J. Pharm. Pharmacol. 1996, 48: 935–939
Received January 29, 1996
Accepted February 29, 1996

Toxicodynamic Analysis of Cardiac Effects Induced by Four Cholinesterase Inhibitors in Rats

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Abstract

The cardiac effect of edrophonium (2–20 μmol kg^{-1}), pyridostigmine (0·5–5 μmol kg^{-1}), neostigmine (0·05–0·5 μmol kg^{-1}) and ambenonium (0·02–0·3 μmol kg^{-1}) was investigated after intravenous administration to rats.

For pyridostigmine and neostigmine, the heart rate decreased in a dose-dependent manner, and then gradually recovered to the basal level at about 10 min. Rapid decrease of heart rate was observed after edrophonium and ambenonium administration, and rapid recovery to the basal level within 1 min. For ambenonium, a dose-dependent tachycardiac response was observed. The time-course of heart rate change was analysed by the effect-compartment model. Significant correlation was observed between bradycardiac EC50 values obtained by effect-compartment model analysis and inhibitory constant (K_i) to acetylcholinesterase in-vitro, suggesting that the bradycardiac response was induced by inhibition of this enzyme and following elevation of acetylcholine concentration in the synaptic cleft. On the other hand, the tachycardiac EC50 values of edrophonium and ambenonium based on the effect-compartment model analysis were similar to dissociation constants (K_d) of these drugs to muscarinic receptors in-vitro, suggesting that the tachycardiac activity of these drugs may be associated with antagonistic activity to postsynaptic muscarinic receptors.

We conclude that, clinically, edrophonium and ambenonium are safer drugs than pyridostigmine and neostigmine, at least as regards muscarinic side-effects, including bradycardia.

Cholinesterase inhibitors are used for the reversal of post-operative neuromuscular blockade or for the treatment of myasthenia gravis. Cholinesterase inhibitors elevate the acetylcholine level in the synaptic cleft by inhibition of acetylcholinesterase (AChE), and potentiate the skeletal muscle contraction. One of the common side-effects of cholinesterase inhibitors is bradycardia. It is suggested that cholinesterase inhibitors produce bradycardia by preventing the hydrolysis of acetylcholine released from the parasympathetic neurons and following stimulation of cardiac muscarinic M_2 receptor. Recently, Backman et al (1993) reported that pirenzepine, a selective M_1 antagonist, could not block bradycardia produced by vagal nerve stimulation at the dose which blocked the neostigmine-induced bradycardia. They concluded that the neostigmine-induced bradycardia may result from the pre-synaptic muscarinic receptor stimulation, which leads to acetylcholine release. Furthermore, ambenonium, a selective AChE inhibitor, has an antagonistic effect on muscarinic receptors (Kenakin & Beek 1985) and induces tachycardia (Brown et al 1982). The mechanism of the chronotropic effect of cholinesterase inhibitors is unclear and the intensity of cardiac action has not been evaluated among cholinesterase inhibitors. It is necessary to know what is the determinant of chronotropic effect of cholinesterase inhibitors for the appropriate drug selection. In this study, toxicodynamics concerning chronotropic effect of four cholinesterase inhibitors (edrophonium, neostigmine, pyridostigmine and ambenonium) after intravenous administration to rats was investigated.

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Methods

Chemicals and reagents

Ambenonium chloride was generously supplied by Nippon Shoji Co. (Osaka, Japan). Edrophonium chloride, neostigmine bromide and pyridostigmine bromide were purchased from Sigma, USA. All other reagents were of analytical grade and used without further purification.

