Effects of atropine on autonomic indices based on electrocardiographic R-R intervals in healthy volunteers

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Abstract

The dose dependent responses to atropine of 11 indices of autonomic function were investigated in 10 healthy volunteers. Five subjects were given cumulative doses of atropine (0.1–2.0 mg/70 g, intravenously). The other five received equivalent volumes of water for injection. The ratio of the longest to the shortest R-R intervals in the electrocardiogram during forced expirations and deep inspirations (respiratory ratio), deviations in the periods of consecutive cardiac cycles, the ratio of the longest to the shortest R-R intervals during a Valsalva manoeuvre (Valsalva ratio), the ratio of the 30th to the 15th R-R intervals after standing up from a low sitting position (30:15 ratio), and the means of R-R intervals recorded in standing or lying positions were calculated with different computer algorithms. Sympathetic orthostatic effects on the above indices were measured by comparing values in supine and standing positions after atropinisation. The recumbent respiratory ratio was the most sensitive test to atropinisation. Its maximum response was a 97% decrease, indicating specificity for the vagal tone. The decrease in other indices in response to atropine ranged from 34% to 94%. The average orthostatic effect on the indices after atropinisation was a 14.6% increase. The Valsalva ratio failed to respond significantly to any degree of muscarinic antagonism. In conclusion, the Valsalva ratio is unlikely to be closely related to the parasympathetic control of the heart. However, the resting vagal tone can be selectively measured in a live and conscious person by using the respiratory ratio.

in an electroencephalogram (ECG),⁴ or as changes in heart rate during special manoeuvres—for example, the Valsalva manoeuvre⁵ or rising from a recumbent position.⁶ Periods of cardiac cycles also vary during inspirations and expirations (respiratory sinus arrhythmia).⁷ The amplitude of this variation is related to the level of parasympathetic control of the heart.⁸ This variation can be quantified as a ratio of R-R intervals during deep breathing exercises.⁹ These tests provide indices of autonomic function.

According to the above indices, the nervous control of the heart in patients with diabetic autonomic neuropathy is reduced.^{10,11} However, the magnitude of abnormalities from the two branches of the autonomic system that can be measured in each test is not known.

The aim of this study was to plot the dose dependent responses to atropine, of the variations in R-R intervals, measured in 11 commonly used tests, in healthy volunteers. The most sensitive test to atropinisation would thus be used to measure specifically the vagal tone in either patients or any other physiological studies on humans. The study elucidates the magnitude of changes attributable to the vagal tone in these indices.

Methods

Subjects

Ten volunteers aged between 20 and 42 were recruited with the approval of the local ethical committee. The procedures were explained to them before obtaining their written consent. Each experiment was started in the morning before the subject had breakfast. Fasting was maintained throughout the experiment. The subjects rested supine for five minutes at the beginning of each experiment, with lead I of the ECG connected. A preliminary measurement of R-R intervals during this bed rest ensured that the subject was relaxed and the ECG was stable. The indices below were measured three times during the resting period using the procedures described by Mubagwa and Adler.¹²

(1) Mean R-R intervals (ms) of 250 consecutive cycles were recorded in supine and standing positions and the ratio of the mean values supine to those standing were calculated.

(2) Deviations in 250 consecutive R-R intervals (ms) were assessed from the SDs of the R-R intervals in supine and standing positions and from the mean of the absolute values

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Cardiac denervation may be one important cause of sudden death in diabetes mellitus.¹⁻³ For this reason non-invasive techniques are needed to assess the degree of autonomic control of the heart in diabetes.⁴ One of the techniques commonly used is to quantify the variation in duration of cardiac cycles. Individual periods are reflexly adjusted beat by beat, creating a variation that depends on the degree of nervous control exerted on the heart. These variations are quantified either as the standard deviation of many R-R intervals

of the beat to beat differences between R-R intervals in supine and standing positions.

(3) Ratios of R-R intervals during special manoeuvres were calculated: (a) Valsalva ratio, the ratio of the longest to the shortest R-R intervals during a Valsalva manoeuvre lasting for 15 s; (b) the 30:15 ratio, the ratio of the 30th to the 15th R-R intervals immediately after standing up from a low sitting position; and (c) the respiratory ratio, the ratio of the longest to the shortest R-R intervals during three cycles of forced expirations and deep inspirations. Each cycle lasted for 10 s, and was recorded in supine and standing positions.

