Reports of Investigation

Heart rate changes in cardiac transplant patients and in the denervated cat heart after edrophonium

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Purpose: The effect of edrophonium on heart rate in cardiac transplant patients and in an animal model of acute cardiac denervation were studied, to evaluate the functional state of the peripheral parasympathetic pathway following cardiac denervation.

Methods: Edrophonium was studied in patients with normally innervated hearts (controls) and in cardiac transplants. Edrophonium was also studied in vagotomized, beta-blocked cats. In Group 1 animals, the vagus nerve was not stimulated. In Groups 2 & 3 the right vagus nerve was electrically stimulated to produce approximately 20% and 40% reductions in baseline heart rate, respectively.

Results: Maximum heart rate reduction in transplants (7.3 ± 0.8 beats·min⁻¹ with 0.6 ± 0.08 mg·kg⁻¹) was less than in controls (13.3 ± 1.6 beats·min⁻¹ with 0.4 ± 0.05 mg·kg⁻¹, $P < 0.01$). In Group 1 animals heart rate decreased maximally by 20.9 ± 2.5 beats·min⁻¹ with 9.0 ± 1.9 mg·kg⁻¹. In Groups 2 and 3, with doses < 1.5 mg·kg⁻¹, reductions in heart rate were greater than in Group 1 and maximal reductions were obtained with lower doses (Group 2: maximum reduction by 20.3 ± 2.8 beats·min⁻¹ with 1.3 ± 0.1 mg·kg⁻¹; Group 3: 22.6 ± 4.0 beats·min⁻¹ with 0.8 ± 0.2 mg·kg⁻¹, $P < 0.001$). Doses > 1.5 mg·kg⁻¹ in Groups 2 and 3 produced increases in heart rate.

Conclusion: Edrophonium produced bradycardia in cardiac transplants suggesting spontaneous release of acetylcholine from parasympathetic postganglionic neurons in the transplanted heart. The magnitude of the bradycardia was less in transplant than in control patients. Findings from animal studies suggest that the reduction in transplants can be attributed to diminution or absence of tonic cardiac parasympathetic drive. At high doses, edrophonium may interfere with parasympathetic neuron activation.

Objectif : Étudier l'influence de l'édrophonium sur la fréquence cardiaque chez le transplanté du coeur et sur une préparation animale de dénervation cardiaque aiguë; évaluer l'état fonctionnel des voies de conduction périphériques parasympathiques après dénervation cardiaque.

Méthodes : L'édrophonium a d'abord été étudié chez des patients à innervation cardiaque normale (contrôles) et chez des transplantés. L'édrophonium a aussi été étudié sur des chats vagotomisés β -bloqués. Dans le groupe des animaux du groupe 1, le vague n'était pas stimulé. Dans les groupes 2 et 3, le vague droit était stimulé dans le but de provoquer une baisse de la fréquence cardiaque initiale de 20% et de 40%.

Résultats : Chez les transplantés, la réduction maximale de la fréquence cardiaque ($7,3 \pm 0,8$ b·min⁻¹ avec $0,6 \pm 0,08$ mg·kg⁻¹) était inférieure à celle des contrôles ($13,3 \pm 1,6$ b·min⁻¹ avec $0,4 \pm 0,05$ mg·kg⁻¹, $P < 0,01$). Chez les animaux du groupe 1, la diminution maximale de la fréquence cardiaque était de $20,9 \pm 2,5$ b·min⁻¹ avec $9,0 \pm 1,9$ mg·kg⁻¹. Dans les groupes 2 et 3, avec des doses $< 1,5$ mg·kg⁻¹, les diminutions étaient plus importantes que dans le groupe 1 et les diminutions maximales étaient enregistrées avec les doses les plus faibles (groupe 2: diminution maximale de $20,3 \pm 2,8$ b·min⁻¹ avec $1,3 \pm 0,1$ mg·kg⁻¹; groupe 3: $22,6 \pm 4,0$ b·min⁻¹ avec $0,8 \pm 0,2$ mg·kg⁻¹, $P < 0,001$). Dans les groupes 2 et 3, des doses $> 1,5$ mg·kg⁻¹ ont provoqué des augmentations de la fréquence cardiaque.

