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INTRA-AMYGDALA INJECTION OF $GABA_A$ AGONIST, MUSCIMOL, REDUCES TACHYCARDIA AND MODIFIES CARDIAC SYMPATHO-VAGAL BALANCE DURING RESTRAINT STRESS IN RATS

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Abstract—At present, little is known about the brain origin of stress-induced cardiac sympathetic drive responsible for stress-induced tachycardia. Our aim was to determine the effect of bilateral microinjections of the $GABA_A$ receptor agonist, muscimol, into the amygdaloid complex on both the heart rate and cardiac autonomic activity during restraint stress. Experiments were performed in male Sprague–Dawley rats (n=9), with pre-implanted electrocardiographic electrodes. Heart rate increased sharply after the onset of the restraint and reached a peak 1–2 min later (from 344±6–440±20 BPM). Subsequently, heart rate began to fall, and during the next 10–15 min approached the steady-state level of 384±11. After vehicle, mean heart rate during each of three 10-min restraint epochs was significantly higher compared with the pre-restraint level. After muscimol, mean heart rate was significantly elevated only during the first 10 min of restraint. There was no difference in the early peak tachycardia between both conditions. Muscimol substantially accelerated the fall of the HR from the peak to the steady-state level, and thus the area under the curve value for muscimol (503±162 BPM×min) was significantly smaller than that for vehicle (1221±231 BPM×min); P<0.05. After vehicle, the high-frequency spectral power of the heart rate decreased and the low-frequency power increased during the restraint, resulting in a significant rise of the low frequency/high frequency ratio from 1.2±0.2–2.8±0.6 (n=9, P<0.05). Muscimol suppressed these stress-induced effects. We conclude that inhibition of the amygdala neurons abolishes the sustained component of tachycardia during the restraint, has no effect on the early tachycardic component, and prevents stress-induced alterations in the heart rate variability indices.

Key words: heart rate, psychological stress, sympathetic, telemetry.

Tachycardia is a hallmark of emotional arousal induced by psychological stressors, and is predominantly mediated by cardiac sympathetic nerves (Barron and Van Loon, 1989; Shapiro et al., 1993). At present, very little is known about the brain origin of stress-induced cardiac sympathetic drive. Some earlier studies (Galeno and Brody, 1983; Gelsema et al., 1987; Lovick, 1990; Soltis and DiMicco, 1991; Soltis et al., 1998) identified a number of brain sites whose stimulation results in tachycardia. However, the definite answer to the question of whether these regions indeed participate in the cardiac control during stress could be obtained only in the experiments conducted in conscious animals, where tachycardiac responses to natural stressful stimuli could be suppressed by blocking relevant brain regions.

Many studies have proven that the amygdala integrates both behavioral and vascular (pressor) responses to acute psychological stressors (see Discussion), but effects of inhibition of the amygdala on stress-elicited *cardiac* responses have not been studied so far. Thus our principal aim was to determine the effect of pharmacological inhibition of the amygdaloid complex on both the heart rate and cardiac autonomic activity (evaluated by the heart rate variability, HRV) during psychological stress. For this purpose, we used restraint, a common and well-described model of stressing a rat, consistently eliciting tachycardia and a rise in arterial pressure (Barron and Van Loon, 1989; Chen and Herbert, 1995; McDougall et al., 2005).

EXPERIMENTAL PROCEDURES

Animals and preliminary surgery

The study was carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and was approved by the Flinders University Animal Welfare Committee. All efforts were made to minimize the number of animals used and their suffering. Experiments were performed on 20 male Sprague–Dawley rats (300–350 g). Preliminary surgery was conducted under isoflurane anesthesia (1.5% in 100% oxygen). Firstly, electrocardiographic (ECG) electrodes were implanted according to the method described by Sgoifo et al. (1996): one electrode was attached to the internal surface of the xyphoid processes, another was positioned in the mediastinum, along the trachea at the level of the left ventricle. Such electrode placement permits the recovery of 95–99% of heartbeats, even in moving animals. The leads of ECG electrodes were tunnelled under the skin to the back of the neck, exteriorized and soldered to a head socket. Animals were then placed in a stereotaxic apparatus, two burr holes were drilled, and stainless steel guide cannulae were placed bilaterally 2.8 mm caudal to bregma and 4.0 mm lateral to the midline, with a tip located 5 mm below the surface of the skull. The head socket and cannulae were fixed to the skull with stainless steel screws and dental cement. Cannulae were closed with obturators protruding 0.3 mm below the tip. Animals recovered from anesthesia and were returned to the animal house for at least 1 week before experimental studies.

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Abbreviations: AUC, area under the curve; ECG, electrocardiographic; HF, high frequency; ΔHR, heart rate change; HRP, horseradish peroxidase; HRV, heart rate variability; LF, low frequency.

