Laboratory Investigations

Bradycardia produced by pyridostigmine and physostigmine

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Purpose: The bradycardia produced by pyridostigmine and physostigmine in an animal model of acute cardiac denervation was examined according to its relation to cholinesterase inhibition and sensitivity to block by cholinergic receptor antagonists.

Methods: Cats were anaesthetised, vagotomised and propranolol-treated. Heart rate was continuously recorded. Erythrocyte cholinesterase activity of arterial blood was measured using a radiometric technique. Nicotinic and muscarinic M_1 receptors were blocked with hexamethonium and pirenzepine, respectively. M_2 receptors were blocked with gallamine, pancuronium and AFDX-116.

Results: With pyridostigmine and physostigmine, the dose-response relationship for the decrease in heart rate (ED_{50} 1.05 ± 0.25 and 0.198 ± 0.03 mg·kg⁻¹, respectively) was shifted to the right of that for the inhibition of cholinesterase activity (ED_{50} 0.094 ± 0.03 and 0.032 ± 0.01 mg·kg⁻¹, respectively). The decrease in cholinesterase activity reached a plateau at a cumulative dose of 0.56 ± 0.08 and 0.32 ± 0.08 mg·kg⁻¹, respectively. In contrast, there did not appear to be a plateau in the bradycardic effect. The bradycardia produced by pyridostigmine and physostigmine was blocked by hexamethonium (ED_{50} 10 ± 1.3 and 15.3 ± 2.4 mg·kg⁻¹, respectively), pirenzepine (ED_{50} 68 ± 16 and 138 ± 32 µg·kg⁻¹, respectively), gallamine (56 ± 11 and 67 ± 17 µg·kg⁻¹, respectively), pancuronium (32 ± 10 and 30 ± 4 µg·kg⁻¹, respectively), and AFDX-116 (31 ± 4 and 28 ± 4 µg·kg⁻¹, respectively).

Conclusion: The bradycardia produced by reversible anticholinesterase drugs containing a carbamyl group is not clearly related to the degree of cholinesterase activity, and has a low sensitivity to nicotinic and muscarinic M_1 and a high sensitivity to muscarinic M_2 receptor antagonists.

Objectif : Examiner si la bradycardie provoquée par la physostigmine et la pyridostigmine sur un modèle de dénervation cardiaque animal est en rapport avec l'inhibition de la cholinestérase et la sensibilité au bloc du récepteur cholinergique par ses antagonistes.

Méthodes : Des chats ont été anesthésiés, vagotomisés et traités au propanolol. Leur fréquence cardiaque a été enregistrée continuellement. Une technique radiométrique a permis de mesurer dans le sang artériel l'activité de la cholinestérase érythrocytaire. On a bloqué les récepteurs nicotiniques avec de l'hexaméthonium et les récepteurs muscariniques M_1 avec de la pirenzépine. Les récepteurs M_2 étaient bloqués avec de la gallamine, du pancuronium et de l'AFDX-116.

Résultats : Avec la pyridostigmine et la physostigmine, la relation dose-effet en rapport avec la baisse de la fréquence cardiaque (ED_{50} respective de 1,05 ± 0,25 et de 0,198 ± 0,03 mg·kg⁻¹) a dévié vers la droite de celle de l'inhibition de l'activité cholinestérasique (ED_{50} respective de 0,094 ± 0,03 et 0,032 ± 0,01 mg·kg⁻¹). La baisse de l'activité cholinestérasique a atteint un plateau à la dose cumulative respective de 0,56 ± 0,08 et 0,32 ± 0,08 mg·kg⁻¹. Par contre, il ne semblait pas y avoir de plateau en ce qui concerne l'effet bradycardisant. La bradycardie provoquée par la pyridostigmine et la physostigmine était bloquée par l'héxaméthonium (ED_{50} respective de 10 ± 1,3 et de 15,3 ± 2,4 mg·kg⁻¹), la pérenzépine (ED_{50} respective de 68 ± 16 et de 138 ± 32 µg·kg⁻¹), la gallamine (respectivement 56 ± 11 et 67 ± 17 µg·kg⁻¹), le pancuronium (respectivement 32 ± 10 et 30 ± 4 µg·kg⁻¹) et l'AFDX-116 (respectivement 31 ± 4 et 28 ± 4 µg·kg⁻¹).