Conclusion : L'édrophonium produit une bradycardie chez les transplantés cardiaques, ce qui suggère une libération spontanée d'acétylcholine à partir des neurones parasympathiques postganglionnaires du greffon. L'importance de la bradycardie était moindre chez les transplantés que chez les contrôles. Les données obtenues à partir des études sur les animaux suggèrent que la bradycardie chez les transplantés peut être attribuée à une diminution ou une absence d'activité tonique parasympathique. À doses élevées, l'édrophonium peut gêner l'activation des neurones parasympathiques.

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DURING cardiac transplantation, the branches of the vagus nerves which contain the cardiac parasympathetic preganglionic axons are sectioned, thus the parasympathetic postganglionic neurones contained within the transplanted heart become denervated.¹ While immunohistochemical studies of the human transplanted heart have demonstrated the presence of parasympathetic postganglionic neurones,² it is not known whether these neurones retain the ability to synthesize and release acetylcholine (ACh) in the absence of synaptic input. Survival of such functional postganglionic neurones may be important with respect to the potential for parasympathetic reinnervation,¹ or in controlling the intrinsic rate of the pacemaker cells^{3,4} of the transplanted heart. The presence of such functional neurones could be inferred by the demonstration of parasympathetic reinnervation during the post-transplant period. Yet, reinnervation of the human transplanted heart is controversial, with only some patients showing signs suggestive of reinnervation.⁵⁻¹¹ On the assumption that functional postganglionic parasympathetic neurones spontaneously release ACh,¹² it may be possible to demonstrate indirectly their presence as well as the persistence of their ability to synthesize and release ACh by testing the ability of anticholinesterases to slow heart rate. Assuming acetylcholinesterase activity is present in the transplanted heart, a cardiac parasympathomimetic action of anticholinesterases would be anticipated because of the prevention of hydrolysis of spontaneously released ACh, leading to subsequent activation of inhibitory muscarinic M_2 receptors at cardiac effector sites. In fact, it has been shown that neostigmine produces a dose-dependent reduction in heart rate in patients who have undergone cardiac transplantation.¹³⁻¹⁵ However, since it has been demonstrated that neostigmine produces bradycardia by a mechanism in addition to its anticholinesterase action,^{16,17} the reduction in heart rate produced by neostigmine does not necessarily demonstrate the presence of functional postganglionic neurones. Edrophonium, however, appears to decrease heart rate solely by cholinesterase inhibition,^{16,17} and thus may be useful for demonstrating the presence of functional cardiac parasympathetic postganglionic neurones in the transplanted heart.

The purpose of the present study was to test whether edrophonium produces a reduction in heart rate in patients after cardiac transplantation. The magnitude of the bradycardia was compared with that produced in patients with normally innervated hearts. Any difference in the magnitude of the bradycardia in the two groups of patients may be a consequence of the presence or absence of cardiac parasympathetic input. This

assumption was verified in animal experiments, in which the effect of edrophonium on heart rate was studied in the absence of cardiac parasympathetic input and when tonic parasympathetic drive was provided by continuous electrical stimulation of the vagus nerve.

Methods

The study was approved by the Ethics Committee for human and animal studies at the Royal Victoria Hospital.

Edrophonium in patients with normally innervated hearts vs cardiac transplants

The effect of edrophonium on heart rate was studied in anaesthetized, paralyzed, mechanically respired patients at the conclusion of non-cardiac related surgery. None of the patients was receiving medications with antimuscarinic effects prior to study, and all were in sinus rhythm. During the study, lead II of the electrocardiogram was continuously monitored to verify the presence of sinus rhythm. The QRS complex triggered a cardiometer for continuous display of heart rate. Agents used for premedication, induction and maintenance of general anaesthesia, and muscle relaxation were at the discretion of the anaesthetist in charge of the case. However, drugs with an antimuscarinic effect, e.g., pancuronium, atropine, glycopyrrolate, were avoided. At the conclusion of surgery and wound dressing, no attempt was made to lighten the level of anaesthesia, change parameters of mechanical ventilation, or reverse neuromuscular blockade. After 10-15 min during which baseline heart rate stabilized, the effect of edrophonium was studied.

