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COMMENTARY

AUTONOMIC NERVOUS SYSTEM ACTIONS OF CARDIAC GLYCOSIDES*

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It has long been known that digitalis drugs have important effects on the autonomic nervous system [1]. The extent and complexities of these interactions have only recently begun to be appreciated[2–4]. These drugs appear to act at all levels of the autonomic reflex arc to influence cardiovascular function. Interactions occur with chemo- and baroreceptors [5, 6], central nervous system structures [7, 8], ganglia [9, 10], pre- and postganglionic nerve endings [11], and cholinergic [12] as well as adrenergic post-synaptic receptors [13]. The complexities of the actions of digitalis drugs on neural tissue become apparent when one attempts to predict the net effect of these drugs on either autonomic outflow or on end organ function. This is because digitalis is capable of exciting nerves within the autonomic reflex arc which produce opposite effects on outflow. For example, these agents can activate afferent nerves feeding into and inhibiting sympathetic cell bodies in the central nervous system (CNS), while at the same time they can enhance activity in central sympathetic neurons [14, 15]. In addition, cardiovascular tissue is innervated by both divisions of the autonomic nervous system and digitalis drugs affect both divisions as well as the cardiovascular tissue itself [16].

Neural sites affected

In terms of the afferent component of the autonomic reflex pathway, digitalis drugs have been demonstrated to increase the discharge rate of neurons in the carotid sinus nerve trunk [5, 6, 17–19]. The discharge rate of both chemoreceptor and baroreceptor fibers within the carotid sinus nerve is increased. Electrical activity in the aortic depressor nerve subserving baroreceptor function is also enhanced [20]. Recordings made from afferent vagal fibers originating in the myocardium and presumably comprising the afferent limb of the Bezold–Jarisch reflex arc indicate that digitalis drugs augment the discharge rate of these nerves as well [21, 22].

The effects of digitalis drugs on electrical activity of neurons within the CNS that exert control over sympathetic and parasympathetic outflow have not been examined. However, recordings from preganglionic fibers of both systems have been made and alterations in neural discharge have been used to indicate the effect of these drugs on CNS neurons controlling autonomic outflow. In our laboratory, we have observed increases in preganglionic sympathetic nerve activity with doses of digitalis that are in the toxic range [14, 15, 23, 24], but these results have not been confirmed by Weaver *et al.* [25]. We have observed increases in activity in preganglionic fibers to the heart, adrenal gland and eye, and in some experiments observed increases in preganglionic sympathetic activity in animals with reflexogenic areas denervated [15]. These latter results led us to conclude that digitalis was acting in the CNS to enhance sympathetic outflow [15]. Weaver *et al.* [25] did not observe an increase in activity monitored from preganglionic splanchnic nerves. Two other studies appear to support our findings that digitalis acts in the CNS to increase sympathetic outflow. In one [26], administration of ouabain was found to augment preganglionic sympathetic discharge elicited by electrical stimulation of the posterior hypothalamus. In the other [27], digoxin was found to cause sympathetically mediated coronary constriction in cross perfusion experiments where the isolated head of a recipient dog was perfused from a donor dog receiving digoxin. No sympathetically mediated coronary constriction occurred when digoxin was restricted to the body of the recipient dog.

Results obtained from measurements of parasympathetic preganglionic nerve discharge are also consistent with digitalis drugs exciting CNS autonomic centers. McLain [28], Gillis *et al.* [24], and Pace and Gillis [15] have demonstrated an increase in the firing rate of neurons in the vagal nerve trunk after the administration of subarrhythmic as well as arrhythmic doses of digitalis. In support of a CNS site of action for the vagomimetic effect of digitalis drugs are the findings of Krayer [8] and Chai *et al.* [29]. Krayer [8] observed vagomimetic effects when digitalis was injected into the arterial supply of the separately perfused dog brain. Chai *et al.* [29] observed vagomimetic effects when digitalis was injected into either the vertebral artery or into a cannula implanted in the fourth ventricle of the cat brain. Interestingly, enhancement in activity of parasympathetic preganglionic fibers can be prevented by prior sectioning of the afferent nerves linking baro- and chemoreceptors

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to the CNS [15]. This suggests the lack of a direct action of digitalis on central parasympathetic areas. On the other hand, another interpretation can be given to these findings. That is, sectioning of afferent nerves abolished incoming neural activity which may have been facilitated at the central level by digitalis, and which would have resulted in an increase in parasympathetic outflow. Thus, prevention of digitalis-induced enhancement of parasympathetic efferent activity by reflex denervation does not rule out a CNS site of action for this response.