Conclusion : La bradycardie provoquée par les anticholinestérases réversibles porteuses d'un groupe carbamyl n'a pas de relation certaine avec le degré d'activité cholinestérasique et est faiblement sensible aux antagonistes du récepteur nicotinique et muscarinique M_1, et fortement sensible aux antagonistes du récepteur muscarinique M_2.

BRADYCARDIA produced by anticholinesterases is a well-known phenomenon.[1,2] We have suggested that the anticholinesterase-induced bradycardia observed in an animal model of acute cardiac denervation may be mediated by at least two mechanisms.[3,4] One, exemplified by the response to the rapidly reversible anticholinesterase edrophonium, has the characteristic of being highly correlated to the level of inhibition of cholinesterase, with the maximum decrease in heart rate, to approximately 85% of the baseline, occurring when cholinesterase activity is maximally inhibited. In addition, whereas this bradycardia is blocked by selective muscarinic M_2 antagonists at doses similar to those which block the bradycardia produced by electrical stimulation of the vagus nerve, it is insensitive to block by selective nicotinic receptor antagonists.[3,4] The properties of the bradycardia produced by edrophonium suggest that it is mediated by the inhibition of cholinesterase which results in the protection from hydrolysis of acetylcholine (ACh) released from cardiac parasympathetic nerve terminals (Figure 1). A second mechanism, exemplified by the response to the reversible carbamate anticholinesterase neostigmine, is not related solely to the level of cholinesterase activity such that even when cholinesterase activity is maximally inhibited, further increases in neostigmine dose result in further reductions in heart rate.[4] The maximal decrease in heart rate produced by neostigmine while a sinus rhythm is maintained is to approximately 50% of the baseline, a decrease more than three times greater than that produced by edrophonium.[3,4] The neostigmine-induced bradycardia, like the bradycardia produced by edrophonium, is blocked by muscarinic M_2 receptor antagonists.[3,4] However, the neostigmine-induced bradycardia is more sensitive to block by the M_2 antagonists than is the bradycardia produced by edrophonium or vagus nerve stimulation.[3,4] In addition, unless the neostigmine-induced bradycardia is blocked by selective nicotinic receptor antagonists, the block occurs at doses much greater than those at which these drugs act as selective antagonists.[3] On the basis of properties of the neostigmine-induced bradycardia we have suggested that neostigmine activates cholinergic receptors of the M_2 sub-type in the cardiac parasympathetic pathway[3,4] (Figure 1).

The purpose of this study was to investigate the properties of the reduction in heart rate produced by the reversible carbamate anticholinesterases pyridostigmine and physostigmine. Of interest was whether the features of the bradycardia produced by these anticholinesterase drugs resembled those of the bradycardia produced by neostigmine or that of edrophonium, as described above. In a first set of experiments, to determine how the anticholinesterase action of pyridostig-
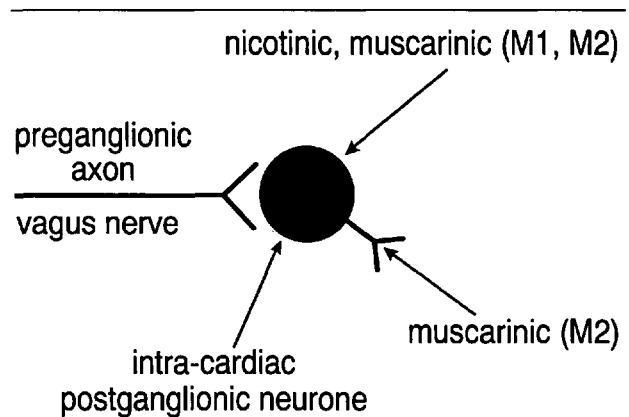

