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Neostigmine decreases heart rate in heart transplant patients

Purpose: This study evaluated the effect of neostigmine on heart rate in cardiac transplant patients.

Methods: Neostigmine ($2.5\text{--}50\text{ }\mu\text{g}\cdot\text{kg}^{-1}$) was administered to ASA 1 or 2 patients with normally innervated hearts (controls), and to patients who had undergone recent (<six months before study) or remote (>six months before study) cardiac transplantation.

Results: Baseline heart rate was $66 \pm 3\text{ beats}\cdot\text{min}^{-1}$ in controls ($n = 10$, mean $\pm$ SEM), which was slower than that observed in recently ($95 \pm 4\text{ beats}\cdot\text{min}^{-1}$, $n = 15$, $P < 0.001$) and in remotely ($88 \pm 3\text{ beats}\cdot\text{min}^{-1}$, $n = 16$, $P < 0.001$) transplanted patients. Neostigmine produced a dose-dependent decrease in heart rate in all patients. Controls were the most sensitive to neostigmine, with a 10% decrease in heart rate produced by an estimated dose of $5.0 \pm 1.0\text{ }\mu\text{g}\cdot\text{kg}^{-1}$. The recently transplanted group was the least sensitive, with the maximum dose producing only an $8.3 \pm 0.9\%$ reduction. The response to neostigmine of the remotely transplanted patients was variable. The estimated dose to produce a 10% decrease in heart rate in this group was $24 \pm 6\text{ }\mu\text{g}\cdot\text{kg}^{-1}$ which was greater than that for controls ($P = 0.008$). Administration of atropine (1.2 mg) reversed the neostigmine-induced bradycardia in all three groups. Reversal of the bradycardia consisted of a transient peak increase in heart rate in controls to $145 \pm 6\%$ of baseline, a value which was greater than that observed in recent ($103 \pm 1\%$, $P < 0.001$) and in remote ($109 \pm 3\%$, $P < 0.001$) transplants.

Conclusions: Neostigmine produces a dose-dependent brady-

cardia in heart transplant patients. Some remotely transplanted patients may be particularly sensitive to the bradycardic effects of neostigmine.

Objectif: Evaluer les effets de la néostigmine sur les transplantés cardiaques.

Méthodes: De la néostigmine ($2,5\text{--}50\text{ }\mu\text{g}\cdot\text{kg}^{-1}$) a été administrée à des patients ASA 1 ou 2 dont le coeur était innervé normalement (contrôles) et à des patients qui avaient subi une transplantation cardiaque récente (<six mois) ou plus ancienne.

Résultats: La fréquence cardiaque de base était de $66 \pm 3\text{ b}\cdot\text{min}^{-1}$ ($n = 10$, moyenne $\pm$ SEM) laquelle était inférieure à celle des transplantés récents ($95 \pm 4\text{ b}\cdot\text{min}^{-1}$, $n = 15$, $P < 0,001$). La néostigmine a produit une baisse de la fréquence proportionnelle à la dose chez tous les patients. Les contrôles ont été plus sensibles à la néostigmine, avec une baisse de 10% produite par une dose estimée de $5,0 \pm 1,0\text{ }\mu\text{g}\cdot\text{kg}^{-1}$. Les transplantés récents ont été les moins sensibles, alors que la dose maximale ne produisait qu'une baisse de $8,3 \pm 0,9\%$. La réponse à la néostigmine des transplantés plus anciens a été variable. La dose estimée, pour produire une baisse de 10% de la fréquence cardiaque, plus élevée que pour les contrôles, était de $24 \pm 6\text{ }\mu\text{g}\cdot\text{kg}^{-1}$ ($P = 0,008$). L'administration d'atropine a neutralisé la bradycardie induite par le néostigmine chez les trois groupes. L'antagonisme de la bradycardie a été caractérisé par une augmentation transitoire de la fréquence cardiaque à $145 \pm 6\%$ de la valeur initiale dans le groupe contrôle, valeur plus importante que chez les transplantés récents ($103 \pm 1\%$, $P < 0,001$) et anciens ($108 \pm 3\%$, $P < 0,001$).

