Clin Auton Res (2003) 13 : 45–50
DOI 10.1007/s10286-003-0074-x

RESEARCH ARTICLE

Matthew R. Farmer
Hamish F. Ross
Saqib Chowdhary
Faisal Osman
Jonathan N. Townend
John H. Coote

GABAergic mechanisms involved in the vagally mediated heart rate response to muscle contraction as revealed by studies with benzodiazepines

Received: 7 May 2002
Accepted: 10 September 2002

M. R. Farmer, PhD · H. F. Ross, BSc, DipAdvMath · Professor John H. Coote (✉)
Department of Physiology
The Medical School
The University of Birmingham
Birmingham B15 2TT UK
Tel.: +44-121/414-6916/5
Fax: +44-121/414-6924
E-Mail: J. H.Coote@bham.ac.uk

Current address:
Dr. M. Farmer
Clinical Study Management
Astra Zeneca
Macclesfield, UK

S. Chowdhary, MB, BS · F. Osman, MB, BCH · J. N. Townend, MD
Department of Cardiovascular Medicine
Division of Medical Sciences
The Medical School
The University of Birmingham
Birmingham, UK

Abstract The aim of this study was to determine if central GABA mechanisms are involved in the cardiac vagal withdrawal at the beginning of exercise in man. We tested whether GABA-enhancing effects of a benzodiazepine could be observed in the HR change (R-R interval) immediately following the onset of a brief (10 s) isometric contraction (60 % maximum) of the biceps muscle. The difference between the change in R-R interval occurring during the same phase of respiration was compared for placebo (Pla) and 10 mg oral diazepam (Dz) treatment in a double blind, crossover trial. ECG, blood pressure, respiration and biceps muscle tension were recorded. The subjects breathed to a metronome and R-R interval measurements were plotted for early and late inspiration and early and late expiration. The mean values of the first late expiration R-R interval immediately following the start of contraction in early expiration were compared to the same measurements without contraction. Contractions initiated following diazepam treatment resulted in a significantly greater reduction in R-R interval ($P < 0.05$) implying that GABAergic suppression of cardiac vagal outflow may be responsible for contraction-induced tachycardia in man.

Key words cardiac vagal inhibition · exercise heart rate · benzodiazepines

Introduction

Upon muscle contraction there is an immediate rapid rise in heart rate [1, 7, 14, 20], that in the initial period is mediated purely by the abrupt withdrawal of cardiac vagal activity [2, 12, 14, 15, 19, 20]. This early effect is followed by a slower sympathetically mediated increase in HR [16, 30].

Several ideas have evolved to account for these effects [5, 6, 14, 27, 32], most focus on possible afferent sources of neural information but do not attempt to explain how this input produces changes in the autonomic outflow. However this was addressed in a series of studies, which used ventral root-evoked muscle contraction in cats, to examine a possible reflex mechanism for the initial cardiac vagal effects [20–22]. These studies showed that a muscle afferent-induced increase in heart rate was greater if cardiac vagal tone was enhanced by increasing baroreceptor input. Such an action suggested that muscle afferents prevented baroreceptor excitatory drive either by an inhibitory action in the nucleus tractus solitarii or at the cardiac vagal neurones. GABA synapses have been described at both of these sites [8, 13, 17, 23, 29, 34], and stimulation of hind limb afferent nerve fibres was shown to inhibit a baroreceptor reflex-induced decrease in heart rate [21, 22]. There are though no studies of these mechanisms following muscle contraction.

To study the possible role of GABA in regulating cardiac vagal sites in the brain we made use of the property of benzodiazepines to associate with a binding site close to the $GABA_A$-binding site on the same receptor protein

[36]. It is this property that is recognised to give rise to the various benzodiazepine actions in the brain [28, 33]. The action of the benzodiazepines is considered to be a cooperative enhancement of the effect of GABA to increase chloride conductance, thus inhibiting neurones to a greater degree [36]. Thus it is argued that the action of benzodiazepines can reveal the effect of GABA at the $GABA_A$ receptor [33].

