Different properties of the bradycardia produced by neostigmine and edrophonium in the cat

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Purpose: *The bradycardia produced by neostigmine and edrophonium was examined according to its relation to cholinesterase inhibition and to its sensitivity to block by muscarinic receptor antagonists. For comparison, the ability of muscarinic antagonists to block the bradycardia produced by electrical stimulation of the vagus nerve was determined.*

Methods: *Cats were anaesthetized, vagotomized and propranolol-treated. Heart rate was continuously recorded. Erythrocyte cholinesterase activity of arterial blood was measured using a radiometric technique. The right vagus nerve was isolated for electrical stimulation. The muscarinic antagonists used were atropine, glycopyrrolate, pancuronium, gallamine, and AFDX-116.*

Results: *Neostigmine produced a dose-dependent decrease in cholinesterase activity which reached a plateau at a cumulative dose of 0.16 mg·kg^{-1} (ED_{50} 0.009 ± 0.003 mg·kg^{-1}). Neostigmine produced a dose-dependent decrease in heart rate with the dose-response relationship (ED_{50} 0.1 ± 0.01 mg·kg^{-1}; P = 0.0006) shifted to the right of that for the inhibition of cholinesterase activity. In contrast to the anticholinesterase effect, the bradycardic effect did not reach a plateau and continued to increase even at doses at which the cholinesterase inhibition was maximal. The maximal decrease in heart rate when the heart was still in sinus rhythm was by 81 ± 13 bpm (49 ± 7% of baseline), which was produced by a dose of 0.32 mg·kg^{-1}. Edrophonium produced dose-dependent decreases in cholinesterase activity and heart rate, which were highly correlated (correlation coefficient r = 0.99, P <0.0001). The ED_{50} of the reduction in heart rate (0.9 ± 0.18 mg·kg^{-1}) and cholinesterase activity (0.89 ± 0.12 mg·kg^{-1}) produced by edrophonium were similar. Moreover, the reduction in heart rate and cholinesterase activity produced by edrophonium reached a plateau at the same dose (6.4 mg·kg^{-1}). At this dose, heart rate decreased by 22 ± 2 bpm (14.6 ± 0.9% of baseline). Compared to the bradycardia produced by stimulation of the vagus nerve, that produced by neostigmine was blocked by muscarinic antagonists at significantly lower doses while that produced by edrophonium was blocked at similar doses.*

Conclusions: *The neostigmine-induced bradycardia is poorly correlated with cholinesterase inhibition compared to that produced by edrophonium, and has a higher sensitivity to muscarinic receptor antagonists compared to that produced by edrophonium or vagus nerve stimulation. These results are consistent with the hypothesis that the neostigmine-induced bradycardia is, in part, the result of neostigmine directly activating cholinergic receptors within the cardiac parasympathetic pathway. The bradycardia produced by edrophonium may be accounted for solely by an anticholinesterase action.*

Key words
ANTAGONISTS, NEUROMUSCULAR RELAXANTS: edrophonium, neostigmine;
HEART: arrhythmia, bradycardia.

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Objectif: *La bradycardie produite par la néostigmine et l'édrophonium fait l'objet de cette communication qui s'intéresse spécifiquement à sa correlation avec l'inhibition cholinestérasique et l'effet des antagonistes des récepteurs muscariniques. Pour fin de comparaison, l'influence des antagonistes muscariniques sur le blocage de la bradycardie induite par la stimulation électrique du nerf vague a été déterminée.*

Méthodes: *Des chats étaient anesthésiés, vagotomisés et traités au propanolol. La fréquence cardiaque était enregistrée en continu. L'activité cholinestérasique érythrocytaire du sang artériel était mesurée par radiométrie. Le nerf vague*

droit était isolé pour la stimulation électrique. Les antagonistes muscariniques suivants étaient utilisés: l'atropine, le glycopyrrolate, le pancuronium, la gallamine et l'AFDX-116.

***Résultats**: La néostigmine a produit une baisse proportionnelle à la dose de l'activité cholinestérasique qui a atteint un plateau à la dose cumulative de 0,16 mg·kg^{-1} (ED_{50} 0,009 ± 0,003 mg·kg^{-1}). La néostigmine a provoqué une baisse proportionnelle à la dose de la fréquence cardiaque avec une relation dose-effet (ED_{50} 0,1 ± 0,01 mg·kg^{-1}; P = 0,0006) déviée à droite de celle de l'inhibition de l'activité cholinestérasique. Contrairement à l'effet anticholinestérasique, l'effet bradycardique n'a pas atteint de plateau et a continué d'augmenter même aux doses d'inhibition cholinestérasique maximale. La baisse maximale dela fréquence cardiaque en rythme sinusal a été de 81 ± 13 bpm (49 ± 7% de la ligne de base) et a été induite par une dose de 0,32 mg·kg^{-1}. L'édrophonium a provoqué des baisses proportionnelles à la dose nettement corrélées de l'activité cholinestérasique et de la fréquence cardiaque, (coefficient de corrélation r = 0,99, P < 0,0001). L'ED_{50} de la baisse de fréquence cardiaque (0,9 ± 0,18 mg·kg^{-1}) et celle de l'activité cholinestérasique (0,89 ± 0,12 mg·kg^{-1}) produites par l'édrophonium étaient identiques. En outre, la baisse de la fréquence cardiaque et de l'activité cholinestérasique produite par l'édrophonium a atteint un plateau à une dose identique (6,4 mg·kg^{-1}). A cette dose, la fréquence cardiaque a diminué de 22 ± 2 bpm (14,6 ± 0,9% de la ligne de base). Comparativement à la bradycardie produite par la stimulation vagale, la bradycardie produite par la néostigmine était bloquée par les antagonistes muscariniques à des doses plus faibles alors que celle produite par l'édrophonium était bloquée à des doses identiques.*

