Diminished Inotropic Response but Unaltered Toxicity to Acetylstrophanthidin in the Senescent Beagle

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SUMMARY The toxic and inotropic effects of a rapid-acting cardiac glycoside, acetylstrophanthidin (ACS), administered as a rapid i.v. bolus was compared in six healthy adult (age 2–3 years) and seven senescent (age 12–14 years) beagles. In the conscious state no age difference was observed in the dosage of ACS at which toxicity, defined as ventricular tachycardia (VT), occurred. Serum levels of ACS at toxicity were 70 ± 15 ng/ml in the senescent and 65 ± 12 ng/ml in the adult (NS). On a separate occasion, the inotropic effect, dP/dt/P_{50}, and the toxic end point were measured in the anesthetized state. As in the awake state, no age difference was seen in the dosage to VT. The control dP/dt/P_{50} measured with a left ventricular Millar catheter during brief periods of right ventricular pacing at 250 beats/min was not age related. However, the increase in contractility in response to ACS was twice greater in the adult than the senescent (p < 0.001). This difference persisted when β-blockade was effected with practolol. Thus, in senescence, while glycoside toxicity is unaltered, the inotropic efficacy of ACS is significantly diminished. This age difference in inotropy cannot be attributed to an age difference in serum levels of the drug or in sympathetic tone. Additional studies indicated that glycoside inhibition of Na^+–K^+ ATPase isolated from these dogs was not age related, suggesting that the mechanism for the diminished inotropic response is distal to the inhibition of this receptor enzyme.

AGE is an important determinant in the response to cardiac glycosides. Most mammalian species exhibit altered sensitivity to the inotropic and toxic effects of digitalis preparations in the neonatal period compared with the young adult.4 These differences have been attributed to both maturational changes in the volume distribution of glycosides5, 6 and to different effects of these agents at the cellular level.7, 8

With advanced age, many physiologic changes occur in the cardiovascular system in both man and animals.9, 10 Although the performance of cardiac muscle isolated from the heart of senescent animals in many ways is indistinguishable from that of its adult counterpart, the response to some interventions that impose a stress on the excitation-contraction system is altered in senescent myocardium.11 In particular, the inotropic response to ouabain is diminished in senescent myocardium when compared with the adult, while the response to an increase in [Ca^{++}] in the bathing fluid or to paired pacing is not age related.12

The purpose of the present study is to extend these observations on the effect of advanced age on the response to cardiac glycosides in isolated cardiac muscle to the intact organism. Specifically, we compare the toxic and inotropic responsiveness to a rapid-acting cardiac glycoside in intact, senescent dogs. While the glycoside is used primarily to elucidate age-related alterations that occur in the cardiovascular system as a result of advanced age, the study may also have practical implications. The marked increase in the prevalence of cardiovascular pathology in aged man necessitates extensive use of cardiac glycosides in this population. However, glycoside toxicity and its relation to inotropic effect in the aged has not been elucidated.13, 14

Materials and Methods

The effects of bolus injections of acetylstrophanthidin (ACS) were studied in six healthy adult (2–3 years old, 12 ± 0.8 kg) and seven senescent (12–14 years old, 11.1 ± 0.6 kg) female beagles that were free of gross cardiovascular pathology. ACS was used because of its rapid onset and short duration of action. The dogs were studied in each of three protocols. We determined 1) the toxic threshold in awake, unanesthetized dogs; 2) the toxic threshold and inotropic response in the anesthetized state; and 3) the inotropic response at toxicity in the presence of β blockade during anesthesia. Individual studies in any single dog were separated by at least 2 weeks. In addition, glycoside inhibition of Na^+–K^+ ATPase was measured in microsomal fractions from many of these hearts when the dog was sacrificed later.

Toxic Threshold in Awake, Unanesthetized Dogs

Standard surface ECG leads were attached to the skin, and lead I or II was monitored. ACS was administered as a rapid bolus through an indwelling peripheral venous catheter. The ACS concentration of the initial bolus was 20 μg/kg, which was increased by 5-μg/kg increments every 30 minutes until ventricular tachycardia (VT) occurred. VT was defined as five or more consecutive premature ventricular complexes. Blood was drawn for the determination of ACS concentration before each injection and at the toxic end point, VT. When VT was reached, diphenylhydantoin was given in order to preserve the dog for future studies. Serum ACS concentration was determined by standard radioimmunoassay technique.15, 16

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Toxic Threshold and Inotropic Response in the Anesthetized State

Dogs were anesthetized with sodium pentobarbital, 30 mg/kg i.v. The surface ECG was displayed on a direct-writing oscilloscope and was continually recorded. A bipolar USCI pacing catheter was positioned in the right ventricle and attached to a Grass SD 9 stimulator. A high-fidelity Millar micromanometer-tipped catheter was inserted in the left ventricle via the femoral artery. Left ventricular pressure (LVP), left ventricular end-diastolic pressure (EDP), and the first derivative of LVP with time (dP/dt) were continually recorded and displayed on a Brush 480 multichannel recorder. The micro- manometer-tipped catheter was calibrated in columns of mercury; zero pressure was taken as the atmospheric pressure immediately after withdrawal from the femoral artery. The pressure signal was differentiated by an R-C network, the response of which was verified as linear through 7500 mm Hg/sec using a triangular wave form produced by a signal generator.

