Laboratory Reports

Synaptic inhibitory effects of edrophonium on sympathetic ganglionic transmission

Reuben D. Stein PhD,*†
 Steven B. Backman MDCM PhD FRCPC,*
 Brian Collier PhD,‡
 Canio Polosa MD PhD,*†

Purpose: To evaluate the effect of edrophonium on synaptic transmission in the superior cervical ganglion.

Methods: In anesthetized rats the effect of edrophonium on synaptic transmission was studied *in vitro* by testing whether it blocks the compound action potential recorded from postganglionic fibres evoked by stimulation of preganglionic axons. The superior cervical ganglion was excised and the cervical sympathetic trunk and internal carotid nerve were used for stimulating and recording, respectively. Drugs superfused included edrophonium (0.1-500 μ M), neostigmine (0.1-10 μ M), and muscarinic M_1 and M_2 antagonists pirenzepine and AFDX-116 (200 nM-10 μ M), respectively. To evaluate a presynaptic action, the effect of edrophonium on basal and high- K^+ (35 mM) evoked release of [3 H]ACh from the superior cervical ganglion was studied *in vitro*. To evaluate a postsynaptic action, edrophonium's effect on postganglionic nerve discharge in response to arterial injection of ACh (100 μ g) into the superior cervical ganglion was determined *in vivo*.

Results: Edrophonium (10-500 μ M) decreased the compound action potential amplitude (ED_{50} 163.5 μ M). A decrease was not produced by neostigmine, nor was it reversed by pirenzepine or AFDX-116. Edrophonium blocked postganglionic cell firing in response to exogenously administered ACh. Although edrophonium did not affect basal or high- K^+ evoked ACh release, when the evoked increase was calculated as a multiple of the basal release, it caused approximately a 30% ($P < 0.005$) reduction.

Conclusions: Edrophonium blocks ganglionic cholinergic transmission postsynaptically and, possibly, presynaptically. The mechanism(s) by which this occurs does not appear to involve inhibition of cholinesterase, or activation of M_1 or M_2 receptor subtypes.

Objectif : Évaluer l'effet de l'edrophonium sur la transmission synaptique dans le ganglion cervical supérieur.

Méthode : On a étudié *in vitro* l'effet de l'edrophonium sur la transmission synaptique chez des rats sous anesthésie en vérifiant s'il y a blocage du potentiel d'action composée, enregistré à partir des fibres postganglionnaires, déclenché par la stimulation des axones préganglionnaires. On a excisé le ganglion cervical supérieur et on a utilisé le tronc sympathique cervical et le nerf carotide interne pour, respectivement, stimuler et enregistrer. Les médicaments superfusés comprenaient l'edrophonium (0.1-500 μ M), la néostigmine (0.1-10 μ M), et les antagonistes muscariniques M_1 et M_2 , la pirenzépine et l'AFDX-116 (200 nM-10 μ M), respectivement. Pour évaluer une action présynaptique, on a étudié *in vitro* l'effet de l'edrophonium sur la libération provoquée de [3 H]ACh du ganglion cervical supérieur en milieu kaliémique basal et en milieu kaliémique élevé (35 mM). Pour évaluer maintenant une action postsynaptique, on a déterminé *in vivo* l'effet de l'edrophonium sur la décharge du nerf postganglionnaire en réponse à une injection artérielle de ACh (100 μ g) dans le ganglion cervical supérieur.

Résultat : L'edrophonium (10-500 μ M) a réduit l'amplitude du potentiel d'action composée (ED_{50} 163,5 μ M). La néostigmine n'a pas produit de diminution de potentiel et la pirenzépine ou l'AFDX-116 ne l'ont pas renversé. L'edrophonium a bloqué la décharge des cellules postganglionnaires en réaction à l'administration exogène d'ACh. Même si l'edrophonium n'a pas eu d'effet sur la libération provoquée d'ACh en milieu kaliémique basal et en milieu kaliémique élevé, l'augmentation provoquée étant calculée comme un multiple de la libération de base, il a causé une réduction d'environ 30 % ($P < 0.005$).

Conclusion : L'edrophonium bloque la transmission ganglionnaire cholinergique postsynaptique et, probablement, présynaptique. Le (ou les) mécanisme par lequel cela se produit ne semble pas comprendre l'inhibition de la cholinestérase ou l'activation des sous-types de récepteurs M_1 ou M_2 .

From the Department of Anaesthesia,* Royal Victoria Hospital, and Departments of Physiology† and Pharmacology,‡ McGill University.