In a recent study using benzodiazepines to enhance GABA transmission, we reported that there are GABAergic mechanisms in the central nervous system, which are able to suppress the cardiac vagal outflow in man [10]. This conclusion was reached by showing a reduction in the variation of the cardiac pulse interval (R-R interval) in subjects following an intravenous dose of the benzodiazepine midazolam, which was reversed by the antagonist flumazenil. We therefore considered a similar approach might reveal whether a GABAergic mechanism was participating in the heart rate increase caused by inhibition of vagal tone at the start of the muscle contraction in man. For this purpose the effect of an oral dose of diazepam on the R-R interval change produced just after the onset of isometric contraction of the biceps muscle [14] was studied during fixed respiration. Diazepam was used in preference to midazolam because it could be given orally and preliminary studies showed at a dose of 10 mg it did not compromise the maximum force of muscle contraction or affect the ability of the subject to voluntarily respond to a verbal or auditory command.

The results show that a benzodiazepine significantly enhances the heart rate change induced by brief isometric muscle contraction. This supports the idea that in man, and probably in other animals, GABA neurones are involved in the muscle-heart rate response of exercise.

Materials and methods

With the approval of the South Birmingham Local Research Ethics Committee, five healthy volunteers were recruited from the students of the University of Birmingham (mean age 19 ± 2.2 years). The subjects provided written informed consent to take part in this study, and all procedures were performed in accordance with the Declaration of Helsinki. All volunteers were examined by a medical practitioner and were considered to have no obvious signs of cardiovascular or neurological diseases, or other relevant medical conditions. Subjects were asked to attend upon two separate occasions (following an acclimatisation session), at least two weeks apart, but at the same hour of the day. Prior to the experiment, all subjects were instructed to refrain from caffeine and tobacco for at least 12 hours, food for at least 2 hours prior to the study, and alcohol for 24 hours before and after the study.

The study was conducted in a placebo-controlled, double blind crossover fashion. On both of the study days, the subjects presented at the laboratory and after five isometric contraction tests were given a single unidentifiable capsule containing either placebo or 10 mg diazepam (Valium, Pharmacy, Queen Elizabeth Hospital, Birmingham), taken orally two hours prior to the study.

Laboratory set-up

The laboratory was kept silent during recordings, and was maintained at a temperature of between 20 and 25 °C with low lighting levels. Subjects were semi-supine bed-rested for 30 minutes prior to the start of the experiment, and during this time were set-up for the data collection. The electrocardiogram (ECG) was obtained by a Lead II arrangement, comprising three silver chloride monitoring electrodes (Red Dot, 3M Health Care, Borken, Germany) placed on the subject's chest. Beat to beat blood pressure was obtained from an index finger of the inactive limb (left arm) resting on the chest at heart level, using the Finapress blood pressure monitoring system (Ohmeda, Louisville, CO, USA). Respiratory movements were recorded from a strain gauge placed around the chest at the level of the base of the sternum. In addition isometric force produced by contractions of the elbow flexors was measured using a strain gauge with the arm held firmly in place in a frame so that the forearm was at right angles to the upper arm as described previously [2, 4].

Data capture

All signals were amplified, and sampled at 500 Hz after analogue to digital conversion. These data were captured on an Apple Power Macintosh (8100/180) running a custom-written suite of programmes within the LabVIEW programming application. The traces were visually checked for aberrant or ectopic events [35], and converted to a series of R-R intervals (calculated from the ECG trace) each with associated values for systolic pressure (SBP), mean blood pressure (MBP), diastolic blood pressure (DBP), respiration and force.

Pre-experimental protocol

Initially subjects were asked to perform a maximum voluntary contraction (MVC) of the right biceps muscle. From this a 60 % MVC was calculated and the subject practised attaining and holding this level of contraction for 10 s periods, aided by a visual display of the force trace on an oscilloscope. The force was checked on each visit and when at the end the study was unblinded the value in Newtons for 100 % MVC was found to be either the same or slightly less (< 2 %) for subjects on diazepam compared to placebo. Therefore for each subject a 60 % MVC was similar throughout the series of tests on placebo and diazepam.