Dogs were given an initial rapid i.v. bolus of 10 µg/kg ACS, which was increased by 10-µg/kg increments at 30-minute intervals until VT occurred. Steady-state hemodynamic measurements were recorded during continuous pacing at 250 beats/min and then pacing was terminated. This overdrive pacing for a short period of time allowed comparison of the inotropic response at a constant ventricular rate through the entire ACS concentration range, including concentrations at which toxicity occurred. Hemodynamic measurements were recorded continuously at low paper speed, and at 200 mm/sec before, during the peak response, and 30 minutes after each ACS injection. Serum was drawn for ACS determination 30 minutes after a given injection and at the toxic end point, VT.

To insure that sodium pentobarbital did not differentially depress cardiac function of senescent dogs over the time course of the experimental period, the dogs were anesthetized on a separate day and catheterized as above, but were given saline injections rather than ACS.

Inotropic Response in the Presence of β Blockade During Anesthesia

After the toxic threshold during anesthesia had been determined in a given dog, the animal was again anesthetized and instrumented. Beta-adrenergic blockade was effected with practolol, 2-4 mg/kg i.v. The efficacy of the block was tested and considered complete when there was no greater than a 5 beats/min increase in heart rate response to a 5-µg bolus i.v. injection of isoproterenol. This concentration of isoproterenol in the absence of β blockade resulted in an increase of heart rate of 35 ± 12 beats/min. During β blockade, ACS was given in a concentration near the previous toxic threshold and increased by 10-µg/kg increments every 30 minutes until VT occurred. Hemodynamic parameters were then measured during pacing at 200 beats/min.

In addition to measurements of EDP, LVP and dP/dt max, the ratio of dP/dt to LVP at a ventricular pressure of 50 mm Hg was calculated (dP/dt/P 50). This ratio has been previously assessed as an index of contractility17-20 and was used in the present study to measure the inotropic response to ACS. The data reported are the mean of at least five beats during the control period, before a bolus of ACS, and at the peak inotropic response to ACS, which occurred 3–10 minutes after the injection.

Na+ -K+ ATPase Inhibition

At a later date, several dogs used in this study were sacrificed and glycoside inhibition of Na+ -K+ ATPase isolated from these hearts was determined. Microsomes containing (Na+ -K+)-adenosine triphosphatase were isolated by the method of Matsui and Schwartz.21 The ouabain-sensitive and total ATPase activities in this preparation were determined at 22 ± 1°C by means of a previously described spectrophotometric method.12

Statistical Analysis

Data are expressed as mean ± SEM and were compared, when appropriate, by the nonpaired t test, Fisher's exact test for tables, or a nested analysis of variance.22

Results

Toxic Threshold in Awake, Unanesthetized Dogs

The heart rate in the resting, awake dogs was not age related and was 88.6 ± 4.2 beats/min in the seven senescent beagles and was 92.9 ± 4.0 beats/min in the six adult beagles. In response to ACS, a series of dysrhythmias, including sinus bradycardia, junctional beats and supraventricular tachycardia, occurred before VT, but there was no age difference in the sequence or magnitude of these subtoxic responses. Table 1 lists the size of the bolus injection at which VT

<table>
<thead>
<tr>
<th>Table 1. Effect of Age on Acetylstrophanthinid (ACS)-induced Ventricular Tachycardia in Awake Dogs</th>
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</thead>
<tbody>
<tr>
<td>ACS bolus (µg/kg)</td>
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<tr>
<td>---------------------------------</td>
</tr>
<tr>
<td>Adult</td>
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<tr>
<td>Sereneest</td>
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</table>

The number at each dose indicates the number of dogs manifesting ventricular tachycardia in response to an intravenous bolus of ACS of that dose.
plotted against cmz six adult and dogs. These was acetylstrophanthidin (ACS) at 45-50 1.