***Conclusions**: La bradycardie induite par la néostigmine est en faible corrélation avec l'inhibition cholinestérasique comparativement à celle qui est produite par l'édrophonium, et est plus sensible aux antagonistes des récepteurs muscariniques comparativement à celle qui est produite par l'édrophonium ou la stimulation vagale. Ces résultats concordent avec l'hypothèse selon laquelle la bradycardie induite par la néostigmine est partiellement causée par l'activation directe de récepteurs cholinergiques empruntant la voie de conduction cardiaque parasympathique. La bradycardie produite par l'édrophonium peut s'expliquer uniquement par un effet anticholinestérasique.*

A common side effect of anticholinesterase drugs is bradycardia, which, on occasion, can be so severe as to result in asystole.[1,2] A previous study suggested that the bradycardia induced by neostigmine involves mechanisms other than the inhibition of cholinesterase.[3] In propranolol-treated, vagotomized cats neostigmine and edrophonium produced dose-dependent reductions in heart rate. However, the dose-response curves were markedly different for the two drugs. For neostigmine, throughout the range of doses tested, the magnitude of the bradycardia varied directly with dose and a plateau in the response was never observed. The decrease in heart rate produced by the highest dose of neostigmine used, while sinus rhythm was maintained, was approximately 50% of baseline. In contrast, edrophonium produced a dose-dependent decrease in heart rate to a plateau of only 15% below the baseline. The shape of the edrophonium dose-response curve was consistent with the expectation that, if the reduction in heart rate was the consequence of protection from hydrolysis of the acetylcholine (ACh) spontaneously released by the parasympathetic pre- and postganglionic terminals (Figure 1), a plateau in the response should occur, presumably at the dose which produces maximum inhibition of the enzyme. This prediction has never been tested. In contrast, the lack of a plateau in the neostigmine dose-response relationship suggests mechanisms additional to the inhibition of cholinesterase. On the basis of properties of the neostigmine-induced bradycardia we have suggested that neostigmine activates cholinergic receptors on cardiac parasympathetic ganglion cells, with subsequent release of ACh from their axon terminals at the sinus node[3] (Figure 1). While the pharmacological identity of the cholinergic receptor(s) activated by neostigmine remains to be characterized, evidence suggests that it may be muscarinic of the M_2 sub-type.[3,4]

The purpose of this study was twofold. First, to determine how the anticholinesterase action of neostigmine and edrophonium is related to the production of the bradycardia, the relationship between the inhibition of cholinesterase and the decrease in heart rate was compared following administration of increasing doses of neostigmine or edrophonium. In a second set of experiments the potency of muscarinic antagonists to block the bradycardia produced by neostigmine or edrophonium, and electrical stimulation of the vagus nerve was compared. If neostigmine or edrophonium produces bradycardia solely by their anticholinesterase action, muscarinic antagonists should block the bradycardia produced by these drugs and that produced by vagus nerve stimulation at similar doses by interfering with the action of ACh (released spontaneously or by vagus nerve stimulation) at the SA node (Figure 1). If, on the other hand, neostigmine or edrophonium also activates muscarinic M_2 receptors on cardiac ganglion cells, leading to subsequent release of ACh and activation of M_2 receptors at the SA node, the bradycardia produced by these drugs may be more sensitive to muscarinic block,

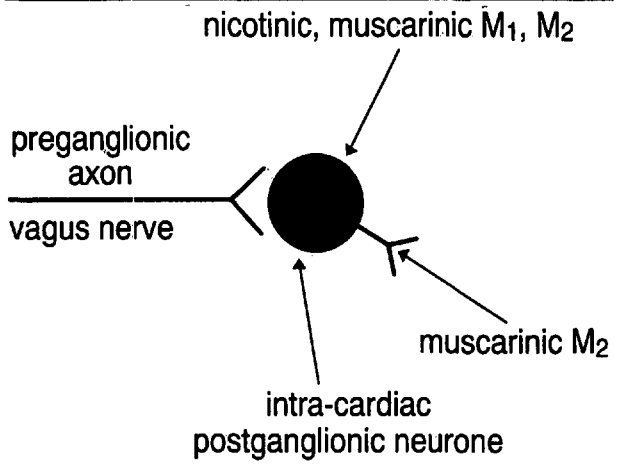

FIGURE 1 Schematic diagram of the peripheral cardiac parasympathetic pathway. Preganglionic axons contained in the vagus nerve synapse onto intra-cardiac postganglionic neurones, which in turn synapse onto cardiac parasympathetic target cells. ACh is released from both the preganglionic and postganglionic terminals. Cholinergic receptors have been identified on the postganglionic neurone as being nicotinic, muscarinic M_1, and M_2.[18] Cholinergic receptors on the target cells have been identified as being M_2.