The response to edrophonium was studied in patients with normally innervated hearts (controls) and in patients who had undergone cardiac transplantation. Subjects selected as controls were ASA 1 or 2 patients who had no history of cardiac or respiratory disease ($n=15$). Cardiac transplant patients ($n=10$) underwent transplantation 8-120 mos (57 ± 10) before this study. Edrophonium was administered in divided doses *iv* to a maximum cumulative dose of $0.8 \text{ mg} \cdot \text{kg}^{-1}$, which constitutes a full reversing dose.¹⁸ Following the initial dose, each subsequent dose was administered when the heart rate response to the previous one had reached steady state, which was typically within two minutes. For control and transplant patients, administration of edrophonium was to cease when heart rate or mean systemic arterial pressure decreased by approximately 20%, when heart rate decreased below 50 beats $\cdot$ min $^{-1}$ or when the maximum cumulative dose was reached. Atropine 1.2 mg was administered routinely following the last dose of edrophonium.

Edrophonium in animals in the presence or absence of cardiac parasympathetic input

Adult cats were anaesthetized with sodium pentobarbitone ($35 \text{ mg}\cdot\text{kg}^{-1}$ *ip* initial dose, maintenance doses $3\text{--}4 \text{ mg}\cdot\text{kg}^{-1}$ *iv* every hour). Parasympathetic transmission to the heart was interrupted by bilateral vagotomy (cervical level). Sympathetic transmission to the heart was blocked by propranolol ($3 \text{ mg}\cdot\text{kg}^{-1}$ *iv*) to avoid reflex changes in heart rate secondary to anticholinesterase administration. Thus, the heart rate approached the intrinsic rate of the sinus pacemaker. Following tracheal cannulation, the lungs of the cats were artificially ventilated with 100% oxygen. End-tidal CO_2 tension was monitored and maintained at $30\text{--}35 \text{ mmHg}$. Core temperature was maintained at 37°C by a thermistor-controlled heating blanket. Arterial pressure was recorded on a Grass model 7 polygraph using a Statham transducer connected to a catheter in a femoral artery. The arterial pressure pulse triggered a cardiometer for continuous recording of heart rate. Lead II of the electrocardiogram was continuously monitored on an oscilloscope to verify the presence of sinus activity. A constant infusion of NaCl solution (0.9% , $7\text{--}8 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{hr}^{-1}$) and drugs were administered through a catheter in a femoral vein. A portion of the distal stump of the sectioned right vagus nerve was separated from the sympathetic trunk and aortic depressor nerve and placed on a bipolar hook electrode connected via a Grass SIU5 stimulus isolation unit to a Grass S88 stimulator. To mimic tonic cardiac parasympathetic input, the distal stump of the sectioned right vagus nerve was continuously stimulated (10 V , 0.5 msec , $1\text{--}3 \text{ Hz}$). The effect of edrophonium on heart rate was studied in three groups of cats. In Group 1, the vagus nerve was not stimulated, and in Groups 2 and 3, the vagus nerve was continuously stimulated to produce decreases in baseline heart rate of approximately 20% and 40%, respectively. In each group, the dose of edrophonium was increased once the heart rate response to the previous dose had reached a steady-state, which typically was within one minute.

Accumulation of consecutive doses of edrophonium was assumed in determination of dose-response curves. The dose to produce 50% of the maximum response was estimated by linear regression analysis using the linear portion of the log dose-response curve (values between 20% and 80% of maximal). The significance of differences between means was evaluated using either an unpaired Student's *t* test or an analysis of variance. Multiple comparisons were made using the Tukey-Kramer multiple comparisons test. A *P* value <0.05 was considered significant. Data are presented as means $\pm$ SEM.

FIGURE 1 Relationship between cumulative dose of edrophonium (abscissa) and change in heart rate from baseline (ordinate) expressed as beats·min (bpm). Each point represents averaged responses of control patients (filled circles, $n=15$) and transplanted patients (open circles, $n=10$). *, ***, minimum dose of edrophonium which evoked a statistically significant fall in heart rate compared to baseline in control ($P<0.05$) and transplanted ($P<0.005$) patients, respectively. Bars indicate SEM.

