Electrophysiological effects of edrophonium in the innervated and the transplanted denervated human heart

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SUMMARY In order to determine the mechanism of action of edrophonium on the cardiac conduction system, we used His bundle recording and pacing techniques to examine the electrophysiological effect of edrophonium in 6 patients undergoing diagnostic evaluation for coronary artery disease and in 9 cardiac transplant recipients who were free of acute graft rejection. After control measurements were made of sinus cycle length, conduction intervals, and conduction system refractory periods, edrophonium was administered by constant intravenous infusion, and all electrophysiological measurements were repeated. In the normally innervated patients, edrophonium significantly increased sinus cycle length from $778 \pm 21$ ms to $883 \pm 36$ ms ($P < 0.01$), AH interval from $88 \pm 11$ ms to $100 \pm 12$ ms ($P < 0.01$), and AV nodal functional refractory period from $351 \pm 44$ ms to $391 \pm 36$ ms ($P < 0.05$). In the patients with transplanted hearts, edrophonium had no electrophysiological effect other than increasing the cycle length of the remnant recipient atrium from $722 \pm 21$ ms to $798 \pm 31$ ms ($P < 0.01$). We conclude that the electrophysiological effects of edrophonium in man are mediated primarily through autonomic innervation of the heart.

Edrophonium is a drug with brief anticholinesterase activity which is useful in the management of supraventricular tachyarrhythmias (Cantwell et al., 1972; Reddy et al., 1971). Though the drug has been used clinically for several decades, the specific electrophysiological effects of edrophonium have not been evaluated in humans.

Anticholinesterase agents produce their parasympathomimetic effects by inhibiting acetylcholinesterase and allowing accumulation of acetylcholine released from nerve endings (Koelle, 1975). Many of these agents, however, also have important pharmacological effects that are at least partly independent of acetylcholinesterase inhibition. For example, the ability of anticholinesterases to reverse the neuromuscular blockade produced by curare is not quantitatively proportional to their anticholinesterase activity (Brimblecombe, 1974). Neostigmine can produce contraction in muscle depleted of anticholinesterase by chronic denervation (Riker and Wescoe, 1946) and neostigmine and physostigmine produce neuromuscular blockade in the rat phrenic nerve diaphragm preparation in the absence of acetylcholine accumulation (Thesleff, 1960). Edrophonium may also have a non-autonomic mediated direct cardiac effect, but this possibility has not been studied.

In order to determine whether the electrophysiological effects of edrophonium are wholly dependent on cardiac autonomic innervation, we studied the effect of edrophonium on both the innervated and transplanted denervated human heart. Previous investigations in cardiac transplant recipients have shown that their hearts remain functionally denervated and devoid of direct autonomic neural influences after transplantation (Cannom et al., 1973, 1975). Thus, the cardiac transplant recipient provides an opportunity to study the possibility of direct cardiac effects of edrophonium.

Methods

We performed electrophysiological studies in 15 patients after obtaining informed written consent. At the time of study, all patients were in New York Heart Association Functional Class I. None was receiving digitalis preparations, antiarrhythmic
agents, or beta-blocking drugs. The 9 post-transplant patients were free from evidence of acute graft rejection or extracardiac disease, and the 6 normally innervated subjects were undergoing catheterisation for diagnostic evaluation of coronary artery disease.

Patients were catheterised in the postabsorptive state under sedation with oral diazepam. A 7F quadripolar recording-stimulating electrode catheter was positioned in the high right atrium for atrial pacing and recording of the right atrial electrogram. In the cardiac transplant patients, the catheter was positioned against the high antero-lateral atrial wall to provide stable pacing of the donor atrium. A 7F tripolar or a 5F bipolar electrode catheter was advanced across the tricuspid valve for His bundle recording. Three surface electrocardiographic leads (I, aVF, and V1) were recorded simultaneously with the intracardiac electrograms and transmitted to the AC input of an electrocardiographic amplifier with filter frequencies set between 40 and 500 Hz. Pacing stimuli of 2 ms in duration were delivered by a programmable digital stimulator (M. Bloom Associates, Ltd., Narberth, Pennsylvania) at an amperage twice the diastolic threshold. Data were recorded on photographic paper at 100 mm/s paper speed on a Hewlett-Packard recorder, Model No. 4568C.

During cardiac transplantation the recipient heart is excised by division of the atria at their mid-level, leaving in situ the posterior walls of the right and left atria to which the donor atria are anastomosed (Stinson et al., 1969). Thus, the transplant patients have both donor and recipient activity which can be recorded by surface and intracardiac techniques (Fig. 1) (Stinson et al., 1972).