Conclusions: Le néostigmine produit une bradycardie proportionnelle à la dose chez le transplanté cardiaque. Quelques uns des transplantés plus anciens peuvent être plus particulièrement sensibles aux effets bradycardisants de la néostigmine.

Key words

ANTAGONIST NEUROMUSCULAR RELAXANT: neostigmine;

HEART: transplantation.

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Although not verified experimentally, it is claimed that neostigmine has no effect on heart rate in heart transplant patients.¹⁻⁴ This claim derives from the observation that parasympathetic reinnervation of the heart following transplantation rarely, if ever, occurs.⁵⁻¹¹ Without reinnervation there is, presumably, insufficient tonic release of acetylcholine from cardiac parasympa-

thetic neurones of the transplanted heart to influence heart rate and, assuming that anticholinesterases produce bradycardia by preventing the hydrolysis of the tonically released acetylcholine,¹² a lack of effect of neostigmine is anticipated. However, in animal studies, neostigmine still produces a reduction in heart rate when tonic autonomic efferent activity to the heart is interrupted. For example, we have shown that when neostigmine is administered to cats with blocked parasympathetic (bilateral vagotomy) and sympathetic (propranolol $3 \text{ mg} \cdot \text{kg}^{-1}$ *iv*) efferent input to the heart, a powerful, dose-dependent bradycardia is evoked.¹³ A mechanism other than the inhibition of cholinesterase appears to be responsible for evoking the bradycardia. Evidence suggests that the bradycardia is produced by neostigmine activating cholinergic receptors in the cardiac parasympathetic pathway.^{13,14,17} From these animal studies, it is predicted that neostigmine would produce a reduction in heart rate in cardiac transplant patients. In fact, in a recent case report, we described the bradycardic effects of neostigmine in a heart transplant patient.¹⁵ The present study has examined the effect of neostigmine on heart rate in patients with normally innervated hearts and in patients who had undergone recent (<six months) or remote (>six months) cardiac transplantation.

Methods

After approval by the Ethics Committee for human studies at the Royal Victoria Hospital, informed consent was obtained from patients included in this study. The effect of neostigmine on heart rate was studied in anaesthetized, paralyzed patients, during mechanical ventilation at the conclusion of surgery, or in fully awake spontaneously-breathing patients. None of the patients was receiving medications with anticholinergic effects before the study, and all were in sinus rhythm. During the study, lead II of the ECG was continuously monitored to verify the presence of sinus rhythm. Heart rate was recorded by a continuous printout at $50 \text{ mm} \cdot \text{sec}^{-1}$. In those patients studied at the conclusion of surgery, 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 anticholinergic effect, e.g., pancuronium, atropine, glycopyrrolate, were avoided. After the conclusion of surgery and wound dressing, a 10–15 min “rest” period was observed during which the patient was not stimulated. During this time, no attempt was made to lighten the level of anaesthesia, change variables of mechanical ventilation, or reverse neuromuscular blockade. Awake patients were studied in the recovery room or in the intensive care unit following a 10–15 min “rest” period.

Neostigmine *iv* was administered in divided doses to a maximum cumulative dose of $50.0 \mu\text{g} \cdot \text{kg}^{-1}$ which constitutes a full reversing dose. Following the initial dose, each subsequent dose was administered when the heart rate response to the previous one had reached a steady state, which was typically within seven minutes. Administration of neostigmine ceased when one of the following occurred: (1) heart rate or mean systemic arterial pressure decreased by approximately 20%, (2) heart rate decreased below 50 beats $\cdot \text{min}^{-1}$, or (3) patients complained of unpleasant side-effects, e.g., abdominal cramps, nausea, salivation, or visual disturbances. Atropine 1.2 mg *iv* was administered within seven minutes after the last dose of neostigmine.

The effect of neostigmine on heart rate was studied in patients with normally innervated hearts (controls) and in patients who had undergone cardiac transplantation. Patients studied within six months of their transplant were classified as “recent” and those studied after six months were classified as “remote” in accordance with a previous investigation.¹⁶ The dose of neostigmine to produce a 10% decrease in heart rate was estimated in individual patients (when more than one dose was given) using linear regression analysis. 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.