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Experimental protocol

On the day of experiments, rats were brought from the animal house, connected to the recording system via a flexible cable attached to a swivel, placed in a dark box (40×40×40 cm) and remained undisturbed for at least 60 min. They subsequently received a bilateral injection of $GABA_A$ receptor agonist muscimol (0.1 nmol in 500 nl sterile Ringer's solution) or, on a different day, vehicle, in a counterbalanced manner. The tip of the injecting cannula was lowered either to the level of 8 mm or 6 mm below the skull surface, for the injections into the amygdala or for control injections into the striatum, respectively. Injections were made using a handheld syringe, with visual control of the injected volume using a calibrated 10 μl glass capillar. After injections, animals were returned to the box for 30 min and then placed into a restrainer for the next 30 min. The restrainer consisted of transparent PVC tubing (I.D. 6 cm). At the end of the experiment we labeled injection sites with horseradish peroxidase (HRP) dissolved in the Ringer's solution (500 nl) using the same injection cannula. Animals were killed, perfused transcardially with a fixative, and the brain was removed and sectioned. Sections were processed for HRP, stained with Neutral Red, and the location of the cannula tip was photographed. Muscimol was from Sigma (St. Louis, MO, USA).

Data acquisition and analysis

Analog ECG signal was digitised at 1 kHz and acquired using PowerLab A/D converter and Chart 5.4 software (ADInstruments, Sydney, Australia). We measured peak stress-induced tachycardia and mean heart rate and heart rate changes (ΔHR) during four 10-min epochs (one pre-stress and three during stress), and computed the area under the curve (AUC) values for three epochs during stress. Delta AUC was defined as ($AUC_{Vehicle} - AUC_{Muscimol}$). HRV analysis in the frequency domain was performed using Chart 5.4 software (ADInstruments). Artifact-free data segments containing at least 1200–1400 heart beats were selected just before restraint ("pre-stress"), during the first 5 min of restraint ("onset-stress") and during the last 10 min of restraint ("end-stress"). We detected total spectral power, the percentage of the low-frequency (LF, 0.4–0.67 Hz) and high-frequency (HF, 0.67–3 Hz) components, and the LF/HF ratio. All values are means±S.E.M. A repeated measures two-way ANOVA (time×drugs) was followed by Tukey HSD post hoc test. Linear regression was used for assessing delta AUC/AUC dependence.

RESULTS

Effects of intra-amygdala muscimol injections on restraint-induced tachycardia

Tachycardia associated with handling during brain injections was short-lasting, so that within 10–15 min after injection the heart rate returned to the basal level after both vehicle and muscimol, in all animals. Before stress, there was no difference in the HR values between vehicle and muscimol conditions. In rats that received intra-amygdala injection of vehicle, restraint stress caused tachycardia that peaked at about 425 BPM within 2–3 min and then declined, approaching steady-state level of 40–45 BPM above the baseline within the next 10–15 min and remaining at this level until the end of restraint (see Fig. 1A for illustrations and data values). Although peak tachycardia did not differ between the two conditions (absolute values: 443±15 and 441±13 BPM and deltas: +94±13 and +86±11 BPM for vehicle and muscimol, respectively; $P>0.05$, $n=9$ for both data sets), increases in heart rate during each of the 10-min restraint epochs were significantly lower after muscimol. For the muscimol condition, mean heart rate was significantly elevated (i.e. different from pre-restraint) only during the first 10 min of the restraint. Mean ΔHR values for each epoch were significantly lower after muscimol (Fig. 1A). Muscimol substantially accelerated the fall of the heart rate from the peak to the steady-state level (Fig. 1A), and thus the AUC value for muscimol (503±162 BPM×min; $P<0.05$, $n=9$) was significantly smaller then that for vehicle (1221±231 BPM×min). The inhibitory effect of muscimol on stress-induced tachycardia (assessed as an AUC) was directly proportional to the initial response (Fig. 1B).

Intra-amygdala locations of microinjection sites in nine animals are illustrated in Fig. 2A. We did not find any dependence of the magnitude of muscimol effect on the location of the actual injection site. Fig. 2A also shows five cases of unsuccessful injections, where we missed the target. In two of these five cases that were closer to the target, we observed some effect of muscimol (the AUC was reduced from 1361 to 1097 BPM×min and from 962 to 887 BPM×min, respectively), whereas at three other sites the drug was without effect. We did not observe any gross behavioral changes after administration of either vehicle or muscimol to the amygdala.

Effects of intra-amygdala muscimol injections on restraint-induced changes in HRV

Restraint stress altered both LF and HF components of the heart rate power spectra. For the vehicle condition, the normalized HF power decreased and the LF power increased at the beginning, but not at the end of the restraint, resulting in a significant rise of the LF/HF ratio during this period. Following pre-treatment with muscimol, rats exhibited no significant changes in the HRV in response to stress. Data values for HRV changes are presented in Table 1.

Effects of intra-striatal muscimol injections on restraint-induced cardiac changes

In six animals (control group) either vehicle or muscimol was injected, on different days, into the ventral striatum, 2 mm dorsal to the amygdalar site. Restraint-induced tachycardic responses and changes in HRV in these rats did not differ between the two conditions (Fig. 3 and Table 1), and did not differ from responses observed in our major experimental group after vehicle injection. Intra-striatal locations of microinjection sites are illustrated on Fig. 2B.

DISCUSSION

This is the first study investigating the effect of pharmacological inhibition of the amygdala on stress-induced tachycardiac and HRV responses. We have demonstrated that activation of inhibitory $GABA_A$ receptors in the amygdala prior to restraint stress reduces the duration, but not the magnitude, of initial transient tachycardia and prevents the increase of the LF/HF ratio of the spectral power. Another novel finding is that restraint stress modifies spectral pa-