compared to that produced by vagus nerve stimulation, because of an additive effect, resulting from an action of muscarinic block at both the cardiac ganglion cell and the SA node (Figure 1).

Methods

Cats (2.0–4.5 kg, $n = 65$) were anaesthetized with sodium pentobarbitone (35 mg·kg^{-1} *ip* initial dose, maintenance doses 3–4 mg·kg^{-1} *iv* every hour). Parasympathetic efferent nerve activity to the heart was interrupted by bilateral vagotomy (cervical level), and sympathetic transmission to the heart was blocked by propranolol (3 mg·kg^{-1} *iv*), in order to avoid reflex changes in heart rate secondary to anticholinesterase administration. A constant infusion of 0.9% NaCl solution (7–8 ml·kg^{-1}·hr^{-1}) and drugs were administered through a catheter in a femoral vein. A catheter was inserted in a femoral artery to withdraw blood samples for determination of erythrocyte cholinesterase activity (see below). Following tracheal cannulation, the cats were artificially respired with 100% oxygen. End-tidal CO_2 was monitored and maintained at 30–35 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 second femoral artery. The arterial pressure pulse triggered a cardiotachometer for continuous recording of heart rate. Lead II of the electrocardiogram was continuously monitored on an oscilloscope to verify the presence of sinus activity following administration of anticholinesterases. The distal end of the cut 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. The effect of cholinesterase inhibitors on erythrocyte cholinesterase activity was used as an index of their effect on activity in cardiac tissue. In this set of experiments, cholinesterase activity was determined on arterial blood samples prior to (control) and following injection of increasing doses of neostigmine or edrophonium, when heart rate had reached a steady-state at each dose. One hundred microlitre aliquots of freshly collected heparinized blood were pre-incubated with the butylcholinesterase inhibitor ISO-OMPA (10 μM, 22°C, 10 min) and acetylcholinesterase activity was measured with acetylcholine (1.5 mM) as substrate. The ACh (acetyl labelled ^{3}H acetylcholine, 223 μCi. mmole^{-1}) was added (10 μl), the reaction proceeded (22°C) for 60 sec, and was terminated by the addition of 1 ml ice-cold isotonic buffer (pH 7.4) containing 1 mM physostigmine. After centrifugation (10,000 g for four minutes), the supernatant was collected and shaken with a solution (10 mg·ml^{-1}) of tetraphenylboron in heptanone. Radioactivity in the organic phase (residual ^{3}H acetylcholine) and in the aqueous phase (^{3}H acetate resulting from the hydrolysis of substrate) were measured. The reversible inhibition of acetylcholinesterase by neostigmine and edrophonium and the need to ensure that the *in vitro* measures reflected the *in vivo* activity, required assay of cholinesterase activity without sample dilution. Each sample was measured in duplicate; assay variability was less than 5%. The specificity of the assay was ensured by choice of substrate, ACh, and by the use of ISO-OMPA so that acetylcholinesterase activity was measured. The sensitivity of the assay was determined by the specific radioactivity of substrate used; the hydrolysis of 1 nmole of ACh could be determined under the conditions used.

In another set of experiments the effect of muscarinic receptor antagonists on the bradycardia produced by anticholinesterases was tested using a dose of the anticholinesterases that produced 50–60% of the maximum decrease in heart rate. To this end, neostigmine 0.25 mg·kg^{-1} and edrophonium 1.0 mg·kg^{-1} were administered, which produced reductions in baseline heart rate of 38 ± 2% ($n = 24$) and 10 ± 1% ($n = 8$), respectively. To study the effect of muscarinic receptor antagonists on the bradycardia produced by vagus nerve stimulation, the right vagus nerve was electrically stimulated

(10 V, 0.5 msec, 1–2 Hz, 10 sec) to produce a reduction in heart rate of 34 ± 1% (n = 23). In this set of experiments, administration of neostigmine or edrophonium, and electrical stimulation of the vagus nerve, were done in different animals, and only one muscarinic antagonist was studied per animal. A dose-response relation was determined for each muscarinic receptor antagonist studied. The dose of the muscarinic receptor antagonist was increased once the response to the previous dose had reached a steady-state.

