Correlation of Antiarrhythmic Effects of Diphenylhydantoin with Digoxin-Induced Changes in Myocardial Contractility, Sodium-Potassium Adenosine Triphosphatase Activity, and Potassium Efflux


ABSTRACT

To clarify the suppressant action of diphenylhydantoin (DPH) on digitalis-induced arrhythmias we studied effects of DPH and digoxin, alone and in combination, on contractile force, sodium-potassium adenosinetriphosphatase (Na⁺-K⁺ ATPase) activity, and potassium (K⁺) balance in dog hearts perfused with Krebs-Ringer's solution. Neither control perfusion nor DPH alone (3 × 10⁻⁵ M) altered Na⁺-K⁺ ATPase activity or produced K⁺ loss; DPH alone depressed contractile force. Digoxin alone (10⁻⁶ M) caused a 59% rise in contractile force at the onset of arrhythmia along with a net rate of K⁺ loss (K⁺ efflux) of 50 ± 12 μmoles/min and a decrease in Na⁺-K⁺ ATPase activity from 13.8 to 5.2 μmoles phosphorus/mg protein hour⁻¹ (P < 0.001). Perfusion with combined DPH and digoxin delayed toxicity by 13 minutes (or 82%), at which time contractile force was higher (92% above base line, P<0.05), K⁺ efflux was greater (87 ± 20 μmoles/min), and Na⁺-K⁺ ATPase activity was lower (2.6 μmoles/mg hour⁻¹, P<0.05) than they were with digoxin alone. Combined DPH and digoxin perfusion lasting only until the time toxicity appeared with digoxin alone, however, yielded higher Na⁺-K⁺ ATPase activity (7.7 μmoles/mg hour⁻¹, P<0.02) and less rise in contractile force (25% above base line, P<0.05). Thus, DPH appeared to retard digoxin-induced inhibition of Na⁺-K⁺ ATPase activity. Nevertheless, digoxin in the presence of DPH ultimately produced greater inhibition of Na⁺-K⁺ ATPase activity, greater increase in contractile force, and greater K⁺ efflux prior to toxicity than did digoxin without DPH. These findings suggest that the antiarrhythmic effects of DPH cannot be attributed solely to prevention of inhibition of Na⁺-K⁺ ATPase activity or to diminution of K⁺ efflux, two changes characteristically accompanying digoxin administration.

KEY WORDS  cardiac glycoside  arrhythmia  ionic flux  perfused hearts  dog

The efficacy of diphenylhydantoin (DPH) in treating ventricular extrasystoles and ventricular tachycardia due to digitalis toxicity is well established (1–8). Whether this antiarrhythmic action represents a specific antagonism of digitalis (9, 10) or whether it merely reflects a nonspecific tendency of DPH to suppress all ventricular ectopic activity, regardless of etiology (11, 12), remains uncertain. To help discriminate between these possible modes of action, we studied the ability of DPH to modify digitalis-induced potassium (K⁺) loss and inhibition of sodium-potassium adenosinetriphosphatase (Na⁺-K⁺ ATPase) activity, two biochemical alterations thought to be involved in the genesis of digitalis-related arrhythmias (13–17). A specific interaction of DPH and digoxin might be expected to affect the development of these alterations during digitalis administration, but a nonspecific action of DPH would leave these aspects of the digitalis-induced effect unchanged.

Methods

Isolated dog hearts were perfused with Krebs-bicarbonate solutions containing either (1) no drug (control perfusion), (2) DPH alone, (3) digoxin alone, or (4) a combination of digoxin and DPH; simultaneously cardiac rhythm, contractile performance, and myocardial potassium balance were evaluated. These measurements were then compared with values of
Na⁺-K⁺ ATPase activity in samples of ventricular myocardium obtained at the termination of the perfusion. In all seven hearts perfused with digoxin alone and in a group of five hearts receiving both digoxin and DPH, perfusion was terminated and tissue was obtained for analysis of Na⁺-K⁺ ATPase activity just at the onset of toxic arrhythmia (ventricular tachycardia or sustained multifocal ventricular extrasystoles). This procedure permitted comparison of enzyme activity (and other parameters) at the onset of digoxin toxicity in the presence and the absence of DPH. However, in every instance, the time required to reach toxicity with combined digoxin and DPH exceeded the longest time of perfusion with digoxin alone. Thus, to compare experiments of equal duration, seven additional combined DPH-digoxin perfusions were terminated prior to toxicity at exactly the same times as the (toxic) end points of the seven digoxin perfusions. Comparisons were then made between paired studies ending at the same time. Each type of perfusion was performed in rotation to minimize the possibility of a systematic error due to possible changes in technique during the course of the studies.