Experimental protocol

The subjects were asked to adjust their respiratory frequency to an auditory signal (at a frequency they found comfortable), and data over a five-minute period were collected. This resting respiratory frequency did not change between visits. Following this, data for each respiratory cycle over a 30 s period were registered, at the end of which the subject performed a 60 % MVC of the right biceps. The contraction was initiated by a signal at a predetermined phase of respiration (see later) and maintained for 10 s. This procedure of tests was repeated five times, each separated by rest periods of at least 10 min. Immediately following this series of baseline measurements the subject was given a capsule containing either placebo or diazepam and 2 h later the tests were repeated. This period was chosen since plasma levels of orally administered diazepam show an initial peak which then declines slightly to plateau at 2 h thereafter remaining high for up to 4 h [31].

Data analysis

Each 5-min record of resting data was initially subjected to time domain analysis. Mean R-R interval (R-R), standard deviation of R-R in-

tervals (SDRR), and the percentage of R-R intervals that deviated from their predecessor by more than 50 ms (pNN50) were calculated in accordance with the recommendations of the Task Force of the European Society of Cardiology and the North American Society of Electrophysiology and Pacing [35]. The magnitude of respiratory sinus arrhythmia was also calculated from the difference between the minimum R-R interval and maximum R-R interval, measured for 50 respiratory cycles at rest. In addition an autoregressive power spectral analysis was performed upon these 5-min records as described previously [9, 10, 36].

Since the R-R interval varies throughout the respiratory cycle, values of the R-R interval were measured at identified phases of the cycle as illustrated in Fig. 2. Phases 1 and 2 relate to early and late inspiration and phases 3 and 4 relate to early and late expiration respectively. For each series of tests, values were calculated for the mean phase 4 R-R interval for 30 s before each contraction (milliseconds), and the first phase 4 R-R interval immediately following the initiation of contraction (post-contraction; milliseconds). The difference in these two R-R interval measurements was then calculated for five tests to give the change in R-R interval upon contraction expressed in milliseconds and as a percentage. Phase 4 in the respiratory cycle was chosen because the change in R-R interval is greatest for this phase when contractions are initiated at the onset of expiration [2, 3]. Mean values for the five subjects were calculated from the means of each set of five contractions.

Statistical analysis

All data are presented as means ± SEM and were analysed using Student's paired t-test for within subject comparisons and Wilcoxian Signed Rank test for the group data. Differences were only considered significant if $P < 0.05$ (5 % confidence level).

Results

Subjects were 5 males of normal height (174 ± 5 cm), mass (63 ± 4 kg), and body mass index (21 ± 0.7 kg.m^2). They were normotensive with systolic blood pressure of 108 ± 2 mmHg and diastolic blood pressure of 71 ± 3 mmHg, and in normal sinus rhythm (69 ± 8 beats per minute).

Rest

Baseline values were documented during the 5-min control period prior to the series of muscle contractions. In resting subjects on placebo, the mean R-R interval was 991 ms ± 44 ms, the standard deviation of successive R-R intervals (SDRR) was 64 ± 13 ms, and the percentage of intervals that differed from the predecessor by more than 50 ms (pNN50) was 39 ± 13 %. Following the oral dose of diazepam during a similar control period, there was a small fall in the mean R-R interval to 958 ms ± 25 ms, an increase in SDRR to 80 ± 15 ms and pNN50 to 55 ± 10 %. These changes did not reach significance ($p > 0.05$). There were no differences between placebo and diazepam with regard to SBP (122 ± 6 and 119 ± 3 respectively), MBP (78 ± 6 and 75 ± 2 respectively) or DBP (61 ± 6 and 59 ± 3 respectively).

All subjects exhibited a respiratory sinus arrhythmia (RSA) (Fig. 1), which was quantified as the mean max-min difference in R-R interval over 50 respiratory cycles. Subjects at rest on placebo had a mean RSA of 120 ms ± 40 ms, and on diazepam the RSA was 140 ms ± 25 ms. The difference was not significant.

