with ^{3}H-QNB binding and displacement by carbachol in the chick heart were interpreted as being consistent with the hypothesis that the pharmacologically active agonist-receptor complex occurred with the receptor in the high affinity state (82).

Changes in muscarinic receptor properties have been implicated in desensitization produced by agonist. When the negative chronotropic effect of carbachol had diminished due to desensitization, the number of muscarinic receptor binding sites had been reduced by about 50% and the concentration of carbachol required to arrest spontaneous beating had increased 12-fold (105). These results, which confirmed those reported earlier by Galper and Smith (80), were attributed to a reduction of muscarinic receptors in the H state without a change in affinity of carbachol for the receptor. [In contrast to the conclusion of Halvorsen and Nathanson (105) that the L state of the muscarinic receptor is the pharmacologically active species, it may be argued that the transition from H to L, which is facilitated by guanine nucleotides is the pharmacological stimulus. This view seems especially appropriate when one considers that desensitization is accompanied by a loss of receptors from the H state.] The loss of muscarinic receptors from the H state occurred rapidly (within 1 to 15 min) and this could be distinguished from a more slowly occurring loss of receptors presumably from the L state (80, 82). It is noteworthy that the slower loss of muscarinic receptors produced by chronic exposure to carbachol was opposed by colchicine and vinblastine, agents that inhibit microtubule function and thereby endocytosis of muscarinic receptors (80).

The ontogenesis of muscarinic receptors has been extensively reviewed (248, 273). Of particular concern are discrepancies reported in the number of receptors detected during development of the heart and in the relationship between receptor number and response. With regard to the former, there is still no satisfactory solution for the discrepancy in which muscarinic receptor number either increases (139, 283) or does not change (81, 268) as a function of age in the developing chick heart. In addition, it seems particularly important to conduct such experiments with specific cardiac tissues rather than with membranes from whole hearts. For example, measurements of the effects of muscarinic agonists in cell cultures prepared from whole hearts revealed only small changes in beating rate (−15%) and K^+ efflux (+33%) at 10^{-3} M carbachol (83). It is conceivable that the K^+ efflux changes occurred only in atrial cells and that much larger effects would have been recorded if K^+ fluxes in the atrial cell were not diluted by those in the ventricle where K^+ fluxes are not sensitive to muscarinic agonists (see section VI B 2). Another illustration of such a discrepancy was reported in the fetal mouse heart on the 13th to 14th gestational days where ACh reduced heart rate by 44% yet no specific binding sites for ^{3}H-QNB could be detected (274). These divergent results were attributed to a dilution of ^{3}H-QNB binding sites in the sinoatrial node by membranes derived from whole heart (274).

VI. Myocardial Responses to Vagal Activity and to Muscarinic Agonists

A. Regional Variations

Acetylcholine and vagal stimulation were thought to inhibit supraventricular (SA node, atrium, AV node) but not ventricular electrical and mechanical activity (Purkinje fibers and ventricular muscle) in mammalian hearts (review, ref. 132). In contrast, in avian and amphibian ventricles, negative inotropic effects of ACh and vagal activity were stronger and therefore were detected earlier (reviews, refs. 22, 247, 248; see also refs. 1, 21).

The first demonstration that mammalian ventricles also showed vagal depression was obtained by DeGeest et al. (50). This result was confirmed recently under in vivo conditions (70). Following sympathetic and parasympathetic decentralization, the slight atropine-sensitive negative inotropic response to trains of high-frequency stimuli applied directly to the ventricular epicardial surface during the absolute refractory period of the cardiac tissue indicated parasympathetic neuroeffector transmission in the ventricular myocardium in vivo. The effect was more obvious when the sympathetic influence was unimpaired. Although these ventricular effects were small, recent studies support the notion that the parasympathetic innervation of the ventricle (section II B) bears physiological and pathophysiological significance. Acetylcholine has direct effects on the electrical properties of Purkinje fibers (5, 40, 79, 196). The problem of whether the ventricular parasympathetic innervation is functionally significant was elucidated when the roles of the sympathetic innervation and the cyclic AMP system in the parasympathetic responses were detected. Exogenously applied ACh and vagal stimulation exerted negative inotropic effects on the ventricle that were markedly increased, or were demonstrable, only when the ventricle had been stimulated by sympathetic adrenergic nerves or by β-adrenoceptor agonists (reviews, refs. 126, 183, 184). Moreover, several phosphodiesterase inhibitors

markedly increased the negative inotropic effect of ACh in the cat ventricular heart muscle (235) indicating that activation of cyclic AMP-dependent pathways may be a prerequisite for the effectiveness of muscarinic receptor activation in the ventricle. For example, ACh reduced the positive inotropic effect but not the accumulation of cyclic AMP caused by the phosphodiesterase inhibitor, isobutylmethylxanthine, in embryonic chick ventricle (12). This view is also consistent with the results of experiments with cholera toxin in embryonic chick ventricular muscle inasmuch as ACh completely and rapidly (<3 min) antagonized the positive inotropic effect of the toxin without affecting the accumulation (2.5-fold) of cyclic AMP (250). In the absence of cholera toxin, ACh had no effect on cyclic AMP content or on contractility in embryonc chick ventricles. The inhibitory effect of muscarinic agonist in the presence of cholera toxin, which irreversibly activates adenylate cyclase (section VI C 2), could result from diminished activation of cyclic AMP-dependent protein kinase (143) or interference with the phosphorylation promoted by cyclic AMP-dependent protein kinase (169). Moreover, vulnerability for ventricular fibrillation was significantly reduced by muscarinic agonists and vagal activity; again, this effect became obvious only during stimulation of β-adrenoceptors and disappeared after administration of β-adrenoceptor blocking drugs (e.g., ref. 320). Muscarinic inhibition of cyclic AMP accumulation caused by β-adrenoceptor agonists is discussed in section VI C 2.

B. Electrophysiological Effects

1. Time-Independent K^+ Current (i_{K_1}). Gaskell (1886–1887) was the first to report that vagal inhibition of the heartbeat was related to changes in the electrical properties of the heart. Vagal stimulation hyperpolarized the mechanically inactive tortoise auricle and this inhibition was opposed by atropine (86). Gaskell (87) also observed that muscarine mimicked the inhibitory effect of vagal stimulation on cardiac contractions (no hyperpolarization was detected in the presence of muscarine) by an action on an atropine-sensitive process; he concluded that muscarine acted primarily on the cardiac muscle cells and not through nerves. The validity of these conclusions was strengthened in 1896 by the experiments of Pickering (253) who observed that application of electric currents inhibited the heartbeat of chick embryos only after it was assumed that nervous elements were present and he proposed that: ". . . these experiments apparently decide that the nervous elements are not only essential, but that they play the more important part in the phenomena of inhibition." (253, p. 203). Pickering also reported that the strength of an electric current needed to inhibit the heartbeat was reduced in the presence of muscarine and that atropine opposed the inhibition caused by electrical stimulation and by muscarine in the embryonic heart.

Experiments with intracellular microelectrodes confirmed and extended Gaskell's observations for the effects of vagal stimulation (133, 142) and muscarinic drugs (37, 336) on the sinoatrial node and the atrium. Burgen and Terroux (37) proposed in 1953 that muscarinic drugs hyperpolarized atrial cells by increasing K^+ permeability. This view was verified by measuring the effect of muscarinic drugs on the rate of ^{42}K efflux (111) and on the K^+-dependence of the reversal potential for muscarinic-induced hyperpolarization (309). The ability of ACh to increase a time-independent K^+ current (i_{K_1}) had generally been accepted as a mechanism for muscarinic inhibition of the sinoatrial pacemaker and atrium (review, ref. 28). (This conclusion has been disputed by the results of experiments on isolated rabbit sinoatrial node cells studied with the patch clamp technique. In this preparation, ACh appears to induce current through a specific class of K^+ channels rather than by increasing current through existing inwardly rectifying K^+ channels (280). This matter is also considered in section VI C 1.) An increase of i_{K_1} has also been invoked to explain the hyperpolarization of canine (79) and rabbit (225) cardiac Purkinje fibers although it cannot account for the effects of ACh on the maximum diastolic potential of sheep cardiac Purkinje fibers (40, 196). When ACh inhibits the slow inward current (i_{si}) in the ventricle of birds and mammals, there is no increase of i_{K_1} detected (128, 144, 156). [The only apparent exception to this view was a hyperpolarization of embryonic chick ventricular myocytes by carbachol (291). While the carbachol-induced hyperpolarization was occasionally accompanied by an increased membrane conductance (291, Fig. 7B), in most cells, there was no change of conductance (291, Fig. 7C). The authors did not rationalize these divergent results.] Therefore, occupation of muscarinic receptors by agonists would not be expected to produce a stereotyped electrophysiological response throughout the heart.

2. Voltage- and Time-Dependent Slow Inward Current (i_{si}). Muscarinic inhibition of the cardiac action potential is also dependent upon a reduction of i_{si} which is carried by Ca^{2+} and Na^+. This mechanism for muscarinic inhibition was first described in the amphibian atrium (90); the reduction of i_{si} occurred at significantly lower concentrations of ACh than those required to increase i_{K_1} (85, 90). In the mammalian atrium, ACh also reduced i_{si}, but at concentrations greater than those needed to increase i_{K_1} (303). Acetylcholine also reduced i_{si} and Ca^{2+}-dependent action potentials in the mammalian (128, 145, 156) and the avian ventricle (11, 144). As in mammalian ventricle, ACh inhibited Ca^{2+}-dependent action potentials in canine cardiac Purkinje fibers when i_{si} had been augmented either by β-adrenoceptor agonist or by phosphodiesterase inhibitor (6). Therefore, ACh can inhibit canine cardiac Purkinje fibers by acting on i_{si} and i_{K_1} as in atrial muscle. Muscarinic inhibition of the ventricular action potential appears to involve a reduction of i_{si} alone.

The inhibition of the SA node by ACh has been reported to result entirely from the activation of a K^+ current induced by muscarinic agonist; no change of i_{si} was detected (240). The observation that ACh acted on i_{K_1} and not on i_{si} is surprising and these experiments have been critically reviewed (28). If the result presented by Noma and Trautwein (240) is correct, one may conclude that the conductances involved in muscarinic inhibition vary greatly as a function of cardiac cell type from i_{K_1} alone (SA node), to i_{si} and i_{K_1} (atrium, canine Purkinje fibers) and to i_{si} alone (ventricle). [Recent experiments with voltage-clamped mammalian AV node indicated the participation of i_{K_1} and i_{si} with the former being dominant (130).] This apparent order of conductance changes produced by ACh in various cardiac cells is complicated by species differences. In sheep Purkinje fibers, ACh reduced i_{K_1} and i_{si} (41, 42) while in the dog, ACh increased i_{K_1} (79) and reduced i_{si} (6).

C. Relationships between Receptor Activation and Cardiac Function

1. Membrane Ionic Conductances. Because ACh modifies at least two ionic conductances, i_{si} and i_{K_1}, it is conceivable that the interaction between muscarinic receptor and these two channels is achieved by qualitatively different mechanisms. It has been speculated that subclasses of muscarinic receptors (section V B) may be coupled to i_{K_1} and i_{si} channels, respectively (review, ref. 114). Results consistent with this view have been presented by Nargeot and Garnier (232) who reported that tetraethylammonium (TEA) inhibited the effect of muscarinic agonists on i_{K_1} but not on i_{si} whereas atropine blocked agonist action on both conductances. Because TEA can block K^+ channels directly, this result is no proof of the existence of distinct subclasses of muscarinic receptors linked to ionic conductances. However, Pott (257) has reported that TEA, at concentrations lower than those needed to block K^+ channels directly, acted like a competitive muscarinic antagonist when applied iontophoretically to guinea pig atrial cells. If this result is correct, it should be possible to demonstrate a displacement of radioactively labeled muscarinic antagonists by TEA. [In this context, it is noteworthy that the density of muscarinic receptors (sites/μm^2), estimated from specific binding of ^{3}H-QNB, averaged 120 [frog interatrial septum (113)] and 110 [embryonic chick atrial and ventricular myocytes (291)]. By contrast, the density of K^+ channels acted upon by a maximally effective concentration of ACh is about $1/\mu m^2$ [rabbit SA node (239)]. The different densities of receptor sites and K^+ channels could be taken as an indication that fractions of the receptor population are coupled to different electrophysiological (i_{si} and i_{K_1} channels) and metabolic (adenylate cyclase, phosphatidylinositol) properties.]

The ability of ACh to increase i_{K_1} was viewed as an effect of mucarinic receptor activation on an existing background K^+ current (85). Although this conclusion seems correct for the action of ACh on atrial muscle cells (296), it may not apply in the cases of ACh action on sinoatrial and atrioventricular node cells. According to recent experiments in the sinoatrial node (review, ref. 310), ACh induces a separate class of K^+-specific membrane channels that are gated by the muscarinic receptor. This proposal has been strengthened by results obtained in patch clamp experiments with isolated heart cells from rabbit sinoatrial and atrioventricular nodes (280). In these nodal cells, ACh was required to elicit a K^+ current that displayed inward-going rectification (280). That SA node cells may not have i_{K_1} channels in the absence of ACh has also been reported by others (290). In rabbit cardiac Purkinje fibers, ACh, acting through muscarinic receptors, increases i_{K_1} by an action on the inwardly-rectifying K^+ current and not by opening a special class of ACh-activated K^+ channels (226). Therefore, atrial muscle cells and Purkinje fibers appear to have "ACh regulated K^+ channels" while SA and AV node cells seem to exhibit "ACh-dependent K^+ channels." The muscarinic receptors may be a component of the specific class of K^+ channels in the SA and AV nodes but not in the atrium or in Purkinje fibers. It has been proposed that the operation of ACh-dependent K^+ channels involves a series of reactions, similar to that triggered by the activation of nicotinic receptors, with appropriate slowing of the reaction rates to accommodate the kinetic features of the muscarinic response (280). Insofar as it is known, such a reaction would include a change in channel gating conformation by muscarinic agonist independent of metabolic intermediate(s). Although cyclic GMP has been suggested as a mediator of muscarinic receptor activation in the heart, this cyclic nucleotide is able neither to mimic the effect of ACh on ^{42}K efflux (75, 234) nor to modify the hyperpolarization of AV node cells by ACh (311). Nevertheless, one cannot exclude a reaction scheme that requires a metabolic intermediate linking muscarinic receptor and K^+ channel (95, 114, 127). Experiments in atrial cells showed that there is a marked delay between ACh application and membrane hyperpolarization; this delay is highly sensitive to temperature (95, 113). These results, along with elegant experiments done with photoisomerizable muscarinic antagonists (233), are consistent with the proposal that an enzymatic-like reaction involving metabolic intermediates is involved in linking muscarinic receptor to K^+ channel.

By contrast, it has been convincingly demonstrated that cyclic AMP is involved in the regulation of i_{si} by ACh in the adult and in the embryonic heart (reviews 221, 249, 251, 269). The inhibitory effect of muscarinic agonists on i_{si} includes not only a reduction of adenylate cyclase activity (221, 266, 331) but also an interference with the effects of accumulated cyclic AMP (116, 248, 250, 327). In the latter, muscarinic inhibition might be achieved by mechanisms discussed above (see section VI A).