FIGURE 1 Schematic diagram of the peripheral cardiac parasympathetic pathway. Preganglionic axons contained in the vagus nerve synapse onto intra-cardiac postganglionic neurones, which in turn synapse onto cardiac target cells. ACh is released from both the preganglionic and postganglionic terminals. Cholinergic receptors have been identified on the postganglionic neurone as being nicotinic, muscarinic M_1, and M_2.[27] Cholinergic receptors on the target cells have been identified as being M_2. Anticholinesterases may produce a bradycardia by protection from hydrolylsis of ACh released from the axon terminals, or by directly activating cholinergic receptors.

mine and physostigmine was 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 either anticholinesterase drug. In a second set of experiments the pharmacological properties of the bradycardia produced by pyridostigmine and physostigmine were studied by comparing the potency of various cholinergic receptor antagonists to block the bradycardia produced by these anticholinesterase drugs or by electrical stimulation of the vagus nerve.

Methods

This study was approved by the McGill University Animal Care Committee. Cats (2.0–4.0 kg, n= 80) were anaesthetised with sodium pentobarbitone (35 mg·kg^{-1} *ip*, initial dose, maintenance doses of 3–4 mg·kg^{-1} *iv* every hour). The transmission of parasympathetic efferent nerve activity to the heart was interrupted by bilateral vagotomy (cervical level), and sympathetic transmission to the heart was blocked by 3 mg·kg^{-1} propranolol *iv*, in order to avoid reflex changes in heart rate secondary to anticholinesterase administration. Following tracheal cannulation, the lungs were artificially ventilated 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. An infusion of 7–8 ml·kg^{-1}·hr^{-1} NaCl 0.9% solution 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). 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 ECG was continuously monitored on an oscilloscope to verify the presence of sinus activity following administration of anticholinesterase drugs. The distal end 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.

The effect of cholinesterase inhibitors on erythrocyte cholinesterase activity was used as an index of their effect on the enzyme activity in cardiac tissue.[4] Cholinesterase activity was determined on arterial blood samples prior to (control) and following injection of increasing doses of pyridostigmine (n = 4) or physostigmine (n = 4) when heart rate had reached a steady-state at each dose. Aliquots, 100 µl, of freshly collected heparinised 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 pyridostigmine and physostigmine 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 < 5%. The specificity of the assay was ensured by the choice of substrate, ACh, and by the use of ISO-OMPA so that only acetylcholinesterase activity was measured. The sensitivity of the assay was determined by the specific radioactivity of the substrate used; the hydrolysis of 1 nM of ACh could be determined under the conditions used.

In another set of experiments the effect of nicotinic and muscarinic receptor antagonists on the bradycardia produced by anticholinesterases was tested using a dose of the anticholinesterases that produced 65–75% of the maximum decrease in heart rate.[4] To this end, 2.1 ± 0.2 mg·kg^{-1} pyridostigmine and 0.5 ± 0.07 mg·kg^{-1} physostigmine were administered, which produced a reduction in baseline heart rate of 33.8 ± 1.8% (n = 24) and 33.8 ± 2.3% (n = 23), respectively . To study the effect of nicotinic and muscarinic receptor antagonists on the bradycardia produced by vagus nerve stimulation, the right vagus nerve was electrically stimulated (10 V, 0.5 ms, 1–2Hz, 10 sec) to produce a reduction in heart rate of 35.4 ± 1.7% (n = 25). In this set of experiments, administration of pyridostigmine or physostigmine and electrical stimulation of the vagus nerve were done in different animals, and only one cholinergic antagonist was studied per animal. The dose of the antagonist was increased once the response to the previous dose had reached a steady-state.

The relationship between dose and effect of either anticholinesterase drug or muscarinic antagonists was determined by constructing dose-response curves. For each cat the ED_{50} of the anticholinesterase drug or cholinergic 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_{90} of the anticholinesterase drug on cholinesterase activity, and the effect of that dose on heart rate, was determined directly from the dose-response curve. The ED_{50} of the anticholinesterase effects on heart rate and cholinesterase activity was compared using a paired Student's t test. The ED_{50} for cholinergic block of each type of bradycardia were compared using the unpaired Student's t test. $P \leq 0.05$ was considered significant. Data are expressed as mean ± SEM.