IN SITU ISOLATED, PERFUSED HEART

Following the administration of sodium pentobarbital (30 mg/kg, iv), 30 mongrel dogs of both sexes (18-25 kg) were ventilated with room air and a midline thoracotomy was performed. A large-bore drainage cannula was introduced into the right ventricle via the right atrial appendage and a similar cannula was placed in the left ventricle through the apex (Fig. 1).

Cannulas for perfusion and for pressure measurement were introduced into the proximal aortic arch and a Walton-Brodie strain-gauge arch was sewn to the right ventricle. Perfusion was then initiated with the control Krebs solution at a rate of 150 ml/min. The two ventricular drains were vented, and ligatures were tied about the distal aortic arch, the brachiocephalic artery, the venae cavae, and the pulmonary trunk. Perfusion fluid exiting via the right ventricular drain was collected for sampling purposes but was not recycled. Siphoning action in the drainage tubing maintained slightly subatmospheric pressures in both ventricles. Once flow rates stabilized, less than 1% of the perfusion fluid exited via the left ventricular drain. After perfusion was initiated the sinoatrial node was crushed and the heart rate was maintained at 120 beats/min by electrical stimulation of the right atrium. The portion of right ventricular muscle under the strain gauge arch was stretched approximately 50% until maximum peak contractile force was attained (18). Control perfusion was continued for 15-25 minutes until stability (defined as less than 5% change in peak contractile force over a 5-minute period) was achieved. Then the definitive perfusion was begun with Krebs solution containing either digoxin, DPH, or both drugs; in the control perfused hearts, perfusion with control Krebs solution was continued until the dogs were killed.

PERFUSION FLUID

The perfusion medium, a Krebs-bicarbonate solution, was warmed and equilibrated with 95% O₂-5% CO₂. Fluid was passed through a column packed with silicone-coated stainless steel sponge. Final fluid temperature averaged 36.3°C. Each solution contained glucose, 5.6 mM, and had the following millimolar composition: K⁺ 3.7, Na⁺ 145, Cl⁻ 139, HCO₃⁻ 25, Ca²⁺ 2.5, H₂PO₄⁻ 1.2, and Mg²⁺ 1.2. In addition, solutions used for drug perfusion contained either 1 μM digoxin (Burroughs Wellcome Company) or 30 μM DPH sodium (Parke, Davis, and Company), or both. To ensure its sustained solubility, each drug was introduced in its commercial solvent. The resulting propylene glycol and ethanol content of solutions containing digoxin alone and those containing both digoxin and DPH were very nearly the same. Thus differences observed in the crucial comparison of the effects of digoxin alone and those of digoxin combined with DPH cannot be ascribed to differences in solvent content of the perfusion medium.

Every 5 minutes simultaneous inflow and outflow samples were obtained for determination of K⁺ concentrations, Po₂, PCO₂, and pH. Using a flame-emission spectrophotometer, four replicate determinations of K⁺ concentration were performed for each sample, and the results were averaged. This technique yielded a mean standard error in net rate of K⁺ loss of ± 5.7 μEq/min.

TISSUE STUDIES

At the termination of each experiment the heart was perfused for an additional 3 minutes with drug-free Krebs-bicarbonate solution chilled to 0°C. The free wall of the left ventricle was excised and a particulate

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microsomal fraction was prepared immediately according to the method of Matsui and Schwartz (19). This method was modified in that the third and the fifth centrifugations were carried out at 100,000 g for 45 minutes and the Na purification steps were omitted.

