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Modulation of the Dose-Dependent Effects of Atropine by Low-Dose Pyridostigmine: Quantification by Spectral Analysis of Heart Rate Fluctuations in Healthy Human Beings

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IZRAELI, S., M. ALCALAY, Y. BENJAMINI, R. WALLACH-KAPON, Z. TOCHNER AND S. AKSELROD. *Modulation of the dose-dependent effects of atropine by low-dose pyridostigmine: Quantification by spectral analysis of heart rate fluctuations in healthy human beings.* PHARMACOL BIOCHEM BEHAV **39**(3) 613–617, 1991.—The interaction between a low-dose cholinesterase inhibitor, pyridostigmine (PYR), and atropine was investigated by spectral analysis of heart rate fluctuations in eight healthy humans. Each subject was given increasing boluses of IV atropine during treatment with PYR (30 mg·3/day) or placebo. PYR attenuated the bimodal dose-dependent changes in the respiratory peak (which represents the parasympathetic control) in response to atropine. We suggest that spectral analysis can be used for quantifying the complex dose-dependent cholinergic agonist-antagonist interactions, and may help to disclose an asymptomatic low-dose intoxication with acetylcholinesterase inhibitors.

Spectral analysis of heart rate Atropine Pyridostigmine Humans Anticholinesterase

POISONING with anticholinesterase (anti-ChE) compounds causes many central and peripheral cholinergic effects which result in autonomic, motor and neurobehavioral symptomatology (12,17). The development of effective therapy requires proper experimental models that allow investigation of the interactions between potential antidotes and anti-ChE agents. The autonomic cardiac control system may serve as such a model. Being controlled by both central and peripheral cholinergic neurons, it may therefore be correlated with neurobehavioral drug effects. Unlike other complex behavioral systems, the autonomic cardiac control is very similar in many animal species, including man (7). This similarity may allow transformation of observations from animal studies to humans. A major obstacle, however, has been the lack of a reliable, sensitive, noninvasive method for measuring pharmacological modulations of the autonomic cardiac control.

Spectral analysis of spontaneous fluctuations of heart rate enables noninvasive differential evaluation of parasympathetic and sympathetic autonomic cardiac control and, as such, may facilitate comparative studies in animals and humans (1,2). The heart rate spectrum displays two predominant regions of power: a low (<0.1 Hz)- and a high (0.2–0.4 Hz)-frequency band. The low-frequency band is due to both sympathetic and parasympathetic activity. The higher-frequency band, or "the respiratory peak" (RP), represents respiratory sinus arrhythmia and is *mainly* of parasympathetic mediation. Therefore, spectral analysis of heart rate fluctuations allows accurate estimation of the parasympathetic tone.

Alteration of vagal tone, e.g., by cholinergic agents, will result in a corresponding change in the RP. Using this method, it has been recently shown (3,21) that atropine has a bimodal effect on the vagal tone: at low dosage, it augments the RP, while at intermediate and high dosages, a progressive decrease in the RP is observed. The finding that low-dose atropine causes vagal stimulation was previously demonstrated in animals only by invasive methods (18).

Chronic exposure to low doses of anti-ChE compounds may not cause overt symptoms, due to the tolerance or to the compensatory capacity of the central nervous system (20,22). An additional pharmacological intervention, such as administration of a cholinolytic agent, may disclose the subclinical poisoning (22). The aim of the present study was to investigate the dose-dependent modulation of parasympathetic tone achieved by the interactions between atropine and a low-dose ChE inhibitor, using HR spectral analysis. PYR was chosen for this study as the

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anti-ChE compound, based on previous experience indicating that, in the selected dosage (30 mg·3/day), no signs or symptoms were observed (11,16).

METHOD

Subjects

Eight healthy male subjects participated in the study (mean age, SD: 29, 1.5 years; body weight, SD: 77.4, 8.8 kg); all gave their informed consent. None took any medication. They were instructed to restrain from smoking as well as consuming beverages containing alcohol or xanthines on the day preceding the study.