Address correspondence to: Dr. S.B. Backman, Department of Anaesthesia, Royal Victoria Hospital, 687 Pine Ave. West, Montreal, Quebec, Canada H3A 1A1. Phone: 514-842-1231 (Ext 4880); Fax: 514-843-1723; E-mail: mdb@musica.mcgill.ca.

This work was supported by grants from the Royal Victoria Hospital Research Institute to S.B.B. and from the Medical Research Council to C.P. and to B.C. Preliminary results described in this paper were presented in part at the Society For Neurosciences 1996 annual meeting in Washington, USA and at the Canadian Anaesthetists' Society 1998 annual meeting in Toronto, Canada.

Accepted for publication July 29, 1998.

ANTICHOLINESTERASE drugs facilitate cholinergic transmission by preventing the hydrolysis of spontaneous or evoked release of acetylcholine (ACh), leading to activation of cholinergic receptors.¹ An important clinical side effect of anticholinesterase drugs is bradycardia, which results from the accumulation of ACh at the cardiac parasympathetic ganglia and SA node (Figure 1). This effect can be blocked by muscarinic antagonists. When comparing equipotent doses of anticholinesterase drugs administered to patients, the dose of muscarinic antagonist required to prevent the bradycardic effect of edrophonium is half of that required for neostigmine or pyridostigmine.^{2,3} We have hypothesized that this difference may be explained by the ability of edrophonium to block ganglionic cholinergic transmission directly, either by interfering with the release of ACh from preganglionic terminals or by blocking cholinergic receptors on the postganglionic neurone, thereby counteracting the facilitating effect of cholinesterase inhibition⁴ (Figure 1). This hypothesis, based on indirect evidence obtained from dose-response studies in cat and man concerned with the effects of edrophonium on heart rate,⁴ has never been directly verified.

The purpose of the present study was to evaluate whether edrophonium interferes with synaptic transmission in autonomic ganglia, and if so, by which mechanisms. This was investigated using the sympathetic ganglion as a model because it shares similar cholinergic mechanisms of transmission with parasympathetic ganglia, but it is easier to work with because it and its postganglionic axons are readily accessible.⁵ We first determined whether edrophonium blocks synaptic transmission in the superior cervical ganglion of the rat, by studying the effect of edrophonium on the evoked compound action potential recorded from postganglionic axons in response electrical stimulation of preganglionic axons. Once such block was demonstrated, we evaluated the effect of another anticholinesterase drug, neostigmine, to help determine if the block produced by edrophonium is a consequence of its anticholinesterase action *per se*. For example, the block may result from a build-up of ACh in the synaptic cleft which in turn has been suggested to produce depolarization block of nicotinic receptors.⁶ Additionally, a build-up of ACh may activate presynaptic M_1 or M_2 autoreceptors to reduce ACh release.^{7,8} Also, overflow of ACh from the synaptic cleft may activate extrasynaptic postganglionic inhibitory M_2 receptors⁹ (Figure 1). In view of this, we also tested the ability of pirenzepine and AFDX-116, selective muscarinic M_1 and M_2 receptor antagonists, respectively, to reverse the inhibitory

effect of edrophonium on the compound action potential. To test specifically for a postsynaptic action we evaluated the ability of edrophonium to block the ganglion cell firing evoked by exogenously administered ACh. As a measure of a presynaptic action, the amount of ACh released from ganglia during stimulation with 35 mM K^+ was compared in the presence and absence of edrophonium.

Methods

Following approval from the Animal Care Committee at McGill University, experiments were done using Sprague-Dawley rats (200-300 g, $n = 41$) anaesthetized with sodium pentobarbital (35 mg·kg⁻¹, *ip*). The effect of edrophonium on synaptic transmission and on ACh release was tested in the superior cervical ganglion *in vitro*. The effect of edrophonium on ganglion cell responsiveness to exogenously administered ACh was tested in the superior cervical ganglion *in vivo*.

1. Recording of the Compound Action Potential

The superior cervical ganglion with the ipsilateral cervical sympathetic trunk carrying preganglionic axons and the internal carotid nerve carrying postganglionic axons were excised *en bloc* and placed in a sylgaard-coated plastic chamber and superfused with Krebs-Ringer solution bubbled with 95% O_2 5% CO_2 (pH 7.4) at room temperature (21°C). The composition of the Krebs-Ringer solution was (in mM): NaCl 130; KCl 4; $MgCl_2$ 1; KH_2PO_4 1; $NaHCO_3$ 12; $CaCl_2$ 2; d-glucose 11. The cervical sympathetic trunk and the internal carotid nerve were drawn into bipolar suction electrodes for stimulating and recording, respectively. The cervical sympathetic trunk was stimulated with single shocks at 0.2 Hz using square wave pulses of twice the intensity which evoked a maximal postganglionic response (0.5 ms, 7.0 - 12.0 V). The amplitude of the compound action potential recorded from the internal carotid nerve was used as a measure of the efficacy of cholinergic transmission. The compound action potential was amplified (bandwidth 3Hz - 3 kHz), displayed on a storage oscilloscope and recorded on magnetic tape. Electrical stimulation of preganglionic axons evokes activation of the postganglionic neurones with a large "safety factor" of transmission, because of maximal spatiotemporal summation of EPSPs.^{10,11} As a result, the compound action potential is at, or near, saturation even at submaximal stimulus intensities. Under these conditions the usefulness of the evoked compound action potential as a tool for demonstrating inhibition or facilitation in sympathetic ganglia may be limited. Therefore, the present experiments were done in the

