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SPECTRAL EVALUATION OF AUTONOMIC CHANGES PRODUCED BY CHRONIC ETHANOL ADMINISTRATION IN RATS

BY

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Mathematical tools have been applied to evaluate the autonomic balance. In particularly, it is widely used the fast Fourier transformer (FFT) that is able to produce a power spectrum of the frequencies from a stationary signal. Nucleus tractus solitarius (NTS) is an important area in the central regulation of autonomic functions. In ethanol addiction, there is an imbalance of autonomic flow but its underlying mechanisms are poorly understood. The purpose of the present study was to investigate the cardiorespiratory responses on acute ethanol application in the NTS and its intravenous administration in normal rats and in a rat model of chronic ethanol consumption (EtOH rats), evaluating, in both conditions, the autonomic balance using a new mathematical tool - wavelets method. Mean arterial blood pressure (MBP), ECG, heart rate (HR) and phrenic nerve activity were monitored. Results show a decrease of all the recorded variables both on normal and addict rats for the moderate ethanol doses applied to the NTS; on intravenous administration of ethanol no changes were observed in cardiorespiratory variables. On evaluation of autonomic nervous system using wavelet method, EtOH rats revealed an elevated sympathetic component in comparison with the normal rats. After i.v. administration of ethanol, an increase on the sympathetic mediated component of normal rats was seen by opposition to the decrease of this component observed in EtOH rats. These results suggest the presence of a neuroadaptation elicited to counteract the autonomic imbalance evoked by ethanol addiction. The wavelet method showed to be a good way of evaluating the transient modification of biological signals.

Introduction

The caudal medulla is a key area of the central nervous system for determining the autonomic outflow to the periphery both in physiological and pathological conditions. In particular, nucleus tractus solitarius (NTS) constitutes the primary autonomic area, that receiving afferent information from receptors located in the cardiovascular system itself or with origin in other central areas belonging to the central autonomic network, is able to integrate and modulate neuronal information. Other two major nucleus of the caudal medulla are those where originates the

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sympathetic information - the rostroventrolateral medulla (RVLM) - and the parasympathetic outflow – the nucleus ambiguus and dorsal motor of the vagus.

Several studies reported that the pathogenesis of ethanol-related neurological disorders is considered to be multifactorial and attributed either to genetic predisposition, nutritional factors or to the neurotoxic effects of ethanol and/or its metabolites [4]. Autonomic involvement, and in particularly, the role of NTS and other central autonomic nucleus in these neurological symptoms are not yet well understood. In a previous work on rats [20], we showed that ethanol microinjections into NTS produced hypothension and bradycardia, which are in opposition to the cardiovascular changes on blood pressure and heart rate observed in acute systemic alcohol administration. Also, brainstem involvement on the genesis or modulation of these effects has been poorly investigated in chronic alcohol administration [9,29,33] despite several studies in alcoholics and social drinkers that reported a decrease on heart rate variability induced by alcohol consumption [15,16,17,23,30].

Attempts to study autonomic function in patients using non-invasive methodology have been the cause of the development of mathematical tools to be applied to biological signals. Heart rate variability was usually assessed in the frequency domain by fast Fourier transform (FFT), autoregressive model (AR), short-term Fourier transform (STFT) and in the time domain by the standard deviation of beat-to-beat intervals (SDRR) and the root mean square of successive beat-to-beat differences in R-R interval durations (rMSSD). The FFT application to heart-rate and blood pressure originates a power spectrum that quantifies both autonomic function and respiratory activity. In human subjects, heart rate variability was described to have two majors oscillatory components [14], one of which, synchronous with respiration, is described as HF ([0.15-0.5]Hz, high frequency) while the other, corresponding to the slow waves of arterial pressure is described as LF (~ 0.1 Hz, low frequency). The power of the LF and HF components evaluated in absolute value, as well in normalized units, together with their ratio (LF/HF) has been specifically designed to estimate the balance between the two branches of the autonomic nervous system. The very-low-frequency (VLF) component, generally below 0.04 Hz, was also identified in human power spectrum, sometimes centred on zero Hz, even if the mean value of the signal has been eliminated. This component accounts for the long-term regulatory mechanisms, mainly related to thermoregulation and humoral factors [27]. Recently, it was shown a significantly better quantitative analysis of heart rate variability using wavelet methods [19, 31]. While FFT, STFT or AR required stationary conditions of the time series and recording intervals greater than 5 minutes, spectral analysis using wavelets gives good approximations of transient or localized phenomena. This monitorization is essential for vital brainstem functional analysis in clinical medicine. Each term in a wavelet series is orthogonal to other which means that there is no redundancy in the representation. Multiresolution analysis provides a simpler and more efficient representation than conventional mathematical representations.