Spinal cord effects of digitalis have also been observed. In the one study in which the effects of these agents were examined on spinal cord neural mechanisms [30], no attempt was made to examine the effects on neural mechanisms involved in controlling autonomic input or output. However, these investigators did observe that the spinal cord was affected by digitalis and that, in general, a hyperexcitable state was produced. This was manifested by: (1) facilitation of high frequency transmission in a mono-synaptic reflex pathway, (2) enhancement in poly-synaptic responses, (3) reduction in pre-synaptic inhibition, and (4) enhancement in the excitability of intraspinal primary afferent nerve endings. Indirect evidence that a spinal cord site may be affected and result in alterations in autonomic neural activity was obtained by Hashimoto *et al.* [31]. They employed an isolated papillary muscle preparation from the dog and perfused it with the arterial blood from a donor dog. They measured simultaneously the contractility and rhythm of the papillary muscle, and heart rate and blood pressure of the donor dog. They administered ouabain i.v. to the donor dog and determined the doses to produce ventricular tachycardia and death. When these end points were reached, they examined the changes that occurred in the blood-perfused isolated papillary muscle. In control animals, a dose of ouabain that produced ventricular tachycardia in the donor dog produced arrhythmic contractions in the papillary muscle. In animals with spinal cords pithed, the dose of ouabain to produce ventricular tachycardia was increased and no arrhythmic contractions in the papillary muscle preparation were observed in five of six dogs studied. Similar results were obtained from adrenalectomized dogs. The authors suggested that ouabain causes excitation of sympathetic preganglionic neurons in the spinal cord and this results in catecholamine secretion from the adrenal glands.

Effects on preganglionic autonomic nerves have been observed by Birks [32]. He measured the spontaneous release of acetylcholine from pre-synaptic nerve endings at ganglia by perfusing the superior cervical ganglia with eserinized choline–Locke solution before and during digoxin administration. He observed that the acetylcholine release was significantly increased in the presence of digoxin. In addition, Ten Eick and Hoffman [11] demonstrated that digitalis administration enhanced the evoked potential recorded from either preganglionic sympathetic or parasympathetic nerves. This enhancement was noted in response to submaximal stimuli applied to decentralized preganglionic autonomic fibers. The enhancement in evoked activity was thought to be due to an increase in the number of fibers responding to each stimulus.

Autonomic ganglia are also affected by digitalis drugs. Konzett and Rothlin [9] demonstrated that digitalis potentiated the contractile response of the nictitating membrane to acetylcholine and potassium administered directly to the superior cervical ganglion. Potentiation of the contractile response to preganglionic nerve stimulation was also observed. The digitalis-induced potentiation was concluded to be on either ganglionic cells or postganglionic fibers since the potentiation of acetylcholine and potassium was still present in preparations after degeneration of the preganglionic fibers. Perry and Reinert [10], using the same preparation, obtained similar results, and, in addition, observed potentiation of the ganglionic stimulatory effect of tetramethylammonium ion. Weaver *et al.* [25] concluded from their studies with recordings of spontaneous activity from postganglionic sympathetic nerve endings that digitalis stimulates sympathetic ganglia. They observed enhancement of sympathetic postganglionic activity but no enhancement in preganglionic sympathetic nerve activity. Finally, Gillis *et al.* [33] reported that digitalis administration may result in activation of muscarinic ganglionic receptors by acetylcholine released by preganglionic nerve endings. They observed an atropine-sensitive augmentation of postganglionic sympathetic nerve discharge after digitalis administration in animals pretreated with a nicotinic ganglionic blocking agent.

