The Effects of Digitalis Glycosides on the Ventricular Fibrillation Threshold in Innervated and Denervated Canine Hearts

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SUMMARY

Digitalis glycosides have been implicated in increased vulnerability to ventricular fibrillation in man. In order to investigate the genesis and occurrence of ventricular fibrillation in the presence of digitalis, the effects of both acetylstrophanthidin and ouabain on the ventricular fibrillation threshold (VFT) were studied in the open-chest anesthetized dog. The current required to induce ventricular fibrillation was determined by passing a train of 12 constant current pulses through epicardial electrodes during the vulnerable period of the cardiac cycle. It was found that an intravenous bolus infusion of acetylstrophanthidin (0.050-0.097 mg/kg) or ouabain (0.035-0.075 mg/kg) in intact innervated dog hearts raised the VFT from 40% to 260% above control values. Continuous infusions of acetylstrophanthidin to toxic levels also resulted in an elevated VFT. Vagotomy alone did not qualitatively change the effects of acetylstrophanthidin on VFT. However, following vagotomy and stellate sympathectomy, infusions of both toxic and subtoxic doses of acetylstrophanthidin resulted in a decrease in the VFT from 40 to 80% below control values. In denervated animals in which the peripheral ramifications of the left stellate ganglion nerves were stimulated, the VFT decreased below control values in the absence of acetylstrophanthidin, but during stellate stimulation in the presence of acetylstrophanthidin the VFT was increased above control values. These studies demonstrated that the increase in VFT by digitalis in the healthy, innervated heart was mediated via an associated increase in sympathetic activity, in the absence of neural influences digitalis decreased the VFT.

Additional Indexing Words:
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DIGITALIS GLYCOSIDES have been implicated in an increased incidence of ventricular fibrillation in man. Several studies on post countershock arrhythmias have shown that digitalis lowers the electrical threshold for the initiation of ventricular arrhythmias. However, studies as to whether or not digitalis facilitates the development of ventricular fibrillation have not been reported. In the present studies, we investigated the occurrence and genesis of ventricular fibrillation in the presence of digitalis.

Methods

Thirty healthy mongrel male dogs weighing 7.27 to 20.9 kg were anesthetized intravenously with 30 mg/kg pentobarbital. After a tracheotomy was performed, each dog was ventilated on room air by means of a Harvard respiratory pump. Tidal volume and rate of respiration were determined by a weight-ventilation nomogram. The femoral artery and vein were cannulated for sampling arterial blood and administration of intravenous digitalis preparations. Blood gases were carefully monitored, and blood pH was kept within the physiologic range. A lead II electrocardiogram was monitored throughout the experiment. Rectal temperature was also monitored and maintained by an electric heating pad. Additional sodium pentobarbital anesthesia was given in doses of 65 mg as necessary throughout the experiment.

A right thoracotomy was performed, and the heart was suspended in a pericardial cradle with the right ventricle exposed. The sinus node was crushed to inactivate its pacemaker activity. The heart was then paced at a cycle length of 350 msec by placing a bipolar intramural electrode on the right atrial appendage.

The fibrillation-inducing electrodes, which consisted of two platinum electrodes 1 mm in diameter separated by 5 mm and embedded by acrylic, were sutured to the myocardium of the right ventricle. A gated train of 12 to 15 pulses 4

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msec in duration and separated by 10 msec was applied to the myocardium during the vulnerable period of the cardiac cycle. The positioning of the train was checked prior to each fibrillation measurement to assure the same pulse train positioning relative to the vulnerable period, regardless of the length of the Q-T interval as influenced by test drugs. Fibrillating current was delivered only after every twelfth heart beat. A stimulator using digital timing circuits programmed the sequence of atrial and ventricular pacing. The amount of fibrillating current delivered to the myocardium was measured directly on a Tektronix 564 memory oscilloscope as the voltage drop across the precision one kilohm resistor. Stimulating current was increased in 1 mA increments until fibrillation occurred; the heart was defibrillated within four seconds using a capacitor discharge direct current defibrillator. The minimum current to cause ventricular fibrillation was determined to be the ventricular fibrillation threshold (VFT) for that particular dog under that particular set of conditions. A minimum of 15 min elapsed between each VFT determination. A baseline fibrillation threshold was determined on each dog prior to the administration of the digitalis preparation, and the VFT was considered stable when four consecutive current readings for initiating fibrillation were within 10% of each other. Previous control studies show that VFT remains stable in dogs for the duration of the experiments. Our technique for determining VFT has been described in a previous publication.