In the present study, we examined the hemodynamic effect of acute ethanol microinjection in the nucleus tractus solitarius (NTS) and its intravenous administration in normal rats and in a rat model of chronic ethanol consumption, evaluating, in both conditions, the autonomic balance using wavelets.

Material and methods

Animals and Chronic Ethanol Consumption

The experiments were carried out in male 12-14 week-old Wistar rats (n=15. Rats were maintained on a 12/12 h light/dark cycle, at constant temperature, with free access to food and water and were divided into two groups. One group, control animals, was tested for central microinjections into the NTS and systemic administration of ethanol. The other group of rats (n=6) received ethanol (EtOH; 25% v/v) *ad libitum* in the drinking water for 12 weeks. Rats were allowed to drink tape water for 1h/day except on week-end.

Surgical procedures

Twelve weeks after the beginning of chronic EtOH consumption, rats were anaesthetized (α-chloralose - 100 mg/Kg, i.p.), treated with a neuromuscular blocker (pancuronium bromide - 4mg/kg/h) and artificially ventilated. The depth of the anaesthesia was maintained by ensuring the absence of a withdrawal reflex before paralyzing the animal and changes on arterial blood pressure and heart rate to pinching a paw after the administration of a muscle blocker.

The femoral artery and vein were cannulated for monitoring arterial blood pressure (Sensonor 840, Lectromed Ltd, and UK) and the administration of drugs, respectively. A tracheostomy was made low in the neck. The animal was ventilated with O_2-enriched air (Harvard Apparatus Ltd, UK) after the muscular blockade and ventilation was regulated to maintain end-tidal CO_2 between 4.5 and 5% (ADC Gas analyzer, UK). Rectal temperature was kept at 36.5°-38°C by a servocontrolled heating blanket (Harvard Apparatus Ltd., UK). The electrocardiogram (ECG) was recorded (Neurolog, Digitimer, UK) from needle-electrodes inserted into three of the four limbs and heart rate derived with the use of an instantaneous ratemeter (Neurolog, Digitimer, UK). The animal head was placed in a stereotaxic head holder (Kopf Instruments, Germany) such that the difference in height between lambda and bregma was zero. The left phrenic nerve was identified and placed in a bipolar silver electrode to record neuronal activity (Neurolog, Digitimer, UK). A craniotomy was carried out to allow the insertion of stimulation microelectrodes in the NTS. Arterial blood pressure, phrenic nerve activity, ECG and heart rate were monitored throughout the experiment. All recorded variables were digitized (Instrutech VR100B, Digitimer, UK) and recorded on videotape. Off-line analysis was made using a computer A/D system with data capture and analysis software (PowerLab 8SP, ChartWindow, UK).

Experimental protocol

A multibarrelled glass microelectrode (tip diameter 40-60μm) was inserted into NTS using stereotaxic coordinates according to Paxinos and Watson [18]. The barrels of the microelectrode were filled with wood's metal for electrical stimulation (50Hz, 1ms, 20-50μA, trains of 5s), glutamic acid (2mM, pH=7.4±0.1), ethanol (50 mM) and artificial cerebrospinal fluid (CSF). The microinjection of ethanol was performed at sites where the previous electrical stimulation and glutamate microinjections have evoked the characteristic hypotension and bradycardia. The volume of each injection (50nl) was controlled using a microscope with a calibrated reticule and CSF microinjections were performed as a control in the beginning of each experiment. Blood pressure, heart rate and phrenic nerve activity changes were recorded on NTS microinjections and intravenously ethanol injection (1g/kg). An electrolytic lesion was performed at the end of experiments to allow stimulation sites to be marked.