That inhibition of i_{si} by muscarinic agonists is achieved by stimulation of guanylate cyclase leading to accumulation of cyclic GMP has not received consistent experimental verification (reviews, refs. 189, 221). However, the importance of measuring cyclic GMP-dependent protein kinase activity, in addition to cyclic GMP content, has been stressed by those who have obtained evidence consistent with the cyclic GMP hypothesis for muscarinic inhibition (188). Moreover, the ability of ACh to inhibit ^{45}Ca influx (234) and the Ca^{2+}-dependent action potential (176) in atrial muscle was mimicked by cylic GMP derivatives. In light of these difficulties, it is of interested to consider the possibility that a reciprocal (Yin Yang hypothesis) relationship may exist between the ratio of cyclic AMP/cyclic GMP on the one hand and cardiac contractile force on the other (96). The work of Flitney and colleagues (76, 77, 292) with the amphibian heart indicated that agents or procedures that increased the ratio of cyclic AMP/cyclic GMP produced positive inotropic effects while those that reduced this ratio had negative inotropic effects. However, the applicability of this hypothesis to the mammalian heart is uncertain. Although a reasonable correlation between cyclic AMP content and contractile force was evident with isoproterenol and forskolin, there was no evidence for an important role of the ratio of cyclic AMP/cyclic GMP in contractile function for these agents or for nitroprusside (272). Moreover, cyclic GMP derivatives did not mimic carbamylcholine insofar as they exerted a positive inotropic action of their own and did not oppose the positive inotropic effects of isoproterenol, norepinephrine, phenylephrine, or cyclic AMP in canine ventricle (66). At present, there seems to be insufficient evidence either to reject or to accept the cyclic GMP hypothesis for muscarinic agonist action on heart cells.

2. Metabolic Effects. It now appears that of the various biochemical effects of muscarinic receptor activation those on phospholipids and on cyclic nucleotides may play a central role in signal transmission.

The receptor molecules, membrane-bound enzymes, and channel proteins are intercalated in the *phospholipid* bilayer of the cell membrane. The lipid heads and tails of the phospholipids interact with membrane proteins. This interaction and those involving internal cellular structures gain a new dimension when we recognize that muscarinic receptor activation controls the metabolism of certain membrane phospholipids.

A quantitatively minor group of membrane phospholipids, namely the inositol phosphoglycerides, has attracted considerable attention (134, 135). About 30 years ago, Hokin and Hokin (135) detected the muscarinic stimulation of phosphatidylinositol breakdown. This so-called "PI effect" was studied in heart tissue (30, 34). Surprisingly, carbachol, but not oxotremorine, increased PI turnover in chick heart cells, whereas both drugs inhibited isoproterenol-stimulated cyclic AMP accumulation (30). The muscarinic receptor detected by carbachol displayed H and L affinity states whereas that detected by oxotremorine displayed only the H state. Conceivably, the H state of the muscarinic receptor is associated with adenylate cyclase and the L state with the PI breakdown (30). The possibility was also considered that the metabolic effects of the two agonists could result from actions on the same receptor state that is efficiently coupled to adenylate cyclase and inefficiently coupled to PI hydrolysis (30). The weak efficacy of oxotremorine in causing the PI effect as compared to the efficacy of carbachol was also found in rat cerebral cortical slices (27). Recently, indirect evidence in favor of a muscarinic stimulation of choline phosphoglycerides was found in cerebral cortex and the perfused heart (46, 47).

Changes in phospholipid metabolism may link receptor activation to the final cellular responses (1) by alterations in the membrane properties (e.g., surface charge, fluidity) (reviews, refs. 4, 129) and/or (2) by formation of a metabolite serving as cellular messenger. Phosphatidate (112, 281), arachidonate metabolites (section III B), myoinositol-D-1,4,5-trisphosphate (301; review, ref. 74), and diacylglycerol (review, ref. 238) are possible candidates for such a molecular link in the response to muscarinic agents. It is an important task to find out whether a phospholipid metabolite is released by muscarinic receptor activation and mimicks a certain effect of acetylcholine in the heart. Another approach would be to identify those enzymes involved in phospholipid turnover that are activated by muscarinic agonists and mimick the acetylcholine response. For example, muscarinic agonists activate the phosphodiesteratic cleavage of phosphoinositides ("PI effect") (review, ref. 74) and possibly the deacylation-reacylation cycle (47, 121).

There is much discussion about a link between muscarinic receptors and cyclic nucleotides in cardiac muscle (reviews, refs. 302, 327, 329). Muscarinic agonists increase cyclic GMP (96) and reduce cyclic AMP. However, the reduction of ventricular adenylate cyclase activity (227, 331) and thereby the accumulation of cyclic AMP (11, 29, 250) occur only when the adenylate cyclase is activated reversibly, e.g., by β-adrenoceptor agonists. The effect is not observed with agents (Gpp(NH)p and cholera toxin) that irreversibly activate adenylate cyclase (250, 331). A positive correlation between the accumulation of cyclic GMP and the negative inotropic effect of either ACh (89) or vagal stimulation (73) was reported in rat atria. By contrast, ACh raised cyclic GMP content but had no effect on cyclic AMP content or phosphorylase "a" activity in isolated rat ventricles (84). Nevertheless, ACh inhibited the accumulation of cyclic AMP and the activation of phosphorylase "a" caused by epinephrine, an effect attributed to muscarinic inhibition of β-adrenoceptor stimulation of adenylate cyclase. These results can explain the inhibition of glycogenolysis caused by ACh in mammalian hearts stimulated by β-

adrenoceptor agonists (125, 322). In guinea pig ventricles, ACh increased cyclic GMP content but had no effect on cyclic AMP content or contractility (328). Because, in the ventricle, a negative inotropic effect of ACh occurs in the presence of substances that raise cyclic AMP (section VI A), it was proposed that the effect of muscarinic agonists was due to cyclic GMP-dependent attenuation of the effects of accumulated cyclic AMP (328).

The above results reinforce the view, advanced with regard to the study of muscarinic receptor identification by receptor-specific ligands (see section V B), that it is essential to examine selected cardiac tissue to ascertain muscarinic agonist action because the mechanisms may differ not only between atria and ventricles (26) but also within a small area.

The importance of guanine nucleotides in regulating muscarinic receptor affinity for agonist has been addressed (section V B) and the molecular details of this phenomenon have begun to emerge particularly by analogy with the role of guanine nucleotides in the activation of adenylate cyclase by stimulatory hormones, transmitters, and drugs. For example, activation of adenylate cyclase requires a stimulatory guanine-nucleotide-binding protein (N_s; 271) to couple β-adrenoceptor to the catalytic unit of adenylate cyclase (271, 278). Enzyme activity (synthesis of cyclic AMP from ATP) is low when guanosine diphosphate (GDP) is bound to N_s. Addition of agonist initially forms a high affinity complex between β-adrenoceptor and agonist and also facilitates the exchange of guanosine triphosphate (GTP) for GDP on N_s. In the presence of GTP, N_s decreases β-adrenoceptor affinity for agonist and increases the catalytic activity of adenylate cyclase. Hydrolysis of GTP to GDP, which results from a GTPase inherent in N_s, reduces the catalytic activity of adenylate cyclase and the cycle can begin again by agonist occupation of β-adrenoceptor (271, 278).

Inhibition of cardiac adenylate cyclase by muscarinic agonists also requires guanine nucleotide (149, 331) and it had been proposed that the guanine-nucleotide-binding protein is a different molecule, termed N_i, for this action (reviewed, ref. 271). Treatment of cardiac membranes with N-ethylmaleimide ($\leq 3 \times 10^{-4}$ M) decreased the affinity of muscarinic receptors for agonist and prevented muscarinic inhibition of adenylate cyclase without modifying basal enzyme activity or its activation by β-adrenoceptor agonist (109). Moreover, guanine nucleotides lost their ability to reduce receptor affinity for agonist in the presence of N-ethylmaleimide. Although these results do not distinguish between separate coupling proteins (N_s vs N_i) or distinct sites on the same protein for stimulation and inhibition of adenylate cyclase, respectively, it seemed reasonable to conclude that either of these possibilities is most consistent with the model advanced by Rodbell (271).

A toxin isolated from *Bordetella pertussis* displays features that are consistent with the existence of N_i as a separate protein entity. Pertussis toxin treatment of rat heart cells in culture prevented the decrease of cyclic AMP content caused by ACh (122) while the accumulation of cyclic AMP caused by β-agonist was augmented. Pertussis toxin (sometimes called islet-activating protein because it potentiates insulin secretion in response to hormonal and nutritional stimuli) also prevented the inhibition of cardiac adenylate cyclase and contractility caused by adenosine (63, 122). Because this effect of adenosine, like that of ACh, has been attributed to an effect of inhibitory receptor agonist on N_i (271), Hazeki and Ui (122) suggested that the site of action of pertussis toxin (presumably N_i) was distinct from that of cholera toxin (N_s). This view has been borne out in an elegant series of experiments by Gilman and colleagues who reported on the purification of N_i, its subunit composition and role as a substrate for ADP-ribosylation catalyzed by pertussis toxin and its function as a mediator of inhibitory drug action on adenylate cyclase of several cell types (20, 162, 163, 164). In this context, it would be of interest to learn whether a guanine nucleotide-dependent protein is involved in the regulation if i_{K_1} by muscarinic agonist.

With biochemical methods, it has been difficult to demonstrate guanylate cyclase activity and its regulation by muscarinic agonists in cardiac membrane preparations (review, ref. 302). However, the localization of guanylate cyclase activity by histochemical procedures has been reported in the sarcolemma and sarcoplasmic reticulum of mammalian and avian hearts (287). Whether the guanylate cyclase distribution, as indicated histochemically, is a reflection of enzyme pools subject to muscarinic receptor regulation is problematic. The cytochemical procedure permitted activation of guanylate cyclase by sodium nitroprusside (287), but it has been emphasized that muscarinic agonists and sodium nitroprusside may act on different pools of guanylate cyclase and cyclic GMP-dependent protein kinase (188).

The importance of phosphorylation-dephosphorylation reactions as ultimate expressions of cyclic-nucleotide-mediated actions of autonomic transmitters has been stressed (reviews, refs. 166, 302, 327). The phosphorylation states of the cardiac proteins phospholamban, troponin-I, and protein C will be considered. For material concerning phosphorylase b kinase, the substrate for phosphorylase, the reader should consult the excellent review by Stull and Mayer (302).

Phospholamban, a 22,000 dalton protein of the sarcoplasmic reticulum membranes, regulated the Ca^{2+}-uptake and Ca^{2+}-dependent ATPase activity of such membranes by virtue of the action of cyclic AMP-dependent protein kinase (174; review, ref. 166). In this connection, Watanabe et al. (330) reported that perfusion of isolated hearts with isoproterenol increased the incorporation of ^{32}P into phospholamban in a time-dependent manner. The increased phosphorylation state of phospholamban was

associated with the positive inotropic and accelerated relaxation effects characteristic of β-adrenoceptor agonists and was blocked by propranolol. Of particular interest is their observation that ACh, by an action on muscarinic receptors, attenuated the positive inotropic effect and the phosphorylation of phospholamban caused by isoproterenol (330). Although the effect of ACh could be explained by a reduction of cyclic AMP synthesis, the ability of muscarinic agonists to activate a protein phosphatase has not been excluded (327).

Autonomic transmitters also regulate the phosphorylation of a 165,000 dalton protein (116), which is thought to be the C-protein of the thick (myosin) filaments found in heart muscle (243). The C-protein, which is a component of the thick filament yet antigenically distinct from myosin (243) is phosphorylated by β-adrenoceptor agonists in a concentration- and time-dependent manner (116). The increased phosphorylation of C-protein and positive inotropic effect are demonstrable within 15 s after addition of isoproterenol and these effects persist for as long as the β-adrenoceptor agonist is present (up to 20 min). Carbachol, acting on muscarinic receptors, reduces the phosphorylation of C-protein and the positive inotropic effect of isoproterenol. However, the relationship between the phosphorylation of C-protein and the negative inotropic effect of carbachol is complex. For example, the muscarinic agonist (10^{-5} M) blocked the positive inotropic effect of isoproterenol within 30 s yet did not reduce the phosphorylation of C-protein until 60 s. had elapsed. In addition, carbachol reduced the positive inotropic effect by more than half at a concentration (10^{-7} M) that had no effect on the phosphorylation of C-protein. The inhibition of C-protein phosphorylation caused by carbachol was not mimicked by dibutyryl cyclic GMP. Moreover, carbachol inhibited the phosphorylation of C-protein caused by 8-bromocyclic AMP indicating that the inhibitory effect depended upon interruption of a cyclic AMP-dependent protein kinase system (116). This conclusion has also been drawn by others (see section V C 2). Whereas there is much evidence to link phosphorylation of C-protein with the positive inotropic effect of β-adrenoceptor agonists (116, 153), the relationship between dephosphorylation of C-protein and the negative inotropic effect of muscarinic agonists is uncertain. Hartzell (115) has recently suggested an alternative hypothesis in which the decay time of twitch tension (rather than peak systolic force) is inversely related to the degree of phosphorylation of C-protein. Thus, isoproterenol increased C-protein phosphorylation and decreased the decay time of the cardiac muscle twitch. Carbachol had the opposite effect. In the presence of carbachol, there was a striking temporal relationship between the decreased incorporation of ^{32}P into C-protein and the increased decay time of the muscle twitch (115). A systematic test of this hypothesis, especially of the concentration-dependence for the effect of carbachol on decay time and ^{32}P incorporation, is needed. Only then can one begin to evaluate the quantitative and qualitative significance of this mechanism for neurotransmitter regulation of contraction in cardiac muscles from different vertebrates.

Although an important role for cyclic GMP in the dephosphorylation of C-protein is unlikely, it has been proposed that muscarinic agonists, acting by accumulation of cyclic GMP, dephosphorylate troponin-I in cardiac muscle and thereby increase the sensitivity of myofilaments to intracellular Ca^{2+} (137). Earlier experiments had indicated that β-adrenoceptor agonists acting on intact (68) or on hyperpermeable (224) cardiac muscle increased the phosphorylation of troponin-I. The increased phosphorylation of troponin-I caused by cyclic AMP-dependent protein kinase was associated with a reduced sensitivity of cardiac myofibrils to Ca^{2+} (224) and to stimulation of myofibrillar ATPase by Ca^{2+} (267). By contrast, cyclic GMP increased myofibrillar Ca^{2+} sensitivity in hyperpermeable cardiac fibers (214). The importance of the regulatory function of troponin-I in the negative inotropic action of muscarinic agonists is not known. It appears unlikely that increased Ca^{2+} sensitivity of the myofibrils can account for the negative inotropic effect of muscarinic agonists. However, it has been suggested that the degree of phosphorylation of troponin-I, which is significantly controlled by cyclic nucleotides, is an important mechanism in determining the tone and the rate of diastolic filling in the heart (214, 224). If this assertion is correct, it is essential to solve the discrepancies observed in the troponin-I hypothesis and in the effects of muscarinic agonists. First, troponin-I phosphorylation was not always associated with the positive inotropic effect of β-adrenoceptor agonists (68). Second, ACh reduced the positive inotropic effect of β-adrenoceptor agonists before dephosphorylation of troponin-I could be detected (68). Third, the increased myofibrillar Ca^{2+} sensitivity produced by methacholine persisted for up to 30 min after washout of the drug (137). Although these discrepancies do not necessarily disprove the troponin-I hypothesis for regulation of contractility, they indicate that the regulatory mechanisms for autonomic transmitter action on the contractile proteins are exceeding complex (review, ref. 166).