Animal experiments

Male Wistar rats purchased from Nippon Ikagaku Dobutsu Co. (Tokyo, Japan), 300–330 g, were used in all experiments. The animals had free access to a standard pellet diet and tap water before the experiments. Anaesthesia was induced by the intraperitoneal administration of urethane 1000 mg kg^{-1} and alpha-chloralose 25 mg kg^{-1}. A polyethylene cannula SP-31 (Natsume, Tokyo) was inserted into the right carotid artery and connected to a pressure transducer (TNF-R, Viggo-Spectramed, Singapore). The left femoral veins were cannulated with PE-50 (Becton Dickinson, USA) for drug administration. Heart rate was enumerated by electrically amplified arterial

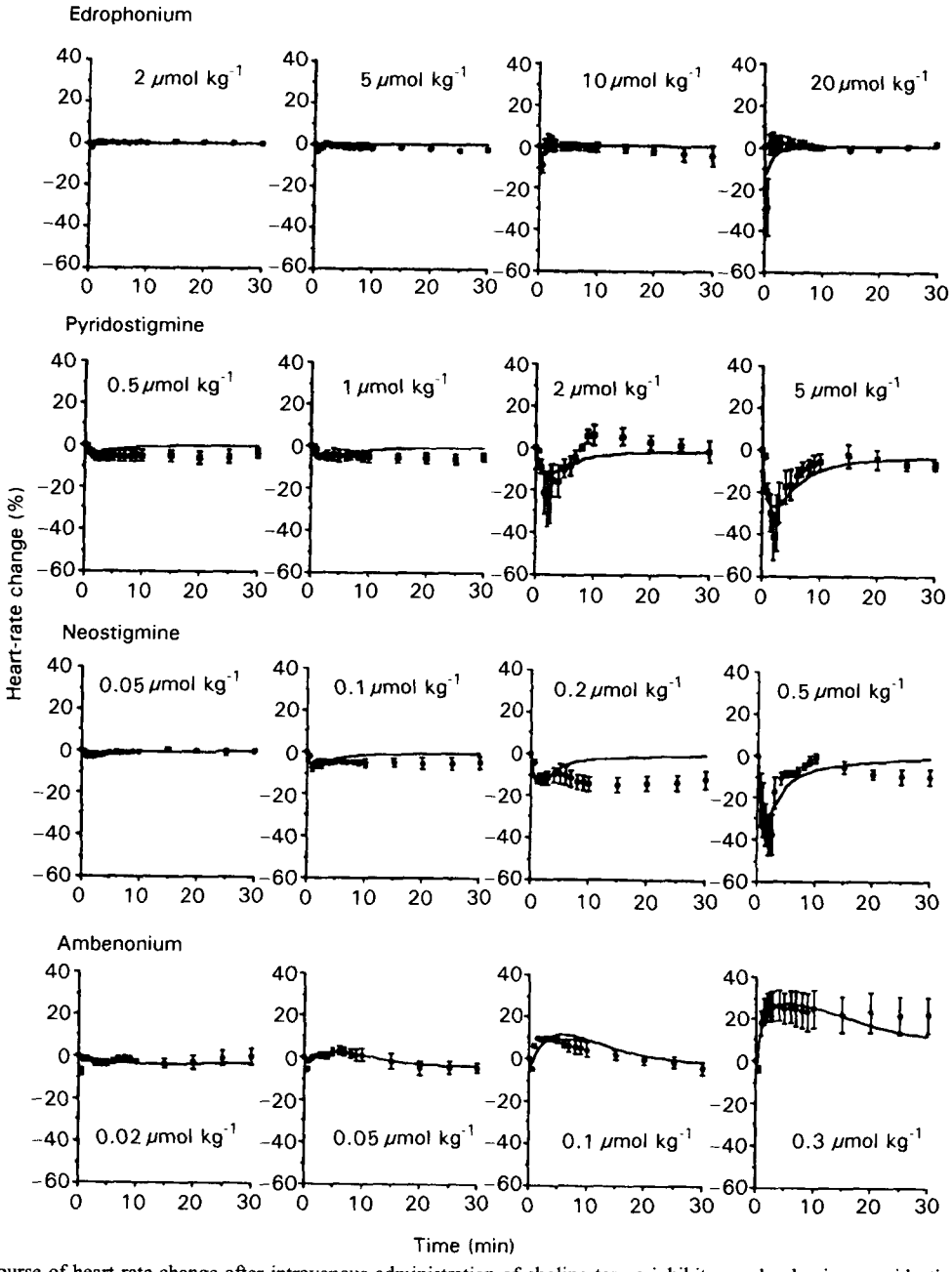

FIG. 1. Time course of heart-rate change after intravenous administration of cholinesterase inhibitors; edrophonium, pyridostigmine, neostigmine and ambenonium. Values are mean ± s.e. of observed data (n = 3). The solid lines are the fitted lines calculated according to equations 27.

pressure signal with a heart-rate counter (AT-621G, Nihon-Kohden, Tokyo). The body temperature was maintained at 37·5 ± 0·3°C. Edrophonium (2–20 μmol kg^{-1}), pyridostigmine (0·5-5 μmol kg^{-1}), neostigmine (0·05–0·5 μmol kg^{-1}) or ambenonium (0·02–0·3 μmol kg^{-1}) was administered intravenously. Higher dose studies could not be performed, since the decrease of heart rate to less than 50% was unacceptable. Blood pressure and heart rate were monitored throughout the experiments.

Data analysis

The chronotropic effect was described as the percent change in heart rate from the basal value. Toxicodynamic analysis was carried out by effect compartment model analysis (Sheiner et al 1979). Plasma-concentration profiles of cholinesterase inhibitors used for the input functions are expressed as:

$$C_p = \text{Dose} \times (A\, e^{-\alpha t} + B\, e^{-\beta t}) \quad (1)$$