Histological procedures

At the end of the experiment the animal was killed with an overdose of anesthetic and the brainstem was removed and fixed in a 4% paraformaldehyde saline with 30% sucrose solution for 48 hours. The tissue was sectioned serially (60μm), stained with neutral red and recording sites were documented with the use of a microscope. Stimulating sites were then identified and drawn onto standard sections taken from the rat atlas of Paxinos and Watson [18].

Data analysis

The mean arterial pressure (MBP) and heart rate (HR) values before and after ethanol administration were measured and the peak changes in both variables (MBP var. and HR var.) were used to determine the effect of ethanol on cardiovascular function. Phrenic nerve activity was analyzed by comparing the area under the average integral of nerve activity of 10 inspiratory cycles before stimulation - baseline values - with the value determined during maximal effect of ethanol microinjection. The presence of an effect was considered when the evoked changes were equal or greater the double of the changes observed in basal conditions.

In order to perform wavelet analysis of cardiovascular signal, implemented software in our laboratory was used [16]. Shortly, the discrete event series that is the plot of R_i-R_{i-1} interval and systolic arterial pressure (SAP) values versus time were re-sampled according to the Shannon theorem and required 2^n samples for good resolution of spectral analysis. Tachogram and SAP signals were decomposed into height wavelet scales (j = 8) using Daubechies 12 (db12) wavelet. We selected wavelets coefficients for details corresponding to signal frequencies between 0.01 and 3 Hz and investigated how the energy in the details signal is distributed in the frequency domain. Low frequency (LF) and high frequency (HF) components of the signals were obtained by merging the detail signals at scale 6 (0.2-0.5 Hz) and at scales 3, 4 and 5 (0.5-3 Hz), respectively. The decomposed VLF signals corresponded to the detail at scale 7. We calculated spectral

components energy by merging positive wavelets coefficients of LF and HF powersignals. Signals of 30s time length recorded before and after EtOH administration into NTS and intravenously, respectively, in control and chronic EtOH fed rats were analysed.

Paired and unpaired t test was used for statistical analysis of ethanol effects on cardiovascular and respiratory parameters both for intra- and intergroup differences. The differences were considered significant where $p \leq 0.05$. Because of large interindividual variation of power spectral values, the Wilcoxon matched pairs test was used for evaluation of ethanol effects on blood pressure variability (BPV) and heart rate variability (HRV).

All the procedures of the present protocol using animals were performed according to national and EU laws on animal experimentation.

Results

Results will refer to two groups of animals (control animals and animals with alcohol addiction) in two experimental conditions: under central activation of NTS neuronal circuits evoked by the microinjection of ethanol and on systemic (i.v.) injection of ethanol through a peripheral vein.

Chronic ethanol effect on blood pressure (BP) and heart rate (HR)

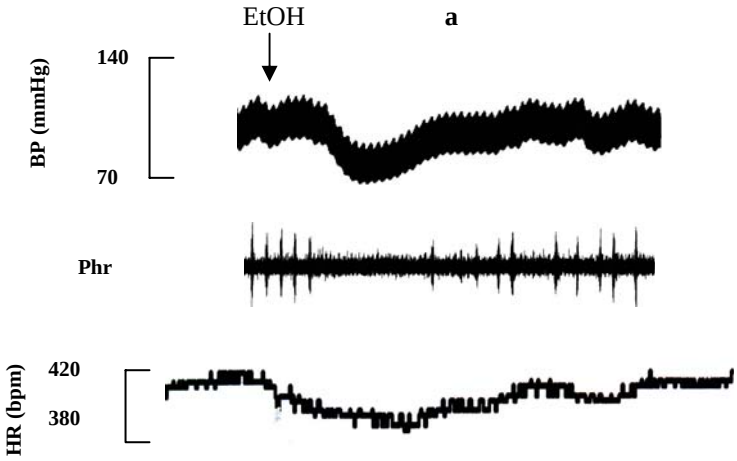
Baseline values for mean arterial pressure, systolic arterial pressure, diastolic arterial pressure and heart rate in control rats were 98±4.9 mmHg, 116±4.2 mmHg, 90±5.7 mmHg (t test, n=9, $p<0.001$) and 413±8.7 bpm (t test, n=9, $p<0.01$), respectively. Rats with chronic ethanol consumption had a slightly increased MBP (104±3.7 mmHg, n=6, t test, $p<0.05$) and decreased HR (392±9.5 bpm, n=6, $p<0.05$) considered not significant when compared to control group (unpaired t test, $p=0.361$ and $p=0.137$ for MBP and HR differences, respectively).The microinjection of ethanol at sites where the previous electrical stimulation and glutamate injection had evoked a decrease in MBP and HR elicited a consistent decrease of MBP and HR of 30± 4.9 mmHg (two tailed paired t test, $p<0.001$, n=6) and 31±9.6 bpm (two tailed paired t test, $p<0.01$, n=6), respectively, with duration of response within 1 to 2 minute in control rats (Fig. 1a). Also the frequency of discharge of phrenic nerve has decreased after microinjection of ethanol that provoked a short-term suppression of respiratory activity (10-30s) immediately after the ethanol injection as is better seen in phrenic nerve activity representation in Fig.1a.