3. Chronotropic and Inotropic Effects. The negative chronotropic effect of ACh in the heart can be explained by the changes produced in i_{K_1} and/or i_{si} of the SA node (reviews, refs. 28, 91, 147, 310). An increase of i_{K_1} and a decrease of i_{si} by ACh seem to participate in the inhibition of automaticity in atrial muscle, AV node, and canine Purkinje fibers (see section VI B 1 and 2). Because ACh acts only to reduce i_{si} in ventricle, it would be expected that muscarinic inhibition of ventricular automaticity depends upon a less complex mechanism. This possibility has not been critically examined.

The negative inotropic effect of ACh in the atrium also

depends indirectly upon an increase of i_{K_1} and directly upon a decrease of i_{si} (see section VI B 1 and 2). Again, a negative inotropic effect of ACh in the ventricle arises from a diminution of i_{si} only. In this tissue, much evidence is available for a critical role of the adenylate cyclase-cyclic AMP system in muscarinic inhibition of ventricular electrical and mechanical activity (see section VI C 1). For example, ACh inhibits ventricular contractility when the force of contraction has been increased by substances that either stimulate adenylate cyclase activity (β-adrenoceptor agonists, histamine, glucagon) or inhibit phosphodiesterase activity (reviews, refs. 184, 248, 327).

It is essential to consider the experimental evidence for a positive inotropic action of muscarinic agonists in the heart. Our discussion will focus only on the catecholamine-independent positive inotropic effect of muscarinic drugs; the catecholamine-dependent positive inotropic effect of ACh is well-known (review, ref. 186). The positive inotropic effect of vagal stimulation or muscarinic agonists has been observed in ventricles of the dog (136), cat (17, 36), and chick (8, 12), and in atria of the rabbit (62). It is unlikely that endogenous catecholamines are mediators of this effect because the positive inotropic action of muscarinic agonists or vagal stimulation is not prevented by adrenergic neuron blockade (17, 36, 136) or by β-adrenoceptor antagonists (17, 62, 136). Moreover, the positive inotropic effect of ACh, which occurs at concentrations $\geq 10^{-5}$ M in all reported studies, was not accompanied by an increase of cyclic AMP content (12). Atropine prevented the positive inotropic effect of vagal stimulation or of ACh in all preparations except the experiments of Buccino et al. (36). This discrepancy is puzzling because atropine opposed the positive inotropic effect of vagal stimulation in the experiments reported by Blinks (17) in which cat heart muscle was also used. There are two mechanisms that may be advanced to explain the positive inotropic effect of vagal stimulation and ACh that is initiated by occupancy of muscarinic receptors. First, the "rebound" positive inotropic effect of vagal stimulation in the rabbit atrium was associated with an increased signal of the luminescent calcium-sensitive protein, aequorin (62). This result indicates that the cytoplasmic concentration of calcium during the positive inotropic effect of vagal stimulation is greater than that occurring during a cardiac muscle twitch under control conditions. [The light signal from calcium-aequorin interaction was less than control during the negative inotropic effect of vagal stimulation which preceded the rebound positive inotropic effect.] A second explanation, which does not exclude the first, is that ACh increases the calcium sensitivity of the myofilaments. This view is consistent with the results of experiments with hyperpermeable cardiac fibers exposed to ACh (137; see section VI C 2). The effect of ACh on calcium sensitivity of the myofilaments, which was also observed when cyclic GMP was present (214), was prevented by atropine. It seems reasonable to conclude that an increased concentration of cytoplasmic calcium (is this from sarcolemmal or sarcoplasmic reticulum stores?) and an increased calcium sensitivity of the myofilaments to calcium contribute to the positive inotropic effect of vagal stimulation and ACh.

It is of interest that α-adrenoceptor agonists also have a positive inotropic effect in the heart (review, ref. 286). The positive inotropic effect of α-adrenoceptor agonists, unlike that of β-adrenoceptor agonists, is not associated with an increase either of cellular cyclic AMP (35, 289) or of the rate of muscle relaxation (181). Additionally, Ca channel antagonists oppose the positive inotropic effect of α-adrenoceptor agonists (65, 181). The ability of α-adrenoceptor agonists to increase the magnitude of Ca^{2+}-dependent action potentials had been attributed to a reduction of a voltage- and time-dependent outward current rather than to an increase of i_{si} (107). However, in voltage clamp experiments, phenylephrine increased i_{si} and delayed the inactivation of this inward current without changing outward current (35). Muscarinic agonists did not interfere with the generation of Ca^{2+}-dependent action potentials caused by α-adrenoceptor agonists (146) and either had no effect (64) or increased slightly (107) the positive inotropic effects in contrast to those inotropic effects that are mediated by the adenylate cyclase-cyclic AMP system (section VI C 2).

VII. Conclusions: The Integrative Nature of the Parasympathetic Neuroeffector Transmission in the Heart

One of the major goals of this review article is to analyze the role of the parasympathetic neuroeffector junction in modulating the neurally mediated signal into a signal of the effector cell. Three aspects are summarized.

A. Integration of the Sympathetic Innervation

A characteristic feature of the sympathetic and parasympathetic modulation of cardiac function are the reciprocal activities of the two nervous systems. Besides the reciprocity of discharge frequencies due to central baro- and chemoreceptor reflex mechanisms (e.g., 44, 56; section III B), activation of the parasympathetic nervous system inhibits sympathetic effects at pre- and postjunctional levels. Axo-axonal muscarinic inhibition of noradrenaline release (section III B) and postsynaptic interaction due to muscarinic receptors being coupled to the β-adrenoceptor-activated adenylate cyclase (section VI) have been described in many studies. The summarizing conclusion is that cholinergic modulation of sympathetic adrenergic effects is intimately integrated into the parasympathetic neuroeffector transmission and that negative chronotropic and, even more so, negative inotropic effects of vagal activity depend on the background level

of the sympathetic activity, particularly in the mammalian heart.

B. Temporal Aspects of Parasympathetic Neuroeffector Transmission

Tonic stimulation of the vagus nerves causes abrupt slowing of the heart within a second or less, and discontinuation of the stimulation is followed by a return to the control level within a few seconds, which is rather long in comparison with the milliseconds (or less) required for the recovery of a twitch of the skeletal muscle and its end-plate potential. Some aspects of the time-course of neuronal activity and of cardiac responses to vagal stimulation have been discussed in sections III and IV. The reader is also referred to electrophysiological analysis of this topic (246, 257, 265).

The relatively fast onset (as compared to the slow termination) of the negative chronotropic response to vagal stimulation presumably is due to the fact that the activation of close neuroeffector junctions (<600 Å) which are frequently found in the sinoatrial and atrioventricular nodes (section II C) determines the rate of onset of the effect. The delay (30 to 100 ms) and early rising phase of the ACh-evoked hyperpolarization were explained by a reaction scheme consisting of at least three subsequent steps between binding of ACh to the receptor and the opening of the K^+ channel (117, 256). In this sequence, the kinetics of the muscarinic receptor-activated channel have to be considered as rate-limiting for the onset and partially for the time to peak of the vagal response (127, 245, 246, 256). This is particularly important for tissues with a high occurrence of close junctions, since in these junctions the changes of the ACh concentration are faster than the kinetics of the K^+ channel. The situation is reversed when the effector cells are more distant to the site of ACh release (see below). Finally, the fact that the ACh receptor of the heart belongs to the muscarinic and not to the nicotinic subtype is another important temporal aspect. Purves (264) pointed out that the kinetic differences in the responses mediated by these receptors have an important physiological meaning whereas the pharmacological distinction may lack any biological relevance.

In contrast to the complicated mechanism of the time to peak, the decay phase terminating the cardiac vagal response is exclusively determined by the ACh concentration, i.e., by the removal of the ACh caused by diffusion that is followed by extracellular hydrolysis or vascular washout. Those molecules that escape hydrolysis will be removed from the extracellular space by vascular washout at a half-time of 10 s. However, most of the ACh released was found to be hydrolyzed after 2.5 s (194). At a diffusion constant of $8 \times 10^{-6} cm^2/s$ for ACh, the bulk of the transmitter molecules diffuses over a distance of several tens of micrometers.

To elucidate the physiological importance of the described temporal aspects of the parasympathetic neuroeffector transmission, the rates of onset and termination of vagal cardiac effects have to be seen in relation to the length of the cardiac cycle (P-P interval) which is 0.3 to 1.5 s in humans. Although the onset of vagal chronotropic effects has been described above as "abrupt," vagal stimulation has no effect on the cycle in which it is delivered, when the stimulation falls within the last 180 ms of the free-running cycle (151, 187). Hyperpolarizing and phase-shifting effects are postponed to the following one or more cycles. The "phase response curves" obtained by plotting the P-stimulus interval versus the P-P interval of that cycle, in which the stimulus was delivered, are also influenced by the rate of junctional clearance of extracellular ACh (section IV). "Phase-response phenomena" are intimately linked to the temporal characteristics of the parasympathetic neuroeffector transmission. Physiological and clinical implications of these phenomena are given by the facts that reflex vagal discharges normally are grouped in discrete bursts within each cycle and that the time of delivery of these bursts is important for the synchronization of the pacemaker. The reader is referred to recent studies in this special field (151, 184).

In this context, some important advances have been made with computer-based models of pacemaker cell membrane. Action potentials of the SA node pacemaker have been reconstructed by computer simulation of experimentally derived ionic currents (24, 219). Bristow and Clark (24) adapted the Purkinje fiber model (213) in their experiments while Michaels and colleagues (219) used a model derived from experiments on the SA node (341). Both groups of investigators (25, 219) have attempted to simulate the effects of vagal stimulation or ACh pulses on SA node pacemaker activity.

In spite of oversimplification (neither potassium accumulation nor electrogenic pump activity is modelled) each model reproduces the time course of vagal or ACh-induced hyperpolarization and the "phase response curves" observed with ACh applied at different times in the diastolic interval. The model used by Michaels and colleagues mimicked the effect of vagal stimuli on P-P interval (deceleration followed by acceleration) while that of Bristow and Clark did not reveal the paradoxical acceleration observed experimentally. It is noteworthy that the "beating mode" model used by Michaels et al. (219) indicated that i_{si} could be greater during diastole and thereby increase the rate of diastolic depolarization. When vagal stimulation caused anomalous acceleration of the SA node pacemaker, the membrane depolarized (223). Neither the depolarization nor the anomalous acceleration was observed in the presence of verapamil and it was tentatively concluded that these phenomena resulted from an increased i_{si} caused by neurally released ACh (223). Neither model showed a significant secondary period of inhibition which has been attributed to extracellular accumulation of potassium (299). [In guinea pig

atria, extracellular accumulation of potassium contributed to the "fade" of ACh-induced hyperpolarization and to the "rebound" of membrane potential when ACh was removed (9, 95). By contrast, the wide extracellular clefts of rabbit Purkinje fibers makes potassium accumulation unlikely even though ACh-induced hyperpolarization displays "fade" and the membrane potential "rebounds" upon removal of ACh (225).] Finally, the entrainment of the SA node pacemaker by vagal stimuli, which has been implicated as a mechanism for vagally induced arrhythmias (150, 220), was demonstrable in the SA node model of Michaels and colleagues (219).

C. Spatial Aspects of Parasympathetic Neuroeffector Transmission

As discussed above, ACh released from parasympathetic terminal fibers can readily diffuse over distances of many tens of micrometers and reaches effector cells of a large area. Theoretically, the diameter of an area "innervated" by a point source of ACh (a single varicosity) may be 100 μm or more, which is about 5 times the average distance between two capillaries (321). This explains why the ultimate fate of released ACh diffusing away from the site of release is not necessarily hydrolysis within the extracellular space but escape as unhydrolyzed ACh into the circulation. Iontophoretic application of ACh for periods of less than 100 ms has been used to analyze temporal aspects of the parasympathetic neuroeffector transmission (see above). However, the spatial aspects are complicated not only by the tonic activity of vagal motor neurons (section III B) but by the multiplicity of point sources of ACh. An area with, for example, a diameter of 100 μm which theoretically could be innervated by a single varicosity (see above) may be controlled by about 30 to 100 cholinergic varicosities depending on the regional density of cardiac innervation (section II C). In other words, the parasympathetic neuroeffector junction of the heart shows a considerable overlap. In this context, the postjunctional effect of neurally released ACh is considered in a simplified manner, that is, heterogeneity in cellular reactivity to ACh is neglected. The importance of heterogeneity among SA node cells to ACh (as well as to epinephrine, calcium, and temperature changes) has been emphasized (207, 208). Shifts of pacemaker activity between superior and inferior portions of the SA node have been implicated as determinants of neurotransmitter action and interaction in vivo (208). A comprehensive discussion of the variations in tissue response to vagal stimulation and to ACh has been given by Levy and Martin (186).

Focal release of ACh into close neuroeffector junctions (<600 Å), which are found most frequently in the sinoatrial and atrioventricular nodes, presumably plays an essential role in the onset of vagally induced effects especially on cardiac automaticity and in the synchronization of the pacemaker to repetitive efferent vagal discharges (see above). Otherwise sustained inotropic and chronotropic vagal effects are determined by the overall concentration of ACh in the extracellular space. In other words, these aspects of vagal effects are imitated by "bath application" of ACh rather than by "focal (e.g. iontophoretic) application" (113). The nonfocal transmission is supported by other features of the vagal cardiac innervation (section II C) such as release of ACh "en passage" from consecutive varicosities of an activated fiber, the random distribution of varicosities in relation to the effector cells, the homogeneous distribution of muscarinic receptors on the myocytial surface of a certain cell (lack of membrane specializations), and finally the low density of receptors and channels even in membranes of sinus cells (239, 245). It seems that the temporal and spatial aspects of the parasympathetic neuroeffector transmission characterize the modulatory role of the vagus in controlling cardiac automaticity and force of contraction.

VIII. Summary

This review has directed attention to the anatomical, biochemical, physiological, and pharmacological features of cardiac parasympathetic neuroeffector transmission. The relationships among these properties have permitted a synthesis of the operation of the parasympathetic nervous system at the subcellular, cellular, tissue, and organ level in the heart. However, the attempt to obtain a comprehensive model of parasympathetic neuroeffector transmission in the heart has not only unmasked many features of junctional activity that are poorly understood, but also indicated significant gaps between in vitro and in vivo experimental situations.

