Cardiac function during mental stress: cholinergic modulation with pyridostigmine in healthy subjects

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ABSTRACT

Mentally or emotionally stressful situations occur throughout our lives and cause physiological haemodynamic responses. In patients with coronary artery disease, such events can also induce myocardial ischaemia and ventricular arrhythmias, increasing mortality rates. The purpose of the present study was to determine the acute effects of the oral administration of pyridostigmine, a reversible cholinesterase inhibitor and thus an indirect cholinomimetic drug, on echocardiographic variables during mental stress in healthy subjects. A total of 18 healthy young volunteers were subjected to mental stress tests (mental arithmetic and the Stroop colour–word test) 2 h after the oral administration of either placebo or pyridostigmine bromide (45 mg), using a balanced-randomized, double-blind, crossover protocol. During mental stress, heart rate (pyridostigmine, 64 ± 1 beats/min; placebo, 70 ± 1 beats/min; \( P = 0.0003 \)) and diastolic blood pressure (pyridostigmine, 66 ± 2 mmHg; placebo, 79 ± 3 mmHg; \( P = 0.01 \)) were lower in the pyridostigmine group, but systolic pressure was not (pyridostigmine, 124 ± 3 mmHg; placebo, 123 ± 3 mmHg; \( P = 0.40 \)). There were no detectable abnormalities in the left ventricular wall motion score during mental stress, but left ventricular outflow tract mean velocity (pyridostigmine, 0.68 ± 0.02 m/s; placebo, 0.64 ± 0.02 m/s; \( P < 0.05 \)) and mitral inflow velocity deceleration (placebo, 4.05 ± 0.18 m/s²; pyridostigmine, 4.41 ± 0.16 m/s²; \( P < 0.05 \)) were higher in the pyridostigmine group. In conclusion, cholinergic stimulation with pyridostigmine seems to increase left ventricular diastolic function during mental stress in healthy subjects.

INTRODUCTION

Mentally or emotionally stressful situations occur throughout our lives, and cause complex organic adjustments related to the well-known ‘fight or flight’ response. Encephalic arousal and the associated activation–deactivation pattern in different cortical regions involved in the response to acute emotional situations [1] trigger the hypothalamic/pituitary/adrenal axis, which mediates a constellation of immune, biochemical, autonomic and haemodynamic responses [2,3]. The latter include increases in heart rate (HR), cardiac output and blood pressure (BP), along with decreased skin and renal blood flow [4], all working in concert towards an increase in skeletal blood flow as a physiological preparation for a ‘defence reaction’. Muscle vasodilation during mental stress is believed to be mediated by nitric oxide released mostly by cholinergic stimulation of the vascular endothelium [5] and by adrenaline acting through \( \beta_2 \)-adrenoceptor

Key words: cholinergic, mental stress, pyridostigmine, ventricular function.
Abbreviations: BP, blood pressure; CAD, coronary artery disease; HR, heart rate.
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stimulation [6]. In healthy subjects, myocardial blood flow also increases in direct proportion to the change in cardiac work provoked by mental stress [7].

Overall autonomic and haemodynamic responses during mental stress in patients with coronary artery disease (CAD) are somewhat different from those of healthy controls, although there may be comparable increases in catecholamine secretion and cardiac work [8]. Vasodilatation of skeletal muscle beds is blunted, causing total peripheral vascular resistance to increase rather than decrease, leading to impaired systolic [9] and diastolic left ventricular function [10], and thus decreased cardiac output. Myocardial blood flow does not increase during stress in patients with CAD as it does in healthy subjects [8], or may even decrease due to coronary spasm, especially in segments with atherosclerotic plaques [11,12]. These effects may cause myocardial ischaemia and ventricular arrhythmias [13–15], with important clinical implications. For example, a recent report of a multicentre trial showed that patients with myocardial infarction who presented with altered ventricular wall motion during a mental stress test had long-term mortality rates that were three times higher [16].

Since mental stress provokes increased sympathetic drive to the heart along with reduced vagal activity [17,18], its potential deleterious effects on the cardiovascular system might be modulated by either adrenergic blockade or cholinergic stimulation. Although β-blockers have been shown to protect against myocardial ischaemia provoked by physical exercise, they do not prevent the wall motion defects induced under conditions of mental stress [19]. On the other hand, cholinergic activation by vagus nerve stimulation [20] or morphine [21] increases the ventricular fibrillary threshold during mental stress and myocardial ischaemia. Thus it is conceivable that cholinomimetic interventions in the clinical setting might have positive effects in patients with cardiovascular disease, such as those with CAD [22,23]. Reversible cholinesterase inhibition by pyridostigmine bromide causes cholinergic stimulation by increasing the concentration of endogenous acetylcholine [24]. It has been shown previously that pyridostigmine increases HR variability [25] and blunts the BP and HR responses to mental stress in healthy volunteers [12], but its effects on cardiac function in this situation are unknown. Accordingly, the purpose of the present study was to determine the acute effects of the oral administration of pyridostigmine on echocardiographic variables during mental stress in healthy subjects.