Ethanol microinjection into NTS in rats with chronic exposure to alcohol produced a decrease of both MBP (of 49±6.5 mmHg, n=5, $p<0.05$) (Fig.1b) and HR (of 35±15.1 bpm, n=5, $p<0.05$) (Fig.1.c). The higher BP response to EtOH microinjections was statistically significant in comparison with control group (unpaired t test $p=0.540$). The cardiac response to NTS EtOH-microinjection did not significantly change in comparison with control group. No changes on respiratory depression were observed

between the two groups of animals. CSF microinjection did not evoke any changes on the recorded variables both in control and ethanol addict rats.

Intravenous ethanol injection in control rats produced a triphasic response: first, a slightly increased of MBP (interval between 1-3 minutes) followed by a decrease and then returning to baseline values (97±4.0 versus 106±3.9 mmHg, $p<0.05$, n=6). No significant changes were observed on HR (396±40.2 versus 408±13.3 bpm, p=0.090, n=6, paired t test).

Ethanol administration intravenously in chronic EtOH fed rats produced a slightly decreased in MBP (118±6.9 versus 120±6.6 mmHg, p=0.087, n=5) (Fig.1.b). Heart rate did not significantly change after central ethanol application (390±8.6 versus 388±8.0 bpm, p=0.178, n=5, paired t test) (Fig.3). Also, no significant changes were recorded on phrenic nerve activity both in control and chronic EtOH fed rats.



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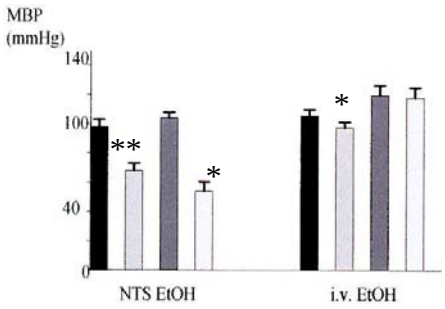

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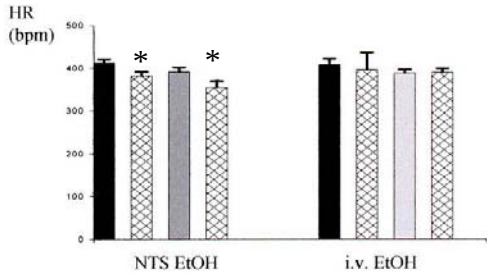

Fig. 1. (a) Representative tracing of the changes on blood pressure (BP), heart rate (HR) and phrenic nerve activity (Phr) evoked by ethanol microinjection into the NTS of a normal rat. (b) Graph bars showing the changes on mean blood pressure (MBP) elicited by ethanol administration, either centrally (NTS) or peripherally (i.v.), in normal and chronic EtOH fed rats. (C) Heart rate (HR) changes produced by EtOH intravenous and central administration in NTS of normal and chronic EtOH rats. Black and dark grey bars are base-line values before EtOH administration either into NTS or intravenously. Open grey and cross hatched bars are changes in MBP and HR, respectively, induced by ethanol when microinjected into NTS or intravenously in normal and chronic EtOH rats. Values are means±SEM. *P<0.05 ,P<0.001 vs. control groups.**