presence of partial nicotinic block with hexamethonium to reduce the high "safety factor" that characterizes ganglionic nicotinic transmission of the evoked, highly synchronized preganglionic input. This allows detection of increases or decreases in the amplitude of the evoked compound action potential which may otherwise be unmeasurable because of the saturated response of the postganglionic cells.^{10,11} Hexamethonium ($150 \pm 15 \mu\text{M}$, $n = 21$, Sigma) was added to the superfusate to reduce the amplitude of the compound action potential to $60 \pm 7\%$ of the baseline value. This reduction in compound action potential amplitude by hexamethonium occurs because of its postganglionic blocking action as well as its presynaptic action to inhibit ACh release.¹² In one set of experiments, edrophonium ($0.1 - 500 \mu\text{M}$, $n = 11$, ICN Canada Ltd.) was superfused, and the range of concentration to produce block of the compound action potential was determined. With this concentration range in mind, in another set of experiments, neostigmine was superfused using a concentration range of $0.1 - 10 \mu\text{M}$ ($n = 4$, Sabex) to produce similar reductions in cholinesterase activity, assuming that neostigmine is approximately 100-200 times as potent as edrophonium on a molar basis to inhibit cholinesterase activity.¹³⁻¹⁵ The effect of superfusion of the muscarinic antagonists pirenzepine ($200 \text{ nM} - 10 \mu\text{M}$, $n = 3$, Sigma), and AFDX-116 ($200 \text{ nM} - 10 \mu\text{M}$, $n = 3$, Boehringer Ingelheim) on the reduction of compound action potential produced by edrophonium $200 \mu\text{M}$ was determined. These dose ranges of pirenzepine and AFDX-116 are consistent with those which block M_1 and M_2 receptors, respectively.^{16,17} To determine whether edrophonium blocks axonal conduction of nerve action potentials, a section of the vagus nerve was excised, superfused with Krebs solution, and each end drawn into suction electrodes for stimulation or recording ($n = 2$). The vagus nerve was chosen as a convenient source of preganglionic axons which is of sufficient length to easily draw into suction electrodes.

2. Measurement of ACh Release.

[^3H]-ACh release was measured using a modification of the technique described by Gonzalez Burgos *et al.*¹⁸ In 14 rats, the superior cervical ganglion was excised bilaterally and the connective sheath was removed. Ganglia from two rats (4 ganglia) were used per experiment. Left and right ganglia from each rat were randomly placed in separate tubes (two ganglia per tube), incubated for 30 min in oxygenated Krebs solution at 37°C and then for five minutes in high- K^+ (35mM) to deplete the existing ACh stores. After washing the ganglia in Krebs solution (normal K^+ , 4 mM), they were incubated for 30 min (normal K^+) in $10 \mu\text{Ci}\cdot\text{ml}^{-1}$

[^3H]-choline ($\text{SA} = 81 \text{ Ci}\cdot\text{mmol}^{-1}$, to give a final concentration of $0.125 \mu\text{M}$) to label the replenished ACh stores and then were washed three times in Krebs before collecting the samples. The total volume of the tube (1 ml) was collected and replaced with Krebs solution every 10 min. Physostigmine was added ($15 \mu\text{M}$) to prevent breakdown of ACh. To determine if edrophonium affected spontaneous release of ACh, after collecting two samples of basal ACh efflux, edrophonium ($500 \mu\text{M}$) was added to one tube, while the other served as a control. This dose of edrophonium reliably blocks the compound action potential (see Results, Figure 2). Again, the total volume of the tube was collected and replaced two times (10 min incubation time). To determine if edrophonium affected evoked release, 35 mM K^+ was added to both tubes (one with edrophonium, one without edrophonium) and samples were collected after 10 min. The [^3H]-ACh was extracted from the samples and assayed as described by Prado *et al.*¹⁹ Results are presented as disintegrations per minute (dpm) of one minute incubation time.