In regard to postganglionic autonomic nerves, there are reports that digitalis administration will enhance the excitability of these nerves [11]. This was concluded from studies in which the evoked action potential produced by submaximal electrical stimulation of postganglionic sympathetic fibers was found to be enhanced. Recordings of spontaneously occurring neural discharges taken from postganglionic sympathetic nerves indicate that large toxic doses of digitalis enhance the activity in these nerves. This may be in part a reflection of enhanced excitability but is probably primarily a reflection of either enhancement in preganglionic neural activity [15] or ganglionic stimulation [9, 10, 25].

Thus far we have stressed that digitalis causes enhanced firing in efferent autonomic nerves. This appears to be the case when the effects of subarrhythmic and arrhythmic doses of digitalis on vagal nerves are examined. However, in sympathetic nerves enhanced firing is seen only with large toxic doses. Smaller, subarrhythmic doses generally result in a decrease in sympathetic discharge [14, 15, 25]. This is due to the fact that subarrhythmic doses activate the afferent limb of the sympathetic reflex resulting in a reduction of central sympathetic outflow. Larger toxic doses presumably exert a CNS effect which over-shadows the peripheral reflex effect and result in an increase in sympathetic outflow [14, 15]. While reflex and CNS effects of digitalis have opposing actions on sympathetic outflow, both effects result in enhanced vagal efferent outflow.

Digitalis also has important actions on the autonomic neuroeffector junction. Studies dealing with the parasympthetic neuroeffector junction of the heart reveal augmentation of cholinergic responses at the sinoatrial node [12, 29, 34–41], and at the atrioventricular node [39, 41, 42–45]. Studies dealing with the sympathetic neuroeffector junction have been made

using cardiac, vascular and nonvascular tissue. Results obtained using cardiac tissue embrace the entire spectrum of effects. Some investigators have reported that the positive chronotropic response (sinoatrial, junctional and ventricular) to injected catecholamines or sympathetic nerve stimulation is blocked [13, 46–52], unaffected [53, 54], or enhanced [53, 55, 56] by digitalis. Possible explanations for these variable responses include non-uniform responses of cardiac tissue and species differences. Evidence for the first is that supraventricular areas of the dog heart exhibit an antagonistic interaction between catecholamines and digitalis [13, 46], whereas ventricular tissue of the dog heart exhibits a synergistic interaction between these two agents [56]. Evidence for the second is that the atria of rats [55] and rabbits [53] exhibit an augmented chronotropic response to norepinephrine in the presence of digitalis whereas other species (cats, dogs and guinea pigs) exhibit a diminished chronotropic response [13, 14, 47–52].

The findings with vascular and nonvascular tissue indicate only enhancement by digitalis of the contractile response to either sympathetic nerve stimulation or catecholamines [57–65]. This has been demonstrated for both arteries and veins and for both alpha- and beta-adrenergic receptor responses.

Mechanism(s) by which digitalis exerts its neural effects

Focusing first on the mechanism of the excitatory effect of digitalis drugs on the afferent limb of the autonomic reflex arc, there is recent evidence to suggest that this effect is related to the capacity of these drugs to inhibit Na^+-K^+-activated ATPase associated with afferent nerve fibers. Saum *et al.* [20] have obtained data which indicate that ouabain prevents the postexcitatory depression of firing in aortic baroreceptor nerves. They postulate that postexcitatory depression is prevented because of ouabain-induced inhibition of a Na^+-K^+-activated ATPase, and propose that this might explain the neuroexcitatory actions of these drugs on baroreceptors.

The approaches taken for determining the mechanism(s) of the central autonomic actions of digitalis drugs have been aimed at: (1) identifying the site(s) within the CNS where these drugs act, and (2) identifying CNS neurotransmitters that mediate the CNS autonomic effects of these drugs. Studies employing the first approach have demonstrated that sympathetic responses occur when digitalis drugs are applied to the posterior hypothalmus [66], ventro-medial hypothalamus [67], lateral ventricle [67, 68], dorsal nucleus of the vagus [69], and nucleus tractus solitarius [69]. The most sensitive area for eliciting a sympathetic response is the posterior hypothalamus [66]. Parasympathetic responses have been shown to occur when these agents are applied to some of the areas listed above (dorsal nucleus of the vagus and nucleus tractus solitarius), as well as when these agents are injected into the fourth ventricle [70]. Thus, it appears that digitalis-induced sympathetic responses result primarily from activation of hypothalamic sites while digitalis-induced parasympathetic responses result primarily from activation of medullary sites.