Toxic Threshold after in adult dogs gg/kg 40 VT age 50 and 40 

Inotropic response in the adult group at the higher ACS concentrations (fig. 3) persisted when the maximum response in each dog was compared independent of the ACS dosage. As shown in table 2A, compared either as absolute change or as a percentage of control, the age difference in the maximum response independent of ACS dosage is highly significant. The maximum inotropic response was reached an ACS bolus less than that at which toxicity occurred in five of seven senescent dogs, but in the adult group, all six dogs had their maximum inotropic response at the toxic dosage (p < 0.02, Fisher’s exact test). When the inotropic response is examined at toxicity (table 2B), dP/dt/Pa increased to 172 ± 9% of control in the adult but only to 130 ± 3.2% of control in the senescent dogs (p < 0.01). This change reflected a 51 ± 5% change dP/dtmax in the young and 18 ± 1.5% change dP/dtmax in the senescent dogs (p < 0.01). The increase in peak LVP was not age related.

Inotropic Response in the Presence of β-blockade During Anesthesia

Practolol reduced the heart rate, LVP, dP/dtmax and dP/dt/Pa to a similar level in both age groups. During β blockade, the heart rate was 128 ± 6 beats/min in the adult dogs and 130 ± 7 beats/min in the senescent dogs. Before ACS administration during pacing at 200 beats/min during β blockade, LVP was 127 ± 68 mm Hg in the adult dogs and 122 ± 13 mm Hg in the senescent dogs, and dP/dt was 1881 ± 286 mm Hg/sec in the adult dogs and 1462 ± 315 mm Hg/sec in the senescent dogs (NS). Practolol did not significantly change EDP in either age group. Table 2C shows the control dP/dt/Pa during β blockade and the response to ACS at toxicity. During β blockade, the adult dogs still had a twofold greater increase in the inotropic response: 174 ± 9% of control vs 135 ± 5% of control in the senescent (p < 0.01). As in the unblocked state, the age difference persisted when analyzed as absolute changes. The change in peak LVP in response to ACS during β blockade was not age related and EDP, as in the unblocked state, did not change from control in response to ACS in either age group.

Na⁺-K⁺ ATPase Inhibition

The total (Mg⁺⁺-dependent) and specific (Na⁺-K⁺)-ATPase activities measured at 22°C in hearts averaged 3.62 ± 0.43 and 2.64 ± 0.38 μmol/mg/hr (n = 5) compared with 3.71 ± 0.43 and 2.44 ± 0.36
Changes in this index have been shown to be relatively independent of preload and afterload changes.27-30 The greater ACS induced increase in dP/dt/P<sub>so</sub> in the adult was accompanied by a similar age-dependent difference in the change in dP/dt<sub>max</sub>, with no age difference in the response in LVP. EDP was not changed in either age group in response to ACS. These changes when examined collectively indicate a greater inotropic response in the adult than in the senescent dog.

The increase in contractility due to cardiac glycosides in the adult dogs during anesthesia in this study is similar to previously reported data.26-27 Vatner et al.27 have shown, however, that the magnitude of this inotropic effect is greater under pentobarbital anesthesia than in the conscious dog. The relatively small increase in contractility caused by cardiac glycosides in the nonfailing heart has been attributed to reflex withdrawal of sympathetic drive.26, 28 However, the age difference in the inotropic response in the present study does not likely result from age differences in sympathetic tone, as this difference persists in the presence of β blockade.

Discussion

VT, as an index of toxicity, has been used previously and the range of ACS concentrations over which VT occurred is similar in this study to that reported for other adult dogs.4, 5 There was no difference in the threshold for ACS toxicity, defined as VT, in the senescent dogs compared with the adult dogs (table 1).

In contrast to the similarity in toxic threshold, the intact senescent dogs had diminished inotropic responsiveness to ACS compared with the adult dogs (fig. 3 and table 2). The inotropic index used in this study was the ratio of left ventricular isovolumic dP/dt to developed pressure at 50 mm Hg (dP/dt/P<sub>so</sub>). The effects of ACS on hemodynamic parameters measured during right ventricular pacing at 250 beats/min in adult (n = 6) and senescent (n = 7) dogs. Saline injections were administered 30 minutes apart and the duration of the experiment was identical to that when the response to acetylstrophanthidin (ACS) was measured. The hemodynamic parameters were stable throughout the experiment and were not age related. B) Hemodynamic parameters measured as in panel A but before each ACS injection. The hemodynamic parameters are at control level and are not age related (n = 6 adult and 7 senescent except at 40 μg/kg, n = 3 adult and 2 senescent). LVP = left ventricular pressure; EDP = left ventricular end-diastolic pressure.
Age differences in factors that alter the bioavailability of glycosides, such as absorption from the gastrointestinal tract, altered volume distribution space and renal clearance of the drug cannot account for the age difference in the present study because a rapid-acting agent was injected via a rapid i.v. bolus. We did not measure the concentration of ACS on the cardiac receptor, and the age difference in inotropic response could possibly result from decreased glycoside delivery to or binding at the receptor site. In the present study as well as in studies of rat myocardium, no age difference was observed in the glycoside inhibition of Na⁺-K⁺ ATPase activity. In addition, the total Na⁺-K⁺ ATPase activity in microsomes from adult and senescent hearts was similar. The mechanism for the age difference in inotropic response does not appear to be attributable to enzyme inhibition, but rather to the steps linking the inhibition of this receptor enzyme to enhanced Ca²⁺ delivery to the contractile proteins upon excitation, or in a diminished contractile response to Ca²⁺ itself in this model. That the latter is not the case, at least during β blockade, may be inferred from table 2. In the senescent group, in the absence of β blockade, dP/dt/P₅₀ in the presence of ACS is nearly twice that during β blockade (table 2B vs 2C). Thus, the diminution in dP/dt/P₅₀ in the presence of ACS in the senescent group during β blockade cannot be related to a non-specific diminution in the maximum dP/dt attainable, and cannot therefore be attributed to an age difference in response to Ca²⁺ at the level of the contractile proteins. In other species, it has been shown in isolated cardiac muscle that the maximum inotropic response to an increased Ca²⁺ or paired pacing is unaltered in senescence.