3. Recording of Postganglionic Response to Exogenous ACh

Rats ($n = 6$) were respired artificially with room air following tracheal cannulation. Edrophonium was administered via a catheter inserted into a femoral vein as a single dose ($5 - 10 \text{ mg}\cdot\text{kg}^{-1}$). This dosage has been suggested to interfere with synaptic transmission in autonomic ganglia.⁴ ACh was injected directly into the blood supply of the superior cervical ganglion. The external carotid artery was cannulated with a polyethylene cannula (gauge PE10), the tip of the cannula was advanced retrogradely and positioned close to the carotid bifurcation. During injections, blood flow into the common carotid artery was temporarily interrupted with a snare clamp. ACh ($100 \mu\text{g}$ in a volume of $100 \mu\text{l}$, Sigma) was injected using an Eppendorf® repeater pipet. The ipsilateral internal carotid nerve was cut, desheathed and pulled into a suction electrode for recording the discharge of postganglionic axons evoked by the exogenously administered ACh. The electrical activity of the internal carotid cervical nerve was amplified (bandwidth $30 \text{ Hz} - 3 \text{ kHz}$) displayed on a storage oscilloscope and recorded on magnetic tape.

The effects of drugs on compound action potential amplitudes were tested with a paired Student's t test. The effect of edrophonium on ACh release was tested with a paired or an unpaired Student's t test, within or between groups, respectively. $P \leq 0.05$ was considered significant. Data are expressed as mean $\pm$ SEM.

Results

1. Effect of Edrophonium on Synaptic Transmission in the Superior Cervical Ganglion

Edrophonium (10 - 500 μ M, $n=7$) caused a concentration-related decrease in the postganglionic compound action potential (Figures 2A & B). Lower concentrations (0.1 - 5 μ M, $n = 4$) had no effect. At each concentration, the compound action potential began to decrease within about 10 min from the time the concentration of edrophonium in the superfusing medium was increased and reached a steady state within 30 min. The ED_{50} for the inhibitory effect was 163.5 ± 25.5 μ M. After wash-out of edrophonium the amplitude of the compound action potential began to recover within two minutes and recovered fully by 30 min ($n = 3$). The muscarinic antagonists pirenzepine (200 nM - 10 μ M) and AFDX-116 (200 nM - 10 μ M) were not effective in reversing the reduction of compound action potential produced by 200 μ M edrophonium (34.8 ± 6.7 % of control amplitude). Edrophonium (500 μ M, 20 min) did not affect the amplitude of the compound action potential of the vagus nerve. In contrast to the effect of edrophonium on synaptic transmission, neostigmine did not reduce the amplitude of the compound action potential. In fact, over the dose range tested, the amplitude appeared to be increased (max increase 122 ± 13 % baseline, $P = 0.17$, 1 μ M) however these increases were not statistically significant.

2. Effect of Edrophonium on ACh Release

In ganglia which were not exposed to edrophonium (control), high- K^+ stimulation produced an increase in release of [3 H]-ACh from a basal level of 41.5 ± 5.9

dpm·min $^{-1}$ to 551.6 ± 138 dpm·min $^{-1}$ ($n = 14$ ganglia, $P < 0.01$). In a different set of ganglia, prior to addition of edrophonium, basal release of [3 H]-ACh was 51.9 ± 6.9 dpm·min $^{-1}$ ($n = 14$), which was similar to that of the control group ($P = 0.3$). Edrophonium did not affect basal release (50.3 ± 9.4 dpm·min $^{-1}$). In the presence of edrophonium, high- K^+ evoked an increase in [3 H]-ACh release to 499.2 ± 134.8 ($n = 14$ ganglia, $P < 0.01$). This release was similar to the high- K^+ evoked release observed in control ganglia. To help control for the variability in ACh content from one ganglion to the next, the evoked increase was calculated as a multiple of the basal release. In this case, edrophonium caused approximately a 30% (12.3 ± 0.8 fold increase compared to 8.6 ± 1.0 fold increase, $P < 0.005$) decrease in the evoked release of [3 H]-ACh (Table).