METHODS

A total of 18 subjects (11 men; age 28 ± 5 years), considered healthy on the basis of clinical, ECG and echocardiographic evaluations, took part in the study. Each subject signed an informed consent form after full explanation of the procedures, risks and discomforts involved in the study, which was approved by the Institutional Research Ethics Committee.

The experiments were conducted on two different days separated by a period of 2–7 days, using a balanced-randomized, double-blind, crossover, placebo-controlled protocol. On each day, after avoiding caffeine, smoking and strenuous exercise for the previous 48 h, the subject arrived at the laboratory in the morning after an overnight fast. A blood sample of 5 ml was withdrawn from an antecubital vein for analysis of cholinesterase activity, and appropriate transducers and electrodes were placed for continuous monitoring of ECG by the Holter system (Rozzin), of HR by telemetry (Polar Vantage®; Electric Oy), and of non-invasive BP by IR photoplethysmography (Finapres 2350®; Ohmeda). Then pyridostigmine bromide (Mestinon®; ICN; 45 mg orally) or placebo was administered, and another 5 ml blood sample was withdrawn 2 h later. The subject rested for 15 min, followed by baseline haemodynamic and echocardiographic evaluations. Then the subject performed two mental stress tests as described previously [26]: mental arithmetic [27] and the Stroop colour–word test [28]. The arithmetic test consisted of displaying sequentially on a computer screen, for a total of 12 min, four panels (3 min each) each with three columns of two-digit random numbers; subjects were asked to sum up mentally the 13 lines of each column and provide the results at the end of the 3-min period. The Stroop colour–word test consisted of a 3-min rapid slide presentation of different names displayed in different colours, and the subject was instructed to report the colour and not the written word. Both mental stress tests were performed with continuous auditory conflict (headphones playing various noises or a taped voice saying different colour names). ECG, HR and BP were monitored continuously throughout the tests. Immediately after the test onset, standard M-mode, bi-dimensional and Doppler images were obtained sequentially and recorded for approx. 1 min each. A commercially available system was used (ATL Apogee CX200; 2.5 MHz transducer), and all off-line measurements were performed in accordance with the criteria established by the American Society of Echocardiography [29].

The patterns of the haemodynamic responses were quite similar during the Stroop test and the mental arithmetic test, with a peak response occurring in the first minute followed by a gradual decrease until the end of the test, at which point the values for HR and BP were still higher than at rest. Therefore the values obtained throughout the two tests were averaged and used for analysis. Since previous studies have shown that pyridostigmine reduces HR [23,30], and that alterations in HR may be responsible for changes in echocardiographic indices of ventricular function [31],
we re-analysed the data by selecting only cardiac cycles during which HR was 70 beats/min. This arbitrary value was chosen because, during observation of the tapes, several cardiac cycles could be identified with this HR occurring during stress both with pyridostigmine and placebo.

All variables were analysed by two-way ANOVA for repeated measures, where drug condition (pyridostigmine or placebo) and time (baseline, mental stress) were the main factors, followed by the Bonferroni post hoc test for pairwise analysis when appropriate. All values are presented as means ± S.E.M., and \( P < 0.05 \) was considered statistically significant.

**RESULTS**

Cholinesterase activity remained unchanged after placebo (\( P = 0.091 \)), but decreased after pyridostigmine (\( P = 0.017 \)), as expected. There were no arrhythmias or ST-segment shifts on the Holter recordings during mental stress with either placebo or pyridostigmine. One subject experienced nausea and salivation after placebo, and another volunteer presented with salivation, shivering and drowsiness. All symptoms were mild and faded spontaneously without the need for medication.

Before the mental stress tests, pyridostigmine led to lower HR values (62 ± 1 beats/min) when compared with placebo (65 ± 2 beats/min; \( P = 0.01 \)), but systolic (pyridostigmine, 117 ± 1.8 mmHg; placebo, 116 ± 2.8 mmHg; \( P = 0.24 \)) and diastolic (pyridostigmine, 64 ± 1.8 mmHg; placebo, 66 ± 1.8 mmHg; \( P = 0.17 \)) BP values remained similar. During mental stress, HR (pyridostigmine, 64 ± 1 beats/min; placebo, 70 ± 1 mmHg; \( P = 0.003 \)) and diastolic BP (pyridostigmine, 66 ± 2 mmHg; placebo, 79 ± 3 mmHg; \( P = 0.01 \)) were lower with pyridostigmine, but systolic pressure was not (pyridostigmine, 124 ± 3 mmHg; placebo, 123 ± 3 mmHg; \( P = 0.40 \)). There were no detectable abnormalities in the left ventricular wall motion score during mental stress after either pyridostigmine or placebo.

