Glucagon in Prevention and Abolition of Ouabain-Induced Ventricular Tachycardia in Normokalemic and Hypokalemic Dogs

By Stanley Einzig, Edward P. Todd, Demetre M. Nicoloff, and Russell V. Lucas, Jr.

ABSTRACT

A recent study suggested that glucagon has antiarrhythmic properties in addition to its positive inotropic effect. The present study was designed to evaluate the efficacy of glucagon in preventing and converting ouabain-induced ventricular tachycardia (VT) in normokalemic and hypokalemic dogs. Paired studies were made in ten normokalemic dogs and in eight dogs rendered hypokalemic by diet and hydrochlorothiazide. All dogs were pretreated with glucagon, and the amount of ouabain required to produce VT was measured. Each dog served as its own control in a second study in which normal saline was substituted for glucagon. When VT was produced during the control study, the response to a bolus injection of glucagon was evaluated. (1) Pretreatment with glucagon delayed the appearance of VT in hypokalemic dogs but not in normokalemic dogs. (2) Glucagon, by bolus injection, converted VT to sinus rhythm in 78% of the normokalemic dogs and in 100% of the hypokalemic dogs. (3) The mechanisms by which glucagon exerts its antiarrhythmic effect are not clearly defined.

KEY WORDS
digitalis toxicity overdrive suppression sinus tachycardia myocardial potassium flux atrioventricular conduction

Glucagon, a polypeptide hormone produced chiefly by the alpha cells of the pancreas, has an essential role in the maintenance of blood glucose levels and is known to affect several other organ systems (1-4). Considerable attention has recently been given to its positive inotropic properties because, unlike other agents that also improve myocardial function, glucagon does not increase myocardial irritability (5-16). Glucagon activates adenyl cyclase in the human heart and other tissues (17) and increases the speed of atrioventricular conduction (16, 18). These effects are apparently not mediated via the beta-receptor sites since they are not blocked by propranolol (5, 6, 16). The potential value of glucagon as an antiarrhythmic agent in converting digitalis (ouabain)-induced arrhythmias has been suggested by a recent study in dogs (19).

The purpose of this study was to determine (1) whether a constant glucagon infusion would protect dogs from ouabain-induced ventricular tachycardia (VT); (2) whether a bolus injection of glucagon would convert ouabain-induced VT to sinus rhythm; and (3) the effect of hypokalemia on the ability of glucagon to prevent or convert ouabain-induced VT.

Methods and Materials

Twenty adult mongrel dogs of both sexes weighing 8.6-24.1 kg were used. Ten were healthy normokalemic stock dogs and the other ten were rendered hypokalemic (arterial potassium < 3 mEq/liter) by hydrochlorothiazide (200 mg b.i.d.) and a potassium-deficient diet. Use of...
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this hypokalemic protocol in previous studies has shown that there are no significant changes in serum sodium, serum calcium, or pH during the first 2 months. A small increase in serum bicarbonate (19.4–23.0 mEq/liter) and a decrease in serum chloride (110–90 mEq/liter) occurs within the first month. All studies reported in the present paper were completed within the first month on the hypokalemic protocol. Hypokalemia occurred after 2–3 weeks on the regimen.

Studies A and B were performed on each dog about 1 week apart. The order of the studies was randomized. Study A was designed to determine if a constant glucagon infusion would protect the dogs from ouabain-induced VT. Study B was designed (1) to serve as a control to study A by infusing normal saline instead of glucagon; and (2) to determine if a bolus injection of glucagon would convert ouabain-induced VT to sinus rhythm.

All dogs were anesthetized with pentobarbital sodium (30 mg/kg iv). Positive pressure respiration was maintained through a cuffed endotracheal tube by a Harvard model 607 respirator.

A lead II electrocardiogram was continuously monitored. In addition, 6- to 10-second recordings of the ECG were made every 5 minutes, or more frequently when appropriate, on a Sanborn two-channel recorder.

The femoral artery and vein were cannulated with standard polyethylene catheters. Systemic blood pressure was measured by a Statham P23AA transducer and was recorded simultaneously with the lead II electrocardiogram. Rectal temperature was recorded during the procedure.

STUDY A

The dogs were ventilated 20 minutes to allow stabilization. Before infusion, blood was drawn and arterial serum potassium was measured in duplicate on a flame photometer. Glucagon2 infusion at a rate of 2.5 μg/kg min⁻¹ was started, and after 10 minutes, blood was again drawn to measure arterial potassium and pH. The animal was then given a bolus injection of ouabain (20 μg/kg) through the venous catheter. After 10 minutes, a ouabain infusion at a rate of 2.5 μg/kg min⁻¹ was started and run simultaneously with the glucagon infusion.