Controls

Subjects selected for study as controls were ASA 1 or 2 patients who had no history of cardiac or respiratory disease and were undergoing non-cardiac related surgery ($n = 10$). All studies were performed in the operating room after the conclusion of surgery. The initial dose of neostigmine was $2.5 \mu\text{g} \cdot \text{kg}^{-1}$ followed by subsequent doses of $2.5 \mu\text{g} \cdot \text{kg}^{-1}$. The maximum cumulative dose given was $7.5 \mu\text{g} \cdot \text{kg}^{-1}$.

Recent transplants

Subjects in this group underwent cardiac transplantation within eight weeks of study ($n = 15$). Four were studied in the operating room immediately after transplantation, and three were studied three to eight weeks after transplantation at the conclusion of non-cardiac related surgery. The remaining eight patients were studied within 48 hr of transplantation, while awake and spontaneously breathing. Neostigmine was administered in doses of $10.0 \mu\text{g} \cdot \text{kg}^{-1}$ to a maximum cumulative dose of $50.0 \mu\text{g} \cdot \text{kg}^{-1}$.

FIGURE 1 Relationship between cumulative dose of neostigmine (abscissa) and decrease in heart rate (ordinate) expressed as per cent baseline heart rate prior to drug administration. Each point represents averaged responses of control patients (solid squares, $n = 10$) and recently transplanted patients (solid circles, $n = 15$), and individual responses of remotely transplanted patients (open triangles, $n = 16$). Remotely transplanted patients #1 and #2 demonstrated enhanced sensitivity to neostigmine. Patient #3 demonstrated intermediate sensitivity. Bars indicate SEM.

Remote transplants

Subjects in this group ($n = 16$) underwent cardiac transplantation 8–152 mo (63 ± 11) before this study. All were studied in the operating room at the conclusion of non-cardiac related surgery. Neostigmine was administered in doses of $10.0 \mu\text{g} \cdot \text{kg}^{-1}$ to a maximum cumulative dose of $50.0 \mu\text{g} \cdot \text{kg}^{-1}$.

Results

Controls

Baseline heart rate was $66 \pm 3 \text{ beats} \cdot \text{min}^{-1}$ ($n = 10$). Neostigmine evoked a dose-dependent decrease in heart rate (Figure 1), with a 10% decrease produced by an estimated dose of $5.0 \pm 1.0 \mu\text{g} \cdot \text{kg}^{-1}$. The decrease in heart rate typically began 60–90 sec after injection and reached a plateau within seven minutes. Within one minute after administration of 1.2 mg atropine, heart rate increased transiently to a maximum of $145 \pm 6\%$ of the baseline level (Figure 2) before returning to control.

Recent transplants

Baseline heart rate was $95 \pm 4 \text{ beats} \cdot \text{min}^{-1}$ ($n = 15$), which was faster than that observed in the control patients ($P < 0.001$). Neostigmine evoked a dose-dependent decrease in heart rate in all patients, with the dose-response curve shifted to the right of that for the controls (Figure 1). The estimated dose of neostigmine to produce a 10% decrease in heart rate was 78 ± 17

FIGURE 2 Maximal increase in heart rate, expressed as per cent baseline heart rate prior to neostigmine injection, in response to atropine 1.2 mg, administered following last dose of neostigmine. Each open symbol represents individual responses of control patients (open diamonds, $n = 10$), recently transplanted (open circles, $n = 15$) and remotely transplanted (open triangles, $n = 16$) patients. Numbers #1–3 indicate patients who had intermediate (#3) or enhanced (#1 and 2) responses to neostigmine (see Figure 1). Filled symbol represents the mean responses of control patients (solid diamonds), recently transplanted (solid circles) and remotely transplanted (solid triangles) patients. ***, significantly different from controls, $P < 0.001$. Bars indicate SEM.