where C_p is the concentration of cholinesterase inhibitors in plasma and t is the time after administration. Pharmacokinetic parameters of edrophonium (5·51, 0·64, 0·59 and 0·049 for A, α, B and β, respectively), pyridostigmine (6·42, 0·25, 0·97, 0·019), neostigmine (5·62, 0·74, 0·83, 0·076) and ambenonium (5·41, 0·18, 1·38, 0·010) after intravenous administration were determined previously (Yamamoto et al 1996). For edrophonium and ambenonium, two effect compartments were assumed, one for the bradycardiac effect compartment and the other for the tachycardiac effect compartment (eqns 2 and 3).

Table 1. Toxicodynamic parameters of cholinesterase inhibitors in rats.

	Edrophonium	Pyridostigmine	Neostigmine	Ambenonium
Bradycardiac effect				
$k_{e0,brady}$ (min^{-1})	11·6 ± 8·5	1·03 ± 0·28	0·761 ± 0·165	0·205 ± 0·043
$E_{max,brady}$ (%)	−100	−100	−100	−100
$EC50_{brady}$ (μM)	314 ± 154	65·6 ± 5·6	3·11 ± 0·29	0·114 ± 0·019
Tachycardiac effect				
$k_{e0,tachy}$ (min^{-1})	12·5 ± 8·5	–	–	0·217 ± 0·034
$E_{max,tachy}$ (%)	14·3 ± 12·4	–	–	143·1 ± 3·3
$EC50_{tachy}$ (μM)	30·4 ± 154	–	–	0·217 ± 0·034

Concentration-effect relationships for both effects were assumed to be represented by the E_{max} model (eqns 4 and 5), and heart rate observed was assumed as the sum of both effects (eqn 6)

$$dC_{e,\,brady}/dt = k_{1e,\,brady} \times Cp - k_{e0,\,brady} \times C_{e,brady} \quad (2)$$

$$dC_{e,tachy}/dt = k_{1e,tachy} \times C_p - k_{e0,tachy} \times C_{e,tachy} \quad (3)$$

$$E_{brady} = E_{max,brady} \times C_{e,brady}/(EC50_{brady} + C_{e,brady}) \quad (4)$$

$$E_{tachy} = E_{max,tachy} \times C_{e,tachy}/(EC50_{tachy} + C_{e,tachy}) \quad (5)$$

$$E = E_{brady} + E_{tachy} \quad (6)$$

where $C_{e,brady}$ and $C_{e,tachy}$ are the drug concentration in the bradycardiac and tachycardiac effect compartments, E_{brady}(%) and E_{tachy}(%) are the bradycardiac and tachycardiac effects, respectively. For pyridostigmine and neostigmine, only the bradycardiac effect compartment was considered for these drugs (eqn 7), since a clear tachycardiac action was not observed.