For the experiments, the dogs were divided into two groups: dogs that had their autonomic nervous system intact throughout the experiment and dogs that had a bilateral vagotomy and bilateral stellate sympatheticctomy before the start of VFT measurements.

In each group, following determination of the baseline VFT values, the intravenous digitalis preparation was administered and VFT measurements were made every 15 min; either acetylstrophanthidin or ouabain was used as the digitalis preparation. The method of administration of acetylstrophanthidin was either as an intravenous bolus with no subsequent dosage or as a drip calculated to provide increasing blood levels of acetylstrophanthidin and consequent toxicity. In the few experiments where ouabain was used, it was always administered as a single bolus.

The administration of toxic doses of ouabain and acetylstrophanthidin resulted in a prolonged P-R interval and ectopic ventricular activity. We controlled the anticipated ectopic pacemaker activity by overdriving the heart at a constant heart rate, from the beginning of the experiment.

When stellate stimulation was performed, both vagi were severed and both stellate ganglia were ablated. Bipolar stimulating electrodes were placed on the left peripheral sympathetic nerves distal to the site of ablation of the left stellate ganglion. Square wave pulses 4 msec in duration at 10 Hz were used for stellate stimulation. The intensity of stimulation was sufficient to increase sinus rate by approximately 20% in a test stimulation before the sinus node was crushed. During VFT determinations the heart was paced at a constant rate fast enough so that control was not lost during stellate stimulation.

Results

Inervated Hearts

Figure 1 shows that the VFT increased in four dogs given a single intravenous bolus of acetylstrophanthidin. The abscissa of figure 1 indicates time in hours. Positive time is the time elapsed after the injected bolus of acetylstrophanthidin while negative time is the time during baseline VFT determination. Positive ordinate values indicate an increase in VFT (heart less vulnerable in the four animals). The earliest effects of acetylstrophanthidin on the VFT occurred 4 min after injection. The VFTs in each animal rose rapidly to peak values between 40 to 98% above baseline within 20 min after acetylstrophanthidin injection, with a steady decline in VFT, returning to predigitalis baseline values within 45–60 min after injection. The time of action of acetylstrophanthidin on the VFT corresponded well with the time course of plasma clearance of acetylstrophanthidin as reported by Selden et al. VFT measurements were made for at least one hour after values returned to baseline, and they remained at their respective pre-drug baseline values. Therefore, figure 1 demonstrates that in dogs with an intact sympathetic nervous system, a bolus of acetylstrophanthidin will cause an elevation in VFT values lasting approximately one hour; VFT values then return and remain at baseline levels.

In order to test whether long infusions of acetylstrophanthidin would cause a maintained elevation in VFT, experiments were performed in six dogs with intact sympathetic nervous systems in which a bolus of acetylstrophanthidin was followed immediately by a continuous intravenous drip of acetylstrophanthidin. Figure 2 is a representative experiment in which we established a VFT baseline, gave an initial intravenous 1 mg bolus of
acetylstrophanthidin (0.058 mg/kg), and then over the next 2½ hours slowly infused an additional 4 mg of acetylstrophanthidin intravenously. The total digitalis administered (5 mg) over the 2½ hours amounted to 0.23 mg/kg. Within 15 min the VFT increased by 110%, and then for the next 2 hours and 15 minutes the VFT fluctuated between a 20% increase and a 129% increase. After 2½ hours the acetylstrophanthidin drip was terminated, and within 70 min the VFT decreased to preinfusion baseline values and remained there until the experiment was terminated. The sudden drop in VFT to 20% at about two hours in this individual experiment after the beginning of infusion is unexplained but probably represents a spurious value.

In the other five dogs, a bolus of acetylstrophanthidin was given followed by a continuous acetylstrophanthidin infusion drip that was maintained so that each dog received 5 mg acetylstrophanthidin over two hours. This total concentration is five times the reported toxic dose of acetylstrophanthidin. Following administration of acetylstrophanthidin the first effects on the VFT occurred at 4 min. The VFTs rose to between 60 and 260% above predrug levels and remained in this range until the experiment was terminated. The experiment was terminated in three cases because the dogs developed complete A-V block and in a fourth dog because the preparation became unstable. These results show a consistent elevation in VFT upon administration of acetylstrophanthidin in doses well into the toxic range.