The selective M_1 muscarinic receptor antagonist studied was pirenzepine (PZP, Sigma). 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 (AFDX-116, Boehringer Ingelheim), pancuronium (Organon) and gallamine (Rhone-Poulenc Rorer). The selective nicotinic receptor antagonist studied was hexamethonium bromide (C6, Sigma). All drugs were dissolved in NaCl 0.9% solution, with the exception of AFDX-116 which was dissolved in dimethyl sulfoxide (DMSO) 10% in saline.

Results

Inhibition of cholinesterase activity and the bradycardia produced by pyridostigmine and physostigmine
The maximal inhibition of cholinesterase achieved with pyridostigmine and physostigmine was by 92.6 ± 1.1% (n = 4) and 94.0 ± 2.0% (n = 4) of baseline

activity, respectively. Inhibition of cholinesterase activity by pyridostigmine and physostigmine reached a plateau (90% of maximal inhibition) when a cumulative dose of 0.56 ± 0.08 mg·kg^{-1} and 0.32 ± 0.08 mg·kg^{-1}, respectively, had been administered (Figures 2A & B). The estimated dose of pyridostigmine and physostigmine which produced 50% of the maximum inhibition was 0.094 ± 0.03 and 0.032 ± 0.01 mg·kg^{-1}, respectively.

Pyridostigmine and physostigmine also produced a dose-dependent decrease in heart rate (Figures 2A & B). Heart rate decreased progressively with each additional dose of anticholinesterase drug until arrhythmias (e.g., ectopic QRS complexes, third degree atrioventricular block) were produced. The maximal decrease in heart rate produced by pyridostigmine and physostigmine when the heart was still in sinus rhythm was by 71.4 ± 9.6 beats·min^{-1} (baseline of 154.6 ± 9.3 beats·min^{-1}) at a dose of 4.56 ± 1.4 mg·kg^{-1} and by 84.6 ± 8.8 beats·min^{-1} (baseline of 164 ± 5.6 beats·min^{-1}) at a dose of 1.0 ± 0.4 mg·kg^{-1}, respectively. The dose-response relationship for the bradycardic effect of both anticholinesterase drugs was shifted to the right of that for the inhibition of cholinesterase activity. For example, the estimated dose of pyridostigmine to produce 50% of the maximum bradycardia was 1.05 ± 0.25 mg·kg^{-1}, approximately 11 times that which produced a 50% reduction in cholinesterase activity ($P = 0.01$). Similarly, the estimated dose of physostigmine to produce 50% of the maximum bradycardia was 0.198 ± 0.03 mg·kg^{-1}, approximately 6 times that which produced a 50% reduction in cholinesterase activity ($P = 0.001$).

Cholinergic antagonism of the bradycardia produced by anticholinesterases and vagus nerve stimulation