EtOH effect on heart rate variability (HRV) and blood pressure variability (BPV)

a) Effect on HRV and BPV power spectra of the EtOh microinjections in the NTS

Representative tracing of systolic arterial pressure (SAP) and RR signals wavelet decomposition can be observed in Fig.2. An increased on HF component both in BPV and HRV is revealed in correspondent details of wavelet decomposed SAP and RR signal to EtOH administration into NTS. In control rats, ethanol microinjection into NTS produced an increase of HF component (parasympathetic mediated) both in HRV and BPV (normalized blood pressure HF=0.956±0.012 versus 0.750±0.089, paired t test, p=0.053, n=6, and normalized R-R HF=0.945±0.017 versus 0.853±0.050, paired t test, p=0.050, n=6) (Fig.3 a and b). Spectral analysis showed an increased LF component power in HRV produced by NTS ethanol application, in two rats, but only in one rat LF/HF ratio increased comparative with base-line control. In this animal, an increase in LF was produced only on first moments of ethanol microinjection, the wavelet decomposition showing that the main change to ethanol microinjection was an increase in HF component. If analysis had been made only in frequency domain this behavioural response of NTS neurons to EtOH microinjection would gave false information about EtOH effect on NTS autonomic modulation. A significant decrease of BPV (1311.500±525.950 versus 2746.601±1148.020 $mmHg^2/Hz$, Wilcoxon test, p=0.008, n=6) was recorded on NTS-EtOH administration. A decreased of HRV was not significant (234.210±180.680 ms^2/Hz versus 286.481±267.940 ms^2/Hz, Wilcoxon test,

p=0.187, n=6), but in two rats an increase in HRV was produced by the rise of HF component.