Na\(^+\)-K\(^+\) ATPase activity present in the particulate fraction was assayed the following morning. The material that had been prepared and frozen the previous day was diluted with distilled water to a protein concentration of approximately 0.5 mg/ml. Five samples of 0.2 ml (0.1 mg of protein) were each added to 0.8 ml of stock solution to produce final concentrations of 2 mM disodium ATP, 5 mM MgCl\(_2\), and 100 mM Tris hydrochloride (adjusted to pH 7.4). After a 4-minute incubation at 37°C the reaction was stopped by the addition of 0.5 ml of a cold solution containing 0.2 ml of 10% ammonium molybdate, 0.2 ml of 10 mM H\(_2\)SO\(_4\), and 0.1 ml of 0.2M silicotungstic acid. The free orthophosphate was extracted with 3 ml of isobutanol, and 1.0-ml samples of the organic layer were assayed for phosphate according to the method of Fiske and Subbarow (20). Protein concentrations were determined by the method of Lowry and co-workers (21). An additional five samples of the myocardial particulate fraction were also assayed in the exact manner just described except that, along with reagents listed above, the incubation mixtures also contained 120 mM NaCl and 30 mM KCl. The mean difference in orthophosphate content between these latter five incubation mixtures (high Na\(^+\) and K\(^+\) ) and the former five (low Na\(^+\), no K\(^+\) ) was used as a measure of Na\(^+\)-K\(^+\) ATPase activity.

At the time of excision of the left ventricle, an approximately 3-g midwall portion of the interventricular septum was also removed to determine water content. The tissue was patted dry, weighed, minced, placed in a tared aluminum foil container and heated by an infrared lamp until it achieved a stable weight (24-48 hours).

**Results**

**Perfusion Fluid Characteristics and Tissue Water Content**

Perfusion fluid entering the coronary arteries had a P\(_{O_2}\) ranging between 450 and 600 mm Hg, a P\(_{CO_2}\) ranging between 32 and 42 mm Hg, and a pH ranging between 7.36 and 7.48. Perfusion fluid pressures (mean 24.3 mm Hg) and temperatures were very similar for all the studies. Values given were measured immediately prior to termination of perfusion. However, perfusion pressure remained within narrow limits (± 10%) throughout the course of each experiment. Because the perfusion rate was held constant, these results indicate that coronary vascular resistance remained stable during the study.

Perfusion fluid exiting from the coronary sinus tended to have a lower P\(_{O_2}\) and pH and a higher P\(_{CO_2}\) in hearts receiving digoxin. In no instance did P\(_{O_2}\) fall below 75 mm Hg, suggesting that oxygen delivery was adequate to meet the metabolic requirements of the myocardium (22).

Tissue water content measured by desiccation averaged 82.5 ± 1.0% (SD) in 16 hearts at the termination of perfusion. This value did not differ from the mean for 4 nonperfused hearts, 82.6 ± 2.6%. There was no difference in tissue water content when brief perfusions were compared with longer perfusions, and digoxin or DPH, alone or combined, did not produce consistent differences. Thus, despite the absence of osmotically active colloidal substances in the perfusion fluid, there was no evidence for a sustained tendency to accumulate tissue water during the course of perfusion.

**Contractile Force**

Peak contractile force (Fig. 2) showed no consistent change during the course of six control perfusions. Five hearts perfused with DPH alone manifested a consistent, sustained reduction in contractile force averaging 29% below base-line values at the time of death. Peak contractile force rose progressively, however, in the seven hearts receiving digoxin alone and attained a mean maximum increment of 59% above base-line values at the onset of toxicity, which occurred after an average of 15.9 minutes of digoxin perfusion. Combined perfusion with both digoxin and DPH produced a delayed rise in peak contractile force, which reached an increment of only 26% by 15.9 minutes. Toxicity did not occur during combined perfusions, however, until much later; the mean perfusion time for these hearts was 28.9 minutes. During these more prolonged perfusions (five hearts), contractile force continued to increase progressively and reached an average increment of 92% just prior to toxicity, a value significantly greater than that achieved with digoxin alone (P < 0.05). Thus, in agreement with results obtained in other preparations (9, 10), suppression of toxic arrhythmias by DPH ultimately permitted digoxin to exert a greater inotropic influence prior to toxicity despite the intrinsic depressant properties of DPH.