Drug treatment

Five subjects chosen at random were given atropine sulphate (Central African Pharmaceutical Society, Harare, Zimbabwe) intravenously in seven separate doses of 0.1, 0.1, 0.2, 0.2, 0.4, 0.5 and 0.5 mg per 70 kg. These doses were achieved by diluting 0.6 mg atropine in standard ampoules so that 0.1 ml was equivalent to 0.1 mg of atropine. A tuberculin 1 ml syringe was then used to administer the drug. The other five subjects were given equivalent volumes of water for injection. All the tests above were done starting five minutes after each intravenous injection. This was enough time lag for the heart rate to stabilise after injections. The injections built up cumulative doses of atropine starting from 0.1, then 0.2, 0.4, 0.6, 1.0, 1.5, and 2.0 mg per 70 kg in each subject. The individual experiments lasted between four and nine hours.

Details of the test procedures and the equipment used in this study are as described by Mubagwa and Adler.¹² The values obtained from tests done after each dose of atropine or water were later expressed as percentages of the values obtained during the resting period. These percentages were used to plot the dose dependent response curves of changes caused by atropine or water.

Statistical analysis

Values obtained from each test were expressed as means, and when appropriate SD and SE were calculated. Two tailed *t* tests were used to compare mean values obtained after each dose of atropine to equivalent control values. The confidence limit for significance was 95% ($p < 0.05$). The coefficients of variation in each index experiment were calculated with values obtained from control subjects.

Results

Ratios of R-R intervals during special manoeuvres

Respiratory ratio recorded in the supine position (RRL) was the most sensitive test in the whole study. The range was between 1.16 and 1.66 (mean 1.37 (SE 0.05)) during the resting period (all volunteers). RRL was between 1.01 and 1.02 (1.012 (0.002)), after atropinisation ($n = 5$). This was a 97% decrease in response to atropine (figure 1). Coefficients of variation were between 2% and 5% (3.0% (0.5%)), in control subjects ($n = 5$). There was no increase in RRL

above the initial value in response to low doses of atropine (figure 1). Recordings made in standing positions (RRS) were between 1.19 and 1.70 (1.35 (0.05)) during the resting period. RRS was between 1.04 and 1.05 (1.048 (0.002)) after atropinisation. This was an 88% decrease in response to atropine (figure 1). The 9% difference between the maximum decrease in RRL and RRS was significant ($p < 0.001$). Coefficients of variation of RRS were between 2% and 5% (3.4% (0.6%)). There was no increase in RRS in response to low doses of atropine (figure 1).

The values of the 30 to 15 ratio were between 1.11 and 1.28 (1.20 (0.02)) during the resting period. They were between 1.04 and 1.05 (1.045 (0.003)) after atropinisation. This was a 78% drop in response to atropine (figure 1). Coefficients of variation in the control subjects were between 2% and 8% (4.3% (1.0%)). There was a significant 64% initial increase ($p < 0.01$) in response to a very low dose of atropine (figure 1).

The Valsalva ratios were between 1.38 and 2.33 (1.82 (0.08)) during the resting period. They were between 1.01 and 1.68 (1.41 (0.12)) after atropinisation. All changes were not significant (figure 1). Coefficients of variation were between 4% and 9% (6.2% (1.3%)).

Deviations of consecutive R-R intervals

The mean of absolute values of the beat to beat variations (BV) recorded in the supine position (BVL) was the most sensitive test in this category. The range of BVL was between 15 and 63 ms (37.9 (5.1) ms) during the resting period. It was between 2 and 3 ms (2.6 (0.2) ms) after atropinisation. This was a 94% decrease in response to atropine (figure 2). Coefficients of variation were between 10% and 19% (13.5% (1.7%)). If recorded in a standing position (BVS) the values were between 6 and 18 ms (12.3 (1.2) ms) during the resting period. BVS was between 2 and 4 ms (3.0 (0.3) ms) after atropinisation. This was a 77% drop in response to atropine (figure 2). The 17% difference between the maximum drop in BVL and BVS was significant ($p < 0.001$). Coefficients of variation of BVS were between 8% and 12% (10.3% (1.0%)).

