Effects of the Anticholinesterase Edrophonium on Spectral Analysis of Heart Rate and Blood Pressure Variability in Humans

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ABSTRACT

Edrophonium, an anticholinesterase, exerts a biphasic effect on cardiovascular autonomic drive in humans (lower doses enhance; higher doses reduce). Twenty-five anesthetized, mechanically respired (10 breaths·min⁻¹, constant tidal volume) patients were given either saline (n = 10) or edrophonium (0.01–1.0 mg·kg⁻¹, n = 15) following surgery. ECG, radial arterial pressure, and respiratory rate were sampled at 250 Hz to obtain time series for consecutive R-R intervals (RRIs), and systolic (SBP) and diastolic blood pressure (DBP). A Wigner distribution was used for time frequency mapping of spectral powers at high (HFP, 0.15–0.5 Hz) and low (LFP, 0.0–0.05 Hz) frequency. Edrophonium produced a dose-dependent decrease in heart rate [baseline 66.8 ± 1.9 (S.E.M.) beats per minute; maximum decrease to 55.8 ± 1.4 beats per minute with 1.0 mg·kg⁻¹, P < 0.01]. HFP of the RRI increased at low doses (0.2–0.4 mg·kg⁻¹; maximum increase to 111.0 ± 58.2% baseline; P < 0.01) but decreased (∼49 ± 35.5% baseline; P < 0.01) at higher (1.0 mg·kg⁻¹) doses. Edrophonium had no effect on SBP and DBP. HFP of SBP decreased with increasing doses (maximal decrease to −26.2 ± 7.5% baseline, P < 0.01, 1.0 mg·kg⁻¹). LFP of SBP was also decreased (∼46.3 ± 10.9% baseline, P < 0.01, 1.0 mg·kg⁻¹). Edrophonium may enhance (lower dose) or reduce (higher dose) cardiovascular autonomic drive in humans, as evidenced by the significant changes it evokes in HFP of the RRI (parasympathetic drive), and in the HFP and LFP of SBP (sympathetic drive). These observations may account for the modest autonomic side effects of edrophonium when this drug is used clinically.

Anticholinesterase drugs are used clinically to facilitate cholinergic transmission. Edrophonium and neostigmine are anticholinesterases commonly used postoperatively to reverse the neuromuscular block induced by nondepolarizing muscle relaxants. An undesirable effect of the anticholinesterase is activation of parasympathetic target organs, resulting in a variety of responses including bradycardia.

It is assumed that the bradycardia results from the anticholinesterase activity, which prevents the hydrolysis of ACh released tonically from parasympathetic neurons innervating the heart (Baraka, 1968). However, recent studies suggest that anticholinesterases may have additional effects at cholinergic synapses within the autonomic peripheral pathway. For example, in vivo and in vitro animal studies suggest that neostigmine and edrophonium directly activate (Backman et al., 1993a, 1996a) and inhibit (Backman et al., 1997; Stein et al., 1998), respectively, these cholinergic receptors. Thus, the parasympathomimetic action of the anticholinesterases must be understood within the context of their anticholinesterase activity on the one hand, and their direct interaction with cholinergic receptors on the other. For example, it is well established clinically that the fall in heart rate produced by edrophonium is significantly smaller than that produced by neostigmine (Fogdall and Miller, 1973; Cronnelly et al., 1982). It has been proposed that the modest bradycardic effect of edrophonium occurs because the enhancement of parasympathetic drive, secondary to the anticholinesterase action, is diminished by the inhibitory effect of the drug on autonomic cholinergic transmission (Backman et al., 1997; Stein et al., 1998). Animal studies suggest that the enhancement (anticholinesterase action) occurs at lower doses compared with the reduction (block of cholinergic receptors) of autonomic cholinergic transmission that occurs at high doses (Backman et al., 1996a; Stein et al., 1998).