Analysis of the power spectral density plots between treatments for each subject failed to reveal any signifi-

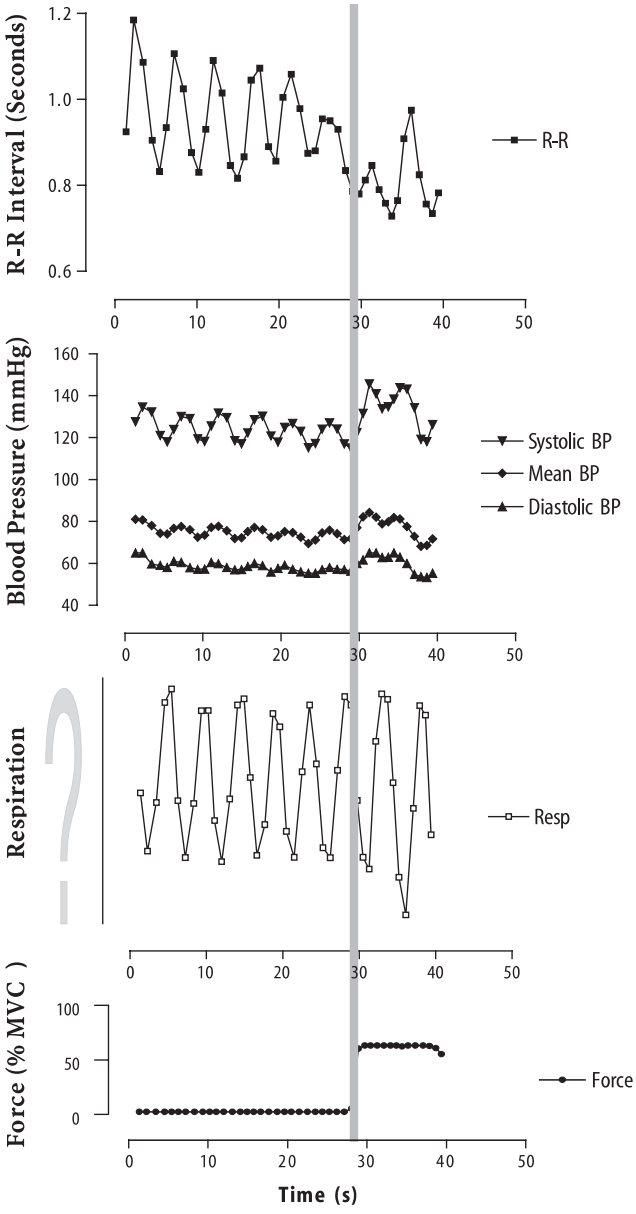

Fig. 1 Example of responses to a typical brief isometric muscle contraction taken from one subject on placebo. Data are given for R-R interval, blood pressure (systolic, diastolic and mean), respiration and force. The line represents the point at which the contraction was initiated. As can be seen from the R-R interval trace within 1.5 s there is an immediate tachycardia upon contraction, which is accompanied by a pressor response (seen as increases in systolic diastolic and mean blood pressures)

cant differences, even when the data were normalised. The high frequency peak was 82.78 ± 5.1 % following placebo pre-treatment and 80.74 ± 1.3 % following diazepam. Low frequency peak was 14.69 ± 4.8 and 14.46 ± 2.5 for placebo and diazepam respectively (ns).

Isometric muscle contraction

Fig. 1 demonstrates the effect of a typical 60 % MVC isometric contraction of the right biceps muscle following placebo pre-treatment in a single subject. For each subject the pre-placebo and pre-drug values (n = 5) were not significantly different from the placebo values ($p < 0.01$). Upon voluntary isometric muscle contraction, elicited at the beginning of expiration (phase 3), the next R-R interval phase 4 decreased so that heart rate increased as is shown in Fig. 2. The change was consistent throughout the five tests repeated on each subject ($P < 0.01$). The group data show that the mean phase 4 R-R intervals declined from 1100 ms ± 39 ms (55 bpm ± 2 bpm) to 950 ms ± 64 ms (64 bpm ± 4 bpm) a change of 150 ms ± 55 ms (5 ± 6.4 %; Fig. 2). This decrease in R-R interval (tachycardia) was significant ($P < 0.05$). Following 10 mg oral diazepam voluntary contraction elicited a significantly larger change in heart rate from 60 bpm ± 1.3 to 73 bpm ± 2.9 bpm and mean phase 4 R-R intervals decreased from 1007 ± 21 ms to 832 ms ± 33 ms a change of 175 ± 42 ms (16.8 ± 3.3 %), which was significantly greater ($P < 0.05$) than the change observed on placebo (Fig. 3). The brief isometric contractions also elicited increases in blood pressure as shown for one subject in Fig. 1. As can be seen from this recording, blood pressure oscillated with respiration and so to enable comparisons, the mean SBP, DBP and MBP were calculated. Typically, the contraction-elicited change in blood pressure occurred some 1.5 s after the heart rate changes. Although the blood pressure changes were usually greater during the contraction period, they varied between tests and between subjects, so that the group data did not reach statistical significance compared to the pre-contraction resting period. There were also no significant differences between placebo and diazepam for SBP (126 ± 6 mmHg and 123 ± 3 mmHg respectively), MBP (83 ± 6 mmHg and 76 ± 3 mmHg respectively), or DBP (66 ± 7 mmHg and 60 ± 3 mmHg respectively).