In addition to the acetylstrophanthidin experiments, figure 3 shows that a single bolus of ouabain in two dogs with intact sympathetic nervous systems caused an elevation in VFT. After the establishment of a VFT baseline, each dog was given a bolus of ouabain at time 0. The abscissa and ordinate are the same as in figure 2. Following the injection of a single bolus of ouabain there was a gradual increase in VFT, reaching a maximum elevation at 55 to 115 min after the injection. The elevated VFT remained relatively constant at approximately 80% above baseline for the duration of the experiment. The rate of rise and duration of the elevated VFT corresponded well with the reported time onset and duration of action of ouabain. The VFT measurements were followed for 3 hours after the ouabain bolus was administered. Ideally we would like to have followed the VFT for the entire duration of action of ouabain, however, the action of ouabain does not begin to regress until 8 to 12 hours after injection, and this is usually beyond the limits of stability of our preparation.

**Derivated Hearts**

In contrast to the previous experiments with intact autonomic innervation, in all dogs in which vagotomies and stellate sympathectomies were performed prior to injection of digitalis, administration of digitalis caused a lowering of the VFT. This is demonstrated in figure 4 where the VFT decreased in four dogs who received a bolus of acetylstrophanthidin plus immediate continuous infusion of acetylstrophanthidin following bilateral vagotomy and stellate sympathectomy. Baseline VFT measurements were taken after bilateral vagotomy and bilateral stellate sympathectomy. At time 0 in figure 4, an intravenous bolus of acetylstrophanthidin was administered and followed immediately by an intravenous drip of acetylstrophanthidin. The first effects on VFT were

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**Figure 2**

The effect of continuous infusion of acetylstrophanthidin on VFT in one dog with an innervated heart. Abscissa, shows time in hours. Ordinate, shows percent change from baseline VFT. Bolus was injected at time 0 with an intravenous drip started immediately thereafter. The drip was terminated at time 2½ hours. Bolus dose was 0.058 mg/kg; total acetylstrophanthidin dose over 2½ hours was 0.23 mg/kg.

**Figure 3**

The effect of a single dose of ouabain on VFT in two dogs with innervated hearts. Abscissa, shows time in hours. Ordinate, shows percent change from baseline VFT. Ouabain bolus was injected at time 0. Solid circles indicate the dog was given a 0.075 mg/kg bolus of ouabain; X's, 0.035 mg/kg bolus of ouabain.
observed after 2 min. Within 15 min after the administration of the drug, the VFT decreased from baseline values to minimum levels between 40 to 80% below baseline. These VFT values remained decreased throughout the duration of the experiment. Three experiments had to be terminated because the dogs could not be defibrillated once fibrillation was induced by a train of pulses. In conclusion, the data from figure 4 shows that in denervated dog hearts, acetylstrophanthidin lowers the VFT.

Figure 5 presents the results of an experiment in which boluses of acetylstrophanthidin were given to a dog before and after bilateral stellate sympathectomy. The ordinate is the percent change in VFT from control value. The abscissa is the elapsed time from the beginning of the experiment. Before the initiation of the VFT determinations, the animal was bilaterally vagotomized. At the time indicated by A, a 0.10 mg/kg bolus of acetylstrophanthidin was given intravenously. Following this bolus the VFT increased to a maximum of 79% above control values and returned to control within one and a half to two hours. At B, a second bolus of 0.05 mg/kg was given. The VFT again increased reaching a maximum VFT of 100% above control values. The preparation returned to control within one and a half hours following the bolus at B. The behavior of the preparation described in figure 5, with stellate innervation intact and vagi cut following boluses, given at A and B is comparable to the data described previously in figures 1 through 3. Again, the decreased vulnerability to fibrillation associated with digitalis administration in sympathetically innervated hearts is demonstrated. In figure 5 at about four and one half hours after the beginning of the experiment, bilateral stellate sympathectomy was performed. Following sympathectomy, at the time indicated by C, a third bolus of acetylstrophanthidin was administered (0.05 mg/kg).

In contrast to the previous two boluses, acetylstrophanthidin in this case caused the VFT to decrease, falling to a minimum of 40% below control value. The behavior of the preparation in figure 5, following the bolus of acetylstrophanthidin at C, is comparable to the data of figure 4, and demonstrates the increased vulnerability to ventricular fibrillation associated with digitalis administration in sympathetically denervated preparations.