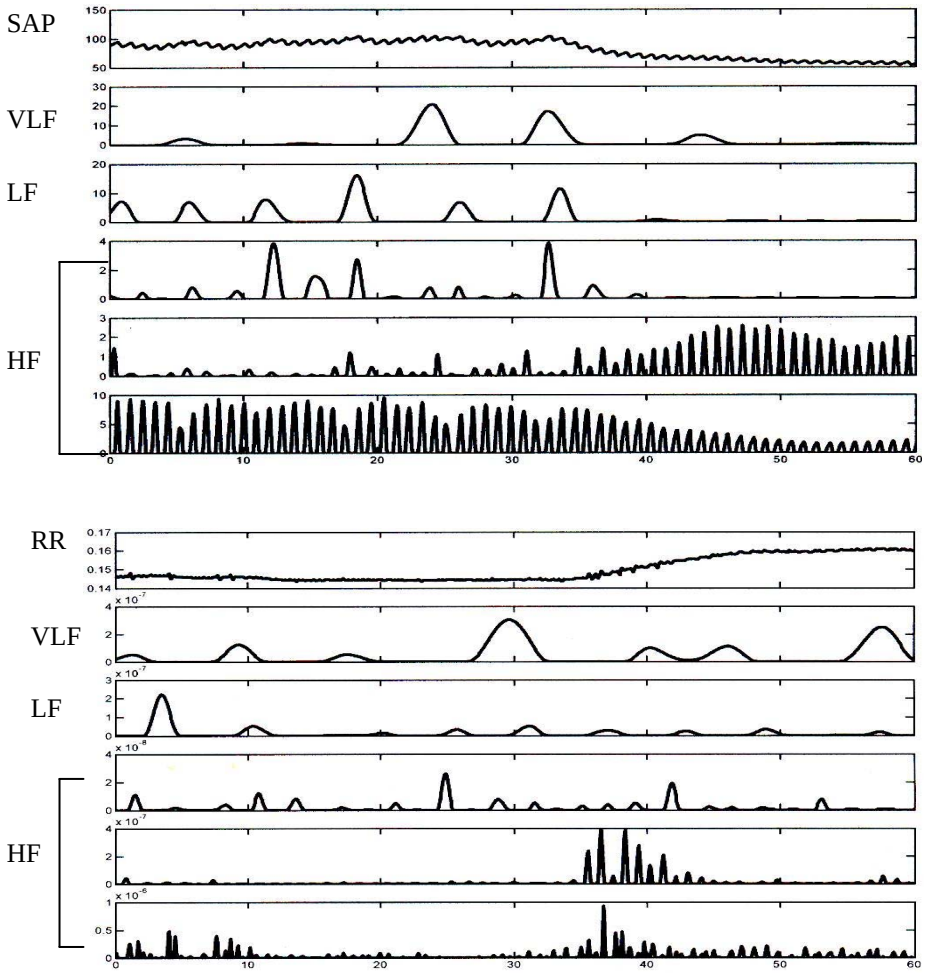

Fig. 2. Representative tracing of wavelet decomposed systolic arterial pressure (SAP) (upper panel) and RR (lower panel) signals recorded on ethanol microinjection into NTS in normal rats. VLF=very low frequency; LF=low frequency and HF=high frequency components.

A similar effect to that evoked in control rats was observed on EtOH microinjection on BPV and HRV in chronic EtOH rats: BPV significantly decreased from 4161.712±1438.010 to 1405.210±360.080 $mmHg^2/Hz$ (Wilcoxon test, p=0.016, n=5) but no changes were seen in HRV (344.09±326.18 versus 754.06±736.89 ms^2/Hz,

Wilcoxon test, p=0.219, n=5). However, in one rat was observed an increased in HRV with an increase in HF power. Ethanol microinjection into NTS produced a rise in parasympathetic flow, reflected especially in BPV. Normalized HF of BPV rose from 0.546±0.077 to 0.794±0.093 (t test, p=0.044, n=5). The higher HF component in HRV was not statistically significant (0.880±0.0674 versus 0.779±0.051 in base-line condition, t test, p=0.362) because of presence of two lower power spectral values.

Fig. 3. Mean changes in normalized high frequency (HF) component of systolic arterial pressure (SAP) and RR signals produced by EtOH microinjection into NTS in normal and chronic EtOH rats. Black and dark grey bars are base-line values in normal and chronic EtOH rats, respectively. Open dark bars and horizontally hatched bars are mean changes in HFn in blood pressure variability (a) and heart rate variability (b), respectively, produced by NTS EtOH. Values are means±S.E.M.

Rats with chronic exposure showed a large interindividual variation of LF/HF ratio. Normalized LF component was higher than in normal rats for blood pressure signal (0.434±0.083, n=5, versus 0.250±0.009, n=6, unpaired t test, p=0.081**).** The spectral analysis of base-line SAP and R-R signal has been shown no significantly changes in BPV and HRV in chronic rats versus control rats.

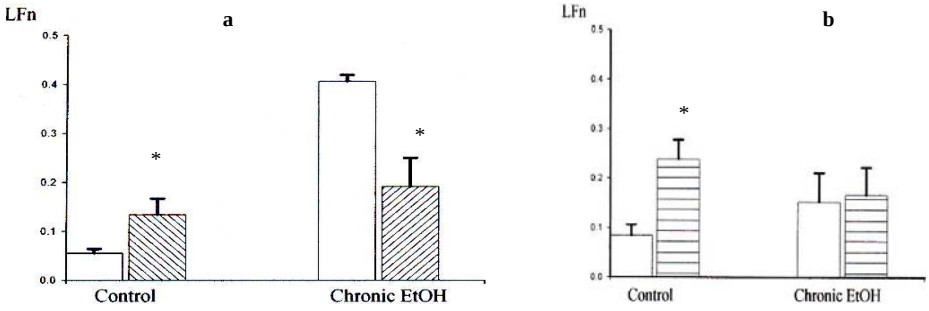

Fig. 4. Changes in normalized low frequency (LF) component of systolic blood pressure and RR signals recorded to ethanol intravenously administered in normal and chronic EtOH fed rats. White bars are base-line values of LF component in normal and chronic EtOH rats. Diagonally and horizontally hatched bars are mean values of LFn in blood pressure variability (a) and heart rate variability (b), respectively, after ethanol intravenously administered. Values are means±SEM.