3. Effect of Edrophonium on Ganglionic Response to Exogenous ACh

In this set of *in vivo* experiments, the ability of *iv* administration of edrophonium to block postganglionic cells activated by exogenously administered ACh was determined. Prior to edrophonium administration, injection of ACh into the arterial supply of the superior cervical ganglion evoked a burst of ganglion cell fir-

TABLE Release of [3 H]-ACh from the superior cervical ganglion

	CONTROL	EDROPHONIUM
BASAL		
RELEASE	41.5 ± 5.9 dpm·min $^{-1}$	51.9 ± 6.9 dpm·min $^{-1}$
EVOKED		
RELEASE	551.6 ± 138 dpm·min $^{-1}$ *	499.2 ± 134.8 dpm·min $^{-1}$ *
EVOKED		
RELEASE		
(% OF BASAL		
RELEASE)	$1232 \pm 80\%$	$862 \pm 100\%\dagger$

BASAL RELEASE: spontaneous release of [3 H]-ACh from control ganglia not exposed to edrophonium, and from ganglia prior to exposure to 500 μ M edrophonium. EVOKED RELEASE: high K^+ (35 mM) evoked an increase in [3 H]-ACh release from control ganglia, and from ganglia exposed to edrophonium. * = significant increase in evoked compared to spontaneous release, $P < 0.01$; † = significant decrease in evoked release in edrophonium-exposed ganglia compared to control, when evoked release is expressed as a multiple of the basal release, $P < 0.005$.

FIGURE 1 Schematic diagram of the peripheral sympathetic and parasympathetic autonomic nervous system. Preganglionic axons synapse onto postganglionic neurones, which in turn synapse onto autonomic effector cells. ACh is released from all preganglionic axon terminals, all parasympathetic postganglionic axon terminals, and a few sympathetic postganglionic axon terminals.³⁵ The majority of sympathetic postganglionic axon terminals release norepinephrine.³⁵ Release of ACh from the terminals may be regulated by presynaptic muscarinic receptors.^{7,8} The postganglionic neurones are activated by stimulation of excitatory nicotinic and muscarinic M_1 and possibly M_2 receptors, and are inhibited by stimulation of inhibitory M_2 receptors.^{5,16,36} Anticholinesterase drugs produce bradycardia by their facilitation of cholinergic transmission in the cardiac parasympathetic pathway, with the subsequent accumulation of ACh and activation of inhibitory muscarinic M_2 receptors at the SA node.

FIGURE 2 Edrophonium causes reduction in synaptic transmission in the *in vitro* rat superior cervical ganglion. **Panel A:** Each trace is composed of three superimposed oscilloscope sweeps depicting the compound action potential recorded from the internal carotid nerve in response to electrical stimulation of cervical sympathetic trunk. Responses are shown (a) prior to superfusion of edrophonium, and after a cumulative dose of (b) 50, (c) 100, (d) 200, and (e) 500 μM . Records are from one experiment. Dots denote stimulus artifacts. **Panel B:** Relationship between the reduction in amplitude of the compound action potential (CAP, ordinate) and the concentration of edrophonium (abscissa). Each point represents the averaged response from seven ganglia. Bars indicate S.E.M.

ing (Figure 3A) while injection of vehicle (100 μl saline) was without effect. The duration of the response varied among different ganglia (15 - 60 sec), however, in any given experiment responses were repeatable having similar amplitudes and time courses (Figure 3C). Following intravenous administration of edrophonium (5 - 10 $\text{mg}\cdot\text{kg}^{-1}$) the ganglion cell firing in response to ACh was blocked (Figure 3B). Recovery of the response to ACh was observed within 15 - 30 min (Figure 3C).

Discussion

Edrophonium, a reversible quaternary ammonium anticholinesterase, produced a marked dose-dependent block of synaptic transmission in the superior cer-

FIGURE 3 Edrophonium blocks the postsynaptic effect of ACh injected into the arterial supply of the *in vivo* rat superior cervical ganglion. Each panel shows a single oscilloscope sweep depicting multi-fibre nerve activity recorded from the internal carotid nerve. **A:** Arterial administration of ACh (100 μg) produced axonal discharge. **B:** Following *in vivo* administration of edrophonium (10 $\text{mg}\cdot\text{kg}^{-1}$), discharge in response to ACh administration is blocked. **C:** Recovery of discharge produced by ACh 30 min after administration of edrophonium. Arrows indicate ACh injection.

vical ganglion of the rat, as shown by its inhibitory effect on the evoked compound action potential recorded from postganglionic axons in response to electrical stimulation of preganglionic axons. Edrophonium appears to interfere with synaptic transmission by a postsynaptic mechanism as demonstrated by the block of the superior cervical ganglion cell response to exogenously administered ACh. Possibly, a presynaptic mechanism may be also involved as shown by the decrease in the high- K^+ stimulated release of ACh from the preganglionic nerve terminals, when this release is expressed as a multiple of basal release. The inhibitory effect of edrophonium was not due to an interference with nerve action potential conduction, as this drug did not affect the amplitude of the compound action potential recorded from the vagus nerve triggered by direct axonal stimulation. The effect of edrophonium on synaptic transmission did not appear to be a consequence of an anticholinesterase action, as inhibition of the compound action potential was not observed with neostigmine at a dose producing a comparable cholinesterase inhibition to that of edrophonium.¹³⁻¹⁵ Nor was it secondary to activation of M_1 and M_2 muscarinic receptor sub-types (see Introduction), as edrophonium's inhibitory effect was not blocked by pirenzepine and AFDX-116, respectively.