Drug Interventions

Pyridostigmine bromide. Tablets (30 mg) were supplied by Duphar (The Netherlands). Identical placebo tablets were supplied by the same manufacturer. Subjects began taking pyridostigmine (PYR) or placebo 24 h before the recording session, a tablet every 8 hours, so that the fourth and last dose was taken 75 minutes before the beginning of the ECG recordings (see protocol below). According to previous experience, this regimen produces a 20 to 40% inhibition of serum and red blood cells' cholinesterase up to six hours following the fourth dose (6, 11, 16).

Atropine sulfate. Intravenous atropine (TEVA Pharmaceutical Industries, Israel) was administered in nine consecutive boluses at 10-minute intervals: First, five small boluses of 1.3 μg/kg, followed by one bolus of 3.9 μg/kg and three boluses of 6.5 μg/kg. Following each dose, the tubing was quickly flushed with 5 ml of normal saline. This protocol takes into consideration the pharmacokinetic properties of IV atropine [$t_{1/2}$(distribution) = 1 min, $t_{1/2}$(elimination) = 140 min] (14) in order to achieve cumulative dose-response curves.

Protocol

The study was designed as a cross-over, placebo-controlled, single-blind investigation. It was approved by the IDF Ethical Committee for Human Investigations. A one-week interval elapsed between the PYR and placebo studies. During the recording session, the subjects were placed in supine position with a 30° upright tilt, connected to an ECG monitor (Mennen) and a respiratory inductive plethysmograph (Respitrace). Then an IV catheter was inserted into the antecubital vein of the arm. Following 30 minutes rest allowed for stabilization, baseline ECG and respiration signals were recorded for 15 minutes. Then the signals were repeatedly recorded for 7 minutes, starting 3 minutes after each new atropine bolus. During the recording sessions, the subjects breathed spontaneously and were instructed to lie quietly.

Data Recording and Analysis

The ECG and respiratory signals were simultaneously recorded onto analog tape using an 8-channel FM magnetic tape recorder (HP-3896A). The recorded signals were then fed into the analog to digital channels of a microcomputer (PDP 11/23 MINC) and sampled at a rate of 0.5 kHz. A fast peak detection algorithm allowed the real-time measurement of R-R intervals. The principles of the software for data acquisition and analysis have been described previously (2).

A typical baseline power spectrum of HR fluctuations is displayed for one of the subjects in Fig. 1. The integrals over various frequency bands of the heart rate (HR) power spectrum were computed. We focused on the integral over the RP because we were interested in pure parasympathetic effects.

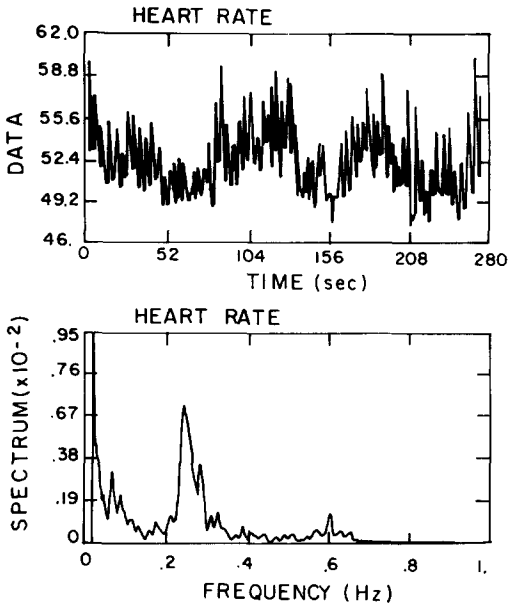

FIG. 1. Heart rate fluctuations (above) and their power spectra (below) in one subject during rest. Note that the heart rate power spectrum displays two predominant regions of power: a low (<0.1 Hz)- and a high (0.2–0.4 Hz)-frequency band.