Control heart rates prior to vagus nerve stimulation (147 ± 4 beats·min^{-1}, n = 25) were similar to those prior to administration of 2.1 ± 0.2 mg·kg^{-1} pyridostigmine (152 ± 4 beats·min^{-1}, n = 24) or 0.5 ± 0.07 mg·kg^{-1} physostigmine (156 ± 4 beats·min^{-1}, n = 23). The dose-response curve of the nicotinic antagonist C6 to block the bradycardia produced by vagus nerve stimulation (ED_{50} 0.6 ± 0.1 mg·kg^{-1}, n = 3) was shifted to the left of those of the block of the bradycardia produced by pyridostigmine (ED_{50} 10 ± 1.3 mg·kg^{-1}, n = 5, $P = .001$) and physostigmine (ED_{50} 15.3 ± 2.4 mg·kg^{-1}, n = 5, $P = .004$, Figure 3A, Table). The dose-response relationship of the muscarinic M_1 antagonist PZP to block the bradycardia produced by vagus nerve stimulation (ED_{50} 115 ± 26 µg·kg^{-1}, n = 5) was similar to that of the block of the bradycardia produced by pyridostigmine (ED_{50} 68 ± 16 µg·kg^{-1}, n = 5, $P = 0.2$) and physostigmine (ED_{50} 138 ± 32 µg·kg^{-1}, n = 5, $P = 0.6$, Figure 3B, Table). The dose- response curves of the muscarinic M_2 antagonists gallamine, pancuronium and AFDX-116 to block the reduction in heart rate produced by vagus nerve stimulation were shifted to the right of those of the block the bradycardia produced by anticholinesterase drugs (Figures 4A, B & C). The ED_{50} of gallamine, pancuronium and AFDX-116 to block the bradycardia produced by vagus nerve stimulation
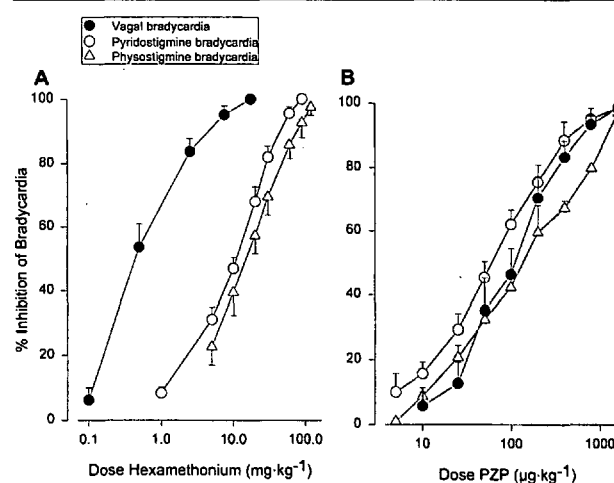
FIGURE 3 Relationship between dose of hexamethonium (abscissa, panel A) or pirenzepine (PZP, abscissa, panel B) and percent inhibition of bradycardia (ordinate) evoked by pyridostigmine (open circles), physostigmine (open triangles) or vagus nerve stimulation (filled circles). Each point is the averaged response of animals given pyridostigmine (n = 5), physostigmine (n = 5), or in which the vagus nerve was stimulated (C6, n = 3; PZP, n = 5). Bars indicate SEM.
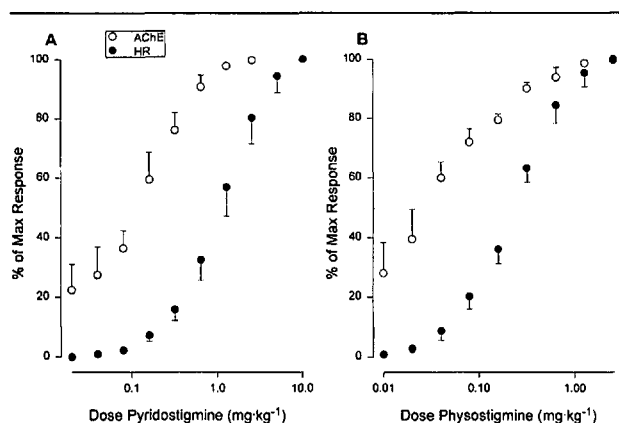

FIGURE 2 Relationship between the decrease in heart rate (HR) and acetylcholinesterase activity (AChE) expressed as percent of maximum response (ordinate) and dose of anticholinesterase (abscissa). Each point represents the average response of animals given pyridostigmine (n = 4) or physostigmine (n = 4). Bars indicate SEM.

TABLE   Estimated doses of C6 (mg·kg⁻¹), PZP (μg·kg⁻¹) and gallamine (Gall, μg·kg⁻¹), pancuronium (Panc, μg·kg⁻¹) and AFDX-116, (μg·kg⁻¹) required to reverse by 50% the bradycardia produced by anticholinesterase drugs (pyridostigmine, physostigmine) and vagus nerve stimulation.

		Pyridostigmine	*Physostigmine*	*Vagus stimulation*
Nicotinic	ED_{50} C6	10 ± 1.3, n = 5§	15.3 ± 2.4, n = 5‡	0.6 ± 0.1, n = 3
M_1	ED_{50} PZP	68 ± 16, n = 5	138 ± 32, n = 5	115 ± 26, n = 5
M_2	ED_{50} Gall	56 ± 11, n = 4‡	67 ± 17, n = 4‡	250 ± 32, n = 6
M_2	ED_{50} Panc	32 ± 10, n = 5*	30 ± 4, n = 4*	80 ± 15, n = 5¶
M_2	ED_{50} AFDX-116	31 ± 4, n = 5*	28 ± 4, n = 5*	86 ± 21, n = 6

P values were determined from statistical comparison of ED_{50} of cholinergic antagonists to reverse the bradycardia produced by anticholinesterase drugs *vs* vagus nerve stimulation.