$\mu\text{g} \cdot \text{kg}^{-1}$, which is greater than the maximum dose of $50 \mu\text{g} \cdot \text{kg}^{-1}$ given to these patients. The maximum dose of neostigmine given to this group produced a decrease in heart rate by $8.3 \pm 0.9\%$ of baseline. The time course of the bradycardia produced by neostigmine was similar to that of the controls. Following administration of atropine (1.2 mg), heart rate increased transiently to a maximum of $103 \pm 1.0\%$ of the baseline, a value which was less than that observed in the control patients ($P < 0.001$, Figure 2).

Remote transplants

Baseline heart rate was 88 ± 3 beats $\cdot$ min⁻¹ ($n = 16$), which was faster than that observed in the control patients ($P < 0.001$), but similar to that observed in the recently transplanted patients ($P > 0.05$). Neostigmine produced a reduction in heart rate in all patients, with a time course similar to that described above. The magnitude of the bradycardia produced by neostigmine was variable (Figure 1). The estimated dose of neostigmine to produce a 10% decrease in heart rate was 24 ± 6 μ g $\cdot$ kg⁻¹, which was greater than that for the control patients ($P = 0.008$). Following administration of atropine (1.2 mg), heart rate transiently increased to $109 \pm 3\%$ of the baseline, which was less than that observed in the control patients ($P < 0.001$) and numerically but not significantly greater than that observed in the recent transplants ($P > 0.05$, Figure 2). The magnitude of this increase was not correlated to the sensitivity to neostigmine, as determined from the estimated dose to produce a 10% reduction in heart rate ($r = 0.15$, $P = 0.6$). Nevertheless, the largest increase in heart rate following atropine administration was observed in patient #2 (133%, Figure 2), who also demonstrated enhanced sensitivity to neostigmine (Figure 1). The next largest increase produced by atropine (128%, Figure 2), was observed in patient #3 who demonstrated an intermediate sensitivity to neostigmine (Figure 1). Patient #1 who had enhanced sensitivity to neostigmine (Figure 1), demonstrated a moderate increase following atropine administration (112%, Figure 2).

Discussion

The main finding of this study is that neostigmine consistently produced a dose-dependent bradycardia in patients having undergone cardiac transplantation. This observation is at odds with the widely-held belief that anticholinesterases are without effect in cardiac transplants,¹⁻⁴ and supports our previous case report describing the bradycardic effect of neostigmine in a heart transplant patient.¹⁵ It is not clear why other investigators did not notice a bradycardic effect of neostigmine because important details concerning the methodology of those studies are lacking. For example, it is not clear whether or not patients had received drugs with antimuscarinic effects (e.g., pancuronium, atropine, or glycopyrrolate) before neostigmine, what dose of neostigmine was administered or how long heart rate was monitored following administration of neostigmine.

The demonstration that neostigmine produces bradycardia in the recently transplanted patients is an important observation because, in this group, cardiac reinnervation can be ruled out. Thus, there is no tonic vagal input to stimulate release of acetylcholine from cardiac

parasympathetic neurones in the transplanted heart. Therefore, in these patients, neostigmine-induced bradycardia is unlikely to be mediated by an anticholinesterase effect on tonically released acetylcholine. By analogy, from the findings in the animal experiments summarized in the introduction, neostigmine may produce bradycardia by direct activation of cholinergic receptors in the peripheral cardiac parasympathetic pathway. While the pharmacological identity of the cholinergic receptor(s) activated by neostigmine remains to be characterized, evidence suggest that it is muscarinic.^{13,14,17} This possibility is consistent with the observation in the current study that neostigmine-induced bradycardia was reversed by atropine.