Statistical Analysis

Logarithmic transformations of HR and of RP were used as the response variables in order to stabilize variances and normalize their distributions. These values were then standardized by subtracting their values at dose 0. The results at parallel dose levels were compared by paired *t*-tests, with adjustment for multiplicity using Holm's modification to the Bonferonni procedure (15).

In order to study the response over the entire range of atropine doses, a third-degree polynomial in 1/(1 + dose) was fitted by least squares. Such a curve was obtained for placebo and PYR treatments. The use of this family of dose-response curves rather than the more familiar Michaelis Menten curves was needed to capture the bimodal nature of the response to atropine (1). The significance of the differences noticed between the fitted curves was judged using a permutation test (4), which is a modification of the permutation test for equality of dose-response curves (10).

RESULTS

The dose-dependent changes in the mean HR for a typical subject is displayed in Fig. 2. At a low cumulative dose of atropine (<5.2 μg/kg), mean HR decreases by about 10%. Higher atropine doses result in tachycardia. When PYR is added to atropine, the mean HR is consistently lower. It is noteworthy that the mean HR at baseline with PYR is not lower than the mean HR with placebo. The RP as a function of atropine dose behaves as a mirror image of the mean HR changes (Fig. 3). It increases in response to low doses of atropine and decreases by two orders of magnitude with higher doses. These changes in the RP, both the increase at low doses and the decrease at higher

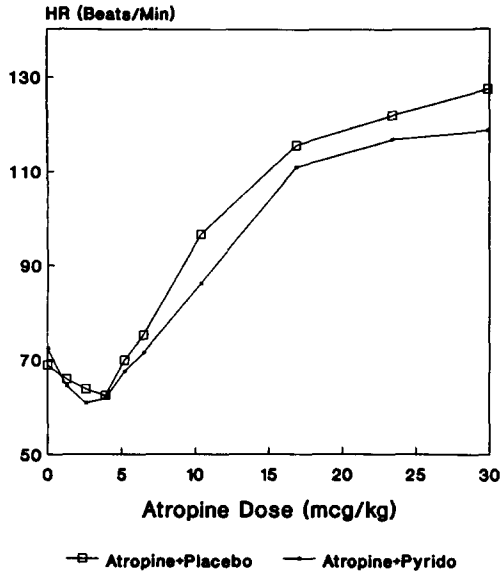

FIG. 2. Dose-response curves of mean HR as a function of atropine dose during treatment with pyridostigmine (full squares) or placebo (empty squares)—one typical subject. Note that the doses on the X-axis are cumulative.

doses, were attenuated during the administration of PYR (Fig. 3).

Similar dose-dependent changes are observed in the other subjects. The standardized logarithmic transformation of mean HR and RP of the whole group, and the corresponding third-degree polynomials, fitted to the dose-response curves (see the Method section) are illustrated in Figs. 4 and 5, respectively. It appears that mean HR is reduced when low-dose PYR is given in conjunction with atropine compared to atropine alone (Fig. 4). Maximal HR is unchanged by PYR. The fitted dose-response curve of HR when PYR is added lies under the curve fitted to the measurements during atropine administration, throughout most of the range of doses—extremes excluded. The difference between the two curves is not statistically significant (mainly due to the minor increase of the fitted PYR curve at the high atropine doses).

When its effect on RP is considered, the addition of PYR causes an attenuation (Fig. 5): PYR reduces the increase in RP caused by low-dose atropine and attenuates the reduction in RP at higher atropine doses. In other words, the absolute change due

