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Acute electrophysiologic consequences of pyridostigmine inhibition of cholinesterase in humans

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Abstract

The cardiovascular electrophysiologic basis for the action of pyridostigmine, an acetylcholinesterase inhibitor, has not been investigated. The objective of the present study was to determine the cardiac electrophysiologic effects of a single dose of pyridostigmine bromide in an open-label, quasi-experimental protocol. Fifteen patients who had been indicated for diagnostic cardiac electrophysiologic study underwent two studies just before and 90-120 min after the oral administration of pyridostigmine (45 mg). Pyridostigmine was well tolerated by all patients. Wenckebach nodal anterograde atrioventricular point and basic cycle were not altered by pyridostigmine. Sinus recovery time (ms) was shorter during a 500-ms cycle stimulation (pre: 326 ± 45 *vs* post: 235 ± 47; P = 0.003) but not during 400-ms (pre: 275 ± 28 *vs* post: 248 ± 32; P = 0.490) or 600-ms (pre: 252 ± 42 *vs* post: 179 ± 26; P = 0.080) cycle stimulation. Pyridostigmine increased the ventricular refractory period (ms) during the 400-ms cycle stimulation (pre: 238 ± 7 *vs* post: 245 ± 9; P = 0.028) but not during the 500-ms (pre: 248 ± 7 *vs* post: 253 ± 9; P = 0.150) or 600-ms (pre: 254 ± 8 *vs* post: 259 ± 8; P = 0.255) cycle stimulation. We conclude that pyridostigmine did not produce conduction disturbances and, indeed, increased the ventricular refractory period at higher heart rates. While the effect explains previous results showing the anti-arrhythmic action of pyridostigmine, the clinical impact on long-term outcomes requires further investigation.

Key words: Cardiac electrophysiology; Parasympathetic nervous system; Cholinesterase inhibitors; Cardiovascular disease; Pyridostigmine bromide; Autonomic nervous system

Introduction

Autonomic dysfunction is a strong and independent predictor of the development of lethal cardiac arrhythmias and sudden death in patients with heart disease (1-4). The concept that adrenergic hyperactivity could be deleterious has led to the widespread clinical use of beta-blockers in patients after acute myocardial infarction (5), with a consequent reduction of overall mortality in these patients (6), as well as in those with heart failure (7). Conversely, few studies have investigated therapeutic alternatives against parasympathetic dysfunction (8), despite the fact that decreased parasympathetic activity represents an independent risk factor in patients after acute myocardial infarction (9,10).

Pyridostigmine, a reversible anticholinesterase agent, i.e., a drug with an indirect vagomimetic effect, seems to be potentially useful in this context. Pyridostigmine has been traditionally used for the treatment of myasthenia gravis due to its action on the motor plate in skeletal muscle (11). In addition, it has been shown to counteract the adverse anti-cholinergic effects of disopyramide, such as decreased baseline saliva and tear production (12). We have previously shown that short-term administration of pyridostigmine may be also potentially useful for cardiovascular protection since in healthy individuals and patients with cardiovascular diseases it results in beneficial effects on markers of cardiovascular risk and dysfunction (8,13-19).

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In patients with coronary artery disease, pyridostigmine improves the autonomic and hemodynamic responses to exercise (13,14,19), prevents the myocardial dysfunction induced by mental stress (16), and reduces QTc dispersion (18). In a randomized, crossover, placebo-controlled, and double-blind study, pyridostigmine administration increased heart rate variability and reduced ventricular arrhythmia density in patients with heart failure (17), an effect mediated, at least in part, by stimulation of the endogenous nitric oxide pathway (20). More recently (19), it was shown that pyridostigmine improves the hemodynamic profile of heart failure patients during dynamic exercise.

Despite the effects of pyridostigmine on these surrogate endpoints (21), the electrophysiologic basis for its action has not been investigated. Since pyridostigmine is a cholinomimetic drug, it is expected to reduce sinusal automatism (22) and chronotropic and dromotropic function (23), increasing the cardiac excitability threshold (24) and the refractory period of sinusal and atrioventricular nodes, but reducing the atrial refractory period (25,26). Therefore, the purpose of the present study was to determine the cardiac electrophysiologic consequences of a single oral dose of pyridostigmine bromide in man.