While it is known that anticholinesterase drugs can depress synaptic transmission in autonomic ganglia,²⁰⁻²³ the present study is the first which documents such an effect of edrophonium. Regarding a possible presynaptic effect of edrophonium, one potential mechanism is that it inhibits ACh synthesis by preventing reuptake of choline resulting in depletion of ACh stores, as has been demonstrated with the anticholinesterase drugs DFP²⁴ and propoxur.²⁵ Another possibility is that edrophonium directly blocks N-type Ca^{++} channels, as has been shown with tacrine,²⁶ and thus interferes with release of ACh from presynaptic terminals. In the present study, considering that the presynaptic effect of edrophonium was only evident when expressing the evoked release as a multiple of the basal release of ACh, and that this effect was observed with a dose of edrophonium which completely blocks ganglionic transmission, a presynaptic mechanism would appear to play a relatively minor role, if any. Regarding the postsynaptic effect, edrophonium may be acting as an antagonist at the nicotinic receptor. In this regard, edrophonium has been shown to block the ion-conducting pore of the neuromuscular nicotinic receptor and to desensitize this receptor expressed in *Xenopus laevis* oocytes.²⁷ In addition, in cultured mouse cerebrovascular tumour cells edrophonium has been shown to interact directly with ACh-activated ion channels to decrease channel open time.²⁸

Edrophonium did not produce a facilitation of synaptic transmission at any dose tested, an effect which may be anticipated as a consequence of its anticholinesterase activity. Indeed, neostigmine caused a small increase in the amplitude of the compound action potential. However, one must be cautious in attributing the effects of neostigmine to its anticholinesterase activity as this drug has been suggested to interact directly with cholinergic receptors in the peripheral autonomic nervous system.^{13,29} In the present study, the simplest explanation to account for the lack of a facilitatory effect of edrophonium is that it was masked by edrophonium's inhibitory effect on synaptic transmission. This would occur if the dose-response relationship for inhibition of cholinesterase activity and for block of ganglionic transmission overlap. In fact, we have demonstrated, in the cat, that the range of dose of edrophonium which blocks the bradycardia produced by electrical stimulation of the vagus nerve⁴ overlaps that which blocks cholinesterase activity.¹³

In a previous study in bilaterally vagotomized, propranolol-treated cats, we reported that edrophonium at a dose $< 1.5 \text{ mg}\cdot\text{kg}^{-1}$ augmented the bradycardia evoked by electrical stimulation of the distal sectioned portion of the vagus nerve, presumably secondary to the facilitation of transmission in the cardiac parasymp-

athetic pathway due to the anticholinesterase action of edrophonium.^{4,13} However, at doses $> 1.5 \text{ mg}\cdot\text{kg}^{-1}$, edrophonium blocked the bradycardia evoked by stimulation of the vagus nerve.⁴ In that study, we argued that the inhibition of the bradycardia was due to block of ganglionic synaptic transmission, either at the level of the preganglionic terminal or the receptor on the postganglionic neurone. The findings from the present study are consistent with these arguments. It is relevant that edrophonium has recently been shown to produce a tachycardia when administered to rats, however in that study it was suggested that this effect is mediated by block of inhibitory cardiac muscarinic receptors.³⁰