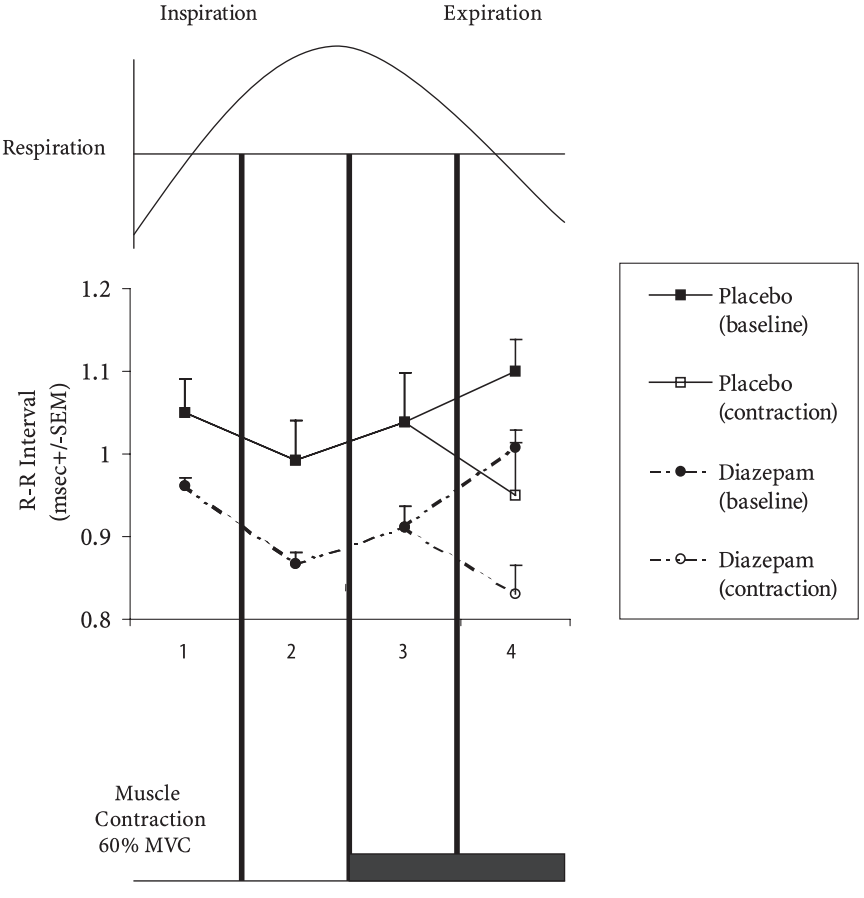

Fig. 2 The effect of isometric muscle contractions on R-R interval during placebo or diazepam treatment. Values of R-R intervals occurring in each of 4 phases of respiration are plotted for a thirty second period before a voluntary contraction was elicited. The values of R-R intervals of the respiratory cycle immediately following this period were measured during which an isometric contraction (60 % MVC) of the biceps muscle was initiated at a signal given at the beginning of phase 3 of respiration shown by the bar in the bottom trace. The mean values of R-R interval for five repeated sequences of the test are plotted. The results obtained during placebo (continuous lines) and during 10 mg diazepam (dashed lines) treatment are shown. The difference between placebo baseline (filled squares) and placebo contraction (open squares) R-R interval values in phase 4 (late expiration) was compared to the difference between diazepam baseline (filled circles) and diazepam contraction (open circles) values