Ouabain was discontinued when VT occurred and lasted 20 seconds. Previous experience indicated that if it lasted 20 seconds, it would persist at least 30 minutes. In each dog the VT did persist 30 minutes, at which time the study was terminated.

The total dose of ouabain (μg/kg) required to produce VT was determined. Blood samples for measuring arterial potassium level were drawn at the onset of the arrhythmia and 10 minutes after arrhythmia began.

STUDY B

The protocol was precisely the same as study A except normal saline was substituted for glucagon. The total dose of ouabain required to produce VT was determined. Five minutes was allowed to ensure that VT was persistent; then glucagon (25 μg/kg) was given as a bolus injection through the venous catheter. If VT persisted or recurred, a second and sometimes a third injection of glucagon (25 or 50 μg/kg) was given.

The only other change in protocol was that a fourth sample for arterial potassium evaluation was drawn 10 minutes after the first bolus injection of glucagon.

The amount of blood withdrawn for studies (60 ml) was less than 10% of the smallest dog's blood volume. There was no significant drop in body temperature in any of the dogs. The solutions of ouabain and glucagon (and normal saline control) were so formulated that the total rate of infusion was 1.8 ml/min.

Hypoxia is known to be an important factor in the production of cardiac arrhythmia (20). Care was taken to maintain adequate oxygenation and pH values were maintained in the normal range (7.37–7.47). During study A, there was a slight increase (4 mm Hg) in systolic blood pressure in all dogs 5 minutes after the glucagon infusion was started. At 10 minutes, just before the ouabain injection, the pressures had returned to control levels in the hypokalemic dogs but remained minimally elevated in the normokalemic dogs. Diastolic pressure had decreased (19 mm Hg) in all dogs after 10 minutes of glucagon infusion. Differences in systolic and diastolic blood pressures between normokalemic and hypokalemic dogs were not statistically significant.

The ouabain doses were in the range other investigators have found to produce a significant, persistent arrhythmia (21). Ouabain induced VT in 16 of the 20 dogs. Two of the remaining four dogs (one normokalemic and one hypokalemic) developed nodal tachycardia, and the other two dogs (both hypokalemic) developed ventricular fibrillation. These four dogs were excluded from statistical analysis.

Results were analyzed by a two-tailed t-test for paired samples, corrected for the number of comparisons (22).

2Crystalline glucagon was provided by Eli Lilly Co. (Lot 258 234 N-167-1). The standard diluent as prescribed for clinical use was utilized and care taken to keep glucagon in solution.
Heart rate recorded on the vertical axis is sinus rate except those points labeled VT (ventricular tachycardia). The mean and standard error of the mean are recorded. Pretreatment with glucagon caused an increase in heart rate in both normokalemic and hypokalemic dogs. Sinus rate recorded immediately prior to VT in the normokalemic dogs was higher than the subsequent VT rate, while in the hypokalemic dogs there was no difference. In the control study saline infusion caused no significant change in sinus rate. The sinus tachycardia (ST), which resulted after the bolus injection of glucagon, was in all cases faster than the preceding VT rate in both groups of dogs.

### Results

**Heart Rate.**—Glucagon infusion caused an increase in sinus rate in both normokalemic and hypokalemic dogs (Fig. 1). The maximum increase in sinus rate was 116 beats/min in the normokalemic dogs and 65 beats/min in the hypokalemic dogs. The sinus tachycardia rates immediately before VT were comparable in both groups of dogs. The VT rate was slower than the immediately preceding sinus tachycardia rate in the normokalemic dogs (Fig. 2), whereas the VT rate in the hypokalemic dogs was equal to the preceding sinus tachycardia rate (Fig. 3).

**Atrioventricular Conduction.**—AV dissociation occurred just before the onset of VT in two normokalemic dogs and one hypokalemic dog. In these three dogs, the atrial rate was slightly less (15 beats/min) than the sinus rate before AV dissociation. In the remaining hypokalemic and normokalemic dogs, the AV conduction time (P-R interval) increased by an average of 0.03 seconds from control sinus rate recorded immediately prior to VT in the normokalemic dogs was higher than the subsequent VT rate, while in the hypokalemic dogs there was no difference. In the control study saline infusion caused no significant change in sinus rate. The sinus tachycardia (ST), which resulted after the bolus injection of glucagon, was in all cases faster than the preceding VT rate in both groups of dogs.