In each of the animals of the preceding experiments the administration of acetylstrophanthidin in the doses described (0.05 to 0.36 mg/kg) caused the appearance of ectopic ventricular activity within about 3 to 8 min. In some cases there was also P-R interval prolongation. These results agree with the data of Klein et al. and Hayes et al. with regard to both toxic dose levels and time of onset of arrhythmias following bolus and infusion of acetylstrophanthidin. In our experiments, the above doses of acetylstrophanthidin produced the initial changes in the VFT within 2 to 5 min after injection. Effects on the VFT could be observed before toxic effects were manifest in the electrocardiogram. That is, toxicity was not re-

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**Figure 4**

The effect on VFT of continuous infusion of acetylstrophanthidin on 4 dogs with denervated hearts. Abscissa shows time in hours. Ordinate, shows percent change of VFT from baseline. At time 0 each dog received a bolus of acetylstrophanthidin, and immediately thereafter an intravenous drip of acetylstrophanthidin was started. Solid circles represent the dog given a 0.091 mg/kg bolus of acetylstrophanthidin plus a 0.363 mg/kg infusion over 2 hours; X's represent the dog given a 0.071 mg/kg bolus plus a 0.126 mg/kg acetylstrophanthidin drip over one hour; open circles represent the dog given a 0.057 mg/kg bolus plus a 0.231 mg/kg acetylstrophanthidin drip over 1½ hours, and dotted circles represent a dog given a 0.097 mg/kg bolus of acetylstrophanthidin.

**Figure 5**

The effect of acetylstrophanthidin on the VFT in a representative dog pre and post stellate sympathectomy. Before the initiation of VFT measurement bilateral vagotomy was performed. Abscissa shows time in hours. Ordinate shows the percent change from control VFT. At Time A a 0.10 mg/kg bolus of acetylstrophanthidin was injected. At Time B a 0.05 mg/kg bolus of acetylstrophanthidin was injected. At 4½ hours the stellate ganglia were severed, at Time C the dog was given a 0.05 mg/kg bolus of acetylstrophanthidin.
required for an effect on the VFT. However, when the preparations exhibited toxicity the VFT was still increased in the innervated group and decreased in the denervated group.

Stellate Nerve Stimulation

The previous experiments have implicated the sympathetic nervous system in the increase of VFT associated with the administration of acetylstrophanthidin or ouabain. While we did not systematically study the influence of the vagus nerves on the VFT in response to digitalis, our preliminary experiments and the experiment depicted in figure 5 demonstrated no qualitative difference between preparations involving digitalis administration carried out with and without vagal innervation. The increase in VFT following digitalis occurred when the stellate ganglia were intact and the decrease in VFT following digitalis occurred when the stellate ganglia were ablated regardless of whether the animals were vagotomized. In order to verify further the role of the sympathetic nervous system with regard to the decreased vulnerability to fibrillation demonstrated in the present studies, we stimulated the stellate nerves in dogs having bilateral vagotomies and ablated stellate ganglia. In these preparations stimulating electrodes were placed on the left peripheral sympathetic nerves distal to the point at which the left stellate ganglion was ablated.

Figure 6 demonstrates the effect of left stellate nerve stimulation on the VFT in the presence and absence of digitalis. The ordinate is the percent change in VFT from control. The abscissa shows pooled data from three animals during control conditions, during stellate nerve stimulation, during acetylstrophanthidin administration, and during both stellate nerve stimulation and acetylstrophanthidin administration. The second column in figure 5 demonstrates that stellate nerve stimulation alone reduced the VFT by 40% of control values. Similar results have been reported previously by Han et al.9 The third column demonstrates that acetylstrophanthidin administration in these same preparations decreased the VFT. This is comparable to our data for denervation preparations presented in figures 4 and 5. The fourth column, however, shows an increase in VFT above control values during stellate nerve stimulation in the presence of acetylstrophanthidin. Therefore, these data demonstrate that stellate nerve stimulation makes the ventricle less vulnerable to fibrillation in the presence of acetylstrophanthidin while both acetylstrophanthidin and stellate nerve stimulation alone make the ventricles more vulnerable to fibrillation.