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REFERENCES

1. ANTONI, H., UND ROTMANN, M.: Zum Mechanismus der negative inotropen Acetylcholin-Wirkung auf das isolierte Froschmyokard. Pflügers Arch. **300:** 67–86, 1968.
2. ALTER, W. A., III, HAWKINS, R. N., PARKHURST, L. J., POULIN, S., PADGETT, D., AND FINK, D.: Barbiturate depression of neurally mediated reflexes to coronary artery occlusion. Proc. Soc. Exp. Biol. Med. **160:** 281–286, 1979.
3. AVISSAR, S., AMITAI, G., AND SOKOLOVSKY, M.: Oligomeric structure of muscarinic receptors is shown by photoaffinity labeling: Subunit assembly may explain high- and low-affinity agonist states. Proc. Natl. Acad. Sci. U.S.A. **80:** 156–159, 1983.
4. AXELROD, J., AND HIRATA, F.: Phospholipid methylation and the receptor-induced release of histamine from cells. Trends Pharmacol. Sci. **3:** 156–158, 1982.
5. BAILEY, J. C., GREENSPAN, K., ELIZARI, M. V., ANDERSON, G. J., AND FISCH, C.: Effects of acetylcholine on automaticity and conduction in the proximal portion of the His-Purkinje specialized conduction system of the dog. Circ. Res. **30:** 210–216, 1972.
6. BAILEY, J. C., WATANABE, A. M., BESCH, H. R., JR., AND LATHROP, D. R.: Acetylcholine antagonism of the electrophysiological effects of isoproterenol on canine cardiac Purkinje fibers. Circ. Res. **44:** 378–383, 1979.
7. BAKER, P. F.: Transport and metabolism of calcium ions in nerve. *In* Calcium Movement in Excitable Cells, ed. by P. F. Baker and H. Reuter, pp. 9–53, Pergamon Press, Oxford, New York, 1975.
8. BAUMANN, F., GIRARDIER, L., AND POSTERNAK, J. M.: Effets inotropes de l'acétylcholine sur le myocarde. Helv. Physiol. Acta **18:** 509–522, 1960.
9. BAUMGARTEN, C. M., SINGER, D. H., AND FOZZARD, H. A.: Intra- and extracellular potassium activities, acetylcholine and resting potential in guinea pig atria. Circ. Res. **54:** 65–73, 1984.
10. BECHEM, M., GLITSCH, H. G., AND POTT, L.: Facilitation of acetylcholine

release from cardiac parasympathetic nerve endings. Effect of stimulation pattern and Mn ions. Pflügers Arch. **391:** 105–111, 1981.
11. BIEGON, R. L., AND PAPPANO, A. J.: Dual mechanism for inhibition of calcium-dependent action potentials by acetylcholine in avian ventricular muscle: Relationship to cyclic AMP. Circ. Res. **46:** 353–362, 1980.
12. BIEGON, R. L., EPSTEIN, P. M., AND PAPPANO, A. J.: Muscarinic antagonism of the effects of a phosphodiesterase inhibitor (methylisobutylxanthine) in embryonic chick ventricle. J. Pharmacol. Exp. Ther. **215:** 348–356, 1980.
13. BIRDSALL, N. J. M., BURGEN, A. S. V., AND HULME, E. C.: The binding of agonists to brain muscarinic receptors. Mol. Pharmacol. **14:** 723–736, 1978.
14. BIRDSALL, N. J. M., BURGEN, A. S. V., AND HULME, E. C.: A study of the muscarinic receptor by gel electrophoresis. Br. J. Pharmacol. **66:** 337–342, 1979.
15. BIRDSALL, N. J. M., AND HULME, E. C.: Muscarinic receptor subclasses. Trends Pharm. Sci. **4:** 459–462, 1983.
16. BLIGH, J.: The level of free choline in plasma. J. Physiol. (Lond.) **117:** 234–240, 1952.
17. BLINKS, J.: Field stimulation as a means of effecting the graded release of autonomic transmitters in isolated heart muscle. J. Pharmacol. Exp. Ther. **151:** 221–235, 1966.
18. BOGUSCH, G.: The innervation of Purkinje fibres in the atrium of the avian heart. Cell Tissue Res. **150:** 57–66, 1974.
19. BOJSEN-MØLLER, F., AND TRANUM-JENSEN, J.: Rabbit heart nodal tissue, sinoatrial ring bundle and atrioventricular connexions identified as a neuromuscular system. J. Anat. **112:** 367–382, 1972.
20. BOKOCH, G. M., KATADA, T., NORTHUP, J. K., UI, M., AND GILMAN, A. G.: Purification and properties of the inhibitory guanine nucleotide-binding regulatory component of adenylate cyclase. J. Biol. Chem. **259:** 3560–3567, 1984.
21. BOLTON, T. B.: Intramural nerves in the ventricular myocardium of the domestic fowl and other animals. Br. J. Pharmacol. Chemother. **31:** 253–268, 1967.
22. BOLTON, T. B.: Nervous system. *In* Avian Physiology, ed. by P. D. Sturkie, pp. 1–28, Springer, New York, Heidelberg, Berlin, 1976.
23. BRISCOE, S. M., AND BURN, J. H.: The formation of an acetylcholine-like substance by the isolated rabbit heart. J. Physiol. (Lond.) **126:** 181–190, 1954.
24. BRISTOW, D. G., AND CLARK, J. W.: A mathematical model of primary pacemaking cell in SA node of the heart. Am. J. Physiol. **243** (Heart Circ. Physiol. **12**): H207–H218, 1982.
25. BRISTOW, D. G., AND CLARK, J. W.: A mathematical model of the vagally driven primary pacemaker. Am. J. Physiol. **244** (Heart Circ. Physiol. **13**): H150–H161, 1983.
26. BROWN, B. S., POLSON, J. B., KRZANOWSKI, J. J., AND WIGGINS, J. R.: Influence of isoproterenol and methylisobutylxanthine on the contractile and cyclic nucleotide effects of methacholine in isolated rat atria. J. Pharmacol. Exp. Ther. **212:** 325–332, 1980.
27. BROWN, E., KENDALL, D. A., AND NAHORSKI, S. R.: Inositol phospholipid hydrolysis in rat cerebral cortical slices. I. Receptor characterization. J. Neurochem. **42:** 1379–1387, 1984.
28. BROWN, H. F.: Electrophysiology of the sinoatrial node. Physiol. Rev. **62:** 505–530, 1982.
29. BROWN, J. H.: Cholinergic inhibition of catecholamine-stimulable cyclic AMP accumulation in murine atria. J. Cyclic Nucleotide Res. **5:** 423–433, 1980.
30. BROWN, J. H., AND BROWN, S. L.: Agonists differentiate muscarinic receptors that inhibit cyclic AMP formation from those that stimulate phosphoinositide metabolism. J. Biol. Chem. **259:** 3777–3781, 1984.
31. BROWN, O. M.: Cat heart acetylcholine: Structural proof and distribution. Am. J. Physiol. **231:** 781–785, 1976.
32. BROWN, O. M.: Cat heart acetylcholine: Unilateral vagotomy studies with pyrolysis-mass fragmentography. Life Sci. **28:** 819–825, 1981.
33. BROWN, O. M., AND SALATA, J. J.: *In vivo* acetylcholine turnover in rat heart. Life Sci. **33:** 213–224, 1983.
34. BROWN, S. L., AND BROWN, J. H.: Muscarinic stimulaton of phosphatidylinositol metabolism in atria. Mol. Pharmacol. **24:** 351–356, 1983.
35. BRÜCKNER, R., AND SCHOLZ, H.: Effects of α-adrenoceptor stimulation with phenylephrine in the presence of propranolol on force of contraction, slow inward current and cyclic AMP content in the bovine heart. Br. J. Pharmacol. **82:** 223–232, 1984.
36. BUCCINO, R. A., SONNENBLICK, E. H., COOPER, T., AND BRAUNWALD, E.: Direct positive inotropic effect of acetylcholine on myocardium. Circ. Res. **19:** 1097–1108, 1966.
37. BURGEN, A. S. V., AND TERROUX, K. G.: On the negative inotropic effect in the cat's auricle. J. Physiol. (Lond.) **120:** 449–464, 1953.
38. BURN, J. H.: Relation of motor and inhibitor effects of local hormones. Physiol. Rev. **30:** 177–193, 1950.
39. CABOT, J. B., AND COHEN, D. H.: Neural control of the avian heart. *In* Hearts and Heart-like Organs, vol. 1, ed. by G. H. Bourne, pp. 199–258, Academic Press, New York, 1980.
40. CARMELIET, E., AND RAMON, J.: Electrophysiological effects of acetylcholine in sheep cardiac Purkinje fibers. Pflügers Arch. **387:** 197–205, 1980.
41. CARMELIET, E., AND RAMON, J.: Effect of acetylcholine on time-independent currents in sheep cardiac Purkinje fibers. Pflügers Arch. **387:** 207–216, 1980.
42. CARMELIET, E., AND RAMON, J.: Effect of acetylcholine on time-dependent currents in sheep cardiac Purkinje fibers. Pflügers Arch. **387:** 217–223, 1980.
43. CECCARELLI, B., AND HURLBUT, W. P.: Vesicle hypothesis of the release of quanta of acetylcholine. Physiol. Rev. **60:** 396–441, 1980.
44. COLERIDGE, J. C. G., AND COLERIDGE, H. M.: Chemoreflex regulation of the heart. *In* Handbook of Physiology. Section 2: The Cardiovascular System, vol. 1, ed. by R. M. Berne, pp. 653–676, American Physiological Society, Bethesda, MD, 1979.
45. CORABOEUF, E., LEDOUARIN, G., AND OBRECHT-COUTRIS, G.: Release of acetylcholine by chick embryo heart before innervation. J. Physiol. (Lond.) **206:** 383–395, 1970.
46. CORRADETTI, R., LINDMAR, R., AND LÖFFELHOLZ, K.: Physostigmine facilitates choline efflux from isolated heart and cortex *in vivo*. Eur. J. Pharmacol. **85:** 123–124, 1982.
47. CORRADETTI, R., LINDMAR, R., AND LÖFFELHOLZ, K.: Mobilization of cellular choline by stimulation of muscarine receptors in isolated chicken heart and rat cortex *in vivo*. J. Pharmacol. Exp. Ther. **226:** 826–832, 1983.
48. COURTNEY, K., AND ROPER, S.: Sprouting of synapses after partial denervation of frog cardiac ganglion. Nature (Lond.) **259:** 317–319, 1976.
49. CROWE, R., AND BURNSTOCK, G.: Fluorescent histochemical localization of quinacrine-positive neurons in the guinea-pig and rabbit atrium. Cardiovasc. Res. **16:** 384–390, 1982.
50. DEGEEST, H., LEVY, M. N., ZIESKE, H., AND LIPMAN, R. I.: Depression of ventricular contractility by stimulation of the vagus nerves. Circ. Res. **17:** 222–235, 1965.
51. DELHAYE, M., DESMET, J. M., TATON, G., DENEEF, P., CAMUS, J. C., FONTAINE, J., WAELBROECK, M., ROBBERECHT, P., AND CHRISTOPHE, J.: A comparison between muscarinic receptor occupancy, adenylate cyclase inhibition, and inotropic response in human heart. Naunyn-Schmiedeberg's Arch. Pharmacol. **325:** 170–175, 1984.
52. DENNIS, M. J., HARRIS, A. J., AND KUFFLER, S. W.: Synaptic transmission and its duplication by focally applied acetylcholine in parasympathetic neurons in the heart of the frog. Proc. R. Soc. Lond. Biol. Sci. **177:** 509–539, 1971.
53. DEVITO, J. L., CLAUSING, K. W., AND SMITH, O. A.: Uptake and transport of horseradish peroxidase by cut end of the vagus nerve. Brain Res. **82:** 269–271, 1974.
54. DIETERICH, H. A., KAFFEI, H., KILBINGER, H., AND LÖFFELHOLZ, K.: The effects of physostigmine on cholinesterase activity, storage and release of acetylcholine in the isolated chicken heart. J. Pharmacol. Exp. Ther. **199:** 236–246, 1976.
55. DIETERICH, H. A., LINDMAR, R., AND LÖFFELHOLZ, K.: The role of choline in the release of acetylcholine in isolated hearts. Naunyn-Schmiedeberg's Arch. Pharmacol. **301:** 207–215, 1978.
56. DOWNING, S. E.: Baroreceptor regulation of the heart. *In* Handbook of Physiology. Section 2: The Cardiovascular System, vol. I, ed. by R. M. Berne, pp. 621–652, American Physiological Society, Bethesda, MD, 1979.
57. ECKBERG, D. L.: Parasympathetic cardiovascular control in human disease: A critical review of methods and results. Am. J. Physiol. **239:** H581–H593, 1980.
58. ECKBERG, D. L., FLETCHER, G. F., AND BRAUNWALD, E.: Mechanism of prolongation of the R-R interval with electrical stimulation of the carotid sinus nerves in man. Circ. Res. **30:** 131–138, 1972.
59. EHINGER, B., FALCK, B., AND SPORRONG, B.: Possible axo-axonal synapses between peripheral adrenergic and cholinergic nerve terminals. Z. Zellforsch. **107:** 508–521, 1970.
60. EHLERT, F. J., ROESKE, W. R., AND YAMAMURA, H. I.: Muscarinic receptor: Regulation by guanine nucleotides, ions, and N-ethylmaleimide. Fed. Proc. **40:** 153–159, 1981.
61. EKSTRÖM, J.: Fall in choline acetyltransferase activity in the ventricles of the rat heart after treatment with a ganglion blocking drug. Acta Physiol. Scand. **102:** 116–119, 1978.
62. ENDOH, M., AND BLINKS, J. R.: Effects of endogenous neurotransmitters on calcium transients in mammalian atrial muscle. *In* Neuronal and Extraneuronal Events in Autonomic Pharmacology, ed. by W. W. Fleming, K.-H. Graefe; S. Z. Langer; and N. Weiner, pp. 221–230, Raven Press, New York, 1984.
63. ENDOH, M., MARUYAMA, M., AND TAIRA, N.: Modification by islet-activating protein of direct and indirect actions of adenosine on rat atrial contraction in relation to cyclic nucleotide metabolism. J. Cardiovasc. Pharmacol. **5:** 131–142, 1983.
64. ENDOH, M., AND MOTOMURA, S.: Differentiation by cholinergic stimulation of positive inotropic actions mediated via α- and β-adrenoceptors in the rabbit heart. Life Sci. **25:** 759–768, 1979.
65. ENDOH, M., AND SCHÜMANN, H. J.: Frequency-dependence of the positive inotropic effect of methoxamine and naphazoline mediated by alpha adrenoceptors in the isolated rabbit papillary muscle. Naunyn-Schmiedeberg's Arch. Pharmacol. **287:** 377–389, 1975.
66. ENDOH, M., AND SHIMIZU, T.: Failure of dibutyryl and 8-bromo-cyclic GMP to mimic the antagonistic action of carbachol on the positive inotropic effects of sympathomimetic amines in the canine isolated ventricular