Baseline recordings of the AH and HV intervals and the sinus cycle length, including the recipient and donor sinus cycles in the transplant patients, were made at the spontaneous and base-paced heart rates (Rosen, 1972). The sinus node recovery time was measured twice after atrial pacing at cycle length of 430 ms for 1 minute, followed by abrupt termination of pacing. Atrial pacing was performed at decreasing cycle lengths of 400, 375, 350, 330, 315, and 300 ms until Wenckebach conduction occurred. The maximal cycle length at which Wenckebach conduction was present was then determined within 5 ms.

The atrial effective refractory period and the effective and functional refractory periods of the atrioventricular node were determined using the extra stimulus technique with an underlying paced rhythm (Rosen, 1972). The atrioventricular nodal effective refractory period was taken as the longest A1-A2 interval at which A3 was not conducted to the His-Purkinje system. The atrioventricular nodal functional refractory period was the shortest H1-H2 interval obtained. The AH interval during rapid atrial pacing was recorded at the most rapid atrial rate at which there was one-to-one atrioventricular
conduction both before and after drug administration.

Edrophonium has a brief duration of action, because of the reversibility of its binding to acetylcholinesterase (Brimblecombe, 1974). Because of the transient effect of a single intravenous dose of edrophonium, we have used a constant infusion of the drug to obtain a stable condition for electrophysiological measurements (Frieden et al., 1971).

After resting control measurements were completed, each patient was given a test dose of 0.03 mg/kg of edrophonium, followed 1 minute later by an additional bolus of 0.07 mg/kg and a constant intravenous infusion of 0.14 mg/kg per min. The drug was infused for 10 minutes to achieve a stable condition. Then during continuous edrophonium infusion, electrophysiological measurements were repeated, using the previous base-pacing cycle length.

A double dose of edrophonium was administered to 1 subject with normal cardiac innervation and 2 transplant recipients; baseline measurement and edrophonium administration were performed as outlined above. Conduction intervals were determined at this first dose of edrophonium, but before pacing studies a second bolus of 0.1 mg/kg was given, and the infusion rate was increased to 0.028 mg/kg per min. After a further 10-minute stabilisation period, electrophysiological measurements were repeated.

All data reported are those obtained at the highest dose of edrophonium. Statistical comparison of results before and during edrophonium was performed using Student's t test for paired data. A linear regression analysis of the H1H2 interval versus A1A2 interval plots in Fig. 2 and 3 was performed to obtain slope and intercept for each group of patients. An unpaired t test was then used to test for significant differences in the derived slopes and intercepts.

**Results**

**INNERVATED PATIENTS**

The electrophysiological effects of edrophonium in the 6 innervated patients are summarised in Table 1. Mean sinus cycle length was increased 13 per cent from 778 ± 21 to 883 ± 36 ms (P < 0.01). Atrial effective refractory period was not significantly changed.

Atrioventricular nodal conduction was slowed and refractoriness increased. The mean AH interval increased from 88 ± 11 ms to 100 ± 12 ms (P < 0.01). The AH interval during rapid atrial pacing increased from 133 ± 22 ms to 156 ± 23 ms (P < 0.05), at a mean pacing cycle length of 372 ms.

The mean maximum paced cycle length at which Wenckebach conduction was present increased from 302 ± 22 ms to 359 ± 28 ms. Thus the mean maximum ventricular rate achieved decreased from 199 to 167 with administration of edrophonium.

The mean atrioventricular nodal effective refractory period was not significantly altered but the mean atrioventricular nodal functional refractory period was prolonged by edrophonium from 351 ± 44 ms to 391 ± 36 ms (P < 0.05). There was no

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**Table 1 Effects of edrophonium on innervated patients**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Edrophonium</th>
<th>No.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Studies at rest</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrial cycle length</td>
<td>778 ± 21</td>
<td>883 ± 36</td>
<td>6</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>AH interval</td>
<td>88 ± 11</td>
<td>100 ± 12</td>
<td>6</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>HV interval</td>
<td>42 ± 2</td>
<td>41 ± 2</td>
<td>6</td>
<td>NS</td>
</tr>
<tr>
<td>QRS duration</td>
<td>82 ± 4</td>
<td>83 ± 5</td>
<td>6</td>
<td>NS</td>
</tr>
<tr>
<td>Pacing studies</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AH interval during</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rapid pacing</td>
<td>133 ± 22</td>
<td>156 ± 23</td>
<td>6</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Longest cycle length</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with Wenckebach</td>
<td>302 ± 22</td>
<td>359 ± 28</td>
<td>6</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>conduction</td>
<td>1022 ± 41</td>
<td>1002 ± 67</td>
<td>5</td>
<td>NS</td>
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<tr>
<td>Sinus node recovery</td>
<td>240 ± 22</td>
<td>248 ± 24</td>
<td>6</td>
<td>NS</td>
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<tr>
<td>time</td>
<td>263 ± 27</td>
<td>290 ± 33</td>
<td>6</td>
<td>NS</td>
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<tr>
<td>Refractory periods</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrial ERP</td>
<td>351 ± 44</td>
<td>391 ± 36</td>
<td>5</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

All intervals given as the mean ± standard error of the mean in milliseconds. ERP, effective refractory period; FRP, functional refractory period.