The relationship between dose and effect of either anticholinesterase drugs or muscarinic antagonists was determined by constructing dose-response curves. For each cat, the ED_{50} of the anticholinesterase or muscarinic receptor antagonist being studied was determined by linear regression analysis using the linear portion of the log dose-response curve (values between 20% and 80% of maximal effect). The ED_{50}s for anticholinesterase effects on heart rate and cholinesterase activity were compared using a paired Student's t test. The ED_{50}s for muscarinic block of each type of bradycardia were compared using the unpaired Student's t test. A P value of less than or equal to 0.05 was considered significant. Data are expressed as mean ± SEM.

Non-selective muscarinic receptor antagonists used were atropine[5] (Sigma,) and glycopyrrolate[5] (Sabex Inc). The selective M_2 muscarinic receptor antagonists studied were 11,2-(Diethylamino)methyl-1-piperidinyl acetyl-5,11-dihydro-6H-pyrido 2,3-b 1,4 benzodiazepine-6-one[6] (AFDX-116, Boehringer Ingelheim), pancuronium[6] (Organon) and gallamine[6] (Rhone-Poulenc Rorer). All drugs were dissolved in 0.9% NaCl solution, with the exception of AFDX-116, which was dissolved in 10% dimethyl sulfoxide (DMSO) in saline.

Results

Inhibition of cholinesterase activity and the bradycardia produced by edrophonium

Edrophonium produced a dose-dependent inhibition of cholinesterase activity (n = 5, Figures 2A and 3A), which reached a plateau when a cumulative dose of 6.4 $mg \cdot kg^{-1}$ had been administered. At the plateau, cholinesterase activity was inhibited by 82 ± 4%. The estimated dose of edrophonium which produced 50% of the maximum inhibition was 0.89 ± 0.12 $mg \cdot kg^{-1}$. Edrophonium also produced a dose-dependent decrease in heart rate (n = 5, Figures 2B and 3A) which closely paralleled the decrease in cholinesterase activity (correlation coefficient r = 0.99, $P < 0.0001$) and reached a plateau at the dose at which inhibition of cholinesterase activity had reached saturation. The maximal decrease in heart rate was 22 ± 2 bpm (14.6 ± 0.9% of baseline). The estimated dose of edrophonium which produced 50% of the maximum decrease in heart rate (0.9 ± 0.18 $mg \cdot kg^{-1}$) was not different from that which produced 50% of the maximum decrease in cholinesterase activity (P = 0.6).

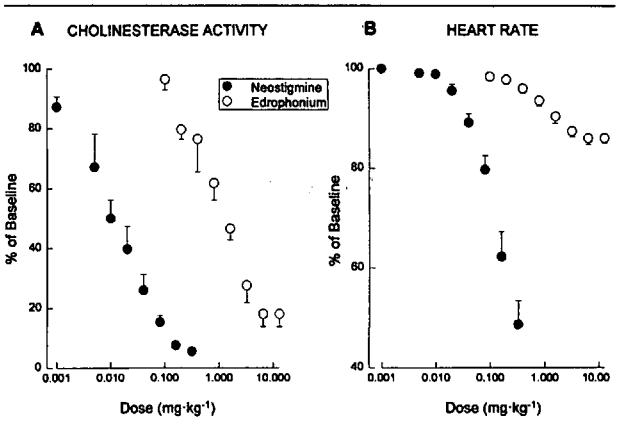

FIGURE 2 Relationship between the decrease in heart rate (panel A) or acetylcholinesterase activity (panel B) expressed as percent of baseline (ordinate) and dose of anticholinesterase (abscissa). Each point represents the average response in five animals. Bars indicate S.E.M.

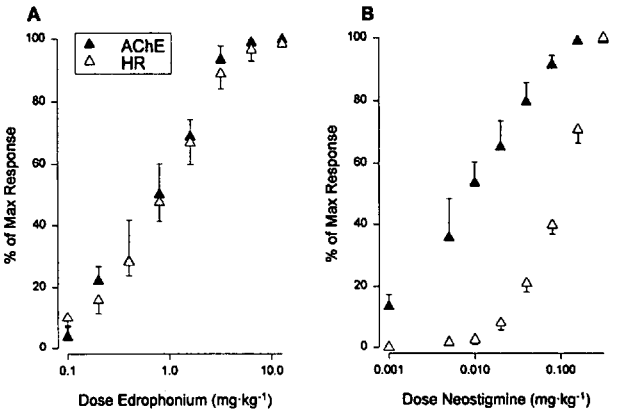

FIGURE 3 Relationship between the decrease in acetylcholinesterase activity (AChE) or heart rate (HR) expressed as percent of maximum response (ordinate) and dose of anticholinesterase (abscissa). Bars indicate S.E.M.

Inhibition of cholinesterase activity and the bradycardia produced by neostigmine

Neostigmine produced a dose-dependent decrease in cholinesterase activity (n = 5, Figures 2A, 3B) which reached a plateau when a cumulative dose of 0.16 $mg \cdot kg^{-1}$ had been administered. At the plateau, cholinesterase activity was inhibited by 93.4 ± 1%.