myocardium. Jpn J. Pharmacol. **29:** 423–433, 1979.
67. ENGEL, U., AND LÖFFELHOLZ, K.: Presence of muscarinic inhibitory and absence of nicotinic excitatory receptors at the terminal sympathetic nerves of chicken hearts. Naunyn-Schmiedeberg's Arch. Pharmacol. **295:** 225–230, 1976.
68. ENGLAND, P. J.: Studies on the phosphorylation of the inhibitory subunit of troponin during modification of contraction in perfused rat heart. Biochem. J. **160:** 295–304, 1976.
69. ERNSTING, J., AND PARRY, D. J.: Some observations on the effects of stimulating stretch receptors in the carotid artery of man. J. Physiol. (Lond.) **137:** 45P–46P, 1957.
70. EULER, D. E.: Release of autonomic neuromediators by local ventricular electrical stimulation. Am. J. Physiol. **238:** H794–H800, 1980.
71. FAWCET, D. W., AND SELBY, C. C.: Observations on the fine structure of the turtle atrium. J. Biophys. Biochem. Cytol. **4:** 63–71, 1958.
72. FIELDS, J. Z., ROESKE, W. R., MORKIN, E., AND YAMAMURA, H. I.: Cardiac muscarinic cholinergic receptors: Biochemical identification and characterization. J. Biol. Chem. **253:** 3251–3258, 1978.
73. FINK, G. D., PADDOCK, R. J., RODGERS, G. M., BUSUTTIL, R. W., AND GEORGE, W. J.: Elevated cyclic GMP levels in rabbit atria following vagal stimulation and acetylcholine treatment. Proc. Soc. Exp. Biol. Med. **153:** 78–82, 1976.
74. FISHER, S, K., VAN ROOIJEN, L. A. A., AND AGRANOFF, B. W.: Renewed interest in the polyphosphoinositides. Trends Biochem. Sci. **9:** 53–56, 1984.
75. FLEMING, B. P., GILES, W., AND LEDERER, J.: Are acetylcholine-induced increases in ^{42}K efflux mediated by intracellular cyclic GMP in turtle cardiac pace-maker tissue. J. Physiol. (Lond.) **314:** 47–64, 1981.
76. FLITNEY, F. W., AND SINGH, J.: Inotropic responses of the frog ventricle to adenosine triphosphate and related changes in endogenous cyclic nucleotides. J. Physiol. (Lond.) **304:** 21–42, 1980.
77. FLITNEY, F. W., LAMB, J. F., AND SINGH, J.: Endogenous 3′,5′-cyclic nucleotides, calcium and the regulation of myocardial contractility: A hypothesis. J. Physiol. (Lond.) **292:** 70P–71P, 1979.
78. FREDHOLM, B. B., AND HEDQUIST, P.: Modulation of neurotransmission by purine nucleotides and nucleosides. Biochem. Pharmacol. **29:** 1635–1644, 1980.
79. GADSBY, D. C., WIT, A. L., AND CRANEFIELD, P. F.: The effect of acetylcholine on the electrical activity of canine cardiac Purkinje fibers. Circ. Res. **43:** 29–35, 1978.
80. GALPER, J. B., AND SMITH, T. W.: Agonist and guanine nucleotide modulation of muscarinic cholinergic receptors in cultured heart cells. J. Biol. Chem. **255:** 9571–9579, 1980.
81. GALPER, J. B., KLEIN, W., AND CATTERALL, W. A.: Muscarinic acetylcholine receptors in developing chick heart. J. Biol. Chem. **252:** 8692–8699, 1977.
82. GALPER, J. B., DZIEKAN, L. C., O'HARA, D. S., AND SMITH, T. W.: The biphasic response of muscarinic cholinergic receptors in cultured heart cells to agonists. J. Biol. Chem. **257:** 10344–10356, 1982.
83. GALPER, J. B., DZIEKAN, L. C., MIURA, D. S., AND SMITH, T. W.: Agonist-induced changes in the modulation of K^+ permeability and beating rate by muscarinic agonists in cultured heart cells. J. Gen. Physiol. **80:** 231–256, 1982.
84. GARDNER, R. M., AND ALLEN, D. O.: The relationship between cyclic nucleotide levels and glycogen phosphorylase activity in isolated rat hearts perfused with epinephrine and acetylcholine. J. Pharmacol. Exp. Ther. **202:** 346–353, 1977.
85. GARNIER, D., NARGEOT, J., OJEDA, C., AND ROUGIER, O.: The action of acetylcholine on background conductance in frog atrial trabeculae. J. Physiol. (Lond.) **274:** 381–396, 1978.
86. GASKELL, W. H.: The electrical changes in the quiescent cardiac muscle which accompany stimulation of the vagus nerve. J. Physiol. (Lond.) **7:** 451–452, 1886.
87. GASKELL, W. H.: On the action of muscarin upon the heart, and on the electrical changes in the non-beating cardiac muscle brought about by stimulation of the inhibitory and augmentor nerves. J. Physiol. (Lond.) **8:** 404–414, 1887.
88. GEIS, G. S., KOZELKA, J. W., AND WURSTER, R. D.: Organization and reflex control of vagal cardiomotor neurons. J. Auton. Nerv. Syst. **3:** 437–450, 1981.
89. GEORGE, W. J., WILKERSON, R. D., AND KADOWITZ, P. J.: Influence of acetylcholine on contractile force and cyclic nucleotide levels in the isolated perfused rat heart. J. Pharmacol. Exp. Ther. **184:** 228–235, 1973.
90. GILES, W., AND NOBLE, S. J.: Changes in membrane currents in bullfrog atrium produced by acetylcholine. J. Physiol. (Lond.) **261:** 103–123, 1976.
91. GILES, W., AND SHIBATA, E.: Autonomic transmitter actions on cardiac pacemaker tissue: A brief review. Fed. Proc. **40:** 2618–2624, 1981.
92. GILLIS, R. A., AND QUEST, J. A.: The role of the nervous system in the cardiovascular effects of digitalis. Pharmacol. Rev. **31:** 19–97, 1980.
93. GIRARDIER, L., BAUMANN, F., AND POSTERNAK, J. M.: Recherches sur les cholinestérases cardiaques. Helv. Physiol. Acta **18:** 467–481, 1960.
94. GLITSCH, H. G., AND POTT, L.: Effect of divalent cations on acetylcholine release from cardiac parasympathetic nerve endings. Pflügers Arch. **377:** 57–63, 1978.
95. GLITSCH, H. G., AND POTT, L.: Effects of acetylcholine and parasympathetic nerve stimulation on membrane potential in quiescent guinea-pig atria. J. Physiol. (Lond.) **279:** 655–668, 1978.
96. GOLDBERG, N. D., HADDOX, M. K., NICOL, S. E., GLASS, D. B., SANFORD, C. H., KUEHL, F. A. JR., AND ESTENSEN, R.: Biologic regulation through opposing influences of cyclic GMP and cyclic AMP: The Yin Yang hypothesis. Adv. Cyclic Nucleotide Res. **5:** 307–330, 1975.
97. GRAEFE, K.-H., ZEITNER, C.-J., FUCHS, G., AND KELLER, B.: Role played by sodium in the membrane transport of ^3H-noradrenaline across the axonal membrane of noradrenergic neurons. *In* Neuronal and Extraneuronal Events in Autonomic Pharmacology, ed. by W. W. Fleming, K.-H. Graefe, S. Z. Langer, and N. Weiner, pp. 51–62, Raven Press, New York, 1984.
98. GREEN, A. L.: The kinetics of enzyme action and inhibition in intact tissues and tissue slices, with special reference to cholinesterase. J. Pharm. Pharmacol. **28:** 265–274, 1976.
99. GREEN, A. L., LORD, J. A. H., AND MARSHALL, I. G.: The relationship between cholinesterase inhibition in the chick biventer cervicis muscle and its sensitivity to exogenous acetylcholine. J. Pharm. Pharmacol. **30:** 426–431, 1978.
100. GREEN, J. P., ALKON, D. L., SCHMIDT, D. E., AND SZILAGYI, P. I. A.: Confirmation of acetylcholine in perfusates of the stimulated vagus nerve by pyrolysis gas chromatography. Life Sci. **9:** 741–745, 1970.
101. GRILLO, M.A.: Electron microscopy of sympathetic tissues. Pharmacol. Rev. **18:** 387–399, 1966.
102. GUSTAFSSON, L. E.: Studies on modulation of transmitter release and effector responsiveness in autonomic cholinergic neurotransmission. Acta Physiol. Scand. Suppl. **489:** 1–28, 1980.
103. GUSTAFSSON, L. E.: Influence of adenosine on responses to vagal nerve stimulation in the anesthetized rabbit. Acta Physiol. Scand. **111:** 263–268, 1981.
104. HALINEN, M. O., HAKUMÄKI, M. O. K., AND SARAJAS, H. S. S.: Suppression of autonomic postganglionic discharges by pentobarbital in dogs, with or without endotoxemia. Acta Physiol. Scand. **104:** 167–174, 1978.
105. HALVORSEN, S. W., AND NATHANSON, M. M.: *In vivo* regulation of muscarinic acetylcholine receptor number and function in embryonic chick heart. J. Biol. Chem. **256:** 7941–7948, 1981.
106. HAMMER, R., BERRIE, C. P., BIRDSALL, N. J. M., BURGEN, A. S. V., AND HULME, E. C.: Pirenzepine distinguishes between different subclasses of muscarinic receptors. Nature (Lond.) **283:** 90–92, 1980.
107. HANDA, Y., WAGNER, J., INUI, J., AVERESCH, H., AND SCHÜMANN, H. J.: Effect of α- and β-sympathomimetic agonists on calcium-dependent slow action potential and force of contraction in the rabbit papillary muscle. Naunyn-Schmiedeberg's Arch. Pharmacol. **318:** 330–335, 1982.
108. HARDEN, T. K., MEEKER, R. B., AND MARTIN, M. W.: Interaction of a radiolabeled agonist with cardiac muscarinic cholinergic receptors. J. Pharmacol. Exp. Ther. **227:** 570–577, 1983.
109. HARDEN, T. K., SCHEER, A. G., AND SMITH, M. M.: Differential modification of the interaction of cardiac muscarinic cholinergic and beta-adrenergic receptors with a guanine nucleotide binding component(s). Mol. Pharmacol. **21:** 570–580, 1982.
110. HARIMAN, R. J., AND HOFFMAN, B. F.: Effects of ouabain and vagal stimulation on sinus nodal function in conscious dogs. Circ. Res. **51:** 760–768, 1982.
111. HARRIS, E. J., AND HUTTER, O. F.: The action of acetylcholine on the movements of potassium ions in the sinus venosus of the heart. J. Physiol. (Lond.) **133:** 58P–59P, 1956.
112. HARRIS, R. A., SCHMIDT, J., HITZEMANN, B. A., AND HITZEMANN, R. J.: Phosphatidate as a molecular link between depolarization and neurotransmitter release in the brain. Science **212:** 1290–1291, 1981.
113. HARTZELL, H. C.: Distribution of muscarinic acetylcholine receptors and presynaptic nerve terminals in amphibian heart. J. Cell Biol. **86:** 6–20, 1980.
114. HARTZELL, H. C.: Physiological consequences of muscarinic receptor activation. Trends Pharmacol. Sci. **3:** 213–214, 1982.
115. HARTZELL, H. C.: Phosphorylation of C-protein in intact amphibian cardiac muscle. J. Gen. Physiol. **83:** 563–588, 1984.
116. HARTZELL, H. C., AND TITUS, L.: Effects of cholinergic and adrenergic agonists on phosphorylation of a 165,000 dalton myofibrillar protein in intact cardiac muscle. J. Biol. Chem. **257:** 2111–2120, 1982.
117. HARTZELL, H. C., KUFFLER, S. W., STICKGOLD, R., AND YOSHIKAMI, D.: Synaptic excitation and inhibition resulting from direct action of acetylcholine on two types of chemoreceptors on individual amphibian parasympathetic neurones. J. Physiol. (Lond.) **271:** 817–846, 1977.
118. HAUBRICH, D. R., GERBER, N. H., AND PFLUEGER, A. B.: Deanol affects choline metabolism in peripheral tissues of mice. J. Neurochem. **37:** 476–482, 1981.
119. HAUBRICH, D. R., WANG, P. F. L., AND WEDEKING, P. W.: Distribution and metabolism of intravenously administered choline [methyl-^3H] and synthesis *in vivo* of acetylcholine in various tissues of guinea pigs. J. Pharmacol. Exp. Ther. **193:** 246–255, 1975.
120. HAUBRICH, D. R., WEDEKING, P. W., AND WANG, P. F. L.: Increase in tissue concentration of acetylcholine in guinea pigs *in vivo* induced by administration of choline. Life Sci. **14:** 921–927, 1974.
121. HAUSER, G.: The involvement of lipids in signal transmission across cell membranes of the nervous system. *In* Neural Membranes, ed. by G. Y. Sun, N. Bazan, J. Y. Wun, G. Porcellati, and A. Y. Sun, pp. 37–65, Humana Press, Clifton, NJ, 1983.