Results

Effect of edrophonium in patients with normally innervated hearts vs cardiac transplants

Baseline heart rate in controls was $71 \pm 3 \text{ beats}\cdot\text{min}^{-1}$ ($n=15$). Edrophonium evoked a dose-dependent decrease in heart rate, with $0.4 \pm 0.05 \text{ mg}\cdot\text{kg}^{-1}$ producing a maximum reduction of $13.3 \pm 1.6 \text{ beats}\cdot\text{min}^{-1}$ ($18.1 \pm 1.6\%$ of baseline, Figure 1). The minimum dose of edrophonium required to produce a statistically significant decrease in heart rate was $0.005 \text{ mg}\cdot\text{kg}^{-1}$ ($P<0.05$). The ED_{50} was $0.049 \pm 0.009 \text{ mg}\cdot\text{kg}^{-1}$. The decrease in heart rate typically began within 30 sec after edrophonium injection and reached a plateau within two minutes. After 1.2 mg atropine, heart rate increased to a maximum of $102.8 \pm 4.9 \text{ beats}\cdot\text{min}^{-1}$ ($152 \pm 6\%$ of baseline, $P<0.0001$). In three of the 15 controls tested, heart rate increased by $3.7 \pm 0.3 \text{ beats}\cdot\text{min}^{-1}$ ($P<0.01$) when the dose of edrophonium was increased from 0.4 to $0.8 \text{ mg}\cdot\text{kg}^{-1}$. The remaining 12 controls showed a plateau in the response.

Baseline heart rate in transplant patients was $84 \pm 3 \text{ beats}\cdot\text{min}^{-1}$ ($n=10$), which was faster than that observed in control patients ($P<0.05$). At each dose of edrophonium the reduction in heart rate was less in the transplant patients than in the controls (Figure 1). The largest decrease, by $7.3 \pm 0.8 \text{ beats}\cdot\text{min}^{-1}$ ($8.7 \pm 1.0\%$ baseline), was produced by $0.6 \pm 0.08 \text{ mg}\cdot\text{kg}^{-1}$. This maximal decrease was smaller than that observed in the control

patients ($P < 0.01$). The minimum dose of edrophonium required to produce a statistically significant decrease in heart rate compared with baseline was $0.005 \text{ mg}\cdot\text{kg}^{-1}$ ($P < 0.005$). The ED_{50} was $0.053 \pm 0.009 \text{ mg}\cdot\text{kg}^{-1}$ which was similar to that observed in the control patients ($P = 0.8$). The time course of the bradycardia produced by edrophonium was similar to that for the control patients. Following administration of 1.2 mg atropine, heart rate increased to a maximum of $88.4 \pm 3.5 \text{ beats}\cdot\text{min}^{-1}$ ($105 \pm 2\%$ of baseline, $P < 0.05$), a value which was less than that observed in control patients ($P < 0.0001$).

Edrophonium in cats in the presence or absence of cardiac parasympathetic input

In Group 1 baseline heart rate was $145 \pm 6 \text{ beats}\cdot\text{min}^{-1}$, $n=10$). Edrophonium produced a dose-dependent decrease in heart rate (Figure 2) by a maximum of $20.9 \pm 2.5 \text{ beats}\cdot\text{min}^{-1}$ ($14.9 \pm 1.9\%$ of baseline) at a dose of $9.0 \pm 1.9 \text{ mg}\cdot\text{kg}^{-1}$. The minimum dose of edrophonium required to produce a statistically significant decrease in heart rate was $0.1 \text{ mg}\cdot\text{kg}^{-1}$ ($P = 0.005$). The dose of edrophonium which produced a decrease in heart rate of $10 \text{ beats}\cdot\text{min}^{-1}$ was $1.5 \pm 0.6 \text{ mg}\cdot\text{kg}^{-1}$.