**Sodium-Potassium Adenosine Triphosphatase Activity**

Assay of Na\(^+\)-K\(^+\) ATPase activity derived from the myocardium at the termination of perfusion yielded indistinguishable values for nonperfused, control-perfused, and DPH-perfused hearts (Fig. 3). Perfusion with digoxin alone until the onset of toxicity produced the expected reduction in Na\(^+\)-K\(^+\) ATPase activity.
Mean peak contractile force just prior to death, expressed as percent change from control value, is shown for the five groups studied. The number of hearts in each group is shown within each bar. Vertical lines intersecting each bar represent standard errors of the mean. The control group was perfused without drug, the DPH group with diphenylhydantoin (30 μM), and the digoxin tox. group with digoxin (1 μM) until onset of toxic arrhythmias. Of the two groups receiving digoxin and DPH (cross-hatched), the match group, killed (prior to toxicity) at the same time as members of the digoxin tox. group, failed to achieve the same contractile response as the digoxin tox. group, but the group receiving digoxin and DPH to toxicity (tox.) greatly exceeded the contractile performance of the digoxin tox. group.

ATPase activity to 5.2 μmoles phosphorus/mg protein hour⁻¹ or 38% of values obtained after control perfusion. Combined perfusion with DPH and digoxin until the onset of toxicity was associated with an even lower mean value, 2.6 μmoles phosphorus/mg hour⁻¹, than that seen with digoxin alone (P<0.05). Hence, the greater contractile response achieved just prior to toxicity with combined DPH and digoxin was accompanied by evidence of greater inhibition of Na⁺-K⁺ ATPase activity. However, combined perfusions with DPH and digoxin halted prior to toxicity yielded slightly but significantly higher values of Na⁺-K⁺ ATPase activity with paired analysis than those observed after an equally long perfusion with digoxin alone. The difference averaged 2.5 μmoles/mg hour⁻¹ (P<0.02). This latter result suggests that DPH did cause a slight delay in digoxin-induced inhibition of Na⁺-K⁺ ATPase activity, although it ultimately permitted the progression of inhibition prior to toxicity far beyond that attainable in its absence.

Na⁺-K⁺ ATPase activity is shown for each heart. Horizontal lines indicate the mean of each group and brackets indicate the associated standard error. Perfused and nonperfused control hearts had nearly identical mean values. Diphenylhydantoin (DPH) perfusion failed to alter enzyme activity but digoxin alone at toxicity reduced Na⁺-K⁺ ATPase activity significantly (13.8 to 5.2 μmoles/mg hour⁻¹, P<0.001). Members of the combined digoxin-DPH group generally had higher values (7.7 μmoles/mg hour⁻¹, P<0.02) than did equally perfused members of the digoxin group (connected by lines). But the group receiving digoxin and DPH until toxicity had significantly lower values (2.6 μmoles/mg hour⁻¹, P<0.05) than did the digoxin group.

If the development of digoxin-induced arrhythmias is mediated through inhibition of Na⁺-K⁺ ATPase, the delayed onset of toxicity in the presence of DPH may possibly be due, at least in part, to a delay in digoxin-induced inhibition of Na⁺-K⁺ ATPase activity. This possibility may be analyzed using a plot of measured values of Na⁺-K⁺ ATPase activity against corresponding duration of perfusion (Fig. 4). Examination of the curve described by the data from combined DPH-digoxin perfusions reveals that the same degree of inhibition attained with digoxin alone (mean Na⁺-K⁺ ATPase activity 5.2 μmoles/mg hour⁻¹) after 15.9 minutes would be attained after approximately 19 minutes with combined DPH and digoxin. Thus retardation of digoxin-induced inhibition of Na⁺-K⁺ ATPase activity could account for only a small
DIPHENYLHYDANTOIN AND DIGOXIN

Figure 4

Na\(^{+}\)-K\(^{+}\) ATPase activity plotted as a function of the length of perfusion preceding death. Enzyme activities are shown for unperfused hearts, hearts receiving digoxin alone, and hearts receiving combined perfusions with digoxin and diphenylhydantoin (DPH). The mean value of Na\(^{+}\)-K\(^{+}\) ATPase activity 5.2 \(\mu\)moles/mg hour\(^{-1}\) observed after digoxin alone was attained after perfusion for an average of 15.9 minutes. The curve approximated by results after combined digoxin-DPH perfusion reaches this same level of Na\(^{+}\)-K\(^{+}\) ATPase activity at about 19 minutes, only 3 minutes later. This interval represents only 23% of the total 13-minute delay in toxicity achieved by DPH. Thus delay in Na\(^{+}\)-K\(^{+}\) ATPase inhibition appears to play only a minor role in the DPH-induced postponement of digoxin toxicity.