In addition to the fact that one must be prudent when extrapolating from observations in animals to humans, it may be difficult to account for the clinical effect of edrophonium on heart rate on the basis of its effect on ganglionic cholinergic transmission, as described in the present study, because in patients with normally innervated hearts, heart rate is controlled by tonic drive from both the sympathetic and parasympathetic nervous systems. However, as the parasympathetic tone is predominant,³¹ it may be predicted that the blocking action of edrophonium on ganglionic cholinergic transmission would result in a relative increase in heart rate in man. Indeed, we have reported that in some patients administration of a cumulative dose of edrophonium to $0.8 \text{ mg}\cdot\text{kg}^{-1}$ produces an increase in heart rate from the nadir obtained with the previous dose of $0.4 \text{ mg}\cdot\text{kg}^{-1}$.⁴ The ability of edrophonium to block ganglionic transmission also may help to explain the clinical observation that less muscarinic antagonist is required to blunt the parasympathomimetic effect of edrophonium than that of other anticholinesterase drugs.^{2,3} In man, assuming a full reversing dose of edrophonium of $1.0 \text{ mg}\cdot\text{kg}^{-1}$,³² a molecular weight of 201.7,³³ and a blood volume of $70 \text{ ml}\cdot\text{kg}^{-1}$, a theoretical peak plasma concentration of edrophonium may be approximated to be $70 \text{ }\mu\text{Mol}$. Pharmacokinetic studies in patients have demonstrated a peak serum concentration which may be calculated to be approx $60 \text{ }\mu\text{Mol}$ following a two minute infusion of $1.0 \text{ mg}\cdot\text{kg}^{-1}$ edrophonium (data from Figure 1 in ref 34). These concentrations are within the range of doses which reduced ganglionic transmission reported in the present animal study. When edrophonium is administered at a full reversing dose, the results from the present study suggest that the facilitating effect on transmission in the peripheral cardiac parasympathetic pathway, secondary to the inhibition of cholinesterase, may be partly antagonized by the direct inhibitory effect on ganglionic cholinergic transmission.

In conclusion, edrophonium blocks ganglionic cholinergic transmission postsynaptically and, possibly,

presynaptically. The mechanism(s) by which this occurs does not appear to involve inhibition of cholinesterase, or activation of M₁ or M₂ receptor subtypes.

Acknowledgments

We are grateful to Ms. A. McNicoll for expert technical assistance. We thank Boehringer Ingelheim Co. Ltd. for the generous supply of AFDX-116.

References

- 1 Brown GL, Dale HH, Feldberg W. Reactions of the normal mammalian muscle to acetylcholine and to eserine. *J Physiol* 1936; 15: 394-424.
- 2 Cronnelly R, Morris RB, Miller RD. Edrophonium: duration of action and atropine requirement in humans during halothane anesthesia. *Anesthesiology* 1982; 57: 261-6.
- 3 Fogdall RP, Miller RD. Antagonism of *d*-tubocurarine- and pancuronium-induced neuromuscular blockades by pyridostigmine in man. *Anesthesiology* 1973; 39: 504-9.
- 4 Backman SB, Stein RD, Fox GS, Polosa C. Heart rate changes in cardiac transplant patients and in the denervated cat heart after edrophonium. *Can J Anaesth* 1997; 44: 247-54.
- 5 Skok VI. *Physiology of Autonomic Ganglia*. Tokyo: Igaku Shoin Ltd., 1973.
- 6 Paton WDM, Perry WLM. The relationship between depolarization and block in the cat's superior cervical ganglion. *J Physiol* 1953; 119: 43-57.
- 7 Brehm G, Lindmar R, Löffelholz K. Inhibitory and excitatory muscarinic receptors modulating the release of acetylcholine from postganglionic parasympathetic neuron of the chicken heart. *Nauyn-Schmiedeberg's Arch Pharmacol* 1992; 346: 375-82.
- 8 Koketsu K, Yamada M. Presynaptic muscarinic receptors inhibiting active acetylcholine release in the bullfrog sympathetic ganglion. *Br J Pharmacol* 1982; 77: 75-82.
- 9 Bachoo M, Polosa C. An AF-DX 116 sensitive inhibitory mechanism modulates nicotinic and muscarinic transmission in cat superior cervical ganglion in the presence of anticholinesterase. *Can J Physiol Pharmacol* 1992; 70: 1535-41.
- 10 Bachoo M, Polosa C. Long-term potentiation of nicotinic transmission by a heterosynaptic mechanism in the stellate ganglion of the cat. *J Neurophysiol* 1991; 65: 639-47.
- 11 Brown TH, McAfee DA. Long-term synaptic potentiation in the superior cervical ganglion. *Science* 1982; 215: 1411-3.
- 12 Liang S-D, Vizi ES. Positive feedback modulation of acetylcholine release from isolated rat superior cervical ganglion. *J Pharmacol Exp Ther* 1997; 280: 650-5.
- 13 Backman SB, Stein RD, Blank DW, Collier B, Polosa C. Different properties of the bradycardia produced by neostigmine and edrophonium in the cat. *Can J Anaesth* 1996; 43: 731-40.
- 14 Randall LO, Conroy CE, Ferruggia TM, Kappell BH, Knoepfel CR. Pharmacology of the anticholinesterase drugs - mestinon, prostigmin, tensilon and TEPP. *Am J Med* 1955; 19: 673-8.
- 15 Smith CM, Mead JC, Unna KR. Antagonism of tubocurarine III: time course of action of pyridostigmine, neostigmine, and edrophonium *in vivo* and *in vitro*. *J Pharmacol Exp Ther* 1957; 120: 215-28.
- 16 Allen TGJ, Burnstock G. M₁ and M₂ muscarinic receptors mediate excitation and inhibition of guinea-pig intracardiac neurones in culture. *J Physiol* 1990; 422: 463-80.
- 17 Vizi ES, Kobayashi O, Töröcsik A, *et al.* Heterogeneity of presynaptic muscarinic receptors involved in modulation of transmitter release. *Neuroscience* 1989; 31: 259-67.
- 18 Gonzalez Burgos GR, Biali FI, Cherksey BD, Sugimori M, Llinás RR, Uchitel OD. Different calcium channels mediate transmitter release evoked by transient or sustained depolarization at mammalian sympathetic ganglia. *Neuroscience* 1995; 64: 117-23.
- 19 Prado MAM, Gomez MV, Collier B. Mobilization of the ready releasable pool of acetylcholine from a sympathetic ganglion by tityustoxin in the presence of vesamicol. *J Neurochem* 1992; 59: 544-52.
- 20 Bronk DW, Tower SS, Solandt DY. Synaptic transmission in the stellate ganglion. *Proc Soc Exp Biol Med* 1935; 32: 1659-61.
- 21 Koketsu K, Nishi S, Noda Y. Effects of physostigmine on the afterdischarge and slow postsynaptic potentials of bullfrog sympathetic ganglia. *Br J Pharmacol* 1968; 34: 177-88.
- 22 Mo N, Dun NJ, Karczmar AG. Facilitation and inhibition of nicotinic transmission by eserine in the sympathetic ganglia of the rabbit. *Neuropharmacology* 1985; 24: 1093-101.
- 23 Volle RL, Koelle GB. The physiological role of anticholinesterase (AChE) in sympathetic ganglia. *J Pharmacol Exp Ther* 1961; 133: 223-40.
- 24 Mattio TG, Richardson JS, Giacobini E. Acute effects of cholinesterase inhibitors on uptake of choline in the rat iris. *Neuropharmacology* 1985; 24: 325-8.
- 25 Kobayashi H, Yuyama A, Ohkawa T, Kajita T. Effect of single or chronic injection with a carbamate, propoxur, on the brain cholinergic system and behavior of mice. *Jpn J Pharmacol* 1988; 47: 21-7.
- 26 Wang S-J, Huang C-C, Gean P-W. Investigations on the mechanism of tetrahydro-9-aminoacridine- induced