b) Effect on HRV and BPV of the peripheral injection of EtOH

In control rats moderate doses of ethanol injected through a peripheral vein produced a higher decrease in BPV (from 7932.508±6560.010 to 746.700±240.520 $mmHg^2/Hz$, Wilcoxon test, p=0.031, n=6) in comparison with the autonomic response elicited, with the same dose, in chronic-EtOH rats (from 2751.743±734.980 to 1756.836.2±828.871 $mmHg^2/Hz$, Wilcoxon test, p=0.031, n=5). In control rats, alcohol injection produced a non-significant decreased of HRV (from 59.284±28.414 to 56.780±27.857 ms^2/Hz, Wilcoxon test, p=0.219, n=6) that was due to the fact that one rat from this group presented an increased HRV (especially the HF component) and this effect was associated with an augmented hypotensive effect produced by ethanol injection.

The stronger decrease of HRV was elicited by ethanol intravenously administered in chronic EtOH rats (20.779±12.186 versus 38.930±21.874 ms^2/Hz, Wilcoxon test, p=0.031, n=5). In chronic animals the intravenous administration of ethanol showed a significant decreased in LF/HF ratio of BPV (normalized LF power of SAP signals decreased from 0.406±0.130 to 0.192±0.058, paired t test, p=0.0453, n=5) (Fig.4a) in comparison with normal rats where ethanol injection produced an increased in LF/HF ratio (from 0.055±0.009 to 0.134±0.033, paired t test, p=0.0464, n=6). Intravenously ethanol produced a rise of sympathetic outflow to the heart mainly in normal rats. Normalized LF component of HRV increased significantly (paired t test, p=0.0428, n=6) from 0.0842±0.022 to 0.239±0.039 in normal rats and from 0.1518±0.059 to 0.166±0.056 (t test, p=0.021, n=5) (Fig.4b) in chronic ETOH rats. One rat showed an opposite profile of its HRV spectrum with a decreased LF component being effect associated with a more hypotensive effect evoked by the intravenous ethanol injection.

Discussions

The primary result of our study was the demonstration that ethanol addict rats have a marked imbalance of its autonomic outflow to the cardiovascular system which is clearly observed when the wavelets methodology is applied to the study of the variability of blood pressure and heart rate of these animals.

Data based on a number of prospective, cohort, cross-cultural, and case-control studies in diverse populations consistently reveal that chronic alcohol consumption is associated with hypertension [12,32], arrhythmias [13], cardiomyopathies [22] and increase nocturnal hypoxemia [28] being the underlying mechanisms of these effects unclear.

In our experiments in normal and ethanol addict rats, ethanol was microinjected in the nucleus tractus solitarius (NTS), a key area of integration of autonomic afferent information located in the caudal medulla. Efferent information originated at NTS level is conveyed to two major medullar areas: the vagal nucleus where is originated the parasympathetic information and the rostroventrolateral medulla where the sympathetic tone is generated. The balance between these two branches of the autonomic nervous

systems is able to affect the cardiovascular and respiratory systems. Direct effects of ethanol microinjections in the nucleus tractus solitarius are poor documented in literature and the existing ones are contradictory. Zhang [33] found in the rat, that low to high ethanol concentrations had no effect on baseline mean arterial pressure, heart rate, or sympathetic efferent discharge when microinjected into nucleus tractus solitarius, the dorsal motor nucleus of the vagus, the rostralventrolateral medulla, or the posterior hypothalamus. Conversely, other authors reported that doses of 25 - 200 nmol/site of ethanol microinjected bilaterally in rat dorsal vagal complex and nucleus tractus solitarius elicited a pressor response [29]. Our results show that ethanol microinjection in the NTS lead to hypotension and bradycardia both accompanied by a transient inhibition of the central respiratory drive. The apparent contradictory results of NTS ethanol effects on cardiovascular variables could be due to the different doses of ethanol centrally microinjected. In fact, in our experiments, a moderate dose of ethanol was used and elicited the same changes on NTS mediated cardiovascular and respiratory responses both in normal and chronic EtOH fed rats. In chronic EtOH rats, the amplitude of hypotension and duration of response was higher than those evoked by the same dose in normal rats. These different responses could be due to a decrease on baroreceptor reflex [1] or to an increase in NMDA receptor function [3] or adenosine receptor modulation as suggested by Diao et al [6].