From the above, it is anticipated that in humans edrophonium produces a dose-dependent biphasic effect on autonomic drive. That is, the drive is augmented by lower doses

ABBREVIATIONS: ACh, acetylcholine; HFP, high frequency power; LFP, low frequency power; RRI, R-R interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; SA, sinoatrial; EDRO, edrophonium-treated patients; CONT, control patients who received saline.
and is reduced with higher doses. In humans, however, there is little information concerning the effect of edrophonium on cardiovascular autonomic responses when this drug is administered without a muscarinic antagonist (van Vlymen and Parlow, 1997). Thus, the purpose of the present study was to characterize the effect of edrophonium on autonomic control of the cardiovascular system in humans. This was achieved by measuring sensitive indices of sympathetically and parasympathetically mediated cardiovascular responses (time-frequency analyses of heart rate and systemic arterial pressure variability) in anesthetized patients following incremental doses of edrophonium. Time frequency mapping based on a Wigner distribution enables simultaneous assessment of signals in both time and frequency domains. High frequency power of R-R intervals (RRIs) is a quantitative indicator of parasympathetic influence on the SA node (Brown et al., 1993; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Novak et al., 1997b). It is established that the low frequency power of the systolic component of systemic arterial pressure may be employed as a quantitative indicator of sympathetic outflow (Pagani et al., 1996). The high frequency power of the systolic component may also be used for this purpose, but it is markedly influenced by mechanical factors such as tidal volume and respiratory rate (Madwed et al., 1989; Pagani et al., 1996; Novak et al., 1997a.)

Materials and Methods

Subjects. This study was approved by the Ethics Committee at the Royal Victoria Hospital, and informed consent was obtained from every subject. This report includes data from 25 patients (13 male, 12 female; 25–63 years old) who underwent abdominal, urological, gynecological, head and neck, or plastic surgery under general anesthesia using a standardized regimen. Exclusion criteria included major cardiovascular, pulmonary, neurological, or endocrine disease, metabolic disorders, recent exposure to medications that could affect heart rate (e.g., beta blockers or agonists, muscarinic agonists or antagonists), age < 18 or > 70 years, patients undergoing emergency procedures, and patients receiving major conduction anesthesia (spinal or epidural) because of possible interference with sympathetic cardiovascular outflow. Patients were also excluded if they demonstrated a cardiac rhythm other than sinus, if premature ventricular beats were present, or if heart rate was less than 55 beats/min prior to the start of the experiment (see below).

Experimental Set-Up. Patients received no premedication. Monitoring in the operating room included ECG (lead II), noninvasive systemic arterial pressure, pulse oximetry, end-tidal carbon dioxide and concentration of volatile anesthetic agents, ventilation pressure, and train-of-four ratio. General anesthesia was induced using fentanyl (1–1.5 \( \mu \text{g} \cdot \text{kg}^{-1} \)) and propofol (1.5–2.0 \( \text{mg} \cdot \text{kg}^{-1} \)). Tracheal intubation was facilitated by muscle paralysis with rocuronium (0.6–1.0 \( \text{mg} \cdot \text{kg}^{-1} \)). Anesthesia was maintained using a mixture of isoflurane (0.4–1.2%) and 50% \( \text{N}_2\text{O} / \text{O}_2 \). Drugs with an antimuscarinic (e.g., pancuronium, atropine, glycopyrrolate) or beta-blocking effects were avoided. The rate of the respirator was set at 10 breaths \( \cdot \text{min}^{-1} \) (0.17 Hz). Tidal volume was initially set at 10 ml \( \cdot \text{kg}^{-1} \) and was then adjusted to maintain end-tidal \( \text{CO}_2 \) between 30 and 35 mm Hg. Respiratory parameters were unaltered during the study period. At the conclusion of surgery and wound dressing, no attempt was made to lighten the level of anesthesia or reverse neuromuscular blockade, and stimulation of the patient (oral suctioning, patient transfer from OR table to stretcher, etc.) was avoided. Fifteen minutes were allotted for stabilization of cardiovascular parameters. After this interval, a 20 gauge catheter was inserted into a radial arterial to record systemic arterial pressure if heart rate was above 55 beats \( \cdot \text{min}^{-1} \) (three patients were excluded for low heart rates).