Although in absolute terms the toxic threshold was not age related, the results may be interpreted to indicate a decrease in the inotropic-toxic ratio to ACS in

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**Figure 3.** The effect of age on the peak response of hemodynamic parameters to bolus concentrations of acetylstrophanthinid (ACS). The number of dogs studied at each concentration is given in parentheses. Control values for each parameter before each ACS bolus are as indicated in figure 2B. The effect of age on the entire dose-response curve was highly significant when compared by a two-way nested analysis of variance and this was due to the marked aged difference at the higher ACS concentrations. LVP = left ventricular pressure.

**Figure 4.** The effect of age on the ouabain inhibition of Na⁺-K⁺ ATPase isolated from canine myocardium. No age effect is observed.
s enescent dogs, for when analyzed at toxicity, the inotropic response was significantly greater in the adult than the senescent group. Stated in other terms, in senescence, the same risk of toxicity for a given serum level existed, but the inotropic benefit from ACS was reduced. In man, however, serum levels of cardiac glycosides for a given dose have been shown to be increased in advanced age. Information regarding the inotropic or toxic effect of a given serum level remains anecdotal.13,14

In summary, in healthy senescent beagles, the threshold for VT was unaltered, but the inotropic response to ACS was diminished when compared with that in the healthy adult beagle. This decrease in the inotropic response was not a function of age differences in drug elimination from the serum, sympathetic tone or glycoside inhibition of Na⁺-K⁺ ATPase.

Acknowledgments

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TABLE 2A. The Effect of Age on the Maximum Inotropic Response to Acetylstrophanthidin (ACS) Independent of Dosage

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Control</th>
<th>ACS</th>
<th>Δ</th>
<th>% Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>6</td>
<td>55.1 ± 6.3</td>
<td>93.0 ± 6.5†</td>
<td>37.9 ± 3.24*</td>
<td>171.5 ± 9.1†</td>
</tr>
<tr>
<td>Senescent</td>
<td>7</td>
<td>47.0 ± 4.7</td>
<td>66.4 ± 6.4</td>
<td>19.4 ± 2.45</td>
<td>142.7 ± 5.9</td>
</tr>
</tbody>
</table>

*p < 0.001, adult vs senescent.
†p < 0.03, adult vs senescent.

TABLE 2B. The Effect of Age on the Inotropic Response to Acetylstrophanthidin (ACS) Measured During Overdrive Pacing at Toxicity

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Control</th>
<th>ACS</th>
<th>Δ</th>
<th>% Control</th>
</tr>
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<tbody>
<tr>
<td>Adult</td>
<td>6</td>
<td>55.1 ± 6.3</td>
<td>93.0 ± 6.5*</td>
<td>37.9 ± 3.2*</td>
<td>172 ± 9.0*</td>
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<tr>
<td>Senescent</td>
<td>7</td>
<td>48.9 ± 5.9</td>
<td>62.0 ± 6.0</td>
<td>13.1 ± 1.7</td>
<td>130 ± 3.2</td>
</tr>
</tbody>
</table>

*p < 0.01, adult vs senescent.

TABLE 2C. The Effect of Age on the Inotropic Response to Acetylstrophanthidin (ACS) Measured During Overdrive Pacing at Toxicity During β Blockade

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Control</th>
<th>ACS</th>
<th>Δ</th>
<th>% Control</th>
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<tr>
<td>Adult</td>
<td>6</td>
<td>29.4 ± 3.6</td>
<td>51.0 ± 6.3*</td>
<td>21.6 ± 3.6*</td>
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<tr>
<td>Senescent</td>
<td>7</td>
<td>26.9 ± 3.9</td>
<td>35.8 ± 4.5</td>
<td>8.9 ± 1.4</td>
<td>135.2 ± 5.0</td>
</tr>
</tbody>
</table>

*p < 0.01, adult vs senescent.
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