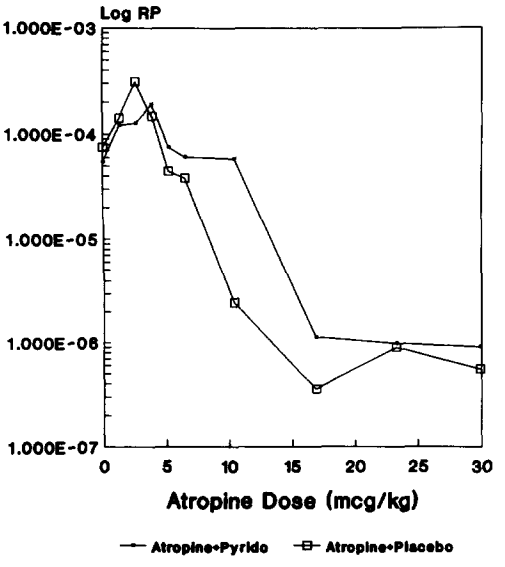

FIG. 3. The same subject as in Fig. 2: Dose-response curves of respiratory peak (RP) as a function of atropine dose during treatment with pyridostigmine (full squares) or placebo (empty squares).

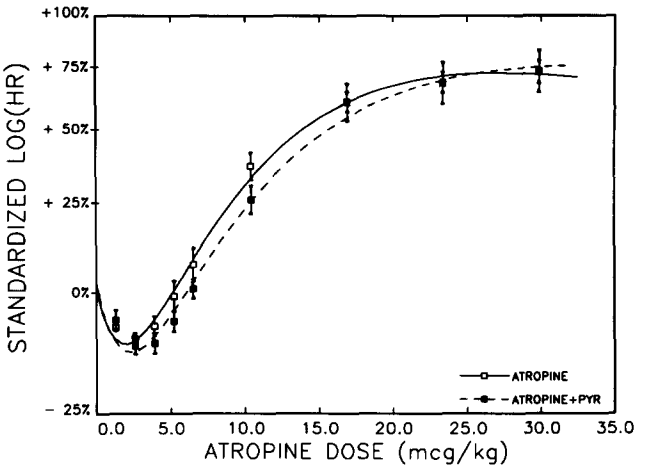

FIG. 4. Dose-response curves of standardized log mean HR (SEM) as a function of cumulative atropine dose for the entire group (n=8) during treatment with pyridostigmine (full squares, dashed line) or placebo (empty squares full line). The line represents the best-fitted 3rd-degree polynomials. The numbers on the Y-axis represent changes from baseline.

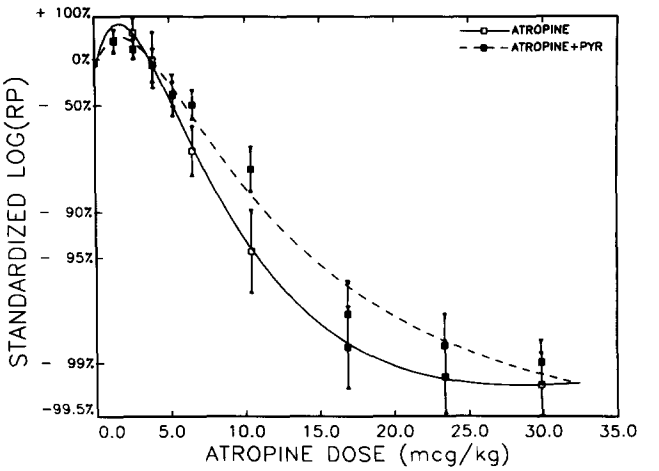

FIG. 5. Dose-response curves of standardized log mean RP (SEM) as a function of cumulative atropine dose for the entire group (n=8) during treatment with pyridostigmine (full squares, dashed line) or placebo (empty squares full line). The line represents the best-fitted 3rd-degree polynomials. The numbers on the Y-axis represent changes from baseline.

to atropine is reduced when PYR is added. The differences between the two fitted dose-response curves is significant ($p<0.01$).

No symptoms attributed to PYR were reported by the subjects, nor could they guess whether placebo or PYR was administered.

DISCUSSION

PYR was elected for this study as a classic anti-ChE compound which, at the chosen dosage, does not cause any significant symptomatology (6, 11, 16). It may therefore serve as a model for a subclinical poisoning with ChE inhibitors. Indeed, no symptoms were reported by the volunteers, and no changes in the mean HR or HR power spectrum were noted after PYR alone. However, this "asymptomatic" dose was enough to attenuate the dose-dependent effects of atropine on parasympathetic tone.