*$P \leq 0.05$, †$P \leq 0.01$, ‡$P \leq 0.005$, §$P \leq 0.001$, ¶data from Backman *et al.*, 1996.

(250 ± 32 μg·kg⁻¹, n = 6; 80 ± 15 μg·kg⁻¹, n = 5; 86 ± 21 μg·kg⁻¹, n = 6, respectively) was greater than those to block the bradycardia produced by pyridostigmine (56 ± 11 μg·kg⁻¹, n = 4, P=.002; 32 ± 10 μg·kg⁻¹, n = 5, P=.03; 31 ± 4 μg·kg⁻¹, n = 5, P=.03, respectively) and physostigmine (67 ± 17 μg·kg⁻¹, n = 4, P=.004; 30 ± 4 μg·kg⁻¹, n = 4, P=.02; 28 ± 4 μg·kg⁻¹, n = 5, P=.02, respectively, Table).

Discussion

Pyridostigmine and physostigmine each produced a dose-dependent bradycardia which resembled that produced by neostigmine[3,4] and differed from that evoked by edrophonium[3,4] in the following manner. The dose-response relationship of the bradycardia produced by pyridostigmine and physostigmine was shifted to the right of that for the inhibition of cholinesterase activity, and when the inhibition of cholinesterase activity had reached a plateau, further increases in the dose of anticholinesterase produced further decreases in heart rate. For example, at the plateau the bradycardic effect was only 37.3 ± 3.3% of the maximum in the case of pyridostigmine and 62 ± 11.8% of the maximum in the case of physostigmine (Figures 2 A & B). These results contrast with the previously reported finding with edrophonium that the dose-response relationship for the bradycardic and anticholinesterase effects are superimposed such that when cholinesterase activity is maximally reduced, further increases in the dose of edrophonium have no effect on heart rate.[4] The maximal decrease in heart rate produced by pyridostigmine and physostigmine while the heart was still in sinus rhythm was to approximately 50% of the baseline. This value is similar to that produced by neostigmine[3,4] and different from the maximum reduction in heart rate evoked by edrophonium to only 85% of the baseline.[3,4] The bradycardia produced by pyridostigmine and physostigmine was more sensitive to block by mus-

Dose Pancuronium (μg·kg⁻¹)   Dose AFDX-116 (μg·kg⁻¹)   Dose Gallamine (μg·kg⁻¹)
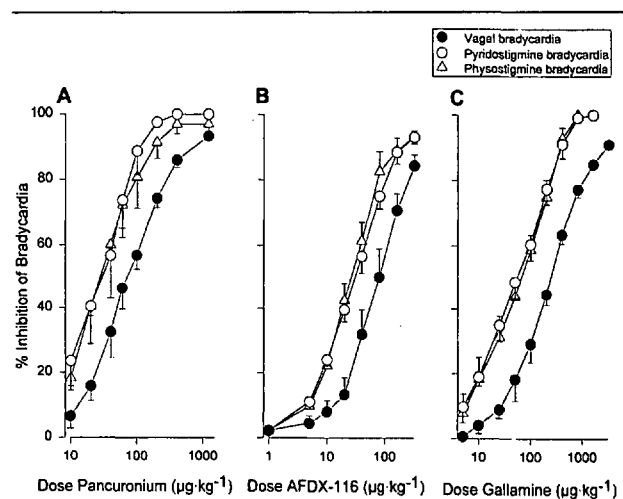
FIGURE 4   Relationship between dose of pancuronium (abscissa, panel A), AFDX-116 (abscissa, panel B), and gallamine (abscissa, panel C) and percent inhibition of bradycardia (ordinate) evoked by pyridostigmine (open circles), physostigmine (open triangles) or vagus nerve stimulation (filled circles). Each point is the averaged response of animals given pyridostigmine (pancuronium & AFDX-116, n = 5; gallamine, n = 4), physostigmine (pancuronium & gallamine, n = 4; AFDX-116, n = 5) or in which the vagus nerve was stimulated (pancuronium, n = 5; AFDX-116 & gallamine, n = 6). Bars indicate SEM.