Figure 5

Time course of the rate of potassium loss (K\(^{+}\) efflux) for each group. Circles and triangles denote mean values and vertical lines indicate associated standard errors. Control and diphenylhydantoin (DPH) perfusion failed to produce significant K\(^{+}\) efflux, unlike digoxin, which resulted in an increasing K\(^{+}\) efflux. Combined digoxin and DPH initially produced less K\(^{+}\) efflux than did digoxin alone but eventually exceeded the K\(^{+}\) efflux of digoxin alone during the more prolonged course of the combined perfusion.

Myocardial Potassium Balance

Control perfusion and perfusion with DPH alone (Fig. 5) caused no consistent alteration in K\(^{+}\) balance. Digoxin perfusion produced a net rate of K\(^{+}\) loss (K\(^{+}\) efflux) which increased steadily with time. When DPH was also present in the perfusate K\(^{+}\) efflux tended to be slightly less after a given length of perfusion. K\(^{+}\) efflux continued to increase, however, throughout the more prolonged period of perfusion after the addition of DPH. At the onset of toxicity during perfusion with DPH and digoxin, K\(^{+}\) efflux tended to be even higher than that observed at toxicity with digoxin alone, particularly during the longest perfusions. The average K\(^{+}\) efflux after 30 minutes of perfusion with DPH and digoxin (109 \(\mu\)Eq/min) was significantly greater \((P<0.01)\) than the average rate of K\(^{+}\) loss just prior to toxicity with digoxin alone (49 \(\mu\)Eq/min). Thus, these data parallel results obtained when measuring Na\(^{+}\)-K\(^{+}\) ATPase activity in that an initial, relatively minor retardation in K\(^{+}\) efflux when DPH accompanied digoxin was ultimately followed by a greater K\(^{+}\) efflux than that attained prior to toxicity with digoxin alone.

The changes in K\(^{+}\) balance presented above reflect the net resultant of several processes affecting K\(^{+}\) uptake and loss. Thus, considered independently, our measurements of net K\(^{+}\) loss do not distinguish between an inhibition of active K\(^{+}\) intake due to depression of the Na\(^{+}\)-K\(^{+}\) pump and an increase in passive K\(^{+}\) loss resulting from a rise in K\(^{+}\) permeability.
Ionic shifts consequent to the digitalis-induced inhibition of Na\(^+\)-K\(^+\) ATPase and the resulting loss of myocardial K\(^+\) are generally thought to be responsible, at least in part, for the arrhythmogenic (13-17) and, perhaps, the inotropic (14, 22-27) properties of the cardiac glycosides. Thus it is possible that an agent that specifically antagonizes digitalis-induced arrhythmias might act either by interfering with glycoside inhibition of Na\(^+\)-K\(^+\) ATPase or by reversing the consequent potassium loss. Scherlag and co-workers (9) have suggested that DPH is a specific antagonist of digitalis; they have reported a reduction in myocardial potassium venoarterial difference when antiarrhythmic doses of DPH are administered to the dogs receiving toxic doses of acetylstrophanthinid. The significance of this finding is greatly circumscribed by the absence of correlative coronary flow data, thereby making calculation of actual K\(^+\) efflux impossible. Indeed, Miller and Gilmore (28) have reported that DPH fails to alter potassium efflux induced by acetylstrophanthinid, and more recently Spain and Chidsey (12) have found that DPH administration does not reverse Na\(^+\)-K\(^+\) ATPase inhibition in animals made toxic with ouabain.