The SD of R-R intervals recorded in supine positions (L-SD) was between 28 and 62 ms (47.5 (3.7) ms) during the resting period. L-SD was between 2 and 7 ms (4.8 (1.0) ms) after atropinisation. It decreased by 90% in response to atropine (figure 2). Coefficients of variation were between 9% and 20% (14.7% (2.8%)). The initial increase of L-SD above the starting value in response to a very low dose of atropine was not significant (figure 2). If recorded in a standing position (S-SD) it was between 19 and 39 ms (30.7 (2.3) ms) during the resting period. S-SD was between 4 and 13 (8.2 (1.7)) after atropinisation. It decreased by 75% (figure 2) in response to atropine. The 15% difference between the maximum decrease in L-SD and S-SD was significant ($p < 0.05$). Coefficients of variation of S-SD were between 11% and 29% (18.4% (3.2%)). There was a significant 37% increase in S-SD ($p < 0.001$)

Figure 1 Dose dependent response to atropine of the ratios of electrocardiographic R-R intervals during special manoeuvres. RRL = ratios of the longest to the shortest R-R intervals during deep breathing exercises in supine positions. RRS = the same ratios recorded in standing positions. 30:15 Ratio = the ratio of the 30th to the 15th intervals immediately after standing up from a low sitting position. Valsalva ratio = the ratio of the longest to the shortest R-R intervals during a Valsalva manoeuvre lasting for 15 s. (Subjects given atropine (◇), and those given equivalent volumes of water for injection (■)). Vertical bars are SEs ($n = 5$). The significance of the differences between values obtained after various doses of atropine and water were tested using two tailed t tests, where * $p < 0.01$ and ** $p < 0.001$. Mean values of RRL and RRS did not vary much throughout the 4 to 9 hours of the experiments in control subjects. Note that 30:15 ratio increased in response to a small dose of atropine and Valsalva ratio did not respond significantly to any dose of atropine.

above the starting value in response to the first small dose of atropine (figure 2). This was followed by an additional 36% increase ($p < 0.01$) in response to a second small dose.

Mean R-R intervals

In the supine position the range of means of R-R intervals recorded in individual volunteers was 876–1340 ms (1026.6 (48.9) ms) during the resting period. It was 490–628 ms (561.8 (28.7) ms) after atropinisation. The means decreased by 46% in response to atropine (figure 3). Coefficients of variation were between 2% and 7% (3.6% (0.9%)). The means in each volunteer when standing were between 617 and 1023 ms (748.9 (43.2) ms) during the resting period and between 439 and 599 ms (496.4 (27.1) ms) after atropinisation. This set of means decreased by only 34%, the lowest response to atropine in the whole study (figure 3). Coefficients of variation were between 2% and 6% (4.1% (0.6%)). The 12% difference between the maximum decrease in the values recorded in supine and standing positions was significant ($p < 0.05$).

Ratios of the mean R-R intervals recorded in supine to those in standing positions (LS-ratio) were between 1.21 and 1.54 (1.38 (0.05)) during the resting period. They were between 1.00 and

1.30 (1.15 (0.06)) after atropinisation. The ratios decreased by 60% in response to atropine (figure 3). Coefficients of variation were between 3% and 5% during the experiment (3.9% (0.5%)). The apparent initial increase of LS-ratio was not significant (figure 3).

Discussion

A clinical index that is closely linked with the parasympathetic control of the heart will respond proportionally to muscarinic antagonism. Graded doses of atropine provide varying degrees of muscarinic antagonism. This can be used to identify the responses of clinical indices that are dose-dependent and therefore genuine responses to muscarinic antagonism (figures 1–3). This procedure is different from the atropine test, in which heart rate is measured before and after a single large dose of atropine. RRL was sensitive to atropine and an accurate indicator of the vagal tone too. The doses of atropine at which RRL was maximally reduced compares well with those which caused maximum haemodynamic¹³ and heart rate changes¹⁴ in healthy volunteers. RRL was reproducible for most of the daytime. Its coefficient of variation over a period of up to nine hours was only 3.0% (0.5%). RRL could be useful as a single clinical

Figure 2 Dose dependent response to atropine of the deviations measured in consecutive R-R intervals of the ECG. BVL = the mean of the absolute difference between consecutive R-R intervals recorded in a supine position. BVS = the same difference recorded in a standing position. L-SD and S-SD are SDs of R-R intervals recorded in supine and standing positions respectively. (Subjects given atropine (◇), and those given equivalent volumes of water for injection (■)). Vertical bars are SEs ($n = 5$). Mean values of all four indices did not vary much during the 4 to 9 hours of the experiments in control subjects. The significance of the differences between values obtained after various doses of atropine and water were tested using two tailed t tests, where $*p < 0.01$, and $**p < 0.001$. Only S-SD increased significantly in response to very low doses of atropine.

index for selectively measuring vagal tone in patients. In addition, the dose dependent responses of RRL to atropine could be used to calculate a linear scale for vagal tone with the formula given by Katona and Jih.¹⁵ Such a scale will have an absolute zero representing no nerve impulses in the cardiac vagal nerve, ideal for comparing parasympathetic regulations of the heart in different groups of people.