carinic M_2 antagonists, by a factor of 2.5–4.5, than was the bradycardia evoked by electrical stimulation of the vagus nerve. Conversely, the anticholinesterase-induced bradycardia was less sensitive to block by the nicotinic receptor antagonist C6, by a factor of 16–25, than was the bradycardia evoked by electrical stimulation of the vagus nerve. These observations are reminiscent both qualitatively and quantitatively of the findings observed with neostigmine.[3,4] The bradycardia produced by edrophonium, in contrast, demonstrates similar sensi-

tivity to block by muscarinic M_2 antagonists compared to that produced by vagus nerve stimulation and is insensitive to block by nicotinic receptor antagonists.[3,4]

Because the anticholinesterases were administered as bolus doses, rather than by continuous infusion, steady-state drug levels were not achieved. Nevertheless, since the objective 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 higher doses of anticholinesterase drugs were required to inhibit cholinesterase activity in the heart than in red blood cells. While this possibility could theoretically account for the observation that the dose-response curve for the anticholinesterase-induced reduction in cholinesterase activity is shifted to the left of that for the reduction in heart rate, it cannot account for the lack of an obvious plateau in the magnitude of the anticholinesterase-induced bradycardia (Figures 2 A & B). In addition, this possibility seems unlikely because of the finding in a previous study that with edrophonium, there is a close correlation between the magnitude of the inhibition of erythrocyte cholinesterase activity and bradycardia suggesting that cholinesterase activity in the heart is inhibited by doses of anticholinesterase which are similar to those required to inhibit red cell cholinesterase.[4]

The similarity between the properties of the bradycardia produced by pyridostigmine and physostigmine and those of the bradycardia evoked by neostigmine suggest that the bradycardia produced by anticholinesterases containing a carbamyl group is mediated by similar mechanisms. Previously, we have hypothesised that the bradycardia produced by neostigmine is caused by direct activation of cholinergic receptors in the peripheral cardiac parasympathetic pathway.[3,4] In this regard, it is relevant that, like neostigmine, pyridostigmine and physostigmine have been shown to activate a variety of nicotinic and muscarinic receptors.[5-18] As has been observed with the neostigmine-induced bradycardia,[3,4] the high doses of nicotinic and muscarinic M_1 receptor antagonists required to block the pyridostigmine- and physostigmine-induced bradycardia suggest that the bradycardia is not mediated by activation of these receptor sub-types.[19,20] On the other hand, the finding in the present study that the bradycardia produced by pyridostigmine and physostigmine is particularly sensitive to block by muscarinic M_2 receptor antagonists, as is the case with neostigmine,[4] is consistent with the suggestion that the

carbamyl anticholinesterase drugs produce their effect by activation of this type of receptor.[4] Possibly, this involves the binding of the carbamyl group to muscarinic receptors, as has been suggested to mediate the increase in inositol monophosphate release from rat tracheal slices induced by these drugs.[21]

In the present investigation, the bradycardia produced by anticholinesterases was studied in the absence of cardiac parasympathetic input (bilateral vagotomy). The experimental paradigm in the present study may be analogous to the effect of anticholinesterase drugs on heart rate in patients who have undergone cardiac transplantation, where the transplanted heart remains functionally denervated.[22] The results from the present study suggest that administration of pyridostigmine or physostigmine to this type of patient could produce a reduction in heart rate, as has been shown to be the case for neostigmine[23-25] and edrophonium.[26]

In conclusion, the findings from this study suggest that the reduction in heart rate produced by anticholinesterases containing a carbamyl group is not clearly related to the degree of cholinesterase block because doses of these drugs greater than those producing maximal cholinesterase inhibition continue to produce further reductions in heart rate. This bradycardia is more sensitive to block by muscarinic M_2 receptor antagonists than is the bradycardia produced by vagus nerve stimulation, and although blocked by nicotinic and muscarinic M_1 antagonists, the doses of the latter are too high to implicate activation of these receptor sub-types.

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