Neostigmine also evoked a dose-dependent decrease in heart rate (Figures 2B and 3B). However, in contrast to the anticholinesterase effect which reached a plateau, the bradycardic effect did not taper off at higher doses. Heart rate decreased progressively with each additional dose of neostigmine until arrhythmias such as ectopic QRS complexes as well as third degree atrioventricular block were produced.[3] At the lowest dose of neostigmine that produced maximal inhibition of cholinesterase activity (0.16 mg·kg^{-1}), heart rate decreased by 62.4 ± 10.9 bpm, which represents 72.0 ± 4% of the maximal decrease in heart rate with preservation of sinus rhythm produced by this agent. The maximal decrease in heart rate when the heart was still in sinus rhythm was produced by a dose of 0.32 mg·kg^{-1}. This dose caused a decrease in heart rate of 81 ± 13 bpm from a baseline value of 162 ± 8 bpm (decrease of 49 ± 7%). The dose-response relationship for the bradycardic effect of neostigmine was shifted to the right of that for the inhibition of cholinesterase activity (n = 5, Figure 3B). For example, the estimated dose of neostigmine to produce 50% of the maximum bradycardia was 0.1 ± 0.01 mg·kg^{-1}, approximately ten times that which produced a 50% reduction in cholinesterase activity (0.009 ± 0.003 mg·kg^{-1}, P = 0.0006).

Muscarinic antagonism of the bradycardia produced by neostigmine and vagus nerve stimulation

Control heart rate prior to neostigmine administration (145 ± 4 bpm, n = 24) was not different from that prior to vagus nerve stimulation (144 ± 4 bpm, n = 23). The dose-response curves of the muscarinic antagonists to block the neostigmine-induced bradycardia were shifted to the left compared with those of the antagonists to block the bradycardia produced by vagus nerve stimulation (Figures 4 and 5). The ED_{50}s of the non-selective muscarinic antagonists glycopyrrolate and atropine to block the bradycardia produced by neostigmine was approximately one-half those required to block the bradycardia produced by vagus nerve stimulation (Table). The ED_{50}s of the selective M_2 muscarinic receptor antagonists AFDX-116, pancuronium and gallamine to block the neostigmine-induced bradycardia were approximately one-half, one-quarter, and one-tenth of those required to block the bradycardia produced by vagus nerve stimulation, respectively (Table).

Muscarinic antagonism of the bradycardia produced by edrophonium and vagus nerve stimulation

Control heart rate prior to edrophonium administration (161 ± 7 bpm, n = 8) was not different from that prior to vagus nerve stimulation (see above). The dose-response

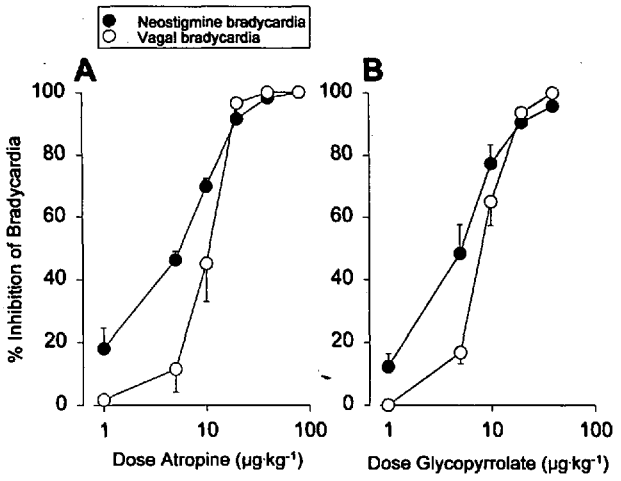

FIGURE 4 Relationship between dose of atropine (abscissa, panel A) or glycopyrrolate (abscissa, panel B) and percent inhibition of bradycardia (ordinate) evoked by neostigmine (filled circles) or by vagus nerve stimulation (open circles). Each point is the averaged response of four or five animals. Bars indicate S.E.M.

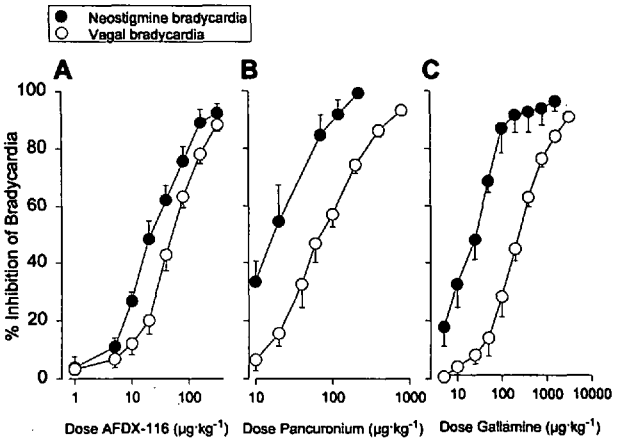

FIGURE 5 Relationship between dose of AFDX-116 (abscissa, panel A), pancuronium (abscissa, panel B) or gallamine (abscissa, panel C) and percent inhibition of bradycardia (ordinate) evoked by neostigmine (filled circles) or by vagus nerve stimulation (open circles). Each point is the averaged response of four to six animals. Bars indicate S.E.M.

curves of the muscarinic antagonists to block the edrophonium-induced bradycardia were similar compared with those of the antagonists to block the bradycardia produced by vagus nerve stimulation (Figure 6). The ED_{50}s for atropine and gallamine block of the edrophonium-induced bradycardia were not different from those for block of the bradycardia produced by vagus nerve stimulation (Table). Further studies using the other muscarinic antagonists to block the edrophonium-induced bradycardia were not done.