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RATE TO SINUS RATE BEFORE VT. ONE NORMOKALEMIC DOG HAD A TRANSIENT PERIOD OF SINUS ARREST WITH NODAL ESCAPE BEATS FOLLOWED BY SINUS TACHYCARDIA AND THEN VT. THE MAJORITY OF DOGS HAD OCCASIONAL PREMATURE VENTRICULAR CONTRACTIONS IMMEDIATELY BEFORE THE ONSET OF VT.

PREVENTION OF OUABAIN-INDUCED VT BY PRETREATMENT WITH GLUCAGON.—Glucagon was not effective in delaying the onset of VT in normokalemic dogs (Table 1). On the other hand, it was effective in delaying the occurrence of ouabain-induced VT in hypokalemic dogs. The hypokalemic dogs pretreated with glucagon required the same amount of ouabain \( (P = 0.10) \) to produce VT as the normokalemic control dogs, whereas hypokalemic control dogs required less ouabain to produce VT.

CONVERSION OF VT TO SINUS TACHYCARDIA BY GLUCAGON BOLUS.—Glucagon \( (25 \mu g/kg) \) bolus injection abolished the ouabain-induced VT in seven of nine normokalemic dogs. Abolition occurred between 45 seconds and 3 minutes after injection. In most animals VT was replaced by sinus rhythm within 1 minute after injection. Duration of sinus rhythm was prolonged (over 10 minutes) in six of the seven animals that responded. The glucagon bolus abolished the ouabain-induced VT in all seven hypokalemic dogs—in most of them within 1 minute of injection. Duration of sinus rhythm was prolonged (over 10 minutes) in six of the seven dogs. In the seventh dog, it persisted for 8 minutes. In all animals, glucagon converted VT to a sinus tachycardia with a rate more rapid than the preceding VT (Figs. 1 and 4).

In four normokalemic dogs, sinus rhythm reverted to VT. A second injection of glucagon \( (25 \text{ or } 50 \mu g/kg) \) did not convert the VT to sinus rhythm. In five hypokalemic dogs, sinus rhythm reverted to VT. In four, a second injection of glucagon \( (25 \mu g/kg) \) resulted in abolition of the arrhythmia. In three dogs in which VT was reestablished, a third injection, \( (25 \mu g/kg) \) resulted in return of sinus rhythm. The duration of sinus rhythm after the second or third injection varied from 1 to over 25 minutes. There was no correlation between the duration of the initial or subsequent conversions to sinus rhythm and the amount of ouabain administered.
Serum Potassium Levels.—Arterial potassium levels in the hypokalemic dogs were significantly less than in normokalemic dogs (2.3 ± 0.1 vs. 3.9 ± 0.2 mEq/liter, P < 0.001) (Fig. 5). Further hypokalemia as a result of the constant glucagon infusion was not seen in either group. The initial arterial potassium level was not significantly different from that in the 10-minute sample.

In the dogs pretreated with glucagon, the arterial potassium levels drawn at the onset of VT were significantly higher than the values before VT in both the normokalemic (P = 0.001) and hypokalemic (P = 0.001) dogs. When saline was substituted for glucagon in the control study, there was no change in potassium level at the onset of VT. The significant rise in serum potassium in the pretreated dogs was not related to hypoxia or hypotension, since neither was present. Potassium values after the bolus injection of glucagon were higher, but the increase was not significant. Hypokalemia was not seen after the bolus injection of glucagon.

Discussion

It was not surprising that hypokalemic control dogs developed VT at lower ouabain dosage than the normokalemic control dogs (Table 1). Digitalis changes the permeability of the cell membrane to potassium and promotes the outflow of potassium from the myocardial cell (23). In the hypokalemic state, the lower cellular potassium results in increased irritability of the myocardium and enhances digitalis toxicity (24-26).

Pretreatment with glucagon did not influence the amount of ouabain required to produce VT in the normokalemic dogs. On the other hand, hypokalemic dogs were protected from VT by glucagon. Thus pretreatment with glucagon appeared to protect the hypokalemic dog from the synergistic effect of hypokalemia on ouabain toxicity. We recall that in these dogs, the level of serum potassium did rise slightly with the development of VT, but not to normokalemic levels.

