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

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 (10s) 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 10mg 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 GABAA 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 acclimatization 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-