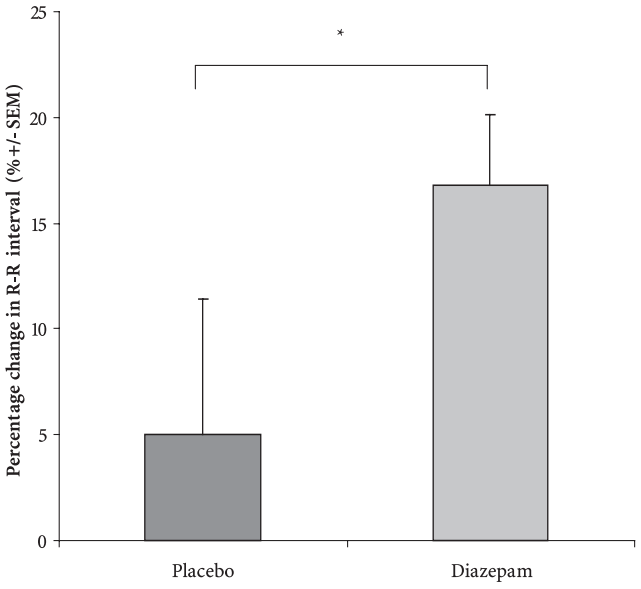

Fig. 3 Enhancement by a benzodiazepine of the exercise-induced change in R-R interval (heart rate). The bar graph shows the group mean data (n = 5) of the percentage change in R-R interval induced immediately after an isometric contraction 60 % MVC of biceps muscle during placebo (solid column) compared to diazepam (hatched column). Standard error bars are shown. *P < 0.05

Discussion

Benzodiazepines work in the brain by amplifying the action of the neurotransmitter GABA at the $GABA_A$ receptor [28, 33]. Therefore we interpret the actions of diazepam as revealing a GABA neurone involvement in the vagally mediated, initial heart-rate response to voluntary skeletal muscle contraction. It is known from previous studies that muscle contraction in man causes an immediate withdrawal of vagal influence on the chronotropic control of the heart, since it is blocked by atropine [12, 15, 19]. This effect has been previously exploited in our laboratory, in order to look at the actions of 60 % MVC muscle contractions upon cardiac vagal tone, in the absence of respiratory variations in cardiac vagal outflow [4].

Previously we have shown that there is a $GABA_A$-mediated suppression of cardiac vagal tone in man [9–11], which is in agreement with existing animal data [13, 21]. In the present study it was found that resting cardiac vagal tone was little affected by 10 mg oral diazepam, as has been noted previously [25], unlike the dramatic reductions seen with the more potent benzodiazepine midazolam [11]. The results of time- and frequency-domain analysis also show no significant differences between diazepam and placebo. This might be expected as diazepam was administered orally rather than intravenously and is also less potent than midazolam [26]. However, it was important to the present study that the benzodiazepine did not produce too much sedation, because we needed the subjects to be able to quickly respond to the signal to contract muscles and to elicit similar forces of contraction for all the treatments. Despite these constraints it is evident that the 10 mg oral dose of diazepam did have a significant influence, which became apparent when vagal tone was challenged. Upon voluntary contraction, the post-contraction R-R interval is reduced to a greater extent following diazepam pre-treatment than during placebo. Placing this in terms of heart rate there was an increase in 9 bpm on placebo at this early stage of contraction which was enhanced by a further 4 bpm by diazepam, an increase of 44 %. Thus we believe this is of physiological significance and we conclude that $GABA_A$-mechanisms can modulate the level of vagal withdrawal initiated by a skeletal muscle contraction in man. This was not due to a greater effort exerted by the subjects on diazepam to maintain the isometric contraction, since absolute force was either the same or slightly less and the same relative force of 60 % MVC was used throughout. We found that a 10 mg dose of diazepam did not affect the absolute force, since very similar forces (in Newtons) were obtained by maximal voluntary contractions throughout the study, even though neither the subject nor the experimenter was aware of the treatment. Furthermore none of the subjects reported finding the contractions more difficult on any visit which may not be surprising since the 60 % MVC only needed to be maintained for two respiratory cycles (around 10 s). We are therefore confident that the changes in vagal tone were not a consequence of increased central command.