Material and Methods

Patients

Fifteen consecutive patients referred to our service by their attending physician for diagnostic cardiac electrophysiologic study were included in the present investigation. The clinical characteristics of these patients are shown in Table 1. All patients continued to use their current medication during the study. Exclusion criteria were bradyarrhythmias, implanted pacemaker, pregnancy, previous use of or allergy to pyridostigmine, bowel dysfunction, urinary retention, and the indication of isoproterenol infusion during the electrophysiologic study. The protocol conformed to the principles outlined in the Declaration of Helsinki and was approved by the Research Ethics Committee on Human Research of the Universidade Federal do Rio Grande do Sul. All patients gave written informed consent to participate in the study after full explanation of the procedures and their potential risks.

Protocol

Following an open-label, quasi-experimental protocol, a single dose of pyridostigmine (45 mg) was orally administered to all patients just before the electrophysiologic study. Ninety to 120 min after administration of the drug, another electrophysiologic study was performed. Both studies were performed under sedation (*iv* midazolam in incremental doses until sedation). Patients were continuously monitored with a chest electrocardiogram and for oxygen saturation and noninvasive blood pressure determination during the study. Patients remained in the hospital for the next 6 h following the study, so that any side effect of the drug could be recorded.

Electrophysiologic study

The electrophysiologic study was performed as described (27). Under fluoroscopic guidance, multipolar electrode catheters were positioned against the upper right atrial wall, near the region of the sinus node, and across the tricuspid valve in the area of the His bundle for pacing and recording of intracardiac electrograms. Basic intracardiac conduction intervals were then measured during sinus rhythm (28). Programmed electrical stimulation of the atrium was performed (UHS 20, Biotronik, Germany) using 2-ms constant current pulses at approximately twice the stimulation threshold. Surface ECG leads and bipolar intracardiac electrograms filtered at a bandpass of 30 to 500 Hz were displayed and recorded simultaneously on a multichannel recorder (Mingograf 7, Siemens, Germany). After control recordings, the atrium was paced during 30-s periods at constant cycle lengths of 600, 500 and 400 ms and sinus node recovery time was measured. The AV nodal Wenckebach cycle was defined as the length of the cycle during incremental atrial pacing in which the AV nodal Wenckebach phenomenon was noted. The anterograde

Table 1. Clinical characteristics of the 15 patients.

Age (years)	56 ± 4
Males	8 (53%)
Body mass index (kg/m^2)	26 ± 2
Indication for the electrophysiologic study	
Syncope	9 (60%)
Palpitations	5 (33%)
Risk stratification for sudden death	1 (7%)
Comorbidities	
Coronary artery disease	4 (27%)
Heart failure	4 (27%)
Hypertension	2 (13%)
Chagas' disease	1 (7%)
No known comorbidities	7 (46%)
Medications	
Amiodarone	2 (13%)
Diuretics	4 (27%)
Angiotensin-converting enzyme	7 (46%)
Calcium channel blockers	2 (13%)
Beta-blockers	4 (27%)
Acetylsalicylic acid	4 (27%)
Nitrates	1 (7%)
Digoxin	1 (7%)
Statins	3 (20%)

Data are reported as means ± SEM or number of patients with percent in parentheses.

effective refractory period of the AV node was measured using an 8-beat drive at a cycle length equal to the sinus cycle length minus 100 ms followed by single premature atrial stimuli introduced decrementally at 10-ms intervals. The ventricular refractory period was measured with the introduction of extrastimuli in 10-ms decrements after an 8-beat drive at cycle lengths of 600, 500, and 400 ms.

Statistical analysis

Descriptive data are reported as means ± SEM. Based on the data of Wit et al. (22), a sample size of 14 patients was required to detect a 20% difference in the refractory ventricular period, with an alpha error of 0.01 and power of 0.80. Electrophysiologic variables obtained in the first study without the effect of pyridostigmine (pre) were compared to those measured in the second study under the action of pyridostigmine (post) using the two-tailed paired Student *t*-test or the Wilcoxon test, when appropriate. Significance was set at $P < 0.05$.

Results

Pyridostigmine was well tolerated by all patients. Table 2 presents the electrophysiologic data before and after pyridostigmine administration. Wenckebach nodal anterograde atrioventricular point and basic cycle were not altered by pyridostigmine. The sinus recovery time was shorter during the 500-ms cycle stimulation but not during 400- or 600-ms cycle stimulation. PA, AH, and HV intervals presented normal values and were not significantly different after pyridostigmine. Pyridostigmine administration significantly increased the ventricular refractory period during the 400-ms cycle stimulation but not during the 500- or 600-ms cycle stimulation (Figure 1). Data from patients who were taking beta-blockers or amiodarone were analyzed separately and showed similar results for sinus recovery time during the 500-ms cycle stimulation (pre: 263 ± 74 *vs* post: 157 ± 61 ms; P = 0.04) and a ventricular refractory period during the 400-ms cycle stimulation (pre: 242 ± 15 *vs* 253 ± 18 ms; P = 0.03).