It is difficult to assess the magnitude of the effect of neostigmine on heart rate in recently transplanted patients. Initially, it was decided to administer neostigmine immediately after transplantation, as patients would be anaesthetized and not experience the unpleasant side-effects. However, it is possible that the chronotropic and inotropic agents which are routinely administered following transplantation, and such factors as global myocardial ischaemia, surgical manipulation and reperfusion injury, interfere with the heart rate response to neostigmine. Therefore, the effect of neostigmine on heart rate was studied in another group within 48 hr following transplantation, once patients had been weaned from chronotropic and inotropic agents. However, these patients were tested when awake and spontaneously breathing, which may additionally influence the response to neostigmine. In three patients, we were able to study the effects of neostigmine within several weeks of transplantation during general anaesthesia at the conclusion of non-cardiac related surgery. It should be noted that the magnitude of the bradycardia produced by neostigmine was not different regardless of whether patients were studied immediately, within 48 hr, or several weeks following transplantation (30 μ g $\cdot$ kg⁻¹ neostigmine produced a decrease in baseline heart rate of 7.0 ± 1.0 , 6.0 ± 1.0 , and $8.0 \pm 1.0\%$, respectively). Moreover, the responses observed in the recently transplanted patients appear to be within the range of those observed with the remotely transplanted group but they are at the low end of the spectrum. This observation suggests that the bradycardic response to neostigmine may change over time after transplantation.

The remotely transplanted patients demonstrated a variable sensitivity to the bradycardic effects of neostigmine. We cannot explain why the remotely transplanted patient may demonstrate a particular sensitivity to the bradycardic effects of neostigmine. Possibly, during the post-transplant period reinnervation of the transplanted heart occurs, enabling neostigmine to exert an anti-

cholinesterase action on tonically released acetylcholine. This possibility is supported by a few reports suggesting sympathetic and parasympathetic reinnervation in transplant patients. Sympathetic reinnervation is supported on the basis of tyramine stimulation^{18,19} and isotopic labelling studies.²⁰ Regarding parasympathetic reinnervation, one transplant patient has been described to have an atropine-sensitive variability in heart rate.²¹ In addition, there are reports of "vaso-vagal" syncope in cardiac transplant patients suggestive of parasympathetic reinnervation.²² Finally, rapid fluctuations in heart rate produced by changes in posture, which reflect changes in parasympathetic tone in the normal heart, have been observed following transplantation.⁷ On the other hand, sympathetic reinnervation has been refuted on the basis of a lack of appropriate change in heart rate in response to exercise⁵⁻⁸ and hypotension.^{7,9} Parasympathetic reinnervation has been denied on the basis of no change in heart rate to atropine^{6,8} and a lack of heart rate variability.⁹⁻¹¹ In the present study, the similar response to atropine among patients recently and remotely transplanted, as well as the lack of a relationship between sensitivity to neostigmine and increase in heart rate produced by atropine, argues against reinnervation. Nevertheless, the largest increase in heart rate produced by atropine was observed in a remotely transplanted patient who also had an enhanced sensitivity to neostigmine (patient #2, Figures 1 and 2). Similarly, a patient with intermediate sensitivity to neostigmine also demonstrated a large increase following atropine administration (patient #3, Figures 1 and 2). These observations raise the possibility that parasympathetic cardiac reinnervation may have occurred in some patients.

Another explanation for the increased sensitivity of the remotely transplanted patients to neostigmine may be a denervation supersensitivity to the cholinergic agonist effect of neostigmine, assuming that neostigmine produces bradycardia by activation of cholinergic receptors in the cardiac parasympathetic pathway (see above). It is relevant that denervation supersensitivity to cholinergic agonists has been reported in animal experiments following prolonged interruption of parasympathetic preganglionic input to the heart.^{23,24} Cardiac reinnervation and denervation supersensitivity are not mutually exclusive phenomena.

Although care was taken to avoid administration of agents with known anticholinergic effects perioperatively, when considering the bradycardia produced by neostigmine in the transplanted patient, this study does not address the potential effects of anaesthesia or perioperative medications. While the mechanism(s) underlying the bradycardic response to neostigmine in the cardiac transplant patients remains to be determined, the

findings from our present study and the recent report of sinus arrest in two cardiac transplant patients following neostigmine administration²⁵ indicate that when neuromuscular blockade is reversed with neostigmine in this unique type of patient, a decrease in heart rate should be anticipated. The bradycardic effect of neostigmine may be particularly pronounced in those patients remotely transplanted, and we caution that a muscarinic antagonist should always be co-administered with anticholinesterases to block the cardiac and other muscarinic side-effects.

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