TABLE Estimated doses ($\mu g \cdot kg^{-1}$) of muscarinic receptor antagonists required to reverse by 50% the bradycardia produced by neostigmine (0.25 $mg \cdot kg^{-1}$), edrophonium (1.0 $mg \cdot kg^{-1}$)and vagus nerve stimulation (10 V, 0.5 msec, 1–2 Hz, 10 sec).

	Neostigmine	*Edrophonium*	*Vagus stim*
ED_{50} Glyco[a]	3.6 ± 0.4† ($n = 4$)	–	8.6 ± 0.4 ($n = 5$)
ED_{50} Atropine	4.7 ± 0.5† ($n = 5$)	10.2 ± 3.1 ($n = 4$)	9.8 ± 1.2 ($n = 4$)
ED_{50} AFDX-116	30 ± 6* ($n = 5$)	–	55 ± 9 ($n = 4$)
ED_{50} Panc[b]	19 ± 5† ($n = 6$)	–	80 ± 15 ($n = 5$)
ED_{50} Gall[c]	28 ± 5‡ ($n = 4$)	186 ± 65 ($n = 4$)	261 ± 37 ($n = 5$)

[a]glycopyrrolate; [b]pancuronium; [c]gallamine.
P values were determined from statistical comparison of ED_{50}s of muscarinic antagonists to reverse the bradycardia produced by anticholinesterases vs vagus nerve stimulation.
*$P \leq 0.05$, †$P \leq 0.01$, ‡$P \leq 0.001$.

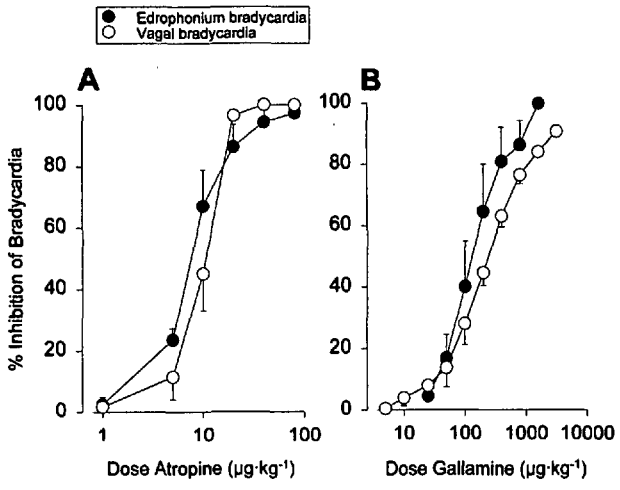

FIGURE 6 Relationship between dose of atropine (abscissa, panel A) or gallamine (abscissa, panel B) and percent inhibition of bradycardia (ordinate) evoked by edrophonium (filled circles) or by vagus nerve stimulation (open circles). Each point is the averaged response of four animals. Bars indicate S.E.M.

Discussion

In the present study, it was found that the shape of the dose-response curve of the bradycardia produced by neostigmine was strikingly different from that of the inhibition of cholinesterase activity. The dose-response relationship of the bradycardia was shifted to the right and the bradycardic effect did not reach a plateau even at doses well-beyond those at which the cholinesterase inhibition was maximal. In contrast, edrophonium produced dose-dependent decreases in cholinesterase activity and heart rate which were highly correlated, and the reduction in heart rate and cholinesterase activity reached a plateau with the same dose. Compared with the bradycardia produced by electrical stimulation of the vagus nerve, that produced by neostigmine was blocked by non-selective and selective M_2 muscarinic antagonists at lower doses while that produced by edrophonium was blocked with similar doses. For edrophonium, the close association of depression of cholinesterase activity with reduction in heart rate is consistent with the hypothesis that it produces bradycardia by preventing hydrolysis of the ACh spontaneously released by axon terminals of cardiac parasympathetic neurones. The finding that the muscarinic antagonists block the bradycardia produced by edrophonium and that produced by vagus nerve stimulation at similar doses is also consistent with this hypothesis. In contrast, in the case of neostigmine, the lack of an association of the magnitude of the bradycardia with the inhibition of cholinesterase activity suggests that the anticholinesterase effect cannot account for the reduction in heart rate. Moreover, this conclusion is supported by the finding that the muscarinic antagonists block the bradycardia produced by neostigmine at significantly lower doses compared to the bradycardia produced by vagus nerve stimulation.