- presynaptic inhibition in the rat amygdala. *Neuroscience* 1996; 70: 409–15.
- 27 *Yost CS, Maestroni E.* Clinical concentrations of edrophonium enhance desensitization of the nicotinic acetylcholine receptor. *Anesth Analg* 1994; 78: 520–6.
- 28 *Wachtel RE.* Comparison of anticholinesterases and their effects on acetylcholine-activated ion channels. *Anesthesiology* 1990; 72: 496–503.
- 29 *Backman SB, Bachoo M, Polosa C.* Mechanism of the bradycardia produced in the cat by the anticholinesterase neostigmine. *J Pharmacol Exp Ther* 1993; 265: 194–200.
- 30 *Yamamoto K, Shimizu M, Ohtani H, Hayashi M, Sawada Y, Iga T.* Toxicodynamic analysis of cardiac effects induced by four cholinesterase inhibitors in rats. *J Pharm Pharmacol* 1996; 48: 935–9.
- 31 *Berne RM, Levy MN.* Cardiovascular Physiology, 4th ed. St. Louis: C.V. Mosby Company, 1981.
- 32 *Breen PJ, Doherty WG, Donati F, Bevan DR.* The potencies of edrophonium and neostigmine as antagonists of pancuronium. *Anaesthesia* 1985; 40: 844–7.
- 33 *Budavari S.* The Merck Index, 12th ed. Whitehouse Station NJ: Merck and Co. Inc., 1996: 594.
- 34 *Morris RB, Cronnelly R, Miller RD, Stanski DR, Fahey MR.* Pharmacokinetics of edrophonium in anephric and renal transplant patients. *Br J Anaesth* 1981; 53: 1311–4.
- 35 *Lefkowitz RJ, Hoffman BB, Taylor P.* Neurotransmission. The autonomic and somatic motor nervous systems. *In:* Hardman JG, Gilman AG, Limbird LE. (Eds.). Goodman & Gilman's The Pharmacological Basis Of Therapeutics, 9th ed. New York: McGraw-Hill, 1996: 105–39.
- 36 *Mihara S, Ikeda K, Nishi S.* Muscarinic M₂ receptors on cardiac ganglion neurons of the guinea-pig heart. *Kurume Med J* 1988; 35: 183–92.