122. HAZEKI, O., AND UI, M.: Modification by islet-activating protein of receptor-mediated regulation of cyclic AMP accumulation in isolated rat heart cells. J. Biol. Chem. **256:** 2856–2862, 1981.
123. HERRON, G. S., AND SCHIMERLIK, M.: Glycoprotein properties of the solubilized atrial muscarinic acetylcholine receptor. J. Neurochem. **41:** 1414–1420, 1983.
124. HERRON, G. S., AND SCHIMERLIK, M. I.: Protein composition of the atrial muscarinic acetylcholine receptor partially purified by wheat germ agglutinin affinity chromatography. Arch. Biochem. Biophys. **230:** 533–542, 1984.
125. HESS, M. E., SHANFELD, J., AND HAUGAARD, N.: The role of the autonomic nervous system in the regulation of heart phosphorylase in the open-chest heart. J. Pharmacol. Exp. Ther. **135:** 191–196, 1962.
126. HIGGINS, C. B., VATNER, S. F., AND BRAUNWALD, E.: Parasympathetic control of the heart. Pharmacol. Rev. **25:** 119–155, 1973.
127. HILL-SMITH, I., AND PURVES, R. D.: Synaptic delay in the heart: An ionophoretic study. J. Physiol. (Lond.) **279:** 31–54, 1978.
128. HINO, N., AND OCHI, R.: Effect of acetylcholine on membrane currents in guinea-pig papillary muscle. J. Physiol. (Lond.) **307:** 183–197, 1980.
129. HIRATA, F., AND AXELROD, J.: Phospholipid methylation and biological signal transmission. Science **209:** 1082–1090, 1980.
130. HIROMASA, S., NISHIMURA, M., TSUJI, Y., AND WATANABE, Y.: Electrophysiologic demonstration of acetylcholine receptors and acetylcholine effects in the rabbit atrioventricular node. J. Am. Coll. Cardiol. **1:** 730, 1983.
131. HIRSCHOWITZ, B. I., HAMMER, R., GIACHETTI, A., KEIRNS, J. J., AND LEVINE, R. R. (EDS.): Subtypes of muscarinic receptors. Trends Pharmacol. Sci., supplement, January, 1984.
132. HOFFMAN, B. F. AND CRANEFIELD, P. F.: Electrophysiology of the Heart, McGraw-Hill, New York, 1960.
133. HOFFMAN, B. F., AND SUCKLING, E. E.: Cardiac cellular potentials: Effect of vagal stimulation and acetylcholine. Am. J. Physiol. **173:** 312–320, 1953.
134. HOKIN, L. E., AND HOKIN, M. R.: Studies on the carrier function of phosphatidic acid in sodium transport. J. Gen. Physiol. **44:** 61–85, 1960.
135. HOKIN, M. R., AND HOKIN, L. E.: Effects of acetylcholine on phospholipids in the pancreas. J. Biol. Chem. **209:** 549–558, 1954.
136. HOLLENBERG, M., CARRIERE, S., AND BARGER, A. C.: Biphasic action of acetylcholine on ventricular myocardium. Circ. Res. **16:** 527–536, 1965.
137. HOROWITS, R., AND WINEGRAD, S.: Cholinergic regulation of calcium sensitivity in cardiac muscle. J. Mol. Cell. Cardiol. **15:** 277–280, 1983.
138. HOSEY, M. M.: Regulation of antagonist-binding to cardiac muscarinic receptors. Biochem. Biophys. Res. Commun. **107:** 314–321, 1982.
139. HOSEY, M. M., AND FIELDS, J. Z.: Quantitative and qualitative differences in muscarinic cholinergic receptors in embryonic and newborn chick hearts. J. Biol. Chem. **256:** 6395–6399, 1981.
140. HOSEY, M. M., AND MCMAHON, K. K.: Effects of magnesium on the affinity of chick heart muscarinic receptor for agonist in native and N-ethylmaleimide treated preparations. Fed. Proc. **43:** 872, 1984.
141. HULME, E. C., BERRIE, C. P., BIRDSALL, N. J. M., AND BURGEN, A. S. V.: Interactions of muscarinic receptors with guanine nucleotides and adenylate cyclase. *In* Drug Receptors and Their Effectors, ed. by N.J.M. Birdsall, pp. 23–34, Macmillan, London, 1981.
142 HUTTER, O. F., AND TRAUTWEIN, W.: Vagal and sympathetic effects on the pacemaker fibers in the sinus venosus of the heart. J. Gen. Physiol. **39:** 715–733, 1956.
143. INGBRETSEN, C. G.: Interaction between alpha and beta adrenergic receptors and cholinergic receptors in isolated perfused rat heart: Effects on cAMP-protein kinase and phosphorylase. J. Cyclic Nucleotide Res. **6:** 121–132, 1980.
144. INOUE, D., HACHISU, M., AND PAPPANO, A. J.: Acetylcholine increases resting membrane potassium conductance in atrial but not in ventricular muscle during muscarinic inhibition of Ca^{2+}-dependent action potentials in chick heart. Circ. Res. **53:** 158–167, 1983.
145. INUI, J., AND IMAMURA, H.: Effects of acetylcholine on calcium-dependent electrical and mechanical responses in the guinea-pig papillary muscle partially depolarized by potassium. Naunyn-Schmiedeberg's Arch. Pharmacol. **299:** 1–7, 1977.
146. INUI, J., BRODDE, O.-E., AND SCHÜMANN, H. J.: Influence of acetylcholine on the positive inotropic effect evoked by α- or β-adrenoceptor stimulation in the rabbit heart. Naunyn-Schmiedeberg's Arch. Pharmacol. **320:** 152–159, 1982.
147. IRISAWA, H., AND NOMA, A.: Pacemaker mechanisms of rabbit sinoatrial node cells. *In* Cardiac Rate and Rhythm, ed. by L. N. Bouman and H. J. Jongsma, pp. 35–51, Martinus Nijhoff, Boston, 1982.
148. IRIUCHIJIMA, J., AND KUMUDA, M.: Activity of single vagal fibers efferent to the heart. Jpn. J. Physiol. **14:** 479–487, 1964.
149. JAKOBS, K. H., AKTORIES, K., AND SCHULTZ, G.: GTP-dependent inhibition of cardiac adenylate cyclase by muscarinic cholinergic agonists. Naunyn-Schmiedeberg's Arch. Pharmacol. **310:** 113–119, 1979.
150. JALIFE, J., FRACCOLA, P., AND MOE, G. K.: Entrainment of the SA nodal pacemaker by brief vagal bursts in relation to AV conduction. *In* Cardiac Rate and Rhythm, ed. by L. N. Bouman and H. J. Jongsma, pp. 577–603, Martinus Nijhoff, Boston, 1982.
151. JALIFE, J., SLENTER, V. A. J., SALATA, J. J., AND MICHAELS, D. C.: Dynamic vagal control of pacemaker activity in the mammalian sinoatrial node. Circ. Res. **52:** 642–656, 1983.
152. JAMES, T. N., AND SPENCE, C. A.: Distribution of cholinesterase within the sinus node and AV node of the human heart. Anat. Rec. **155:** 151–162, 1966.
153. JEACOCKE, S. A., AND ENGLAND, P.: Phosphorylation of a myofibrillar protein of M_r 150,000 in perfused rat heart, and the tentative identification of this as C-protein. F.E.B.S. Lett. **122:** 129–132, 1980.
154. JENDEN, D. J.: An overview of choline and acetylcholine metabolism in relation to the therapeutic uses of choline. *In* Nutrition and the Brain, vol. 5, ed. by A. Barbeau, J. H. Growdon and R. J. Wurtman, pp. 13–24, Raven Press, New York, 1979.
155. JOPE, R. S.: High affinity choline transport and acetyl CoA production in brain and their roles in the regulation of acetylcholine synthesis. Brain Res. Rev. **1:** 313–344, 1979.
156. JOSEPHSON, I., AND SPERELAKIS, N.: On the ionic mechanism underlying adrenergic-cholinergic antagonism in ventricular muscle. J. Gen. Physiol. **79:** 69–86, 1982.
157. JUNSTAD, M., AND WENNMALM, Å.: Release of prostaglandin from the rabbit isolated heart following vagal nerve stimulation or acetylcholine infusion. Br. J. Pharmacol. **52:** 375–379, 1974.
158. KALIA, M.: Brain stem localization of vagal preganglionic neurons. J. Auton. Nerv. Syst. **3:** 451–481, 1981.
159. KARCZMAR, A. G.: History of the research with anticholinesterase agents. *In* Anticholinesterase Agents, vol. 1, ed. by A. G. Karczmar, pp. 1–44, Pergamon Press, New York, 1970.
160. KARCZMAR, A. G.: Overview: Cholinergic drugs and behavior—what effects may be expected from a "cholinergic diet". *In* Nutrition and the Brain, vol. 5, ed. by A. Barbeau, J. H. Growdon, and R. J. Wurtman, pp. 141–175, Raven Press, New York, 1979.
161. KARNOVSKY, M. J.: The localization of cholinesterase activity in rat cardiac muscle by electron microscopy. J. Cell Biol. **23:** 217–232, 1964.
162. KATADA, T., BOKOCH, G. M., NORTHUP, J. K., UI, M., AND GILMAN, A. G.: The inhibitory guanine nucleotide-binding regulatory component of adenylate cyclase: Properties and function of the purified protein. J. Biol. Chem. **259:** 3568–3577, 1984.
163. KATADA, T., NORTHUP, J. K., BOKOCH, G. M., UI, M., AND GILMAN, A. G.: The inhibitory guanine nucleotide-binding regulatory component of adenylate cyclase: Subunit dissociation and guanine nucleotide-dependent hormonal inhibition. J. Biol. Chem. **259:** 3578–3585, 1984.
164. KATADA, T., BOKOCH, G. M., SMIGEL, M. D., UI, M., AND GILMAN, A. G.: The inhibitory guanine nucleotide-binding regulatory component of adenylate cyclase: Subunit dissociation and the inhibition of adenylate cyclase in S49 lymphoma cyc$^-$ and wild type membranes. J. Biol. Chem. **259:** 3586–3595, 1984.
165. KATONA, P. G., POITRAS, J. W., BARNETT, G. O., AND TERRY, B. S.: Cardiac vagal efferent activity and heart period in the carotid sinus reflex. Am. J. Physiol. **218:** 1030–1037, 1970.
166. KATZ, A. M.: Response of the contractile proteins and sarcoplasmic reticulum in the response of the heart to catecholamine: An historical review. Adv. Cyclic Nucleotide Res. **11:** 303–343, 1979.
167. KATZ, B., AND MILEDI, R.: The binding of acetylcholine to receptors and its removal from the synaptic cleft. J. Physiol. (Lond.) **231:** 549–574, 1973.
168. KATZ, B., AND MILEDI, R.: Transmitter leakage from motor nerve terminals. Proc. R. Soc. Lond. B Biol. Sci. **196:** 59–72, 1977.
169. KEELY, S. L., JR., LINCOLN, T. M., AND CORBIN, J. D.: Interaction of acetylcholine and epinephrine on heart cyclic AMP-dependent protein kinase. Am. J. Physiol. **234:** H432–H438, 1978.
170. KENT, K. M., EPSTEIN, S. E., COOPER, T., AND JACOBOWITZ, D. M.: Cholinergic innervation of the canine and human ventricular conducting system. Anatomic and electrophysiologic correlations. Circulation **50:** 948–955, 1974.
171. KILBINGER, H.: Pre- and postsynaptic effects of muscarinic agonists and antagonists at peripheral cholinergic synapses. *In* Abstracts of the 3rd Congress of the Hungarian Pharmacological Society, p. 45, Hungarian Pharmacological Society, Budapest, 1979.
172. KILBINGER, H., AND LÖFFELHOLZ, K.: The isolated perfused chicken heart as a tool for studying acetylcholine output in the absence of cholinesterase inhibition. J. Neural Transm. **38:** 9–14, 1976.
173. KIRBY, M. L., AND STEWART, D. E.: Neural crest origin of cardiac ganglion cells in the chick embryo. Identification and extirpation. Dev. Biol. **97:** 433–443, 1983.
174. KIRCHBERGER, M. A., TADA, M., AND KATZ, A. M.: Adenosine 3′:5′-monophosphate-dependent protein kinase-catalyzed phosphorylation reaction and its relationship to calcium transport in cardiac sarcoplasmic reticulum. J. Biol. Chem. **249:** 6166–6173, 1974.
175. KOELLE, G. B.: Cytological distributions and physiological functions of cholinesterases. *In* Cholinesterases and Anticholinesterase Agents. Handbuch der Experimentellen Pharmakologie, vol. 15, ed. by G. B. Koelle, pp. 187–298, Springer, Berlin, 1963.
176. KOHLHARDT, M., AND HAAP, K.: 8-Bromo-guanosine-3′,5′-monophosphate mimics the effect of acetylcholine on slow response action potential and contractile force in mammalian atrial myocardium. J. Mol. Cell. Cardiol. **10:** 573–586, 1978.
177. KOLLAI, M., AND KOIZUMI, K.: Reciprocal effects of the steroid anesthetic

(Althesin) on autonomic nerves innervating the heart. J. Auton. Nerv. Syst. 4: 93–100, 1981.
178. KUNTSCHEROVÁ, J.: Effect of short-term starvation and choline on the acetylcholine content of organs of albino rats. Physiol. Bohemoslav. 21: 655–660, 1972.
179. KUNZE, D. L.: Reflex discharge patterns of cardiac vagal efferent fibres. J. Physiol. (Lond.) 222: 1–15, 1972.
180. KUNZE, D. L.: Regulation of activity of cardiac vagal motoneurons. Fed. Proc. 39: 2513–2518, 1980.
181. LEDDA, F., MARCHETTI, P., AND MUGELLI, A.: Studies on the positive inotropic effect of phenylephrine. A comparison with isoprenaline. Br. J. Pharmacol. 54: 83–90, 1975.
182. LEITZ, F. H., AND STEFANO, F. J. E.: Effect of ouabain and desipramine on the uptake and storage of norepinephrine and metaraminol. Eur. J. Pharmacol. 11: 278–285, 1970.
183. LEVY, M. N.: Sympathetic-parasympathetic interactions in the heart. Circ. Res. 29: 437–445, 1971.
184. LEVY, M. N.: Neural control of cardiac rhythm and contraction. *In* Cardiac Therapy, ed. by M. R. Rosen and B. F. Hoffman, pp. 73–94, Martinus Nijhoff, Boston, 1983.
185. LEVY, M. N., AND BLATTBERG, B.: Effect of vagal stimulation on the overflow of norepinephrine into the coronary sinus during cardiac sympathetic nerve stimulation in the dog. Circ. Res. 38: 81–84, 1976.
186. LEVY, M. N., AND MARTIN, P. J.: Neural control of the heart. *In* Handbook of Physiology. Section 2: The Cardiovascular System, vol. 1, ed. by R. M. Berne, pp. 581–620, American Physiological Society, Bethesda, MD, 1979.
187. LEVY, M. N., MARTIN, P. J., AND STUESSE, S. L.: Neural regulation of the heart beat. Annu. Rev. Physiol. 43: 443–453, 1981.
188. LINCOLN, T. M., AND KEELY, S. L.: Effects of acetylcholine and nitroprusside on cGMP-dependent protein kinase in the perfused rat heart. J. Cyclic Nucleotide Res. 6: 83–91, 1980.
189. LINDEN, J., AND BROOKER, G.: The questionable role of cyclic guanosine 3′:5′-monophosphate in heart. Biochem. Pharmacol. 28: 3351–3360, 1979.
190. LINDMAR, R., AND LÖFFELHOLZ, K.: The neuronal efflux of noradrenaline: Dependency on sodium and facilitation by ouabain. Naunyn-Schmiedeberg's Arch. Pharmacol. 284: 93–100, 1974.
191. LINDMAR, R., LÖFFELHOLZ, K., AND POMPETZKI, H.: Acetylcholine overflow during infusion of a high potassium-low sodium solution in the perfused chicken heart in the absence and presence of physostigmine. Naunyn-Schmiedeberg's Arch. Pharmacol. 299: 17–21, 1977.
192. LINDMAR, R., LÖFFELHOLZ, K., AND WEIDE, W.: Inhibition by pentobarbital of the acetylcholine release from the postganglionic parasympathetic neuron of the heart. J. Pharmacol. Exp. Ther. 210: 166–173, 1979.
193. LINDMAR, R., LÖFFELHOLZ, K., WEIDE, W., AND WEIS, S.: Evidence for bilateral vagal innervation of postganglionic parasympathetic neurons in chicken heart. J. Neural Transm. 56: 239–247, 1983.
194. LINDMAR, R., LÖFFELHOLZ, K., AND WEIDE, W.: Interstitial washout and hydrolysis of acetylcholine in the perfused heart. Naunyn-Schmiedeberg's Arch. Pharmacol. 318: 295–300, 1982.
195. LINDMAR, R., LÖFFELHOLZ, K., WEIDE, W., AND WITZKE, J.: Neuronal uptake of choline following release of acetylcholine in the perfused heart. J. Pharmacol. Exp. Ther. 215: 710–715, 1980.
196. LIPSIUS, S. L., AND GIBBONS, W. R.: Acetylcholine lengthens action potentials of sheep cardiac Purkinje fibers. Am. J. Physiol. 238 (Heart Circ. Physiol. 7): H237–H243, 1980.
197. LLINAS, R. R., AND HEUSER, J. E.: Depolarization-release coupling systems in neurons. Neurosci. Res. Program Bull. 15: 557–687, 1977.
198. LOEWY, A. D., AND MCKELLAR, S.: The neuroanatomical basis of central cardiovascular control. Fed. Proc. 39: 2495–2503, 1980.
199. LÖFFELHOLZ, K.: Release of acetylcholine in the isolated heart. Am. J. Physiol. 240: H431–H440, 1981.
200. LÖFFELHOLZ, K., BREHM, R., AND LINDMAR, R.: Hydrolysis, synthesis and release of acetylcholine in the isolated heart. Fed. Proc. 43: 2603–2606, 1984.
201. LÖFFELHOLZ, K., LINDMAR, R., AND WEIDE, W.: The isolated perfused heart and its parasympathetic neuro-effector junction. *In* Model Cholinergic Synapses, Progress in Cholinergic Biology, ed. by I. Hanin and A. M. Goldberg, pp. 95–111, Raven Press, New York, 1982.
202. LÖFFELHOLZ, K., AND MUSCHOLL, E.: A muscarinic inhibition of the noradrenaline release evoked by postganglionic sympathetic nerve stimulation. Naunyn-Schmiedeberg's Arch. Pharmacol. 265: 1–15, 1969.
203. LÖFFELHOLZ, K., AND MUSCHOLL, E.: Inhibition by parasympathetic nerve stimulation of the release of the adrenergic transmitter. Naunyn-Schmiedeberg's Arch. Pharmacol. 267: 181–184, 1970.
204. LÖFFELHOLZ, K., AND WEIDE, W.: Aminopyridines and the release of acetylcholine. Trends Pharmacol. Sci. 3: 147–149, 1982.
205. LUND, D. D., SCHMID, P. G., KELLEY, S. E., CORRY, R. J., AND ROSKOSKI, R., JR.: Choline acetyltransferase activity in rat heart after transplantation. Am. J. Physiol. 235: H367–H371, 1978.
206. LUND, D. D., SCHMID, P. G., AND ROSKOSKI, R. JR.: Choline acetyltransferase activity in rat and guinea pig heart following vagotomy. Am. J. Physiol. 236: H620–H623, 1979.
207. MACKAAY, A. J. C., BLEEKER, W. M., OP'T HOF, T., AND BOUMAN, L. N.: Temperature dependence of the chronotropic action of calcium. Functional inhomogeneity of the rabbit sinus node. J. Mol. Cell. Cardiol. 12: 433–443, 1980.
208. MACKAAY, A. J. C., OP'T HOF, T., BLEEKER, W. M., JONGSMA, H. J., AND BOUMAN, L. N.: Interaction of adrenaline and acetylcholine on cardiac pacemaker function. Functional inhomogeneity of the rabbit sinus node. J. Pharmacol. Exp. Ther. 214: 417–422, 1980.
209. MACINTOSH, F. C.: Are acetylcholine levels related to acetylcholine release. *In* Choline and Lecithin in Brain Disorders, Nutrition and the Brain, vol. 5, ed. by A. Barbeau, J. H. Growdon, and R. J. Wurtman, pp. 201–217, Raven Press, New York, 1979.
210. MARK, A. L.: The Bezold-Jarisch reflex revisited: Clinical implications of inhibitory reflexes originating in the heart. J. Am. Coll. Cardiol. 1: 90-112, 1983.
211. MARVIN, W. J., HERMSMEYER, K., MCDONALD, R. I., ROSKOSKI, L. M., AND ROSKOSKI, R., JR.: Ontogenesis of cholinergic innervation in the rat heart. Circ. Res. 46: 690–695, 1980.
212. MCALLEN, R. M., AND SPYER, K. M.: Two types of vagal preganglionic motoneurones projecting to the heart and lungs. J. Physiol. (Lond.) 282: 353–365, 1978.
213. MCALLISTER, R. E., NOBLE, D., AND TSIEN, R. W.: Reconstruction of the electrical activity of cardiac Purkinje fibres. J. Physiol. (Lond.) 251: 1–59, 1975.
214. MCCLELLAN, G. B., AND WINEGRAD, S.: The regulation of the calcium sensitivity of the contractile system in mammalian cardiac muscle. J. Gen. Physiol. 72: 737–764, 1978.
215. MCCLOSKEY, D. I., AND POTTER, E. K.: Excitation and inhibition of cardiac vagal motoneurones by electrical stimulation of the carotid sinus nerve. J. Physiol. (Lond.) 316: 163–176, 1981.
216. MCEWEN, L. M.: The effect on the isolated rabbit heart of vagal stimulation and its modification by cocaine, hexamethonium and ouabain. J. Physiol. (Lond.) 131: 678–689, 1956.
217. MCMAHAN, U. J., AND KUFFLER, S. W.: Visual identification of synaptic boutons on living ganglion cells and of varicosities in postganglionic axons in the heart of the frog. Proc. R. Soc. Lond. B Biol. Sci. 177: 485–508, 1971.
218. MCMAHON, K. K., AND HOSEY, M. M.: Potentiation of monovalent cation effects on ligand binding to cardiac muscarinic receptors in N-ethylmaleimide treated membranes. Biochem. Biophys. Res. Commun. 111: 41–46, 1983.
219. MICHAELS, D. C., MATYAS, E. P., AND JALIFE, J.: A mathematical model of the effects of acetylcholine pulses on sinoatrial pacemaker activity. Circ. Res., 55: 89–101, 1984.
220. MICHAELS, D. C., SLENTER, V. A. J., SALATA, J. A., AND JALIFE, J.: A model of dynamic vagus-sinoatrial node interactions. Am. J. Physiol. 245: (Heart Circ. Physiol. 14): H1043–H1053, 1983.
221. MIRRO, M. J., BAILEY, J. C., AND WATANABE, A. M.: The role of cyclic AMP in regulation of the slow inward current. *In* The Slow Inward Current and Cardiac Arrhythmias, ed. by D. P. Zipes, J. C. Bailey, and V. Elharrar, pp. 111–126, Martinus Nijhoff, Boston, 1980.
222. MOLENAAR, P. C., AND POLAK, R. L.: Acetylcholine synthesizing enzymes in frog skeletal muscle. J. Neurochem. 35: 1021–1025, 1980.
223. MOORE, E. N., SPEAR, J. F., AND KRONHAUS, K. D.: The effects of brief vagal and sympathetic stimulation on rate and rhythm changes in the sinus node. *In* Cardiac Rate and Rhythm, ed. by L. N. Bouman and H. J. Jongsma, pp. 543–561, Martinus Nijhoff, Boston, 1982.
224. MOPE, L., MCCLELLAN, G. B., AND WINEGRAD, S.: Calcium sensitivity of the contractile system and phosphorylation of troponin in hyperpermeable cardiac cells. J. Gen. Physiol. 75: 271–282, 1980.
225. MUBAGWA, K., AND CARMELIET, E.: Effects of acetylcholine on electrophysiological properties of rabbit cardiac Purkinje fibers. Circ. Res. 53: 740–751, 1983.
226. MUBAGWA, K., VEREECKE, J., AND CARMELIET, E.: Does the acetylcholine-induced potassium current in rabbit cardiac Purkinje fibers show relaxation? Arch. Int. Pharmacodyn. Ther. 262: 322–323, 1983.
227. MURAD, F., CHI, Y. M., RALL, T. W., AND SUTHERLAND, E.: Adenylcyclase. III. The effect of catecholamines and choline esters on the formation of adenosine 3′:5′-phosphate by preparations from cardiac muscle and liver. J. Biol. Chem. 237: 1233–1238, 1962.
228. MUSCHOLL, E.: Peripheral muscarinic control of norepinephrine release in the cardiovascular system. Am. J. Physiol. 239: H713–H720, 1980.
229. MUSCHOLL, E.: From the cholinergic link to the cholinergic antilink in adrenergic transmission: The muscarinic inhibitory mechanism. Trends Pharmacol. Sci. 1: 381–382, 1980.
230. MUSCHOLL, E., AND MUTH, A.: The effect of physostigmine on the vagally induced muscarinic inhibition of noradrenaline release from the isolated perfused rabbit atria. Naunyn-Schmiedeberg's Arch. Pharmacol. 320: 760–783, 1982.
231. NAPOLITANO, L. M., WILLIAM, V. L., HANLON, C. R., AND COOPER, T.: Intrinsic innervation of the heart. Am. J. Physiol. 208: 455–458, 1965.
232. NARGEOT, J., AND GARNIER, D.: The action of muscarinic agonists and antagonists on frog atrial fibers: Electrophysiological studies. J. Pharmacol. (Paris) 13: 431–445, 1982.
233. NARGEOT, J., LESTER, H. A., BIRDSALL, N. J. M., STOCKTON, J., WASSERMANN, N. H., AND ERLANGER, B. F.: A photoisomerizable muscarinic