Studies employing the second approach have focused primarily on the possibility that either a central adrenergic or serotonergic mechanism may be involved in mediating digitalis-induced autonomic effects. Stickney and Lucchesi [71] obtained data which suggested that an adrenergic mechanism was involved in the cardiovascular responses induced by centrally administered acetylstrophanthidin. They reported that centrally administered *d,l*-propranolol in a dose that did not affect peripheral beta-adrenergic receptors (1 mg) attenuated some of the cardiovascular effects (primarily the arrhythmias) produced by acetylstrophanthidin in dogs. The *d*-isomer of propranolol which does not possess beta-blocking activity did not exhibit this effect. Saxena and Bhargava [66] also reported that centrally administered beta-adrenergic blocking agents (propranolol, practolol and sotalol) prevented the centrally induced cardiovascular effects of ouabain in dogs and cats. The doses of the blocking agents employed in this study were much higher than in the previous study; however, two of the agents were shown not to have any peripheral beta-adrenergic blocking effects. In addition, Saxena and Bhargava [66] obtained evidence to indicate that central alpha-adrenergic receptors might be involved in mediating the central effects of ouabain. They found that piperoxan was effective in preventing the ouabain-induced cardiovascular responses.

Additional evidence for a role of central catecholaminergic mechanisms was obtained by Saxena and Bhargava [66] by employing other drugs known to impair activity of central adrenergic neurons. For example, when administered centrally Ro 4-1284, 6-hydroxydopamine (agents known to reduce the brain norepinephrine concentration), and bretylium (an agent known to prevent release of norepinephrine) were each found to prevent the cardiovascular responses induced by centrally administered ouabain in dogs and cats.

In contrast, Holloway *et al.* [72] reported that central administration of propranolol or phenoxybenzamine had no effect on centrally induced cardiovascular effects produced by ouabain in the dog. Similarly, Saito *et al.* [73] reported that centrally administered 6-hydroxydopamine had no effect on the cardiotoxic changes produced by intravenously administered ouabain in guinea pigs. Indeed, data reported by Ram and Hess [74] indicate that centrally administered 6-hydroxydopamine actually increases the susceptibility of rabbits to ouabain-induced ventricular arrhythmias. In their study, ouabain was also administered by the intravenous route.

In summary, it is difficult to draw conclusions as to whether a central adrenergic mechanism is involved in the CNS effects of digitalis drugs. It is difficult because of inconsistencies in existing data and because some of the crucial experiments required for obtaining the necessary information have not been performed. Data that does exist have been obtained by using different doses of adrenergic blocking agents and also by using doses which have not been shown either to exert blockade of central alpha and beta receptors [72], or to exert selective effects on these receptors [66]. A glaring inconsistency in the data is the differing results obtained in evaluating the role

of a central adrenergic mechanism in the action of centrally versus peripherally administered digitalis. For example, destruction of CNS adrenergic nerve endings by intraventricularly administered 6-hydroxydopamine has a profound effect on centrally induced cardiovascular changes cuased by digitalis [66] but has either no effect [73] or the opposite effect [74] on cardiovascular changes induced by peripherally administered digitalis. These conflicting results call into question the relevance of data obtained from studies in which digitalis drugs are administered into the CNS. Bircher *et al.* [75] have pointed out that drugs which modify the cardiovascular effects of centrally administered digitalis have no effect on cardiovascular responses produced by intravenously administered digitalis. More meaningful data might be obtained on the role of a central adrenergic mechanism by measuring biochemical correlates of central adrenergic neuronal activity during intoxication with peripherally administered digitalis. Likewise, electrophysiological studies should be performed correlating unit firing of central adrenergic neurons with increasing doses of digitalis administered by the intravenous route.