**Table 2 Effects of edrophonium on transplanted denervated patients**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Edrophonium</th>
<th>No.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Studies at rest</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recipient atrial</td>
<td>722 ± 21</td>
<td>798 ± 31</td>
<td>6</td>
<td>&lt; 0.01</td>
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<tr>
<td>cycle length</td>
<td>664 ± 35</td>
<td>674 ± 38</td>
<td>7</td>
<td>NS</td>
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<tr>
<td>Donor atrial cycle</td>
<td>86 ± 6</td>
<td>85 ± 6</td>
<td>9</td>
<td>NS</td>
</tr>
<tr>
<td>length</td>
<td>43 ± 4</td>
<td>43 ± 3</td>
<td>9</td>
<td>NS</td>
</tr>
<tr>
<td>QRS duration</td>
<td>101 ± 8</td>
<td>103 ± 7</td>
<td>9</td>
<td>NS</td>
</tr>
<tr>
<td>Pacing studies</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AH interval during</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rapid pacing</td>
<td>122 ± 6</td>
<td>122 ± 7</td>
<td>9</td>
<td>NS</td>
</tr>
<tr>
<td>Longest cycle length</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with Wenckebach</td>
<td>324 ± 16</td>
<td>331 ± 17</td>
<td>8</td>
<td>NS</td>
</tr>
<tr>
<td>conduction</td>
<td>976 ± 124</td>
<td>903 ± 79</td>
<td>8</td>
<td>NS</td>
</tr>
<tr>
<td>Sinus node recovery</td>
<td>229 ± 18</td>
<td>236 ± 15</td>
<td>8</td>
<td>NS</td>
</tr>
<tr>
<td>time</td>
<td>290 ± 14</td>
<td>301 ± 11</td>
<td>6</td>
<td>NS</td>
</tr>
<tr>
<td>Refractory periods</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrial ERP</td>
<td>354 ± 15</td>
<td>359 ± 13</td>
<td>8</td>
<td>NS</td>
</tr>
</tbody>
</table>

For explanation see Table 1.
Electrophysiological effects of edrophonium
evidence of altered His-Purkinje conduction since
the mean HV interval and QRS configuration and
duration were unchanged.

TRANSPLANT PATIENTS
Table 2 summarises the effect of edrophonium in
the denervated hearts. Whereas the spontaneous
recipient atrial cycle length was increased from 722
±21 ms to 798 ±31 ms (P < 0.01), the resting
donor atrial cycle length and atrial effective refrac-
tory period were unaffected. There was no change
in the AH interval during spontaneous rhythm and
rapid pacing, the longest pacing cycle length show-
ing Wenckebach conduction and the atrophicentric-
ular nodal refractory periods. As in the innervated
patients, His-Purkinje conduction remained un-
changed.

Fig. 2 illustrates the mean H1H2 interval at each
A1A2 interval for both the control and transplant
patients before drug administration. Each point
represents the mean for at least 4 patients for which
data at the given A1A2 interval (±5 ms) were avail-
able. The values for the control and transplant
groups give linear regression curves (R=0·829 and
R=0·968, respectively) which are significantly
different in slope and intercept (P < 0·01). The
lower position of the refractory curve in the trans-
plant patients is consistent with their lack of resting
vagal tone.

Fig. 3 shows the change in mean H1H2 response
during edrophonium infusion. The linear regres-
sion curves in the innervated patients before
(R=0·829) and during (R=0·874) edrophonium are
not significantly different, but two individual data
points at a premature A1A2 interval of 375 ms and
450 ms show a significant prolongation of H1H2
with edrophonium (P < 0·05). There were no sig-
nificant differences in the transplant patients before
and after edrophonium.

SIDE EFFECTS
The known side-effects of edrophonium include
nausea, abdominal pain, sweating, muscle fasci-
culations, visual blurring, increased bronchial
secretions, and salivation (Randall et al., 1955). Only
5 of our 15 patients experienced no side effects
(3 from the innervated group and 2 from the
denervated group). The other 11 patients all noted

![Graph 1](image1)

**Fig. 2** A plot of the mean H1H2 and A1A2 intervals in
innervated and denervated patients. The innervated
patients have significantly longer H1H2 intervals
consistent with the influence of resting vagal tone. See
text for details.