Our results indicate that EtOH microinjection into the NTS increased the HF parasympathetic mediated component of the BP power spectrum, which is in accordance with the observed bradycardia and hypotension that are vagally-mediated. The present work suggests that the impaired autonomic balance produced by chronic EtOH exposure, which is revealed by an increase of the sympathetic flow, is a consequence of a neuroadaptation provoked by the acute increase of parasympathetic flow evoked by each ethanol intake. This hypothesis is sustained by the fact that acute microinjection into NTS returns the power of HF component to normal levels of blood pressure variability spectrum. The effect of ethanol microinjection into NTS on heart rate variability is not clear, being necessary a larger number of data to avoid data distortion that could caused by a complex number of factors such as, among others, diffusion of ethanol in adjacent nucleus, non-specific effect on cell membrane, different modulation of excitatory effect mediated by neurotransmitters and/or neuromodulators.

Our analysis of cardiovascular parameters in rats with chronic EtOH exposure shows an imbalance between cardiovascular excitatory and inhibitory neurons. This is revealed by a slight increase of blood pressure, in baseline conditions of chronic EtOH rats, that is resetted to a new balance with new dose of ethanol administration, an effect that is clearly observed in the blood pressure response to intravenous alcohol administration. Furthermore, spectral analysis revealed an augmented low frequency (LF) sympathetic mediated spectral component, especially in BPV, that decreases until reaching normal levels by ethanol administration thus showing the existence of a neuroadaptation to chronic EtOH exposure.

Ethanol effects on neuronal network are similar with addictive drugs. The majority of the systems that are acutely affected by ethanol are also affected by chronic exposure, resulting in an adaptative or maladaptive response that can cause tolerance and dependence. In particular, chronic actions of ethanol likely require changes in signaling by glutamate and GABA receptors and intracellular systems such as protein kinase C [5]. There is an increase in NMDA receptor function after chronic alcohol ingestion, which may contribute to the central nervous system hyperexcitability and neurotoxicity seen during ethanol withdrawal [3]. Arginine vasopressin, acting on V1 receptors, maintains tolerance to ethanol in laboratory animals even after chronic ethanol administration has ceased [10]. Also there are studies that demonstrated the importance of adenosine in mediating the acute and chronic effects of ethanol at multiple levels (i.e., molecular receptors) in the central nervous system [6, 7].

The main result of our study is the non-invasive evaluation of pharmacological induced autonomic changes in a short-term, non-stationary cardiovascular signal analysis. In general, removal of the non-stationary part of the data is necessary before applying other methods such as AR or FFT, STFT. The few existing studies on spectral analysis of cardiovascular signals of the rat, the majority of them using the FFT algorithm, show no clear low frequency (LF) bandwidth delimitation in correlation with sympathetic autonomic modulation. Different authors consider different frequency limits for the LF component in the rat: 0.2-0.8 Hz [2, 25]; 0.047-0.305 Hz [26]; 0.01-0.2 Hz [8]; 0.02-0.6 Hz [11] or an interval with a central frequency of 0.43±0.02 Hz [24]. In our study, we considered LF frequency between 0.2-0.5 as a result of data that showed that frequency between 0.5-1 Hz are highly correlated with centrally induced respiratory rate [21]. However, if a short-time window is used for spectral analysis, it is likely that the estimations are dominated by parasympathetic modulation. Nevertheless, the situation when drug-induced changes are present - where the response to an external input in an open-loop model is analyzed - is different from spontaneously oscillations in a closed-loop system.

In conclusion, our results show that the wavelets technique allows the detection of transient events and the onset of autonomic changes with very high temporal resolution. Also, the acutely ethanol effects on cardiovascular function in rats with chronic exposure to EtOH may be the result of reinforcement processes and long-lasting neuroadaptative changes that modulate autonomic balance after short- and long-time chronically ethanol exposure.

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