It is unlikely that these differences between neostigmine and edrophonium can be accounted for simply by their pharmacokinetic properties as they have previously been shown to be similar.[7] Because the anticholinesterases were administered as bolus doses, rather than by continuous infusion, steady-state drug levels were not achieved. Nevertheless, since the object was to correlate two biological end-points, namely the reduction in heart rate and inhibition of cholinesterase activity, the actual drug levels were irrelevant. Interpretation of the findings in the present study may be complicated by the possibility that cholinesterase activity measured in erythrocytes does not reflect that in the heart. If this were the case, it could be argued that with neostigmine, higher doses were required to inhibit cholinesterase activity in the heart compared to that in red blood cells. While this possibility could theoretically account for the observation that the dose-response curve for the neostigmine-

induced reduction in cholinesterase activity is shifted to the left of that for the reduction in heart rate (Figure 3B), it cannot account for the lack of a plateau in the magnitude of the neostigmine-induced bradycardia and for the much larger decreases in heart rate produced by neostigmine compared to edrophonium (Figure 2B). In addition, this possibility seems unlikely because of the findings with edrophonium, for which the close correlation between the magnitude of the inhibition of erythrocyte cholinesterase activity and bradycardia suggests that cholinesterase activity in the heart is inhibited by doses of anticholinesterase which are similar to those required to inhibit red cell cholinesterase.

Although there is no obvious relation between the reduction in cholinesterase activity and the bradycardia produced by neostigmine, it seems reasonable to assume that the anticholinesterase action of neostigmine is partly responsible for producing a reduction in heart rate. This action may be estimated by comparing the bradycardic effects of neostigmine and edrophonium under conditions of similar cholinesterase inhibition, assuming that the bradycardia produced by edrophonium results entirely from its anticholinesterase action. The reduction in cholinesterase activity by approximately 80% provides a convenient reference point. In the case of edrophonium, when cholinesterase activity was reduced by this amount (which was the maximal effect) the heart rate decreased by 22 ± 2 bpm (14.6 ± 0.9% of baseline). When cholinesterase activity was reduced by the same amount by neostigmine, the decrease observed in heart rate was very similar (29 ± 12 bpm, 17 ± 7% of baseline, $P = 0.6$). This similar decrease in heart rate raises the possibility that up to the point when anticholinesterase activity is approximately 80% reduced, the effect of neostigmine on heart rate may be accounted for by its anticholinesterase action. However, Figure 3 shows that for reductions in cholinesterase activity of <80%, a smaller bradycardic effect was observed with neostigmine compared to edrophonium. Thus, at present, it is difficult to determine to what extent the anticholinesterase action of neostigmine contributes to the reduction in heart rate.

Previously, we suggested that the neostigmine-induced bradycardia was mediated by ACh, rather than by a direct action of neostigmine, on SA node cells, based on the finding that the bradycardia was markedly attenuated following depletion of ACh stores in the cardiac parasympathetic pathway.[3] Moreover, the neostigmine-evoked bradycardia was not diminished after degeneration of the parasympathetic preganglionic terminals, suggesting that the ACh was released by cardiac ganglion cells. We proposed that neostigmine activates excitatory cholinergic receptors on cardiac parasympathetic postganglionic cells, producing ACh release from their terminals and subsequent activation of inhibitory cardiac muscarinic receptors.[3] Neostigmine was first suggested to act as a cholinergic agonist in cat skeletal muscle[8] and subsequent studies have indicated that it may behave as a cholinergic agonist at a variety of cholinoceptive sites, including mammalian autonomic ganglia.[9–15]

Regarding the identity of the cholinergic receptor activated by neostigmine, we have shown that the neostigmine-induced bradycardia is not blocked by selective nicotinic and muscarinic M_1 antagonists at doses consistent with activation of these receptor subtypes.[3] Moreover, in studies using other models to investigate cardiac actions of cholinesterase inhibitors, it was demonstrated that neostigmine evokes the release of ACh from presumed cardiac parasympathetic neurones in the isolated chicken atrium, and that this effect was blocked by the non-selective muscarinic antagonist atropine but not by selective nicotinic and M_1 muscarinic receptor antagonists.[4] The observation of excitatory effects mediated by M_2 muscarinic receptors in guinea-pig cardiac parasympathetic ganglion cells[16] suggests the possibility that neostigmine produces its effect on heart rate by activating this type of receptor on cardiac parasympathetic postganglionic neurones. The demonstration of high levels of m_2 mRNA in rat parasympathetic cardiac ganglia[17] is consistent with this possibility. It should be noted, however, that M_2 muscarinic receptors are generally thought to mediate inhibitory actions.[18]

In the present study, the bradycardia produced by neostigmine was particularly sensitive to block by non-selective and selective M_2 muscarinic receptor antagonists when compared to that produced by vagus nerve stimulation, with doses ranging from one-half to one-tenth. This range may reflect the fact that muscarinic antagonists exert different actions (e.g., release of neuroactive substances, inhibition of ionic channels) or bind to receptors in a different manner (e.g., at allosteric sites or at active sites competitively or non-competitively).[6] Assuming that neostigmine produces bradycardia via activation of M_2 muscarinic receptors on cardiac parasympathetic postganglionic neurones leading to subsequent release of ACh from their terminals, the greater sensitivity of the neostigmine-induced bradycardia to muscarinic block may be due to an additive blocking effect, resulting from an action of the muscarinic antagonist at both the cardiac ganglion cell and at the sinoatrial node (see Figure 1). Additionally, neostigmine may have a relatively weak affinity for muscarinic receptors on cardiac ganglion cells compared to endogenously-released ACh at the SA node.