In Groups 2 and 3, baseline heart rate prior to vagus nerve stimulation was $158 \pm 5 \text{ beats}\cdot\text{min}^{-1}$ ($n=10$) and $162 \pm 11 \text{ beats}\cdot\text{min}^{-1}$ ($n=8$), respectively, which was not different from that of Group 1. During continuous electrical stimulation of the right vagus nerve, heart rate was reduced in Group 2 to $125 \pm 3 \text{ beats}\cdot\text{min}^{-1}$ ($21 \pm 1\%$ decrease) and in Group 3 to $101 \pm 6 \text{ beats}\cdot\text{min}^{-1}$ ($38 \pm 1\%$ decrease). Edrophonium 0.05 – $1.5 \text{ mg}\cdot\text{kg}^{-1}$ produced dose-dependent decreases in heart rate by a maximum of $20.3 \pm 2.8 \text{ beats}\cdot\text{min}^{-1}$ ($15.5 \pm 2.3\%$ decrease) at a dose of $1.3 \pm 0.1 \text{ mg}\cdot\text{kg}^{-1}$ in Group 2 and by $22.6 \pm 4.0 \text{ beats}\cdot\text{min}^{-1}$ ($21.5 \pm 5\%$ decrease) at a dose of $0.8 \pm 0.2 \text{ mg}\cdot\text{kg}^{-1}$ in Group 3. The doses required to produce the maximal effect were lower in Groups 2 and 3 than in Group 1 ($P < 0.001$). The minimum dose of edrophonium required to produce a statistically significant decrease in heart rate in either group was $0.05 \text{ mg}\cdot\text{kg}^{-1}$ ($P < 0.005$, Group 2; $P < 0.01$, Group 3). The reductions in heart rate in Groups 2 and 3 were greater than those produced in Group 1 at any given dose within this range (0.01 – $1.5 \text{ mg}\cdot\text{kg}^{-1}$) as indicated in the leftward shift in the dose-response curves (Figure 2). The estimated dose which produced a decrease in heart rate of 10 beats was less in Group 2 and Group 3 (0.25 ± 0.06 and $0.20 \pm 0.08 \text{ mg}\cdot\text{kg}^{-1}$, respectively) than in Group 1 ($P < 0.05$).

Unexpectedly, administration of edrophonium at doses $>1.5 \text{ mg}\cdot\text{kg}^{-1}$ produced an increase in heart rate (Figure 2). The maximal increase in heart rate from the

FIGURE 2 Relationship between cumulative dose of edrophonium (abscissa) and change in heart rate from baseline (ordinate) expressed as beats·minute (bpm). Each point represents the averaged responses of cats in which the vagus nerve was not stimulated (Group 1, $n=10$) or in which the vagus nerve was stimulated to produce an approx. 20% (Group 2, $n=10$) or 40% reduction in heart rate (Group 3, $n=8$). In Groups 2 & 3 baseline heart rate was considered to be that produced by continuous vagus nerve stimulation prior to administration of edrophonium. **, ***, minimum dose of edrophonium which evoked a statistically significant fall in heart rate compared to baseline in Group 3 ($P < 0.01$) and Groups 1 & 2 ($P < 0.005$), respectively. +++, maximum increase in HR is significantly different from nadir in Groups 2 & 3 ($P < 0.005$). Bars indicate SEM.

nadir, by $22.3 \pm 3.9 \text{ beats}\cdot\text{min}^{-1}$ ($P < 0.005$) and by $58.8 \pm 6.6 \text{ beats}\cdot\text{min}^{-1}$ ($P < 0.005$) in groups 2 & 3, respectively, was observed at a dose of $20 \text{ mg}\cdot\text{kg}^{-1}$. Larger doses produced profound hypotension and arrhythmias. The estimated dose to produce 50% of the maximal increase in heart rate was $5.3 \pm 0.7 \text{ mg}\cdot\text{kg}^{-1}$ (Group 2) and $5.4 \pm 0.6 \text{ mg}\cdot\text{kg}^{-1}$ (Group 3). If the heart rate change following edrophonium administration is expressed as the percent of baseline heart rate *prior* to vagus nerve stimulation (Figure 3), rather than as the change in heart rate *during* stimulation of the vagus nerve (Figure 2), it is apparent that heart rate never returns to the pre-vagal stimulation value, but approaches a level similar to that produced by high-dose edrophonium in the absence of vagus nerve stimulation.