Studies examining the role of central serotonergic neurons in response to digitalis were begun by Buterbaugh and Spratt [76]. They demonstrated the importance of a serotonin mechanism in the lethal effects (i.e. respiratory depression) of intravenous digitoxigenin in the rat. However, no information was provided regarding the role of serotonin in the central autonomic effects of digitalis. To obtain information on this point, we pretreated cats with the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine (p-CPA) and found that these animals required higher doses of digitalis to produce cardiac arrhythmias. The same was true when animals were pretreated with the serotonin blocking drug methysergide [77]. In addition, we found that drugs which antagonize serotonin at the receptor level (e.g. methysergide, cinanserin and cyproheptadine) counteracted digitalis-induced ventricular arrhythmias [78]. We initially postulated that a central serotonergic mechanism was involved in the arrhythmigenic action of digitalis. Our postulate was based on two findings: first, destruction of central serotonergic neurons by centrally administered 5,7-dihydroxytryptamine seemed to increase the dose of digitalis required to cause ventricular arrhythmias [77], and second, intravenously administered deslanoside seemed to cause an increase in 5-hydroxyindoleacetic acid (5-HIAA) content and tryptophan hydroxylase activity [79] of several brain areas. However, we have since repeated the biochemical experiments of Morgenroth *et al.* [79] and have found no alteration in either the content of brain 5-HIAA or the activity of tryptophan hydroxylase.* Furthermore, the data that were used from the 5,7-dihydroxytryptamine experiments were selected on the basis of biochemical data that we now suspect were erroneous, showing serotonin depletion in some animals but not in other animals. Ignoring the biochemical data, an examination of the physiological responses in a larger series of animals indicates no effect of 5,7-dihydroxytryptamine on the dose of digitalis required to produce ventricular arrhythmias.

Additional evidence for a role of central serotonergic mechanisms in the arrhythmias produced by digitalis was sought by comparing the antiarrhythmic dose of methysergide when administered by the following routes: (1) intravertebral, (2) intracarotid (via the internal carotid arteries), and (3) intravenously. The doses required for exerting an antiarrhythmic effect for all three routes were similar.† Taken together, our data do not support the notion that central serotonergic mechanisms are involved in the arrhythmias produced by digitalis. Studies are underway to investigate the possible involvement of a peripheral serotonergic mechanism in this toxic response.

It should be pointed out that the provocative results of Buterbaugh and Spratt [76], wherein p-CPA was employed to test the role of central serotonin, could also be interpreted as a test for the role of peripheral serotonin, as this agent depletes the entire organism of this substance. The only strong evidence for a serotonergic mechanism in the central effects of digitalis is the recent study of Gaitonde and Joglekar [80]. These investigators studied the effects of p-CPA and 2-bromolysergic acid diethylamide on toxic effects produced by centrally administered digitalis in the cat. They found that both agents antagonized the toxic effects of digitalis. Furthermore, they reported that perfusion of the ventricular system of the brain with digitalis caused release of serotonin from brain tissue into the perfusate. Again, the problem with this type of study is relevance. Does systemic administration of digitalis excite central serotonergic mechanisms? Our own data indicate that it does not.

Only one study has dealt with the role of central cholinergic mechanisms in the CNS autonomic effects of digitalis. Rozear *et al.* [81] tested the effects of centrally administered anticholinergic agents (L-hyoscyamine and ethybenztropine) on the cardiovascular changes produced by administration of deslanoside into the fourth ventricle of dogs. Deslanoside administered by this route causes cardiovascular changes mediated primarily by vagus nerves. Doses of these antagonists that were demonstrated not to have peripheral vagolytic effects were shown to prevent the deslanoside-induced cardiovascular changes.

The role of a central dopaminergic mechanism(s) has also been examined in the central effects of digitalis. However, no data are available on the role of this neurotransmitter in the centrally mediated autonomic effects of digitalis. Digitalis has been reported to induce vomiting in cats by stimulating dopamine receptors in area postrema [82] and to produce catalepsy in mice and block apomorphine-induced gnawing behavior in rats by antagonizing central dopamine receptors [83]. In addition, there are three studies in which digitalis administration has been reported to alter the synthesis of brain dopamine. These studies [84–86] indicate an increase in dopamine levels. One other study indicates no change in dopamine level [87]. (No data are available on the influence of digitalis on the turnover of dopamine.)