Discussion

Our experiments demonstrate that digitalis increases the threshold for ventricular fibrillation (makes fibrillation more difficult) in dog hearts which have an intact autonomic nervous system, and that digitalis lowers the threshold for ventricular fibrillation (facilitates fibrillation) in dogs which have been vagotomized and stellactomized. Our results imply that there is a dual action of digitalis affecting the VFT. First, there is a direct effect of digitalis on the heart which tends to lower VFT when the heart is denervated. In addition, there is an indirect effect of digitalis on VFT which is protective, and is centrally mediated via the autonomic nervous system. We feel that this protective effect is mediated primarily via the sympathetics. In preliminary experiments we observed that bilateral vagotomy alone did not eliminate the ability of digitalis to increase the VFT (figure 5).

Digitalis has a direct effect on pacemaker activity of the heart, causing increased diastolic depolarization leading to ectopic activity.8 This effect plus an indirect action via increased autonomic tone10 may lead to ventricular premature beats and tachycardia.

Digitalis toxicity has been responsible for overt arrhythmias and also latent arrhythmias exposed by electrical shock. The mechanism by which digitalis sensitizes the heart to electrical shock is unclear. Wittenberg and Lown11 found that the beta-blocking doses of propranolol do not block either the overt or latent arrhythmias. However, Ten Eick8 found that
propranolol decreased the number of arrhythmias produced by countershock in digitalized animals. Erlij and Mendez\textsuperscript{13} noted that adrenalectomy and sympathectomy increased the dose of digitoxin required to cause death. Roberts et al.\textsuperscript{14} has shown that catecholamine depletion with reserpine decreased ouabain induced ectopic beats.

In our experiments, digitalis made the heart less vulnerable to fibrillation even under conditions of digitalis toxicity, and sympathectomy eliminated this protective action.

It is important to discuss our observation that digitalis increases the VFT while also inducing ectopic ventricular activity. Han has shown that short coupling intervals and repetitive ventricular activity lead to a decrease in VFT.\textsuperscript{8} However, in our experiments heart rate was held constant by electrically over-driving ectopic activity induced by digitalis. Under these conditions of constant pacing, any digitalis-induced changes in cycle length were prevented from influencing the VFT. We confirmed the experiments of Han et al.\textsuperscript{8} that increases in sympathetic nerve activity do decrease the VFT in paced hearts (figure 6). However, our experiments showed in addition that stellate stimulation in the presence of digitalis increases the VFT in paced hearts.

The basis for the synergism between stellate nerve stimulation and digitalis in increasing the VFT cannot be determined from our experiments. However, we do believe that our observed increase in VFT induced by digitalis in the innervated paced heart (figures 1, 2, 3, and 5) is due to the associated increase in the autonomic activity induced by digitalis.\textsuperscript{10} When the heart is denervated the autonomic influence is lost. The primary effect of digitalis in decreasing the fibrillation threshold is unmasked (figs. 4 and 5). An increase in the ease with which ventricular fibrillation is initiated is associated with an increased temporal dispersion of recovery of cardiac muscles. Han and Moe\textsuperscript{18} have shown that there is increased temporal dispersion of recovery with ouabain intoxication in autonomically denervated dogs and an increase in temporal dispersion with sympathetic nerve stimulation in the absence of digitalis. However, they found that with subtoxic doses of ouabain there is a little change in temporal dispersion. Our observation that both toxic and nontoxic doses of ouabain or acetylstrophanthidin in innervated dog hearts increase the VFT suggests that this should be accompanied by a decrease in temporal dispersion. In contrast the drop in VFT in denervated hearts should be associated with an increase in temporal dispersion. We are currently exploring these possibilities that complex changes in the temporal dispersion of excitability may parallel our changes in ventricular fibrillation threshold in response to digitalis in innervated and denervated hearts.

We are also currently investigating the effect on VFT of cardiac glycosides in combination with beta-adrenoceptor blocking agents. Preliminary data suggest that there is a decrease in VFT in dogs administered digitalis and propranolol.

We would like to emphasize that our experiments were performed on dogs with healthy hearts, paced at a constant heart rate. The cardiovascular system of such animals is in a much more controlled state than encountered in the complex clinical conditions in patients with ischemic heart disease or congestive heart failure. It is difficult to make broad applications of our data to the clinical setting of diseased hearts. However, our findings do strongly suggest that, although the neural component of digitalis' action may act to increase arrhythmias in some instances, it also definitely acts to decrease vulnerability to fibrillation. Our present studies suggest that it may be more beneficial to treat digitalis-induced arrhythmias with agents that do not interfere also with autonomic activity.

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