antagonist. Studies of binding and of conductance relaxations in frog heart. J. Gen. Physiol. **79:** 657–678, 1982.
234. NAWRATH, H.: Does cyclic GMP mediate the negative inotropic effect of acetylcholine in the heart? Nature (Lond.) **267:** 72–74, 1977.
235. NAWRATH, H., GRUSCHWITZ, P., AND ZONG, X.: A negative inotropic effect of acetylcholine in the presence of several phosphodiesterase inhibitors. Experientia (Basel) **37:** 755–756, 1981.
236. NEEF, K. J. DE, JANSEN, J. R. C., AND VERSPRILLE, A.: Attenuation of the negative chronotropic effect of myelinated fibers by non-myelinated fibers in the vagus nerve of the rabbit. Circ. Res. **52:** 508–514, 1983.
237. NILSSON, E., AND SPORRONG, B.: Electron microscopic investigation of adrenergic and non-adrenergic axons in the rabbit SA-node. Z. Zellforsch. **111:** 404–412, 1970.
238. NISHIZUKA, Y.: Protein kinases in signal transduction. Trends Biochem. Sci. **9:** 163–166, 1984.
239. NOMA, A., PEPER, K., AND TRAUTWEIN, W.: Acetylcholine-induced potassium current fluctuations in the rabbit sino-atrial node. Pflügers Arch. **381:** 255–262, 1979.
240. NOMA, A., AND TRAUTWEIN, W.: Relaxation of the ACh-induced potassium current in the rabbit sinoatrial node cell. Pflügers Arch. **377:** 193–200, 1978.
241. NOSAKA, S., YAMAMOTO, T., AND YASUNAGA, K.: Localization of vagal cardio-inhibitory preganglionic neurons within rat brain stem. J. Comp. Neurol. **186:** 79–92, 1979.
242. ÖBERG, B., KENDRICK, E., THORÉN, P., AND WENNERGREN, G.: Reflex cardiovascular responses to graded stimulations of low- and high-threshold afferents in the carotid sinus and aortic nerves in the cat. Acta Physiol. Scand. **113:** 129–137, 1981.
243. OFFER, G.: C-protein and the periodicity in the thick filaments of vertebrate skeletal muscle. Cold Spring Harbor Symp. Quant. Biol. **37:** 87–93, 1972.
244. OKONEK, S., AND KILBINGER, H.: Determination of acetylcholine, nitrostigmine and acetylcholinesterase activity in four patients with severe nitrostigmine (E 605 forte®) intoxication. Arch. Toxicol. **32:** 97–108, 1974.
245. OSTERRIEDER, W., NOMA, A., AND TRAUTWEIN, W.: On the kinetics of the potassium channel activated by acetylcholine in the S-A node of the rabbit heart. Pflügers Arch. **386:** 101–109, 1980.
246. OSTERRIEDER, W., YANG, Q.-F., AND TRAUTWEIN, W.: The time course of the muscarinic response to ionophoretic acetylcholine application to the S-A node of the rabbit heart. Pflügers Arch. **389:** 283–291, 1981.
247. PAPPANO, A. J.: Ontogenetic development of autonomic neuroeffector transmission and transmitter reactivity in embryonic and fetal hearts. Pharmacol. Rev. **29:** 3–33, 1977.
248. PAPPANO, A. J.: The development of postsynaptic cardiac autonomic receptors and their regulation of cardiac function during embryonic, fetal and neonatal life. *In* Function of the Heart in Normal and Pathological States, ed. by N. Sperelakis, pp. 355–375, Martinus Nijhoff, Boston, 1984.
249. PAPPANO, A. J., AND BIEGON, R. L.: Mechanisms for muscarinic inhibition of calcium-dependent action potentials and contractions in developing ventricular muscle. The role of cyclic AMP. *In* Normal and Abnormal Conduction of the Heartbeat, ed. by A. P. Paes de Carvalho, B. F. Hoffman, and M. Lieberman, pp. 327–344, Futura Press, Mount Kisco, NY, 1982.
250. PAPPANO, A. J., HARTIGAN, P. M., AND COUTU, M.D.: Acetylcholine inhibits the positive inotropic effect of cholera toxin in ventricular muscle. Am. J. Physiol. **243:** (Heart Circ. Physiol. **12**): H434–H441, 1982.
251. PAPPANO, A. J., AND INOUE, D.: Development of different electrophysiological mechanisms for muscarinic inhibition of atria and ventricles. *In* Parasympathetic Neuroeffector Mechanisms in the Heart, Fed. Proc. **43:** 2607–2612, 1984.
252. PETERSON, G. L., HERRON, G. S., YAMAKI, M., FULLERTON, D. S., AND SCHIMERLIK, M. I.: Purification of the muscarinic acetylcholine receptor from porcine atria. Proc. Natl. Acad. Sci. U.S.A. **81:** 4993–4997, 1984.
253. PICKERING, J. W.: Experiments on the hearts of mammalian and chick-embryos, with special reference to action of electric currents. J. Physiol. (Lond.) **20:** 165–222, 1896.
254. PICKERING, T. G., GRIBBIN, B., PETERSEN, E. S., CUNNINGHAM, D. J. C., AND SLEIGHT, P.: Effects of autonomic blockade on the baroreflex in man at rest and during exercise. Circ. Res. **30:** 177–185, 1972.
255. POLAK, R. L., SELLIN, L. C., AND THESLEFF, S.: Acetylcholine content and release in denervated or botulinum poisoned rat skeletal muscle. J. Physiol. (Lond.) **319:** 253–259, 1981.
256. POTT, L.: On the time course of the acetylcholine-induced hyperpolarization in quiescent guinea-pig atria. Pflügers Arch. **380:** 71–77, 1979.
257. POTT, L.: Effects of fast and slowly acting antagonists on the time course of the muscarinic response in guinea-pig atrial muscle. Pflügers Arch. **399:** 35–41, 1983.
258. POTTER, E. K.: Inspiratory inhibition of vagal responses to baroreceptor and chemoreceptor stimuli in the dog. J. Physiol. (Lond.) **316:** 177–180, 1981.
259. POWIS, D. A.: Cardiac glycosides and autonomic neurotransmission. J. Auton. Pharmacol. **3:** 127–154, 1983.
260. PRIOLA, D. V., O'BRIEN, W. J., DAIL, W. G., AND SIMPSON, W. W.: Cardiac catecholamine stores after cardic sympathectomy, 6-OHDA, and cardiac denervation. Am. J. Physiol. **240:** H889–H895, 1981.
261. PRIOLA, D. V., AND SPURGEON, H. A.: Cholinergic sensitivity of the denervated canine heart. Circ. Res. **41:** 600–606, 1977.
262. PRIOLA, D. V., SANCHEZ-WILSON, C., AND CURTIS, M. B.: Pattern of cholinergic sensitivity changes following partial cardiac denervation. Proc. West. Pharmacol. Soc. **26:** 165–167, 1983.
263. PRYSTOWSKY, E. N., JACKMAN, W. M., RINKENBERGER, R. L., HEGER, J. J., AND ZIPES, D. P.: Effect of autonomic blockade on ventricular refractoriness and atrioventricular nodal conduction in humans: Evidence supporting a direct cholinergic action on ventricular muscle refractoriness. Circ. Res. **49:** 511–518, 1981.
264. PURVES, R. D.: Function of muscarinic and nicotinic acetylcholine receptors. Nature (Lond.) **261:** 149–150, 1976.
265. PURVES, R. D.: The time course of cellular responses to iontophoretically applied drugs. J. Theor. Biol. **65:** 327–344, 1977.
266. RANDALL, W. C., AND ARMOUR, J. A.: Gross and microscopic anatomy of the cardiac innervation. *In* Neural Regulation of the Heart, ed. by W. C. Randall, pp. 13–41, Oxford University Press, New York, 1977.
267. RAY, K. P., AND ENGLAND, P. J.: Phosphorylation of the inhibitior subunit of troponin and its effect on the calcium dependence of cardiac myofibril adenosine triphosphatase. F.E.B.S. Lett. **70:** 11–16, 1976.
268. RENAUD, J. F., BARHANIN, J., CAVEY, D., FOSSET, M., AND LAZDUNSKI, M.: Comparative properties of the *in ovo* and *in vitro* differentiation of the muscarinic cholinergic receptor in embryonic heart cells. Dev. Biol. **78:** 184–200, 1980.
269. REUTER, H.: Calcium channel modulation by neurotransmitters, enzymes and drugs. Nature (Lond.) **301:** 569–574, 1983.
270. RICKENBACHER, J., AND MÜLLER, E.: The development of cholinergic ganglia in the chick embryo heart. Anat. Embryol. **155:** 253–258, 1979.
271. RODBELL, M.: The role of hormone receptors and GTP-regulatory proteins in membrane transduction. Nature (Lond.) **284:** 17–22, 1980.
272. RODGER, I. W., AND SHAHID, M.: Forskolin, cyclic nucleotides and positive inotropism in isolated papillary muscles of the rabbit. Br. J. Pharmacol. **81:** 151–159, 1984.
273. ROESKE, W. R., AND WILDENTHAL, K.: Responsiveness to drugs and hormones in the murine model of cardiac ontogenesis. Pharmacol. Ther. **14:** 55–66, 1981.
274. ROESKE, W. R., AND YAMAMURA, H. I.: Maturation of mammalian myocardial muscarinic cholinergic receptors. Life Sci. **23:** 127–132, 1978.
275. ROPER, S., AND KO, C.-P.: Impulse blockade in frog cardiac ganglion does not resemble partial denervation in changing synaptic organization. Science **202:** 66–68, 1978.
276. ROSKOSKI, R., JR., MCDONALD, R. I., ROSKOSKI, L. M., MARVIN, W. J., AND HERMSMEYER, K.: Choline acetyltransferase activity in heart: Evidence for neuronal and not myocardial origin. Am. J. Physiol. **233:** H642–H646, 1977.
277. ROSKOSKI, R., JR., MEYER, H. E., AND SCHMID, P. G.: Choline acetyltransferase activity in guinea-pig heart *in vitro*. J. Neurochem. **23:** 1197–1200, 1974.
278. ROSS, E. M., AND GILMAN, A. G.: Biochemical properties of hormone-sensitive adenylate cyclase. Annu. Rev. Biochem. **49:** 533–564, 1980.
279. ROTHSCHUH, K. E.: Vorkommen and Funktion des Acetylcholins im Herzen. Klin. Wochenschr. **32:** 1–7, 1954.
280. SAKMANN, B., NOMA, A., AND TRAUTWEIN, W.: Acetylcholine activation of single muscarinic K^+ channels in isolated pacemaker cells of the mammalian heart. Nature (Lond.) **303:** 250–253, 1980.
281. SALMON, D. M., AND HONEYMAN, T. W.: Proposed mechanism of cholinergic action in smooth muscle. Nature (Lond.) **284:** 344–345, 1980.
282. SAMMET, S., AND GRAEFE, L.-H.: Kinetic analysis of the interaction between noradrenaline and Na^+ in neuronal uptake: Kinetic evidence for co-transport. Naunyn-Schmiedeberg's Arch. Pharmacol. **309:** 99–107, 1979.
283. SASTRE, A., GRAY, D. B., AND LANE, M. A.: Muscarinic cholinergic binding sites in the developing avian heart. Dev. Biol. **55:** 201–205, 1977.
284. SCHMID, P. G., DYKSTRA, R. H., MAYER, H. E., ODA, R. P., AND DONNELL, J. J.: Evidence of nonuniform sympathetic neural activity to heart regions in guinea pigs. Am. J. Physiol. **237:** H606–H611, 1979.
285. SCHMID, P. G., GREIF, B. J., LUND, D. D., AND ROSKOSKI, R., JR.: Regional choline acetyltransferase activity in the guinea pig heart. Circ. Res. **42:** 657–660, 1978.
286. SCHOLZ, H.: Effects of beta- and alpha-adrenoceptor activators and adrenergic transmitter releasing agents on the mechanical activity of the heart. *In* Handbook of Experimental Pharmacology, vol. 54, Adrenergic Activators and Inhibitors, Part 1, ed. by L. Szekeres, pp. 651–733, Springer-Verlag, New York, 1980.
287. SCHULZE, W., AND KRAUSE, E.-G.: Cytochemical demonstration of guanylate cyclase activity in cardiac muscle: Preferential localization at sarcolemma and junctional sarcoplasmic reticulum. Histochemistry **77:** 243–254, 1983.
288. SCHUMACHER, K. A., AND STARKE, K.: Bradykinin-induced stimulation of cardiac parasympathetic ganglia. Experientia (Basel) **34:** 223–224, 1978.
289. SCHÜMANN, J. H., ENDOH, M., AND BRODDE, O. E.: The time course of the effects of β- and α-adrenoceptor stimulation by isoprenaline and methoxamine on the contractile force and cAMP level of the isolated rabbit papillary muscle. Naunyn-Schmiedeberg's Arch. Pharmacol. **289:** 291–302, 1975.
290. SHIBATA, E. F., AND GILES, W.: Cardiac pacemaker cells from bullfrog sinus