The classical bimodal effect of atropine on HR (13) is observed in this study: low doses cause slight bradycardia, while higher doses result in tachycardia. Concomitantly, we have shown that low doses of atropine enhance the RP and thus the net parasympathetic cardiac tone. A sharp decrease in the magnitude of the RP is observed at higher doses of atropine, reflecting a reduction of parasympathetic tone. Based on animal (18) and human studies (19,21), we assume that the increase in the RP, observed at low doses of atropine, is the result of central vagal stimulation, while the diminished parasympathetic activity, observed at higher doses, results from the increased blockade of postsynaptic muscarinic receptors at the sino-atrial node.

The seemingly "asymptomatic" presence of PYR is unmasked by administration of atropine. PYR tends to attenuate the increase in HR when intermediate doses of atropine are given.

This tendency disappears at higher atropine doses, probably reflecting saturation of the postsynaptic muscarinic receptors by atropine. Similarly, the spectral analysis of HR fluctuations reveals that PYR attenuates the progressive decrease in parasympathetic tone (reflected by the RP) caused by moderate to high doses of atropine. Thus, at each dose of atropine, the resulting magnitude of the measured vagal tone is probably determined by the agonist-antagonist competitive equilibrium at the synapse of the sino-atrial node.

The effects of PYR on the response to low-dose atropine are more complex. The increased concentration of acetylcholine (Ach) at the peripheral vagal synapse induced by PYR causes a further reduction in the mean HR when low cumulative doses of atropine are administered. However, surprisingly, PYR attenuates the increase of the respiratory peak. We therefore suggest a possible explanation for the attenuation of the respiratory peak despite the apparent increased vagal stimulation revealed by mean HR: The RP represents the respiratory sinus arrhythmia (1,2), i.e., the difference between the vagal tone during inspirium and expirium. Prolonging the half-life of Ach by inhibition of acetylcholinesterase may increase the mean concentration of Ach while blurring the differences in concentration between inspirium and expirium. Hence, the mean HR may decrease without the expected corresponding increment in the magnitude of the respiratory peak. Further studies are needed to clarify this interesting observation.

The quantification of the interactions between ChE inhibitors and anticholinergic drugs is important not only for designing an optimal therapeutic regimen, but also as a potential way to unmask latent poisoning by anti-ChE drugs. The symptomatology caused by acute or chronic exposure low doses of anti-ChE is not always clearcut. A pharmacological challenge with anticholinergic drugs could be used to discern the latent muscarinic effect of an anti-ChE compound (9,22). Recently, Birnbaum et al. (5,8) demonstrated an attenuation of the dose-dependent reduction in the amplitude of respiratory sinus arrhythmia in response to atropine in monkeys exposed to low-dose pyridostigmine. Similar observations were reported in dogs exposed to the organophosphate dichlorovos (9). These findings are similar to our present observations in humans.

We used PYR as a model of low-dose anti-ChE intoxication. However, PYR does not penetrate the central nervous system. Organophosphorus poisons have profound neurobehavioral effects, and can cause complex alternation in the cardiac autonomic control through their central nervous system activity (7,17). Obviously, studies of organophosphate poisoning are largely limited to experimental animals. However, since the modulation of the HR power spectrum by the combination of low-dose PYR and moderate to high doses of atropine is similar in humans and in Rhesus monkeys, it is thus suggested that spectral analysis of heart rate fluctuations in poisoned animals may be an appropriate experimental model and can help in transforming cholinergic toxicological data from animals to humans.

We conclude, therefore, that measuring the power spectrum of HR may prove to be a useful aid in diagnosis of low-dose poisoning with anti-ChE agents, both in humans and in animals, by quantifying their latent muscarinic effects.

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