$$E = E_{brady} \quad (7)$$

Maximal bradycardiac effect was fixed to 100% in all cases. Heart-rate change from basal level was fitted to equations 5 or 6 by nonlinear least-squares regression (Yamaoka et al 1981) to estimate the pharmacodynamic parameters.

Results

The mean value of heart rate, systolic and diastolic pressure of rat before drug administration under urethanechloralose anaesthesia were 400·5 ± 37·5 beats min^{-1}, 109·4 ± 12·9 mmHg and 49·0 ± 10·6 mmHg (n = 48, means ± s.d.), respectively. The profiles of heart rate change after intravenous administration of cholinesterase inhibitors are shown in Fig. 1. Rapid and dose-dependent decrease of heart rate was observed after edrophonium administration, and rapidly recovered to the basal level within 1 min. After administration of pyridostigmine and neostigmine, the heart rate decreased dose-dependently, followed by gradual return to the basal level within 15 min. Sustained and dose-dependent tachycardia was observed after the transient bradycardia induced by ambenonium. From these findings, two effect compartments regarding bradycardia and tachycardia were considered for edrophonium and ambenonium, while only the bradycardiac effect compartment was assumed for pyridostigmine and neostigmine. The estimated toxicodynamic parameters are listed in Table 1, and the simulation lines calculated with these parameters are shown as solid lines in Fig. 1. The profiles of heart-rate change seemed to be expressed by these effect compartment models. As shown in Fig. 2, the EC50 values of four drugs for bradycardiac effect determined by in-vivo toxicodynamic studies were significantly correlated with the slope of unity to their inhibitory constants to AChE determined by in-vitro enzyme kinetic study determined previously (Yamamoto et al 1996). The EC50 values of edrophonium and ambenonium for tachycardiac effect determined by in-vivo toxicodynamic study were similar to the dissociation constants of these drugs to the muscarinic receptor (Brown et al 1982) determined by in-vitro binding studies (Fig. 3).

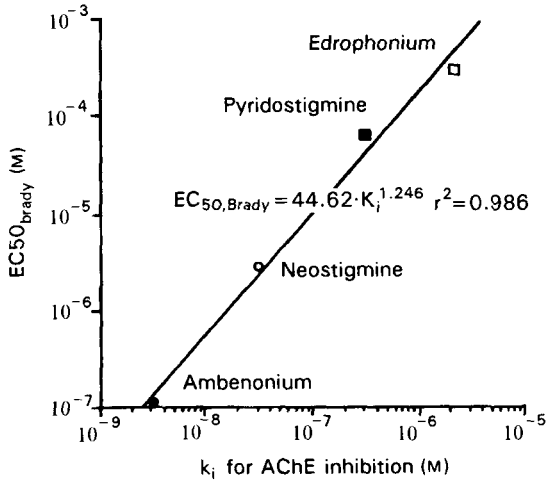

FIG. 2. Relationship between the inhibitory constants of cholinesterase inhibitors to AChE determined by in-vitro enzyme kinetic studies (Yamamoto et al 1996) and $EC50_{brady}$ estimated by the in-vivo pharmacodynamic study. □ Edrophonium, ■ pyridostigmine, ○ neostigmine and ● ambenonium.