Discussion

The observation that edrophonium produces a reduction in heart rate in cardiac transplant patients suggests the presence of parasympathetic postganglionic neurones in the transplanted heart which are capable of synthesizing and releasing ACh. This is based on the

FIGURE 3 Relationship between cumulative dose of edrophonium (abscissa) and change in heart rate (ordinate) expressed as per cent baseline heart rate. Each point represents the averaged responses of cats in Group 1 (no vagal stimulation, $n=10$) Group 2 (vagus nerve stimulation to produce an approx 20% decrease in heart rate, $n=10$) and Group 3 (vagus nerve stimulation to produce an approx 40% decrease in heart rate, $n=8$). In Groups 2 & 3, baseline heart rate was taken as that prior to vagus nerve stimulation. Bars indicate SEM.

assumption that the bradycardia was a result of protection from hydrolysis, by edrophonium, of the ACh spontaneously released by the parasympathetic postganglionic terminals with subsequent activation of inhibitory M_2 receptors at the SA node (Figure 4). The evidence that edrophonium produces bradycardia exclusively by its anticholinesterase action derives from the observation that when it is administered to cats with blocked parasympathetic (bilateral vagotomy) and sympathetic ($3 \text{ mg} \cdot \text{kg}^{-1}$ propranolol *iv*) input to the heart, a dose-dependent bradycardia is evoked which is highly correlated to the inhibition of cholinesterase activity; the maximum reduction in heart rate being produced by a dose of edrophonium which also produced the maximum inhibition of enzyme activity.¹⁷ In addition, muscarinic antagonists block the bradycardia produced by edrophonium and by electrical stimulation of the vagus nerve at similar doses, presumably by interfering with the action of ACh (released spontaneously or by vagus nerve stimulation) at the SA node¹⁷ (Figure 4). By analogy, the observation that the bradycardic effect of edrophonium in the transplanted patients approached a plateau presumably reflects the maximal inhibition of cholinesterase activity. In addition, the antagonism of the edrophonium-induced bradycardia by atropine is consis-

FIGURE 4 Schematic diagram of the peripheral cardiac parasympathetic pathway. Preganglionic axons contained in the vagus nerve synapse onto intra-cardiac postganglionic neurones, which in turn synapse onto cardiac parasympathetic target cells. ACh is released from both the preganglionic and postganglionic terminals. Cholinergic receptors have been identified on the postganglionic neurone as being nicotinic, muscarinic M_1 , and M_2 .³⁰ Cholinergic receptors on the target cells have been identified as being M_2 .

tent with the hypothesis that the bradycardia was mediated by activation of inhibitory muscarinic receptors at the SA node by ACh released from the postganglionic terminals. The finding that following administration of atropine, heart rate was higher, albeit slightly, compared with baseline is consistent with this interpretation. However, one cannot rule out the possibility that cells other than postganglionic neurones become capable of synthesizing and releasing ACh when the preganglionic input is interrupted, as has been suggested for Schwann cells at the amphibian myoneural junction following axotomy.¹⁹ The bradycardic effect of edrophonium in the cardiac transplant patients observed in the present study contradicts previous claims that anticholinesterases have no effect on heart rate in this type of patient.²⁰⁻²⁴ In the only study in which the cardiac effects of edrophonium in heart transplant patients were specifically investigated,²⁴ it was reported that this drug had no effect on heart rate, conduction intervals, and conduction system refractory periods. That study differs from the present investigation in two ways which may help to explain the different findings. First, patients were tested when awake, with the majority experiencing unpleasant parasympathomimetic side-effects. Conceivably, the anxiety associated with these side-effects may have produced release of catecholamine from the adrenal glands, which may have masked a bradycardic effect. Second, edrophonium was administered with a different dosing regimen (initial dose of $0.03 \text{ mg} \cdot \text{kg}^{-1}$ followed 1 min later by $0.07 \text{ mg} \cdot \text{kg}^{-1}$ and by an infusion at a rate of 0.14

$\text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) and cardiac effects were recorded only 10 min after the start of the infusion.

The edrophonium-induced bradycardia observed in the cardiac transplant patients was smaller in magnitude than that in the normally innervated patients. This difference could be accounted for by the additional ACh released from the postganglionic terminals, in patients with normally innervated hearts, as a consequence of tonic parasympathetic drive, over and above the amount released by the denervated postganglionic neurones of the transplanted heart. This suggestion is based on the finding in the cats that, for a given dose of edrophonium ($<1.5 \text{ mg}\cdot\text{kg}^{-1}$), the bradycardia was greater in the presence than in the absence of tonic activity in the parasympathetic pathway, and that in the presence of tonic activity, the bradycardia was greater the greater the level of activity. Another factor contributing to the smaller magnitude of the edrophonium-induced bradycardia in transplants than in controls may be a diminution of acetylcholinesterase activity in the transplanted heart, as has been demonstrated in the cat superior cervical ganglion in which acetylcholinesterase activity is almost completely lost within a few days of preganglionic denervation.²⁵