Although animal studies have long pointed to GABA as a mediator of the exercise-induced vagal withdrawal, it has not been directly tested with muscle contraction in an experimental model. Our study in man is the first to indicate that such a mechanism does play a part in the heart rate response to exercise. Our understanding of how and where this action occurs in the brain is limited but the medullary sites such as the NTS and cardiac vagal motonuclei seem the most likely. Studies in cats and rats show that muscle afferent fibres ascend in the spinal cord to the caudal medulla where they project to and suppress neurones in the NTS [18, 21, 24, 25, 29]. In addition it is also possible they project to the nucleus ambiguus where GABAergic synapses have also been described [13, 23]. It is therefore tempting to suggest that in our rather limited study in man the benzodiazepine was enhancing the action of GABA at one of these medullary sites resulting in an increased efficacy of the exercise-induced change in heart rate. Whether these effects are due to central command or a reflex depending on mechanoreceptors which we have recently described [14] originating in the contracting muscle remains to be determined by a more extensive study.

Acknowledgements Matthew Farmer was a British Heart Foundation Ph. D. student. Their financial support is gratefully acknowledged.

References

1. Alam M, Smirk FH (1938) Observations in man on a pulse-accelerating reflex from the voluntary muscles of the legs. J Physiol 92:167–177
2. Al-Ani M, Forkins AS, Townend JN, Coote JH (1996) Respiratory sinus arrhythmia and central respiratory drive in humans. Clin Sci 90:235–241
3. Al-Ani M, Powell L, West J, Townend JN, Coote JH (1995) Exercise and diving: two conflicting stimuli influencing cardiac vagal tone in man. J Physiol 489:603–612
4. Al-Ani MN, Robins K, Al-Khalidi AH, Vaile JC, Townend JN, Coote JH (1997) Isometric contraction of arm flexor muscles as a method of evaluating cardiac vagal tone in man. Clin Sci 92:175–180
5. Coote JH (1975) Physiological significance of somatic afferent pathways from skeletal muscle and joints with reflex effects on the heart and circulation. Brain Res 87:139–144
6. Coote JH (1995) Cardiovascular responses to exercise: central and reflex contribution. In: Jordan D, Marshall JM (eds) Cardiovascular Regulation. Portland Press Ltd, pp 93–111
7. Coote JH, Hilton SM, Perez-Gonzalez JF (1971) The reflex nature of the pressor response to muscular exercise J Physiol 215:789–804
8. DiMicco JA, Gal K, Hamilton BL, Gillis RA (1979) GABA Receptor control of parasympathetic outflow to heart: characterisation and brainstem localisation. Science 204:1106–1109
9. Farmer MR (2000) GABA inhibitory mechanisms in cardiac control in Humans. PhD Thesis. The University of Birmingham
10. Farmer MR, Chowdhary S, Vaile JC, Ross HF, Townend JN, Coote JH (2000) GABAergic effects on baroreflex cardiac vagal outflow assessed by cross-spectral analysis of R-R interval and systolic blood pressure in man. Clin Auton Res 10:90–91
11. Farmer MR, Vaile JC, Osman F, Ross HF, Townend JN, Coote JH (1998) A central γ -Aminobutyric acid mechanism in cardiac vagal control in man revealed by studies with intravenous midazolam. Clin Sci 95:241–248
12. Freyschuss U (1970) Cardiovascular adjustment to somatomotor activation. The elicitation of increments in heart rate, aortic pressure and venomotor tone with the initiation of muscle contraction. Acta Physiol Scand 342(Suppl):5–63
13. Gilbey MP, Jordan D, Spyer KM, Wood LM (1985) The inhibitory actions of GABA on cardiac vagal motoneurones in the cat. J Physiol 361:49P
14. Gladwell VF, Coote JH (2002) Heart rate at the onset of muscle contraction and during passive muscle stretch in humans: a role for mechanoreceptors. J Physiol 540:1095–1102