![Graph 2](image2)

**Fig. 3** The effect of edrophonium on the mean H1H2
interval at various premature A1A2 intervals in the
innervated and denervated patients. Edrophonium has
no effect on the denervated group of patients, but tends
to prolong the H1H2 intervals in the innervated group.
See text for details.
at least mild nausea. In addition, 1 had stomach cramps, 2 complained of a tingling sensation in the lower extremities, and 5 became diaphoretic. In no patient were the side effects severe enough to stop the study.

Discussion

Our results show that, at the dosage used in this study, the effects of edrophonium on the cardiac conducting system are solely autonomically mediated; no direct effect of edrophonium on the denervated human heart could be detected. Slowing of the innervated recipient atrial rate in the transplant patient group indicates that a pharmacologically active dose of the drug was administered. However, the possibility that a higher dose may have significant, direct electrophysiological effects cannot be excluded.

The electrophysiological effects of edrophonium which we determined in this study are consistent with the known effects of cardiac parasympathetic stimulation: slowing of the sinoatrial rate, decrease of atrioventricular nodal conduction velocity, and increase in atrioventricular nodal refractoriness (Higgins et al., 1973). These effects are also in keeping with the cardiac distribution of cholinergic fibres which are richly supplied to the sinoatrial and atrioventricular nodal areas. Though cholinergic fibres have also been shown to supply the ventricular specialised conducting system, their experimentally measurable effect has been primarily on the ventricular fibrillation threshold rather than conduction velocity per se (Kent et al., 1974). The lack of any measured effect of edrophonium in the denervated heart suggests the absence of any functionally active post-ganglionic cholinergic fibres. Thus, there is no local accumulation of acetylcholine to produce the parasympathomimetic effect seen in the innervated heart.

In patients subjected to remeasurement of atrioventricular nodal refractory periods, the functional refractory period has been reported to vary significantly with time (Reddy et al., 1975). Our finding that the atrioventricular nodal functional refractory period was affected by edrophonium more than the atrioventricular nodal effective refractory period suggests that the functional refractory period is more susceptible to autonomic influences, which may account for its previously observed spontaneous variation.

The slow rate of the normally innervated heart compared with the rapid resting rate of the denervated heart is consistent with a predominantly parasympathetic effect of resting autonomic tone. The present study provides further evidence for the predominant influence of resting vagal tone in the innervated heart. Fig. 2 shows a significantly larger H1H2 interval for each premature stimulus interval in the innervated group of patients compared with the denervated group. Thus, under control conditions, at any given premature pacing interval (A1A2) there is a slower conduction of the premature stimulus (longer A2H2) in the innervated patients.1 This slowed conduction is characteristic of enhanced parasympathetic stimulation (Higgins et al., 1973).

Kolman et al. (1975) have emphasised that the effect of a vagotonic intervention is influenced greatly by pre-existing autonomic tone. For example, vagal stimulation increases ventricular fibrillation threshold only during a period of increased sympathetic activity. Similarly, the electrophysiological effects of edrophonium may be more pronounced when the resting sympathetic input is raised, and less pronounced in a state of low sympathetic tone.

The duration of action of a single intravenous bolus of edrophonium is brief because of its competitive displacement from acetylcholinesterase by acetylcholine (Brimblecombe, 1974). In this investigation, we assumed that a stable condition was achieved by administration of a loading bolus of edrophonium followed by a 10-minute infusion. However, plasma concentrations of edrophonium were not measured to document a steady-state condition. Previous studies in normal Wistar rats showed the plasma half-life of the slow disposition phase of edrophonium elimination to be 68 minutes (Back and Calvey, 1974). On this basis, prolonged infusion of the drug could possibly cause accumulation and toxic plasma levels. However, in our studies, no late toxicity was noted during constant intravenous infusion of up to one hour in duration.

This investigation describes the electrophysiological effect of edrophonium on the innervated human heart and shows that an identical dose produces no significant effect in the transplanted denervated heart, confirming for the first time that the cardiac conduction system effects of edrophonium in man are mediated by autonomic innervation of the heart.

References


Since H1H2 = A1A2 + A2H2 = A2H2

1Since H1H2 = A1A2 + A2H2 = A2H2
Electrophysiological effects of edrophonium


Requests for reprints to Dr Jay W. Mason, Division of Cardiology, Stanford University Medical Center, Stanford, California 94305, USA.
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