Continuous ECG (lead II), systemic arterial blood pressure, and ventilation pressure were recorded on a personal computer via an analog-to-digital converter at a sampling rate of 250 Hz per channel (Windaq; Dataq Instruments, Akron, OH). After the 15-min stabilization interval (see above), a 5-min period of recording of baseline physiological parameters was initiated (baseline = condition 1). Following recording of baseline parameters, in one set of patients (edrophonium, EDRO, \( n = 15 \)) edrophonium chloride (Zeneca Pharma, Mississauga, Ontario, Canada) was injected at 5-min intervals to obtain cumulative doses of 0.01, 0.05, 0.1, 0.2, 0.4, and 1.0 \( \text{mg} \cdot \text{kg}^{-1} \) (conditions 2–7, respectively) with the maximal dose constituting a full reversing dose (Breen et al., 1985). Five-minute intervals were chosen because in a previous study in humans, we demonstrated that the edrophonium-induced bradycardia began within 30 sec after edrophonium injection and reached a plateau within 2 min (Backman et al., 1997). Atropine sulfate (Abbott Laboratories, Ltd., Montreal, Quebec, Canada) 10 \( \mu \text{g} \cdot \text{kg}^{-1} \) was administered 5 min after the last dose of edrophonium. In a second set of patients (control, CONT, \( n = 10 \)), following an initial 5-min period for recording of baseline data (condition 1), six doses of saline (conditions 2–7, respectively), each of a volume matched to the corresponding dose of edrophonium, were injected at 5-min intervals. In this CONT group, 1.0 \( \text{mg} \cdot \text{kg}^{-1} \) edrophonium and 10 \( \mu \text{g} \cdot \text{kg}^{-1} \) atropine were coadministered 5 min following the last dose of saline.

Data Management. Consecutive RRRIs, and systolic and diastolic blood pressures (SBP, DBP) were acquired from the ECG and blood pressure signals and converted to their respective time series. These were linearly interpolated at 4 Hz to obtain equal samples within each time series. A moving fourth order polynomial function, comprising a nonlinear bandpass filter, was used to remove baseline trends. This filtering excludes the very low frequency nonstationary components (<0.005 Hz) but leaves the faster frequencies of interest intact. Time frequency mapping and beat-to-beat spectral estimation was achieved using a discrete Wigner distribution that decomposes signals expressed as a function of time into signals expressed as a function of both time and frequency. The modified Wigner distribution has been demonstrated to be a good estimation method for short nonstationary time series, and its resolution was enhanced by independent time and frequency smoothing using a moving 128-event data window. The time-frequency distributions were computed with the same parameter set for all signals. A detailed description of the Wigner function can be found elsewhere (Novak and Novak, 1993). The cross-frequency distributions were calculated for each signal and each condition to determine common frequency contents between consecutive RRRIs and SBPs. The spectral power was calculated at high frequency (HF, 0.15–0.50 Hz) and low frequency (LF, 0.0–0.05 Hz). Spectral powers were averaged over 4-min intervals starting 1 min following each injection of edrophonium or saline.

Statistics. Heart rate [HR], SBP, DBP and RRI changes, in addition to the high- and low-frequency power of RRI and SBP following injection of either edrophonium or saline, were assessed using a two-way repeated-measures analysis of variance. Log-transformed high- and low-frequency powers were analyzed to ensure a normal distribution of data. When differences between the groups were significant (\( P \) value ≤ 0.05), a Tukey test was performed for post hoc comparison. Data are expressed as mean ± S.E.M. unless indicated otherwise. To permit direct comparison of the magnitude of change in high and low frequency between individuals, percentage changes from baseline were used for graphical representation of these data.
Results

In the EDRO and CONT groups, the distribution of males/females (8:7, 5:5, respectively) and ages (47.3 ± 10.7, 43.7 ± 16.2, respectively) were similar.