If it is true that digitalis drugs do stimulate neurones in the CNS that exert control over autonomic

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activity, then the question arises as to the mechanism by which this stimulation is brought about. Saxena and Bhargava [66] suggest that digitalis depolarizes neurones probably through inhibition of Na^+-K^+-activated ATPase. There is no evidence that digitalis directly depolarizes neural membranes in quiescent structures of vertebrates [18, 30], but evidence does exist for this action in invertebrate systems [88]. Yarbrough [89] has suggested that a central neuroexcitatory effect of ouabain can occur by this agent acting to prevent norepinephrine and dopamine from stimulating Na^+-K^+-activated ATPase. Stimulation of ATPase by these neurotransmitters had previously been suggested as resulting in inhibition of neuronal firing [90, 91]. These proposed mechanisms involving inhibition of Na^+-K^+-activated ATPase are not supported by a recent study indicating that systemically administered toxic doses of digitalis have no effect on brain Na^+-K^+-ATPase [92].

Another possible explanation for the enhancement of central autonomic activity by digitalis is inhibition of neurotransmitter reuptake by nerve endings. It has been demonstrated that digitalis drugs will inhibit uptake of both norepinephrine and serotonin using brain slices or synaptosome preparations *in vitro* [93, 94]. Studies *in vivo* are needed to ascertain the relevance of these observations. Since inhibition of reuptake of these transmitters by digitalis *in vitro* is generally attributed to inhibition of Na^+-K^+-ATPase [95, 96], it is doubtful that inhibition of reuptake would occur *in vivo* by this mechanism, as no inhibition of Na^+-K^+-ATPase in the brain has been demonstrated under these circumstances [92].

In terms of a mechanism to explain some of the other neural effects of digitalis, especially spinal cord and efferent autonomic neuronal effects, inhibition of Na^+-K^+-activated ATPase has been invoked again [11, 30]. The rationale for invoking this mechanism appears to be that this enzyme is known to be inhibited by digitalis [97] and that the neural effects produced are consistent with inhibition of Na^+-K^+-activated ATPase [11, 30].

The mechanisms underlying digitalis action at neuroeffector autonomic junctions have been explored to some extent, but the findings have largely been descriptive. In terms of the cholinergic synapse, enhancement of cardiac responses (usually chronotropic) to both electrical stimulation of cholinergic nerves and injected acetylcholine has been observed. These responses to both types of stimuli were affected indicating that the mechanisms do not appear to be solely on the processes involved in acetylcholine release from the nerve ending. Thus, the action of digitalis to amplify the effects of both types of cholinergic stimuli on the heart appears to be due to an increase in excitability of the post-synaptic cell membrane. At one time, inhibition of cholinesterase had been suggested to be involved [98–106], but this effect has since been ruled out by numerous investigators [9, 10, 107–116].

The effect of digitalis at the postganglionic sympathetic synapse may involve not only alteration of the post-synaptic receptor sensitivity to released norepinephrine, but also an effect on: (1) the reuptake of norepinephrine into the nerve ending, (2) the synthesis and enzymatic breakdown of norepinephrine, (3) the "store" of norepinephrine, and (4) the release of norepinephrine from nerve endings.

The effect of digitalis on uptake mechanisms at the sympathetic neuroeffector junction has been studied by numerous investigators. Those that have examined the effect on uptake across the neuronal membrane have observed: an inhibition [95, 96, 117–126], no effect [127–129], and, in one instance, an increase [128]. The reasons for these variable results appear to be 2-fold. The primary reason appears to be the dose of the digitalis preparation employed. If the dose is high, as it usually is for studies *in vitro* (i.e. above 5×10^{-6} M), uptake can be significantly inhibited [125]. One should keep in mind that the toxic dose *in vivo* is much lower (i.e. 3×10^{-8} M in the case of the dog [130]). The other reason relates to the state of the animals. While normal animals may exhibit either no effect or an inhibition of uptake in the presence of digitalis, animals in heart failure exhibit an enhancement of norepinephrine uptake [128]. Those investigators that have examined the effect of digitalis on uptake of catecholamines into storage granules of nerve and chromaffin tissue have observed no effect [131–133]. The inability of digitalis to affect the granule transport system does not appear to be due to an inadequate dose, as doses of digitalis which inhibit uptake at the neuronal membrane were tested and found to be ineffective [133].