Discussion

It has been generally considered that cholinesterase inhibitors induce bradycardia by preventing the hydrolysis of acetylcholine released from parasympathetic neurons and following stimulation of cardiac M_2 receptors. Gardner & Allen (1977) also reported that cholinesterase inhibitors reduce the muscarinic receptor-mediated cAMP production to induce bradycardia. Recently, the role of cholinesterase inhibitors as

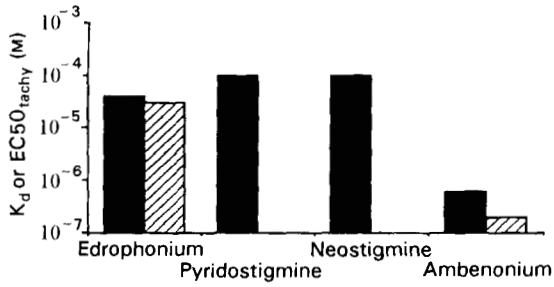

FIG. 3. Comparison between the dissociation constant (K_d) of cholinesterase inhibitors to muscarinic receptors (□) determined by in-vitro binding studies (▨).

agonists for the muscarinic receptor has been recognized. Backman et al (1993) reported that bradycardia was attenuated after acetylcholine depletion in the cardiac parasympathetic pathway, suggesting that the release of acetylcholine from parasympathetic neuron is essential for bradycardia induced by cholinesterase inhibitors. They suggested that neostigmine evokes bradycardia by activation of muscarinic receptors on cardiac ganglion cells producing acetylcholine release. Since atropine and pirenzepine blocked neostigmine-induced bradycardia but McN-A-343, a muscarinic M_1 agonist did not produce bradycardia, the receptor on cardiac ganglion cells appears not to be an M_1 receptor. Further, the dose of pirenzepine which blocked the neostigmine-induced bradycardia was lower than that for blocking bradycardia produced by vagal nerve stimulation, and the pharmacological identity of this receptor remains unclear. Ambenonium, a selective AChE inhibitor, acts as an antagonist to muscarinic receptors on postsynaptic membranes (Kenakin & Beek 1985), and Brown et al (1982) showed the tachycardiac activity of ambenonium after intraperitoneal administration to rats, which is consistent with the results of our study. Bradycardiac response after administration of pyridostigmine or neostigmine lasted for about 15 min, while the bradycardia induced by edrophonium and ambenonium disappeared within one minute. For ambenonium, a dose-dependent tachycardiac change was observed thereafter. In order to describe these chronotropic profiles, two effect compartments were assumed for edrophonium and ambenonium, while only a bradycardiac effect compartment was considered for pyridostigmine and neostigmine. Significant correlation with the slope of unity was observed between the EC50 value estimated in this study and inhibitory constants to AChE of these drugs determined by previous in-vitro enzyme kinetic studies (Yamamoto et al **1996**) as shown in Fig. 2, suggesting that the bradycardiac activity of these drugs may result from AChE inhibition and following elevation of acetylcholine concentration in the synaptic cleft. The dose-dependency of bradycardiac response after intravenous administration of ambenonium was unclear, which may be due to substantially complete inhibition of AChE by ambenonium in the dose range studied.

Tachycardiac EC50 values of edrophonium and ambenonium were close to the dissociation constants of these drugs for muscarinic receptors (Fig. 3), suggesting that the tachycardiac activity of these drugs may be associated with antagonistic activity to postsynaptic muscarinic receptors. Dissociation constants of neostigmine and pyridostigmine (140 μM) were much higher than the plasma concentration in this study, and it is not unreasonable to evoke an undetectable tachycardiac effect for these drugs at the dose studied. The inhibitory potency to AChE was smaller, and the value for $k_{e0,\,brady}$, a parameter representing transfer rate of drug from the central compartment to the bradycardiac effect compartment was also smaller. For edrophonium and ambenonium, $k_{e0,brady}$ and $k_{e0,tachy}$ values were essentially the same, suggesting the site of action to be the same. For neuromuscular-blocking agents,