We have elucidated the proportions of the vagal tone measurable by different tests (see results and figures 1–3). They may be useful for the interpretation of these clinical tests. With power spectral analysis of heart rate, the sympathetic control of the heart was found to be negligible in a person resting in a supine position; it increased during sustained active standing.¹⁶ The average increase in the nervous control of the heart due to this sympathetic orthostatic regulation was 14.6% in our study. This average was derived from the 17%, 15%, and 12% differences between lying and standing values of SD, BV, and mean R-R intervals respectively at full atropinisation (see results). It may look as if the respiratory ratio (respiratory sinus arrhythmia) had no sympathetic component because it was 97% vagal in a supine position (figure 1). It was suggested in earlier studies that the sympathetic system does not contribute to respiratory sinus arrhythmia in the cat.¹⁷ But we found a 12% ($p < 0.001$) non-vagal component when our volunteers were standing. The 9% difference between

RRL and RRS at full atropinisation was significant ($p < 0.001$). Neurograms recorded from the thoracic sympathetic trunk innervating the heart in anaesthetised cats showed phasic discharges, which only came during the expiratory phase of respiration.¹⁸ All these show a sympathetic component of the respiratory sinus arrhythmia. There is also a basal rhythmic variation in the periods of cardiac cycles after all nerve supplies have been cut.¹⁷ It had little effect on RRL here but may contribute to the variations in the other tests.

The technique of choice for measuring the parasympathetic control of the heart would be by quantifying the variations in the periods of cardiac cycles as opposed to the average of the periods (see maximum responses to atropine in figures 1–3). Algorithms and manoeuvres can be optimised for measuring specific variations of the heart periods. SD measures the total variation (constant, slow, or fast) in a time space equal to the duration of data collection. It was 3 minutes or more in this study, depending on the heart rate. This is inbuilt in the statistical algorithm. BVL and BVS measure the fast variation that causes detectable differences between immediate consecutive heart periods.¹² This variation is caused by the action of arterial baroreceptors on the sinoatrial node during the brief ejection period of each cardiac cycle.¹⁹ RRL and RRS measure the maximum variation of heart period synchronised with deep inspirations and forced expirations. This

Figure 3 Dose dependent response to atropine of the means of R-R intervals in the ECG. LS-ratio = the ratio of the means recorded in supine to that in standing positions. (Subjects given atropine ($\diamond$), and those given equivalent volumes of water for injection ($\blacksquare$). Vertical bars are SEs ($n = 5$). The mean values recorded in supine position were constant throughout the 4 to 9 hours of the experiments in control subjects. The significance of the differences between values obtained after various doses of atropine and water were tested using two tailed t tests, where * $p < 0.01$ and ** $p < 0.001$.

variation is caused by the modulation of excitability of vagal motor neurons in nucleus ambiguus by the inspiratory centre.²⁰ The 30:15 ratio measures the immediate cardiac nervous response to active standing within 30 cardiac cycles. It was previously thought to be entirely vagal in origin.⁶ All these manoeuvres and algorithms had different efficiencies for measuring the parasympathetic control of the heart. The range was from 75% to 97% of the parasympathetic regulation (see results and figures 1–3).

One advantage of BVL is that no active participation by the patient is required to measure it. Being 94% responsive to atropine, it could be useful in anaesthesiology for measuring vagal tone in unconscious patients. Cardiorespiratory arrests due to abnormal cardiovascular reflexes are common in diabetic patients during anaesthesia.²¹ BVL could be used to assess the vagal tone in diabetic patients during anaesthesia. It was also interesting that only two indices, the 30:15 ratio and S-SD, increased significantly in response to low doses of atropine. Low doses of atropine are thought to excite the central nervous system, including the vagus nerve.²² RRL, BVL, and other indices that had high sensitivities to atropine did not increase significantly in response to low doses of atropine, as was expected.

The Valsalva ratio was not significantly affected by any degree of muscarinic antagon-

ism produced by atropine. It also failed to significantly respond to the muscarinic antagonism of chloroquine in healthy volunteers.¹² The Valsalva ratio is unlikely to be closely related to the parasympathetic control of the heart. This needs to be remembered because the Valsalva ratio is a popular test in diabetology.^{5 11 23}

In conclusion, RRL was a specific test for the vagal tone on the heart. It could be useful for selective measurement of vagal tone in humans. The degree of parasympathetic regulation measured by other tests was variable and lower. The Valsalva ratio was probably not closely related to the parasympathetic control of the heart, and caution should be taken when interpreting this index in diabetology.

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