Some of those investigators that have observed inhibition of uptake have been able to relate this effect to inhibition of Na^+-K^+-activated ATPase [95, 96, 119, 120, 125], while others have not [123, 124, 129]. A crucial question regarding a digitalis effect on uptake is whether the effect can occur when toxic doses of digitalis are given to the whole organism. A recent preliminary study indicates that inhibition of uptake into the heart does occur *in vivo* in guinea pigs [134].

Digitalis has also been found to influence the synthesis of norepinephrine as well as one of the enzymes that is involved in the catabolism of this transmitter. Anagnoste and Goldstein [84] have reported that ouabain decreases the formation of norepinephrine in the hypothalamus, brain stem and cerebellum. This was later confirmed by Goldstein *et al.* [85], using slices of cerebral cortex. Initially, there was an increase in the synthesis of norepinephrine but, with a longer incubation time for the slices, there was a decrease in synthesis. Goldstein *et al.* in the same paper [85] reported that digitalis had no effect on tyrosine hydroxylase activity. Two investigators have reported that digitalis will inhibit monoamine oxidase activity [135, 136].

The effect of digitalis on tissue content of catecholamines has been studied by numerous investigators and again the results obtained cover the entire spectrum of possible effects. The number of papers reporting either a decrease or no change in tissue catecholamine content is about equally divided, while only two studies report an increase in tissue catecholamine content. Those reporting a decrease observed this effect in cardiac tissue [137—144], adrenal glands [136, 139], spleen [145], and brain [87, 140]. Those who reported no change also investigated effects on cardiac tissue [87, 128, 136, 146–148], adrenal glands [143], and brain [149]. In the two reports

where an increase was observed, it occurred in the heart with a lethal dose of digitalis administered to dogs [143], and in the heart and brain of rats given a LD_{50} dose of digitalis [140]. These disparate results do not appear to be related to the dose of digitalis employed, condition of the animals, or to the species studied.

The effect of digitalis on release of catecholamines from tissue again covers the entire gamut of possible changes. Most investigators have seen an enhanced release from cardiac [144, 150, 151], adrenal [152, 153] and splenic [145] tissue. Those that have reported a decrease were studying nerve granules or chromaffin granules [132, 152]. However, Koch-Weser [148] reported no change in the spontaneous release of norepinephrine from isolated perfused cat heart. Those investigators that have reported a decrease in release have observed this with either a high dose [144] or with prolonged perfusion of the organ with digitalis [145].

In summary, a great deal of investigative work has been performed to examine the effects of digitalis on the autonomic nervous system. In spite of the plethora of studies, only a few conclusions can be drawn. First, digitalis drugs clearly excite baroreceptors, chemoreceptors and the parasympathetic nervous system. Digitalis drugs inhibit the sympathetic nervous system, and although not as firmly established, appear, under certain circumstances, to excite the sympathetic nervous system. Multiple sites are involved in the effects of digitalis drugs on parasympathetic and sympathetic activity and knowledge of these sites of action is necessary for predicting the net effect of digitalis on sympathetic activity. It is controversial as to whether autonomic effects of digitalis drugs occur as a result of an interaction with CNS structures. Additional information bearing on this question should be forthcoming from experiments in which the effects of these drugs are studied on biochemical and electrophysiological correlates of CNS neuronal function. There is a great deal of data on the autonomic effects of centrally administered digitalis drugs. However, the value of this information is uncertain unless it can be demonstrated that systemic administration of these drugs produces the same kinds of changes in autonomic function as central administration, and that the peripheral autonomic responses can be modified by the same autonomic blocking drugs.

Little can be concluded on the effect of digitalis drugs on uptake, storage, and release of autonomic neurotransmitters. The data that have been obtained are contradictory, and relevance should be established wherever possible by performing studies using preparations *in vivo*. Finally, there appears to be a large body of evidence that digitalis drugs affect every tissue directly by inhibiting Na^+-K^+-ATPase [154]. However, according to one of the leading proponents of this hypothesis [154], "evidence in favor of this hypothesis is not completely secure." Caution dictates that this view should not be taken as dogma and used to confirm or refute an action of digitalis drugs in neural tissues [92].

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