Why was glucagon effective in protecting the hypokalemic dog? Did glucagon, by increasing blood glucose levels and increasing insulin release, move glucose and potassium into the myocardial cell? Glucagon would thus tend to antagonize digitalis-induced outflow of potassium from the myocardial cell (27). If this were the case, we would expect glucagon to protect against digitalis toxicity in normokalemic dogs as well as in hypokalemic dogs. It does not. However, the same increase in
myocardial potassium flux might be more effective in the hypokalemic state in protecting against ouabain-induced arrhythmias. The fact that serum potassium levels did not decrease does not rule out an increase in myocardial potassium. Greenberg (11) has shown that the arterial-venous difference of glucose across the heart is not greater after glucagon administration than before. However, the increased coronary blood flow after glucagon administration (4, 11, 28) would result in greater glucose utilization by the myocardium in the face of unchanged glucose arterial-venous difference. Although our data do not define the role of potassium shift into the myocardium in the antiarrhythmic effect of glucagon, this theory remains a plausible explanation.

It has been shown that the level of serum potassium influences the myocardial uptake of tritiated digoxin. Acute hyperkalemia in dogs decreases digoxin uptake (29), whereas hypokalemia increases myocardial uptake (30). Could the protective effect of glucagon be explained by an increase in serum potassium, which did occur in both groups of dogs pretreated with glucagon? This is unlikely since: (1) the normokalemic dogs, which also demonstrated an increase in arterial potassium levels, were not protected by pretreatment with glucagon; and (2) the serum level of potassium (6-9 mEq/liter) that resulted in reduced myocardial uptake of tritiated digoxin (29) was much greater than the level reached by either our hypokalemic (3.2 ± 0.1 mEq/liter) or normokalemic (5.1 ± 0.3 mEq/liter) dogs.

Does pretreatment with glucagon protect from ouabain-induced VT by producing a more rapid sinus tachycardia which competes with the ectopic ventricular rhythm? If overdrive suppression (31, 32) is an important factor in the ability of glucagon to protect against the occurrence of VT, we would expect a sinus tachycardia higher than VT in the hypokalemic dogs that were protected, and an absence of a more rapid sinus tachycardia in the unprotected normokalemic dogs. In fact, the opposite occurred. In seven of nine normokalemic dogs, the glucagon infusion resulted in a sinus tachycardia more rapid than the subsequent ouabain-induced VT, whereas in seven of eight hypokalemic dogs, the sinus tachycardia immediately before VT was equal to the VT rate. Thus it does not appear that we can explain the protective effect of glucagon simply on the basis of rapid sinus rate produced by the glucagon infusion.

We have shown that a bolus injection of glucagon (25 μg/kg) converted ouabain-induced VT in 78% of the normokalemic dogs and 100% of the hypokalemic dogs to sinus tachycardia at a rate greater than the VT rate. Cohn et al. (20) abolished VT with glucagon in 72% of their normokalemic dogs. They also noted that VT was followed by a sinus tachycardia at a higher rate. In this situation, overdrive suppression has been suggested as a mechanism of conversion of VT. We recall, however, that pretreatment with glucagon offered no protection to normokalemic dogs even though it resulted in a sinus tachycardia faster than the subsequent VT.

Glucagon alters the myocardial membrane action potential, i.e., abbreviation of phase 2 without change in total duration or amplitude (33). The resultant impact of glucagon plus ouabain in the presence or absence of

### Table 1

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<thead>
<tr>
<th></th>
<th>Normokalemic (9 dogs)</th>
<th>Hypokalemic (7 dogs)</th>
</tr>
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<tbody>
<tr>
<td>Ouabain</td>
<td></td>
<td></td>
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<tr>
<td>(μg/kg to produce VT)</td>
<td>68</td>
<td>58</td>
</tr>
<tr>
<td>SE</td>
<td>3.3</td>
<td>3.3</td>
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<tr>
<td>P</td>
<td>0.32</td>
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hypokalemia on the action potential is not known. Further investigation in this area might define the antiarrhythmic effect of glucagon.

At this time we cannot define the major mechanism by which glucagon exerts its antiarrhythmic effect. Glucagon does appear to have a definite antiarrhythmic "normalizing" effect in the presence of ouabain toxicity. This effect is more pronounced in hypokalemic dogs. Unpublished observations suggest that glucagon's inotropic effect is enhanced in hypokalemic dogs.

The antiarrhythmic effect of glucagon, particularly in the hypokalemic state, has potential clinical usefulness in man. But one must be cautious in extrapolating from animal studies to human conditions. We must also note that pretreatment with glucagon and bolus injection resulted in a sinus tachycardia more rapid than the ouabain-induced VT in the normokalemic dog. In the hypokalemic dog, bolus injection resulted in a sinus tachycardia greater than the preceding VT, whereas pretreatment with glucagon caused a sinus tachycardia equal to the subsequent VT. If glucagon results in a more rapid sinus tachycardia than VT in man, its clinical usefulness would be limited.

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