Discussion

Pyridostigmine is an acetylcholinesterase

Table 2. Electrophysiologic effects of pyridostigmine.

	Pre-pyridostigmine	Post-pyridostigmine
Basic cardiac cycle (ms)	906 ± 37	914 ± 39
Wenckebach nodal anterograde ventricular point (ms)	400 ± 20	398 ± 17
Sinus node recovery time (ms)		
400-ms cycle stimulation	275 ± 28	248 ± 32
500-ms cycle stimulation	326 ± 45	235 ± 47*
600-ms cycle stimulation	252 ± 42	179 ± 26
PA interval (ms)	36 ± 2	36 ± 1
AH interval (ms)	88 ± 8	93 ± 9
HV interval (ms)	48 ± 3	50 ± 2
Atrial refractory period (ms)		
500-ms cycle stimulation	147 ± 32	123 ± 31
600-ms cycle stimulation	147 ± 32	123 ± 31
Atrioventricular refractory period (ms)		
500-ms cycle stimulation	323 ± 16	316 ± 20
600-ms cycle stimulation	327 ± 20	345 ± 23
Ventricular refractory period (ms)		
400-ms cycle stimulation	238 ± 7	245 ± 9*
500-ms cycle stimulation	248 ± 7	253 ± 9
600 ms cycle stimulation	254 ± 8	259 ± 8

Data are reported as means ± SEM for 15 patients. Measurements were made before and 90-120 min after receiving 45 mg pyridostigmine orally. *P < 0.05 compared to pre-pyridostigmine (two-tailed paired Student *t*-test).

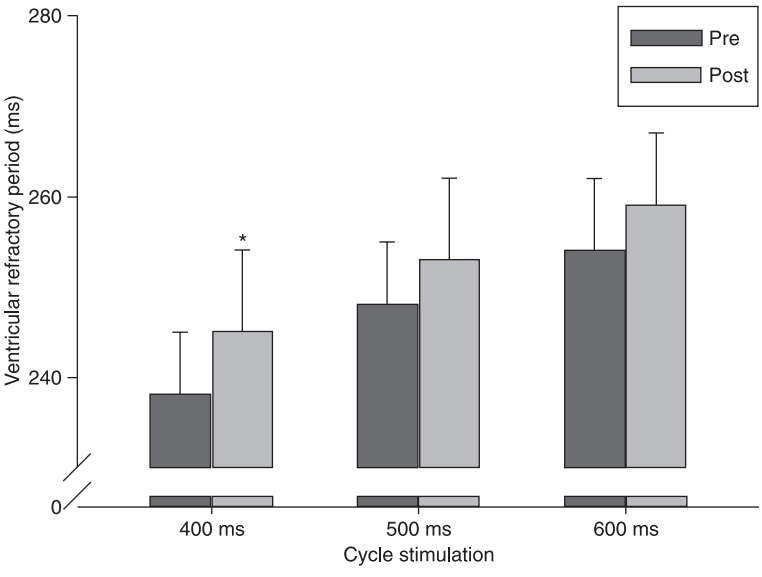

Figure 1. Ventricular refractory periods (means ± SEM) before and after a single dose of pyridostigmine during stimulation with 400-, 500-, and 600-ms cycles. *P < 0.05 compared to the pre-pyridostigmine value (two-tailed paired Student *t*-test).

reversible inhibitor whose cardiovascular activity has been studied systematically for more than a decade. Studies including healthy volunteers and patients with cardiovascular disease have shown that pyridostigmine shifts the autonomic balance to parasympathetic dominance and may reduce arrhythmia density (14,17,29). A single dose of pyridostigmine elicited bradycardia and reduced QTc dispersion in healthy young subjects (18). When administered to patients with coronary artery disease, pyridostigmine reduced the QTc interval at rest (29) and its recovery from maximal effort (14). Heart rate variability, an established method for analysis of autonomic modulation, was also increased when pyridostigmine was used by healthy volunteers (15) and heart failure patients, reducing ventricular arrhythmia density in the latter (17). The electrophysiologic mechanisms involved in these effects are currently unknown. Despite these promising findings with pyridostigmine, the potential of atrioventricular block, bradycardia leading to hemodynamic instability, and other undesirable parasympathetic effects on cardiac conduction should not be underestimated even when small doses are administered. The present study was undertaken in order to further analyze the mechanistic basis for the potential beneficial and hazardous effects of pyridostigmine. Several previous studies had investigated the autonomic and electrophysiologic properties of another cholinesterase inhibitor, edrophonium hydrochloride. When administered at low doses, edrophonium enhances the parasympathetic drive (30), an effect primarily mediated by autonomic innervations of the heart (31). As a consequence, the atrioventricular conduction and firing rate of the sinoatrial node are inhibited proportionally (32) with no adverse effects (33), and with a reduction of ventricular arrhythmias (34), although inducibility of ventricular fibrillation was not changed in patients with implantable defibrillators (35). Albeit exhibiting promising effects, edrophonium needs to be administered intravenously, precluding its prescription for regular use. Therefore, pyridostigmine, which can be administered orally and causes a dose-dependent vagomimetic effect with few side effects (13-19), is a preferable candidate for the treatment of impaired parasympathetic modulation in heart disease.