In the cats in which the vagus nerve was continuously stimulated to provide tonic parasympathetic drive, edrophonium at doses $>1.5 \text{ mg}\cdot\text{kg}^{-1}$ caused an increase in heart rate. A similar effect was observed in three control patients. The simplest explanation for these observations is that edrophonium in high doses blocks the evoked activation of the cardiac parasympathetic neurones. This effect of high-dose edrophonium is clearly antagonistic to its bradycardic effect at lower doses and may account for the observation that in the cats in which the vagus nerve was stimulated, the dose of edrophonium which produced the maximal bradycardia was lower than when the vagus nerve was not stimulated. The failure of heart rate to return to baseline following administration of high-dose edrophonium during continuous vagus nerve stimulation may be the consequence of the anticholinesterase effect on the spontaneous (as opposed to evoked) release of ACh from the cardiac parasympathetic postganglionic neurones. How can high-dose edrophonium interfere with activation of the cardiac parasympathetic pathway? Possibly, this results from more ACh being available (through edrophonium's anticholinesterase action) to act pre-synaptically and subsequently decrease the amount of ACh released from the presynaptic terminals.^{26,27} However, this seems unlikely for two reasons. First, we have demonstrated in cats that the inhibition of cholinesterase activity produced by edrophonium is

maximal with $6.4 \text{ mg}\cdot\text{kg}^{-1}$.¹⁷ Thus, the increases in heart rate observed with higher doses of edrophonium in the present study cannot be secondary to an anticholinesterase action. Second, examination of Figures 2 and 3 shows that the heart rate began to increase with the same dose of edrophonium regardless of whether vagal stimulation decreased heart rate by approx 20% (Group 2) or 40% (Group 3). Presumably, to produce the 40% decrease in heart rate more ACh was released than that required to produce the 20% decrease. It is unlikely that in high doses edrophonium acts as a cholinergic antagonist to block the M_2 receptor at the SA node because, if this was the case, in the absence of vagal input, the bradycardic effect produced at lower doses would be blocked by the higher doses. Similarly, in conditions of vagal input, heart rate never returns to baseline (pre-vagus nerve stimulation) following administration of high-dose edrophonium (Figure 3). A similar argument can be made to rule out edrophonium interfering with release of ACh from the postganglionic terminal. The remaining possible sites where edrophonium can interfere with evoked activation of parasympathetic neurones are the preganglionic terminal or receptors on the postganglionic neurones.

If the transplanted heart does contain functional parasympathetic postganglionic neurones, then the paucity of evidence for functional cardiac reinnervation during the post-transplant period¹ cannot simply be a consequence of a lack of target cells for the preganglionic terminals. Although the bradycardic effect of edrophonium was observed in every transplanted patient, the changes in heart rate were smaller and much less variable than those produced by neostigmine in the same type of patient.¹⁴ This likely reflects the finding that in the denervated heart, neostigmine and edrophonium appear to produce a reduction in heart rate via different mechanisms.^{16,17} Although the potential for severe bradycardia following administration of edrophonium without a muscarinic antagonist to a transplanted patient appears to be less compared to neostigmine, it seems prudent to always administer a muscarinic antagonist to block the cardiac (and non-cardiac) side effects of any type of anticholinesterase administered to this unique type of patient. The finding that higher doses of edrophonium appear to interfere with evoked parasympathetic activation may help to explain the clinical observation that less muscarinic antagonist is required to blunt the parasympathomimetic effect of edrophonium²⁸ than that of neostigmine.²⁹

In conclusion, edrophonium produces a reduction in heart rate when administered to patients having undergone cardiac transplantation. Studies in an animal

model of acute cardiac denervation suggest that the smaller bradycardic response of edrophonium observed in cardiac transplants than in patients with normally innervated hearts is a consequence of diminished or absent endogenous cardiac parasympathetic input. At high doses, edrophonium may interfere with activation of the peripheral parasympathetic nervous system.

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