R-R Intervals. Baseline RRI of patients in the CONT (936 ± 36.0 ms; 65.3 ± 2.7 beats · min⁻¹) and EDRO (897 ± 23.9 ms; 66.8 ± 1.9 beats · min⁻¹) groups were similar. With the patients who received saline (CONT), RRI did not change significantly during the study. In contrast, with the patients who received edrophonium, RRI increased with increasing doses of edrophonium and a plateau was reached after the fifth dose of edrophonium. The maximum increase, to 1072 ± 30.4 ms (P < 0.01), occurred after the last dose of edrophonium (cumulative dose of 1.0 mg · kg⁻¹, condition 7; Fig. 1).

Power analysis of the RRI at the high frequency band (HFP, 0.15–0.50 Hz) in patients receiving anticholinesterase demonstrated significant increases following the fourth (cumulative dose of 0.2 mg · kg⁻¹, condition 5) and fifth doses (maximal increase 111.0 ± 58.2% baseline, P < 0.05, cumulative dose 0.4 mg · kg⁻¹, condition 6, Fig. 2). However, a sharp decrease to −49.5 ± 35.5% baseline, P < 0.01, occurred following the last dose (1.0 mg · kg⁻¹, condition 7). In contrast, the HFP of the CONT group demonstrated no significant changes during the study.

Systemic Arterial Blood Pressure. Baseline SBP and DBP of patients were similar in the two groups, CONT (113.5 ± 18.9 mm Hg, 60.4 ± 13.3 mm Hg, respectively) and EDRO (109.9 ± 17.2 mm Hg, 61.2 ± 11.8 mm Hg, respectively). Neither edrophonium nor saline infusion had a significant effect on SBP and DBP (Fig. 3). Spectral analysis of SBP, however, demonstrated significant differences between the two groups. Spectral powers at low frequency tended to increase, although not significantly, after the third to fifth doses of edrophonium, followed by a precipitous decrease after the final dose (1.0 mg · kg⁻¹, condition 7, −46.3 ± 10.9% baseline, P < 0.01, Fig. 4). This decrease was not observed in the CONT group (Fig. 4).

Spectral powers at high frequency decreased with edrophonium in a dose-dependent manner following the fourth (0.2 mg · kg⁻¹, condition 5, P < 0.01) to sixth doses (1.0 mg · kg⁻¹, condition 7; maximal decrease to −26.2 ± 7.5% baseline, P < 0.01, Fig. 5). This decrease contrasts greatly from the changes observed in the CONT group, where the power did not change significantly (Fig. 5).

Discussion

The main finding of this study is that clinically relevant doses of edrophonium block cardiovascular autonomic drive in humans, as evidenced by the significant decrease it produces in HFP of the RRI, and HFP and LFP of SBP. The observations are at odds with the widely held view that anticholinesterase drugs simply augment cardiac autonomic drive (Cronnelly et al., 1982). However, the findings from the present study are in accordance with results obtained from previous animal studies which demonstrated that edrophonium blocks autonomic ganglionic cholinergic transmission and which predicted such a diminution of cardiovascular
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The evidence for a ganglion-blocking effect of edrophonium was obtained from studies in the rat sympathetic superior cervical ganglion, where clinically relevant doses of edrophonium (10–500 μM, ED_{50} 163 μM; clinical peak serum concentration 50–60 μM with bolus dose of 1.0 mg · kg⁻¹ edrophonium [Stein et al., 1998; Wachtel, 1990]) decreased the compound action potential amplitude recorded from the postganglionic axons in the internal carotid nerve in response to electrical stimulation of preganglionic axons in the cervical sympathetic trunk (Stein et al., 1998). Block of the synaptic transmission was shown to occur postsynaptically, as edrophonium inhibited postganglionic cell firing in response to exogenously administered ACh (Stein et al., 1998). It is relevant that in other models of cholinergic transmission, mouse tumor cells (Wachtel, 1990), and Xenopus laevis oocytes (Yost and Maestrone, 1994) with expressed nicotinic receptors, clinically relevant doses of edrophonium (ED_{50} 3.8 and 82 μM, respectively) decrease ACh-activated channel open time (Wachtel, 1990) and DMPP (a selective nicotinic agonist)-activated currents (Yost and Maestrone, 1994), indicating a postsynaptic blocking effect. Edrophonium may also block transmission in the peripheral autonomic pathway by blocking release of ACh from preganglionic terminals in the autonomic ganglia (Stein et al., 1998) and by blocking inhibitory ganglionic transmission (Bachoo and Polosa, 1998; Shannon et al., 1998). Although the findings from the present study offer no further insight into the mechanisms by which edrophonium blocks autonomic cardiovascular drive, they are the first demonstration that such blockage occurs when this drug is administered to patients and are entirely consistent with the results from previous animal and cellular studies cited above.