As a drug that elicits a parasympathetic effect, pyridostigmine was expected to cause bradycardia in the present study. However, it was quite surprising to observe that the basic cycle was not altered after the use of pyridostigmine. Although cholinesterase activity was not measured in the present protocol, previous studies had shown that even doses smaller than 45 mg were capable of inhibiting cholinesterase serum activity (36). Thus, it is unlikely that a lack of anticholinesterase activity could explain the absence of bradycardia. Sedation could have affected the pharmacodynamics of the drug, altering the time needed for its absorption and for its peak action, but there are no studies that specifically addressed the interactions between midazolam and pyridostigmine. Nevertheless, this is not the first study where bradycardia is not seen after pyridostigmine administration. Indeed, Raj et al. (37) were unable to detect a reduction of heart rate in the seated position 2 h after pyridostigmine ingestion.

The atrioventricular node is densely innervated with both cholinergic and adrenergic neurons (38) and thus plays an important role in the pathophysiology of arrhythmias, not only protecting ventricles during atrial tachyarrhythmias, but also assuming the pacemaker's role during sinoatrial node failure. The dense cholinergic innervation of the atrioventricular node would make it more susceptible to the occurrence of conduction disturbance secondary to vagomimetic drugs, such as pyridostigmine (39). In the present study, pyridostigmine did not change the Wenckebach point or the atrioventricular conduction intervals, including the AH interval. A possible explanation is that pyridostigmine, as a systemic drug acting on the heart as a whole, probably acts synchronously on the right and left vagal nerve endings. As previously shown by Schiereck et al. (40), asynchronous right and left stimulation of parasympathetic efferent preganglionic axons causes a greater change in atrioventricular conduction.

The most important finding of the present study is that pyridostigmine increased the ventricular refractory period measured during 400-ms cycle stimulation. This effect was not observed when the ventricular refractory period was studied during longer cycles (500 and 600 ms). Therefore, pyridostigmine presents a potential anti-arrhythmic effect at higher heart rates, as occurs during physical exercise and mental stress. Accordingly, previous studies have shown a modulatory action of pyridostigmine on cardiovascular reactivity to exercise and stress, both in healthy subjects (18) and in patients with cardiovascular diseases (13,14,19). Whether adverse outcomes can be hampered by pyridostigmine or the sustained release version of the drug leads to a better effective action with lower side affects remains to be determined.

The results of the present study must be interpreted considering its limitations. Due to obvious ethical reasons, only patients with medical indication for electrophysiologic study were enrolled, composing a heterogeneous group. For the same reasons, there was no difference between the electrophysiologic study performed in volunteers of the present study and the procedure routinely performed at our hospital. A 400-ms period is not routinely used for all measurements during electrophysiologic study in our hospital and that is why data regarding the cycle length of 400 ms for atrial and AV refractory period were not presented. Also because of ethical limitations, the volunteers were not submitted to a second electrophysiologic study on a different day that would be needed for a placebo-controlled experiment. Nevertheless, a baseline electrophysiologic study was conducted before the administration of pyridostigmine. Considering that pyridostigmine achieves its peak of action at 90-120 min, it is very unlikely that any vagomimetic effect of the

drug was operating during the first electrophysiologic study. Our data were also obtained under sedation and some of the patients were taking medications that act on the autonomic nervous system. Therefore, baseline sympathetic activity was probably lower than during daily activities. As shown in the Results section, the use of beta-blockers or amiodarone did not modify the results.

These findings not only agree with the previously demonstrated anti-arrhythmic effects of pyridostigmine (17) but also extend the knowledge about pyridostigmine electrophysiologic effects. The oral administration of a low dose of pyridostigmine was well tolerated and did not produce conduction disturbances. In addition, pyridostigmine increased the ventricular refractory period at higher heart rates.

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