venosus lack an inwardly rectifying background K^+ current. Biophys. J. **45:** 136a, 1984.
291. SIEGEL, R. E., AND FISCHBACH, G. D.: Muscarinic receptors and responses in intact embryonic chick atrial and ventricular heart cells. Dev. Biol. **101:** 346–356, 1984.
292. SINGH, J., AND FLITNEY, F. W.: Inotropic responses of the frog ventricle to dibutyryl cyclic AMP and 8-bromo cyclic GMP and related changes in endogenous cyclic nucleotide levels. Biochem. Pharmacol. **30:** 1475–1481, 1981.
293. SLAVIKOVÁ, J., AND TUČEK, S.: Choline acetyltransferase in the heart of adult rats. Pflügers Arch. **392:** 225–229, 1982.
294. SMITH, R. B.: Intrinsic innervation of the avian heart. Acta Anat. **79:** 112–119, 1971.
295. SMYTH, H. S., SLEIGHT, P., AND PICKERING, G. W.: Reflex regulation of arterial pressure during sleep in man. Circ. Res. **24:** 109–121, 1969.
296. SOEJIMA, M., AND NOMA, A.: Mode of regulation of the ACh-sensitive K-channel by the muscarinic receptor in rabbit atrial cells. Pflügers Arch. **400:** 424–431, 1984.
297. SOROTA, S., ADAM, L. P., AND PAPPANO, A.: Dissimilar mechanisms for muscarinic inhibition of chick atrium and ventricle are not explained by differences in antagonist binding. Fed. Proc. **42:** 1146, 1983.
298. SOROTA, S., ADAM, L. P., AND PAPPANO, A.: Unpublished observations.
299. SPEAR, J. F., KRONHAUS, K. D., MOORE, E. N., AND KLINE, R. P.: The effect of brief vagal stimulation on the isolated rabbit sinus node. Circ. Res. **44:** 75–88, 1979.
300. STANLEY, R. L., CONATSER, J., AND DETTBARN, W.-D.: Acetylcholine, choline acetyltransferase and cholinesterases in the rat heart. Biochem. Pharmacol. **27:** 2409–2411, 1978.
301. STREB, H., IRVINE, R. F., BERRIDGE, M. J., AND SCHULZ, I.: Release of Ca^{2+} from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-trisphosphate. Nature (Lond.) **306:** 67–69, 1983.
302. STULL, J. T., AND MAYER, S. E.: Biochemical mechanisms of adrenergic and cholinergic regulation of myocardial contractility. *In* Handbook of Physiology. Section 2: The Cardiovascular System, vol. I, ed. by R. M. Berne, pp. 741–774, American Physiological Society, Bethesda, MD, 1979.
303. TEN EICK, R., NAWRATH, H., MCDONALD, T. F., AND TRAUTWEIN, W.: On the mechanism of the negative inotropic effect of acetylcholine. Pflügers Arch. **361:** 207–213, 1976.
304. THAEMERT, J. C.: Atrioventricular node innervation in ultrastructural three dimensions. Am. J. Anat. **128:** 239–264, 1970.
305. THESLEFF, S.: Aminopyridines and synaptic transmission. Neuroscience **5:** 1413–1419, 1980.
306. THOENEN, H., AND TRANZER, J. P.: Chemical sympathectomy by selective destruction of adrenergic nerve endings with 6-hydroxydopamine. Naunyn-Schmiedeberg's Arch. Pharmacol. Exp. Path. **261:** 271–288, 1968.
307. TODA, N., AND WEST, T. C.: The influence of ouabain on cholinergic responses in the sinoatrial node. J. Pharmacol. Exp. Ther. **153:** 104–113, 1966.
308. TODA, N., AND WEST, T. C.: Interaction between Na, Ca, Mg and vagal stimulation in the S-A node of the rabbit. Am. J. Physiol. **212:** 424–430, 1967.
309. TRAUTWEIN, W., UND DUDEL, J.: Zum Mechanismus der Membranwirkung des Acetylcholin an der Herzmuskelfaser. Pflügers Arch. **266:** 324–334, 1958.
310. TRAUTWEIN, W., OSTERRIEDER, W., AND NOMA, A.: Potassium channels and the muscarinic receptor in the sino-atrial node of the heart. *In* Drug Receptors and Their Effectors, ed. by N. J. M. Birdsall, pp. 5–22, Macmillan, London, 1981.
311. TRAUTWEIN, W., TANIGUCHI, J., AND NOMA, A.: The effect of intracellular cyclic nucleotides and calcium on the action potential and acetylcholine response of isolated cardiac cells. Pflügers Arch. **392:** 307–314, 1982.
312. TRAUTWEIN, W., AND UCHIZONO, K.: Electron microscopic and electrophysiologic study of the pacemaker in the sino-atrial node of the rabbit heart. Z. Zellforsch. **61:** 96–109, 1963.
313. TRAUTWEIN, W., WHALEN, W. J., UND GROSSE-SCHULTE, E.: Elektrophysiologischer Nachweis spontaner Freisetzung von Acetylcholin im Vorhof des Herzens. Pflügers Arch. **270:** 560–570, 1960.
314. TUČEK, S.: Changes in choline acetyltransferase activity in the cardiac auricles of dogs during postnatal development. Physiol. Bohemoslov. **14:** 530–535, 1965.
315. TUČEK, S.: Acetylcholine synthesis in neurons. Chapman and Hall, London, 1978.
316. TUČEK, S.: The synthesis of acetylcholine in skeletal muscles of the rat. J. Physiol. (Lond.) **322:** 53–69, 1982.
317. TUČEK, S.: The synthesis of acetylcholine. *In* Handbook of Neurochemistry, vol. 4: Enzymes in the Nervous System, ed by A. Lajtha, 2nd ed., pp. 219–249, Plenum Press, New York, London, 1984.
318. TUČEK, S., ZELENA, J., GE, I., AND VYSKOČIL, F.: Choline acetyltransferase in transected nerves, denervated muscles and Schwann cells of the frog: Correlation of biochemical, electron microscopical electrophysiological observations. Neuroscience **3:** 709–724, 1978.
319. VENTER, J. C.: Muscarinic cholinergic receptor structure: Receptor size, membrane orientation, and absence of major phylogenetic structural diversity. J. Biol. Chem. **258:** 4842–4848, 1983.
320. VERRIER, R. L., AND LOWN, B.: Sympathetic-parasympathetic interactions and ventricular electrical stability. *In* Neural Mechanisms in Cardiac Arrhythmias, ed. by P. J. Schwartz, A. M. Brown, A. Malliani, and A. Zanchetti, pp. 75–85, Raven Press, New York, 1978.
321. VETTERLEIN, F., AND SCHMIDT, G.: Effects of propranolol and epinephrine on density of capillaries in rat heart. Am. J. Physiol. **246:** H189–H196, 1984.
322. VINCENT, N. H., AND ELLIS, S.: Inhibitory effect of acetylcholine on glycogenolysis in the isolated guinea pig heart. J. Pharmacol. Exp. Ther. **139:** 60–68, 1963.
323. VINCENZI, F. F., AND WEST, T. C.: Modification by calcium of the release of autonomic mediators in the isolated sinoatrial node. J. Pharmacol. Exp. Ther. **150:** 349–360, 1965.
324. VIZI, E. S.: Presynaptic modulation of neurochemical transmission. Prog. Neurobiol. **12:** 181–290, 1979.
325. VYSKOČIL, F., NIKOLSKY, E., AND EDWARDS, C.: An analysis of the mechanisms underlying the non-quantal release of acetylcholine at the mouse neuromuscular junction. Neuroscience **9:** 429–435, 1983.
326. WAELBROECK, M., ROBBERECHT, P., CHATELAIN, P., AND CHRISTOPHE, J.: Rat cardiac muscarinic receptors. I. Effects of guanine nucleotides on high- and low-affinity binding sites. Mol. Pharmacol. **21:** 581–588, 1982.
327. WATANABE, A. M.: Cholinergic agonists and antagonists. *In* Cardiac Therapy, ed. by M. R. Rosen and B. F. Hoffman, pp. 95–144, Martinus Nijhoff, Boston, 1983.
328. WATANABE, A. M., AND BESCH, H., JR.: Interaction between cyclic adenosine monophosphate and cyclic guanosine monophosphate in guinea pig ventricular myocardium. Circ. Res. **37:** 309–317, 1975.
329. WATANABE, A. M., JONES, L. R., MANALAN, A. S., AND BESCH, H. R., JR.: Cardiac autonomic receptors: Recent concepts from radiolabeled ligand-binding studies. Circ. Res. **50:** 161–174, 1982.
330. WATANABE, A. M., LINDEMANN, J. P., JONES, L. R., BESCH, H. R., JR., AND BAILEY, J. C.: Biochemical mechanisms mediating neural control of the heart. *In* Disturbances in Neurogenic Control of the Circulation, ed. by F. M. Abbound, H. A. Fozzard, J. P. Gilmore, and D. J. Reis, pp. 189–203, Waverly Press, Baltimore, 1981.
331. WATANABE, A. M., MCCONNAUGHEY, M. M., STRAWBRIDGE, R. A., FLEMING, J. W., JONES, L. R., AND BESCH, H. R., JR.: Muscarinic cholinergic receptor modulation of β-adrenoceptor receptor affinity for catecholamines. J. Biol. Chem. **253:** 4833–4836, 1978.
332. WEI, J.-W., AND SULAKHE, P.: Regional and subcellular distribution of myocardial muscarinic cholinergic receptors. Eur. J. Pharmacol. **52:** 235–238, 1978.
333. WEIDE, W., AND LÖFFELHOLZ, K.: 4-Aminopyridine antagonizes the inhibitory effect of pentobarbital on acetylcholine release in the heart. Naunyn-Schmiedeberg's Arch. Pharmacol. **312:** 7–13, 1980.
334. WEISS, G. K., AND PRIOLA, D. V.: Brain-stem sites for activation of vagal cardioaccelerator fibers in the dog. Am. J. Physiol. **223:** 300–304, 1972.
335. WENNMALM, Å, AND HEDQUIST, P.: Inhibition by prostaglandin E_1 of parasympathetic neurotransmission in the rabbit heart. Life Sci. **10:** 465–470, 1971.
336. WEST, T. C., FALK, G., AND CERVONI, P.: Drug alteration of transmembrane potentials in atrial pacemaker cells. J. Pharmacol. Exp. Ther. **117:** 245–252, 1956.
337. WETZEL, G. T., AND BROWN, J. H.: Relationships between choline uptake, acetylcholine synthesis, and acetylcholine release in isolated rat atria. J. Pharmacol. Exp. Ther. **226:** 343–348, 1983.
338. WETZEL, G. T., AND BROWN, J. H.: Presynaptic modulation of acetylcholine release from cardiac parasympathetic neurons. Am. J. Physiol., **248** (Heart and Circ. Physiol. **17**) H33–H39, 1985.
339. WHITE, H. L., AND WU, J. C.: Choline and carnitine acetyltransferases of heart. Biochemistry **12:** 841–846, 1973.
340. WURTMAN, R. J., HEFTI, F., AND MELAMED, E.: Precursor control of neurotransmitter synthesis. Pharmacol. Rev. **32:** 315–335, 1981.
341. YANAGIHARA, K., NOMA, A., AND IRISAWA, H.: Reconstruction of sinoatrial node pacemaker potential based on the voltage clamp experiments. Jpn. J. Physiol. **30:** 841–857, 1980.
342. YOUNG, D. A. B.: Factors controlling the washout of the interstitial space of the isolated, perfused rat heart. J. Physiol. (Lond.) **196:** 747–759, 1968.
343. ZEISEL, S. H.: Dietary choline: Biochemistry, physiology and pharmacology. Annu. Rev. Nutr. **1:** 95–121, 1981.
344. ZIMMERMAN, H.: Commentary: Vesicle recycling and transmitter release. Neuroscience **4:** 1773–1803, 1979.