The decrease edrophonium produces in HFP of the RRI, and HFP and LFP of SBP, as shown in the present study, may be accounted for by the inhibitory effect this anticholinesterase drug has on transmission in the peripheral autonomic nervous system (see above). However, at cumulative doses less than 1.0 mg · kg⁻¹, augmentation of the HFP of the RRI was apparent (Fig. 2). Presumably, this reflects the enhancement of cholinergic transmission in the parasympathetic autonomic ganglia, or at the SA node, as a consequence of the inhibition of cholinesterase and subsequent accumulation of ACh at these sites. This is reminiscent of observations in animal studies in which lower doses of edrophonium augment the bradycardia evoked by electrical stimulation of the vagus nerve, whereas higher doses block the bradycardia (Backman et al., 1997). It may be anticipated that cholinergic transmission in the peripheral cardiac parasympathetic pathway is particularly sensitive to the facilitatory effect of cholinesterase inhibition because of an additive effect at both the ganglion and the SA node. Moreover, blockage of cholinesterase activity may affect the ACh released spontaneously from intrinsic cardiac parasympathetic postganglionic neurons in addition to that released as a consequence of ongoing cardiac parasympathetic drive (Backman et al., 1993a, 1996a, 1997). In contrast, the peripheral sympathetic pathway has only the one cholinergic synapse, at the autonomic ganglion, and this may account for the lack of a clear facilitatory effect of edrophonium on the HFP and LFP of SBP (Figs. 4 and 5), which are indices of sympathetic outflow. An additional consideration may be that as a consequence of inhibition of cholinesterase, overflow of ACh from the synaptic cleft may activate extrasynaptic postganglionic inhibitory M2 receptors in the sympathetic ganglion, which would block ganglionic cholinergic transmission (Bachoo and Polosa, 1992). Finally, data from animal studies indicate that the dose-response relationships for inhibition of cholinesterase activity and for block of cholinergic ganglionic transmission

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Figure 4. Relationship between condition (abscissa) and high frequency power of systolic blood pressure (HFP, ordinate) expressed as percentage change from baseline value. Each histogram represents the average response from patients who received saline (control, n = 10, black) or edrophonium (n = 15, white). *, change in power that was significantly different from control (P < 0.05).

Figure 5. Relationship between condition (abscissa) and low frequency power of systolic blood pressure (LFP, ordinate) expressed as percentage change from baseline value. Each histogram represents the average response from patients who received saline (control, n = 10, black) or edrophonium (n = 15, white). *, change in power that was significantly different from control (P < 0.05).
Edrophonium was administered as bolus doses and steady state plasma concentrations were not achieved. However, since the objective of this study was to assess the effect of clinically relevant doses of edrophonium on cardiovascular autonomic outflow, a dose-response study best mimics the clinical situation where the drug is administered as a bolus. Yet, the data from the present study do not reveal the dose at which edrophonium reduces cardiac autonomic outflow. In all likelihood, with the experimental paradigm used, the dose-response data provided in Figs. 2, 4, and 5 overestimate this dose. After the initial injection of edrophonium, each subsequent injection was separated by an interval of ~5 min. Given a t\textsubscript{1/2}\textsubscript{192} of approximately 7 min (Morris et al., 1981), it is anticipated that the final plasma concentration of edrophonium achieved with the cumulative dose of 1 mg kg\textsuperscript{-1} is considerably less than that which would be produced by the single bolus dose of 1 mg kg\textsuperscript{-1} that is used in clinical practice. Incremental doses of edrophonium, as used in the present study, were necessary to be able to demonstrate any dose-response changes in cardiovascular autonomic outflow. Had edrophonium been administered as a single bolus dose of 1 mg kg\textsuperscript{-1}, responses could only have been compared with baseline values, and changes in autonomic parameters may have been missed. Of course, there are issues of patient safety that have to be considered; although we have administered edrophonium in incrementally increasing bolus doses in previous clinical studies without adverse effect (Backman et al., 1997), this may not be the case with a larger bolus dose.