Standard analysis of ventricular function during mental stress (Table 1) revealed higher values for left ventricular outflow tract mean velocity and mitral inflow velocity deceleration after pyridostigmine.

The results for cardiac cycles with the same HR (70 beats/min) confirmed that pyridostigmine increased left ventricular inflow velocity deceleration (pyridostigmine, 4.68 ± 0.76 m/s²; placebo, 4.28 ± 0.83 m/s²; \( P = 0.05 \)). In addition, at the same HR, peak early filling velocity (E wave; pyridostigmine, 1.01 ± 0.13 m/s; placebo, 0.92 ± 0.17 m/s; \( P = 0.02 \)) and its ratio to peak atrial filling velocity (E/A ratio; pyridostigmine, 1.93 ± 0.30; placebo, 1.76 ± 0.31; \( P = 0.03 \)) were higher after pyridostigmine compared with placebo.

**DISCUSSION**

The novel finding of the present study is that cholinergic stimulation with oral pyridostigmine modified left ventricular dynamics during mental stress, increasing both left ventricular inflow velocity deceleration and outflow tract mean velocity.

Since pyridostigmine also blunts the chronotropic response to mental stress, the increased ventricular filling velocity could have been caused by the well-known effect of a longer diastolic period [32]. However, when average ventricular function was evaluated in cardiac cycles during mental stress when HR was similar (70 beats/min) after placebo and pyridostigmine, the results did not differ. In other words, pyridostigmine increased peak mitral flow velocity even when HR values were comparable. Therefore it seems that other mechanisms could be involved in the effects of cholinergic...
stimulation with pyridostigmine on ventricular diastolic function during mental stress. One potential mechanism is a decrease in atrial contraction induced by cholinergic activation, causing increased atrial pressure and leading to a higher atrial–ventricular gradient during mental stress. This effect would facilitate early ventricular filling, without obligatory enhanced ventricular relaxation. A previous study involving anaesthetized dogs showed that vagal stimulation increased the atrial–ventricular pressure gradient by 42% due to reciprocal changes in left atrial and ventricular pressures [33]. However, in the present study, a main index of left atrial contraction (A wave) did not change, making this mechanism less likely.

Another potential mechanism is increased ventricularlusitropism caused by pyridostigmine, since a greater deceleration of the early-diastole velocity wave has been proposed as an index of ventricular diastolic function [32]. This effect could have been caused by pyridostigmine activating endothelial or inducible NO synthase expressed in the endothelium, endocardium or myocytes themselves. Paulus et al. [34] detected greater ventricular relaxation and distensibility following intracoronary infusion of NO in humans. Since pyridostigmine effectively inhibits cholinesterase activity even at a low dose (see Results), it will increase the concentration of endogenous acetylcholine, a major activator of NO, which may have caused the observed effect on ventricular diastolic function [35].

It is also conceivable that the effects of pyridostigmine on cardiac dynamics during mental stress were an indirect consequence of a vascular action of the enhanced acetylcholine concentration. It has been shown previously that muscle vasodilatation during mental stress can be mediated by NO release, which is known to be promoted by muscarinic stimulation [5]. Pyridostigmine may have caused greater vasodilatation during mental stress, representing a lower afterload with respect to the left ventricle. In the face of a lower impedance for ventricular ejection, echocardiographic indices of systolic and diastolic function can be enhanced [32,36]. Therefore it is possible that pyridostigmine decreased left ventricular afterload, leading to increases in left ventricular inflow velocity deceleration and outflow tract mean velocity. Although it was not the purpose of the present study to determine the exact mechanisms involved in the cardiac changes provoked by pyridostigmine during mental stress, the possibility that the left ventricular afterload was lowered could have a direct impact on patients with cardiovascular disease. In this group of patients, vasodilatation in skeletal muscle during mental stress is blunted and peripheral vascular resistance increases, in contrast with the decreased resistance seen in healthy subjects [9]. This paradoxical response leads to impaired systolic and diastolic left ventricular function, thus blunting the cardiac output response [9,10].

It is worth noting that pyridostigmine increased the index of ventricular diastolic function with no deleterious effects on systolic function. In addition, continuous electrocardiographic tracings did not show any signs of myocardial ischaemia, arrhythmia or conduction abnormalities after placebo or pyridostigmine. These recordings were a necessary precautionary measure, since mental stress may provoke coronary spasm and myocardial ischaemia even in healthy subjects [37]. In addition, cholinergic stimulation may facilitate the occurrence of bradyarrhythmias, including various degrees of atrioventricular blockade. In fact, the observed safety profile of pyridostigmine administration we have seen in healthy subjects supports the design of experiments involving patients with cardiovascular disease, in order to test the hypothesis that cholinergic stimulation by pyridostigmine may have protective effects in these subjects. More specifically, future studies should investigate whether pyridostigmine may prevent the cardiac changes induced by mental stress, since ventricular wall abnormalities during a mental stress challenge may predict overall mortality in patients with CAD [16].

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REFERENCES