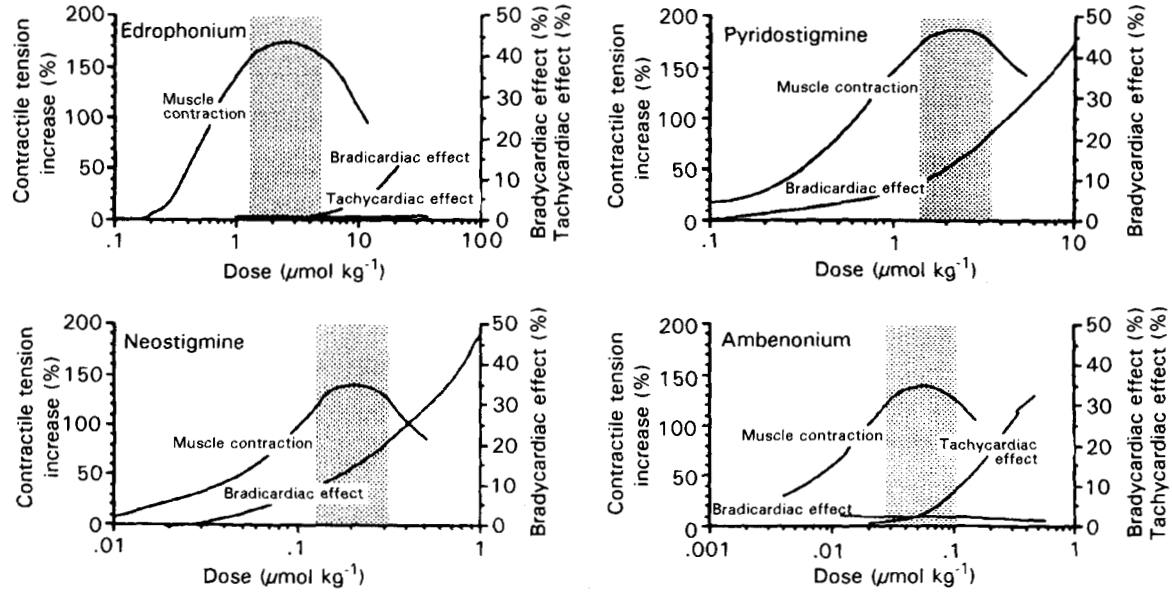

FIG. 4. Dose-response relationship of cholinesterase inhibitors for muscle contraction, bradycardiac and tachycardiac effect. The maximum value of each response is plotted. Dose-muscle contractile response is simulated according to the pharmacodynamic analysis (Yamamoto et al **1996**).

good correlation is reported between the onset of action and the affinity to acetylcholine receptors (Kopman 1989; Donati & Meistelman 1991; Min et al 1992), and their site of action is also on the postsynaptic membrane in the synaptic cleft. It is not the association–dissociation rate but the diffusion rate of drugs in the synaptic cleft that is considered as the rate limiting step for these drugs (Glavinovic et al 1992). A neuromuscular blocking agent with higher affinity interacts with acetylcholine receptor more frequently, and the diffusion rate through the synaptic cleft and onset time of action is slower. Assuming a similar process for cholinesterase inhibitors, the transfer rate of a drug with higher affinity to AChE from the central compartment to the effective site may be delayed.

Clinical implication

Fig. 4 shows the dose–response relationship of cholinesterase inhibitors for bradycardiac and tachycardiac effect as side-effects, and muscle contraction as a pharmacological effect estimated in our previous pharmacodynamic study (Yamamoto et al **1996**). Maximum values of contractile-tension increase are essentially the same among the drugs, suggesting the same potency as the anti-myasthenic drugs. Increase of contractile tension is also associated with the AChE inhibition; therefore bradycardia is considered as an inevitable side-effect for cholinesterase inhibitors. Change of heart rate was, however, not induced by edrophonium and ambenonium at the dose which causes the maximum increase of contractile tension, since they have anti-muscarinic potency. From these findings, edrophonium and ambenonium may be safer drugs than pyridostigmine and neostigmine as far as bradycardia is concerned.

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