Spectral components of autonomic target organ responses may be influenced by a variety of factors including gender, age (Bigger et al., 1995; Jensen-Urstad et al., 1998), and choice of volatile anesthetic agent (Galletly et al., 1994; Widmark et al., 1998). The influences of these factors were balanced in the present study by using the same anesthetic agent for each patient in each group and by the equal distribution of age and gender between the groups. Possibly, variations in power observed over time in the controls and in those who received edrophonium may have been additionally influenced by a time-dependent effect of the anesthetic agent (Galletly et al., 1994; Widmark et al., 1998). Graphical representation of the power spectral data as a percentage change from baseline helps to compare the magnitude of the changes for each individual studied as well as between the groups.

There is ample evidence to suggest that HFP of heart rate variability may be used as an index of parasympathetic outflow at the sinoatrial node (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). With regard to LFP of systolic blood pressure, there is good evidence to suggest that it reflects sympathetic outflow, which may control vasomotor tone (Novak et al., 1997a; Pagani et al., 1996). HFP of systolic blood pressure appears to reflect sympathetic outflow, although this has been shown to be strongly influenced by mechanical factors such as respiratory rate and tidal volume on blood pressure (Novak et al., 1997a; Madwed et al., 1989; Rimoldi et al., 1990; Pagani et al., 1996). In the present study, the effects of these mechanical influences over time were controlled because respiratory frequency, tidal volume, and end-tidal CO\textsubscript{2} were kept constant during the study period.

The diminution of cardiovascular autonomic drive by edrophonium may explain the clinical observation of the modest cardiac parasympathomimetic side effect of this drug (Cronnelly et al., 1982). In addition, it may account for the observation that less muscarinic antagonist is required to blunt the bradycardia produced by edrophonium than is required for neostigmine (Fogdall and Miller, 1973; Cronnelly et al., 1982). Although neostigmine has been shown to block cholinergic activation of nicotinic receptors expressed in mouse tumor cells (Wachtel, 1990) and in X. laevis oocytes (Yost and Maestroni, 1994), and to block cholinergic responses in the rat sympathetic superior cervical ganglion (S. B. Backman, R. D. Stein, and C. Pulosa, unpublished observations), these effects are observed at concentrations (ED\textsubscript{50} 4.6, 46, and 82.0 \mu M, respectively) much higher than those achieved clinically (peak serum concentration 0.9 \mu M (Wachtel, 1990)). In fact, unlike edrophonium, neostigmine appears to directly activate cholinergic muscarinic receptors in the peripheral cardiac parasympathetic pathway (Backman et al., 1993a, 1996a; Endou et al., 1997), and this may contribute to the enhanced parasympathomimetic action of neostigmine compared with edrophonium. Such direct cholinergic activation may also account for the bradycardia neostigmine produces in patients who have undergone cardiac transplantation (Backman et al., 1993b, 1996b,c). Consideration should be given to the possibility that the direct interaction of anticholinesterase drugs with cholinergic receptors may mediate other effects such as analgesia, which are assumed to be produced as the consequence of cholinesterase inhibition (Eisenach, 1999).

In summary, data from the present study demonstrate that clinically relevant doses of edrophonium block cardiovascular autonomic drive in humans, as evidenced by the significant decrease it produces in HFP of the RRI, and HFP and LFP of SBP. These observations may account for the modest autonomic side effects of edrophonium.

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**References**


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