To obtain a more complete evaluation of these hypotheses regarding the action of DPH, we examined the influence of an effective antiarrhythmic concentration of DPH on digoxin-induced augmentation of contractility, on myocardial potassium loss, and on the highly specific, digoxin-induced inhibition of Na\(^+\)-K\(^+\) ATPase in an isolated, perfused canine heart preparation. This preparation was similar to that used by Besch and co-workers (22), who were able to relate the increment in contractile performance during ouabain infusion to the degree of reduction in Na\(^+\)-K\(^+\) ATPase activity present in tissue fractions of the same hearts. Similar results have been obtained by Akera and co-workers (29) using the intact, open-chest dog. Thus digitalis-induced inhibition of Na\(^+\)-K\(^+\) ATPase, readily demonstrable in vitro, appears to be accurately reflected by the behavior of Na\(^+\)-K\(^+\) ATPase activity measured in myocardium perfused with pharmacologically active concentrations of ouabain.

Caution must be observed, of course, in extrapolating in vitro assay results to arrive at conclusions concerning enzyme activity in vivo. As Lee and Klaus (13) have indicated, the ionic environment and other factors modulating Na\(^+\)-K\(^+\) ATPase activity may be radically different in vitro and in vivo. Nevertheless, the consistently parallel course of Na\(^+\)-K\(^+\) ATPase inhibition, as assessed in vitro, and the physiological consequences of Na\(^+\)-K\(^+\) ATPase inhibition, namely myocardial K\(^+\) loss, support the assumption that in vitro assay results tend to reflect functionally significant enzyme inhibition in vivo.

The antiarrhythmic efficacy of the concentrations of DPH used in our study was evident. Perfusion with DPH combined with digoxin succeeded in postponing the development of toxic arrhythmias from an average of 15.9 minutes to an average of 28.9 minutes (an 82% increase). However, rather than attenuating the metabolic concomitants of digoxin action, our data suggest that DPH combined with digoxin resulted in an even greater degree of inhibition of Na\(^+\)-K\(^+\) ATPase and a more rapid loss of myocardial K\(^+\) before the development of toxic arrhythmias. Thus, in our preparation, DPH appeared to act primarily by nonspecifically suppressing toxic arrhythmias rather than by specifically interfering with the potentially arrhythmogenic metabolic alterations induced by digitalis. By measuring Na\(^+\)-K\(^+\) ATPase activity in matched preparations we were able to demonstrate a delay in digoxin-induced Na\(^+\)-K\(^+\) ATPase inhibition in the presence of DPH. In our studies, however, this delay appeared to play only a minor role in postponing toxicity.

The foregoing conclusions are based on the assumption that the arrhythmogenic actions of digoxin are closely related to myocardial K\(^+\) balance and Na\(^+\)-K\(^+\) ATPase activity. It is possible that the sites at which digoxin affects these myocardial metabolic parameters and the sites which mediate the toxic influence of digoxin are distinctly different in their pharmacologic properties. In that case, DPH could interact in a highly specific manner with digoxin with regard to arrhythmias and not affect digoxin-induced changes in the myocardial K\(^+\) balance or Na\(^+\)-K\(^+\) ATPase activity. At present, however, there is little evidence available to substantiate this possibility.

The results of our studies are somewhat at variance with those of Godfraind and co-workers (30), who found that DPH attenuated digitalis-induced reduction in tissue K\(^+\) content of guinea pig atria. However, these workers measured tissue K\(^+\) only after toxicity had been present for some time. Since toxic arrhythmias may themselves induce K\(^+\) loss (31), DPH possibly attenuated reduction in tissue K\(^+\) simply by shortening the period of arrhythmia rather than by primarily...
antagonizing the K⁺ losing influence of toxic doses of cardiac glycosides.

In addition to their significance relative to the antarrhythmic action of DPH, our results also demonstrate that digoxin-induced metabolic alterations are consistently linked to contractile enhancement even when longer perfusions with digoxin are permitted by suppression of toxic arrhythmias. The fact that the greater enhancement of contractility observed after more prolonged digoxin administration was accompanied by a greater inhibition of Na⁺-K⁺ ATPase activity and more rapid K⁺ efflux is consonant with the hypothesis that Na⁺-K⁺ ATPase inhibition and myocardial K⁺ loss mediate the inotropic actions of digitalis.

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References


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