15. Hollander AP, Bouman LN (1975) Cardiac acceleration in man elicited by a muscle-heart rate reflex. J Appl Physiol 38:272–278
16. Iellamo F, Massaro M, Raimandi G, Peruzzi G, Legramante JM (1999) Role of muscular factors in cardiorespiratory responses to static exercise: contribution of reflex mechanisms. J Appl Physiol 86:174–180
17. Jordan D, Mifflin SW, Spyer KM (1988) Hypothalamic inhibition of neurones in the nucleus tractus solitarius of the cat is GABA mediated. J Physiol 399:389–404
18. Kalia M, Mei SS, Kao FF (1981) Central projections of ergoreceptors (c fibres) in muscle involved in cardiopulmonary responses to static exercise. Circ Res 48:148–162
19. Maciel BC, Gallo L, Marin Neto JA, Martins LEB (1987) Autonomic nervous control of the heart rate during isometric exercise in normal man. Pflügers Archiv 408:73–177
20. McMahon SE, McWilliam PN (1992) Changes in R-R interval at the start of muscle-contraction in the decerebrate cat. J Physiol 447:549–562
21. McMahon SE, McWilliam PN, Robertson J, Kaye JC (1992) Inhibition of carotid sinus baroreceptor neurones in the nucleus tractus solitarius of the anaesthetised cat by electrical stimulation of hindlimb afferent fibres. J Physiol 452:224P
22. McWilliam PN, Yang T (1991) Inhibition of cardiac vagal component of baroreflex by stimulation of group III and IV afferents. Am J Physiol 260:730–734
23. Maqbool A, Batten TFC, McWilliam PN (1991) Ultrastructural relationship between GABAergic terminals and cardiac vagal preganglionic motoneurones and vagal afferents in the cat: a combined HRP tracing and immunogold labelling study. Eur J Neurosci 3:501–513
24. Nyberg G, Blomquist A (1984) The central projections of muscle afferent fibres to the lower medulla and spinal cord. J Comp Neurol 230:99–109
25. Potts JT, Fuchs IE, Li J, Leshnower B, Mitchell JH (1999) Activation of skeletal muscle afferents release substance P in the nucleus tractus solitarii in cats. J Physiol 514:829–843
26. Reves JG, Fragen RJ, Vinik R, Greenblatt DJ (1985) Midazolam: pharmacology and uses. Anaesth 62:310–324
27. Rowell LB, O'Leary DS, Kellogg DL. (1996) Integration of CV control systems in dynamic exercise. In: Rowell LB, Shepherd JT (eds) Handbook of Physiology. Section 12. Exercise: Regulation and Integration of Multiple Systems. American Physiological Society. Oxford University Press, pp 770–838
28. Rudolph U, Crestani F, Mohler H (2001) $GABA_A$ receptor subtypes: dissecting their pharmacological functions. TIPS 22:188–194
29. Saha S, Batten FC, McWilliam PN (1995) Glutamate, γ-aminobutyric acid and tachykinin immunoreactive synapses in the cat nucleus tractus solitarii. J Neurocytol 24:55–74
30. Sato A, Sato Y, Schmidt RF (1981) Heart rate changes reflecting modifications of efferent cardiac sympathetic outflow by cutaneous and muscle afferent volleys. J Auton Nerv Sys 4:231–247
31. Schwartz MA, Koechlin BA, Postma E, et al. (1965) Metabolism of diazepam in rat, dog and man. J Pharmacol Exp Ther 149:423–435
32. Secher NH (1999) Cardiovascular function and oxygen delivery during exercise. In: Whipp BJ, Sargeant AJ (eds) Physiological Determinants of Exercise Tolerance in Humans. Portland Press London, pp 93–114
33. Sieghart W (2000) Unraveling the function of $GABA_A$ receptor subtypes. TIPS 21:411–413
34. Sved JC, Sved AF (1989) Cardiovascular responses elicited by γ-aminobutyric acid in the nucleus tractus solitarius: evidence for action at the $GABA_B$ receptor. Neuropharmacol 28:515–520
35. Task Force of the European Society of Cardiology and the North American Society of Electrophysiology and Pacing (1996) Heart rate variability: standards of measurement, physiological interpretation and clinical use. Circ 93:1043-1065
36. Wisden W, Stephens DN (1999) Towards better benzodiazepines. Nature 401:751–752