It can be argued that the greater potency of the M_2 muscarinic receptor antagonists to block the bradycardia produced by neostigmine compared with that produced by vagus nerve stimulation is because neostigmine interacts with muscarinic receptors at the SA node and depresses their affinity for ACh.[19] This explanation is unlikely, however, because in such a situation, more ACh would be needed to obtain a given decrease in heart rate in the presence of neostigmine than in its absence. Since, in the present study, the magnitudes of the bradycardia produced by neostigmine and vagus nerve stimulation were similar any depression of ACh binding to the M_2 muscarinic receptor in the presence of neostigmine must have been compensated by a greater ligand concentration. Moreover, the bradycardia produced by edrophonium (which has a similar displacing effect on binding of the muscarinic antagonist QNB as does neostigmine[20]) did not show a greater sensitivity to muscarinic receptor antagonists than the response to vagus nerve stimulation. Another possibility is that neostigmine binds to, and activates muscarinic receptors at the SA node. Due to possible heterogeneity of the muscarinic receptor population on SA node cells,[21] the sets of receptors activated by the synaptically released agonist (vagal stimulation) and the blood borne agonist (neostigmine) may be different and have different affinity for ACh and/or for the agonists. However, our previous observation, discussed above, that the neostigmine-induced bradycardia is severely attenuated after depletion of ACh in the cardiac parasympathetic pathway argues against this possibility.[3] In addition, it has been reported that neostigmine[22] and other anticholinesterases[20] have an atropine-like effect when applied directly to atrial tissue leading to positive inotropy and chronotropy, an effect which is opposite to that observed in the present study.

In the present investigation, the bradycardia produced by anticholinesterases was studied in the absence of cardiac parasympathetic input (bilateral vagotomy). In patients with normal cardiac innervation, in which ACh is released from preganglionic and postganglionic neurons as a consequence of on-going vagus nerve activity, anticholinesterases may affect heart rate in a manner which is not analogous to that when there is no cardiac autonomic efferent input. This may account for the discrepancy of the findings in the present study, in which there is enhanced sensitivity of the neostigmine-induced bradycardia to muscarinic antagonism, with the clinical observation that less muscarinic antagonist is required to block the bradycardic effect of edrophonium[23] than to block that of neostigmine.[24] On the other hand, the experimental paradigm in the present study may be analogous to the effect of anticholinesterases on heart rate in patients who have undergone cardiac transplantation. Recently, we have shown that neostigmine[25,26] and edrophonium[27] evoke a reduction in heart rate in cardiac transplant patients and the findings of the present study may help to account for these observations.

An unexpected finding of the present study was that glycopyrrolate and atropine had similar potencies on a weight basis in their ability to block bradycardia produced by vagus nerve stimulation. It has been claimed that glycopyrrolate has a smaller effect on basal heart rate than that of atropine, based on clinical studies which were complicated by previous administration of drugs with muscarinic effects (e.g., atropine, pancuronium and anticholinesterases), varying depths of anaesthesia, or unequal doses of glycopyrrolate and atropine.[28–33] Undoubtedly, the fact that glycopyrrolate is prepared as a 0.2 $mg \cdot ml^{-1}$ solution whereas atropine is 0.6 $mg \cdot ml^{-1}$, further contributes to the clinical impression that glycopyrrolate is less potent than atropine on basal heart rate. It is important to realise that, in clinical studies which were more specifically designed to compare the effects of glycopyrrolate and atropine on basal heart rate, it has been shown that glycopyrrolate is at least as potent (if not more so) as atropine.[34–39]

In conclusion, the bradycardia produced by anticholinesterases may involve at least two mechanisms. One, exemplified by the response to edrophonium, is likely to involve only inhibition of cholinesterase. This bradycardia is highly correlated with the level of inhibition of cholinesterase, with the maximum decrease in heart rate occurring when cholinesterase activity is maximally inhibited. In addition, the bradycardia is blocked by muscarinic receptor antagonists at doses which are similar to those required to block the bradycardia produced by vagus nerve stimulation. A second mechanism, exemplified by neostigmine, is not clearly related to the level of cholinesterase activity such that even when cholinesterase activity is maximally inhibited, anticholinesterases continues to produce further reductions in the heart rate. This bradycardia is more sensitive to block by muscarinic receptor antagonists than is the bradycardia produced by vagus nerve stimulation.

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