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running title: cerebellum and cardiac function

Effect of stimulation of sublobule IX-b of the cerebellar vermis over cardiac function

Isabel Rocha, Victor Gonçalves, Maria Joaquina Bettencourt and Luís Silva-Carvalho.

Institute of Physiology, Faculty of Medicine of Lisbon, Av. Prof. Egas Moniz, 1649-028 Lisbon, Portugal, Unit of Autonomic Nervous System, Instituto de Medicina Molecular, Lisbon, Portugal

Corresponding author:

Professor Isabel Rocha

Address: Instituto de Fisiologia, Faculdade de Medicina de Lisboa, Av. Prof. Egas Moniz, 1649-028 Lisbon, Portugal.

Phone: 00 351 217999434

Fax: 00 351 217999436

E-mail: isabelrocha@fm.ul.pt

Summary

Activation of sublobule IX-b of the cerebellar vermis evokes hypotension, bradycardia and decrease of the phrenic nerve activity in the anaesthetized animal. Cardiac performance during the isovolumic phases of systole and relaxation can be evaluated by dP/dt_{max}, Vpm, $dP/dt/DP_{40}$ and τ, respectively. In the present study, we evaluated the changes on cardiac function evoked by the stimulation of sublobule IX-b. New Zealand white rabbits were anaesthetised, paralysed and artificially ventilated. A posterior craniotomy was made to reveal and stimulate the cerebellar uvula (4s train; 50Hz; 1ms; 20μA). The femoral artery and veins were canulated and a Swan-Ganz catheter was advanced in the upper abdominal aorta to control afterload when inflating the balloon. The left ventricle was catheterized with a Millar catheter. Blood pressure, heart rate, left ventricular pressure were monitored. Results showed a significant decrease on IX-b stimulation of all the indices of systolic function and an increase of τ indicating a decrease on the speed of the relaxation. These data provide the first evidence of the influence of sublobule IX-b on cardiac function. They may contribute to the understanding of the origin the cardiovascular changes that were observed in two patients with vermian and paravermian haemorrhage.

Key-words: Cerebellum, autonomic nervous system, vermis, cardiac function

Introduction

The assessment of ventricular function is an important task in the evaluation of patients with known or suspected heart disease. There are several parameters that can be used to evaluate ventricular function but most of them are relatively dependent of afterload and preload. The maximum rate of rise of ventricular pressure [dP/dt max], largely independent of afterload changes provided these occur before aortic valve opening, is influenced both by large changes of preload and by acute changes of contractility [Gleason and Braunwald 1962]. An index that is less affected by pre-load and is not affected by afterload is $dP/dt/DP_{40}$ which is the ratio between dP/dtmax and the developed ventricular pressure computed at a DP of 40mmHg [Braunwald 1988], where developed pressure is defined as the left ventricular pressure minus the end-diastolic pressure. To access directional changes of contractility, an other index relatively independent of afterload and preload, the peak of dP/dt/TP [TP being the total pressure development], which is also designated as Vpm, can be applied [Braunwald 1988]. Previous work by several authors had shown that the time-course of the fall of left ventricular pressure after dP/dtmin has an exponential profile that defines an index- τ – which allows the characterisation of the isovolumic relaxation phase [Leite-Moreira 1997, Weiss *et al* 1976].

Stimulation of sublobule IX-b of the posterior vermis of the cerebellum provokes in the anaesthetized animal a cardiovascular response characterized by hypotension and bradycardia, and an accompanying decrease of phrenic nerve activity [Bradley *et al* 1987a]. Previous studies [Bradley *et al* 1987a, Bradley *et a,* 1987b, Gonçalves *et al* 2002, Rocha *et al* unpublished observations] have shown that this depressor response is due to a decrease n sympathetic activity rather than an increase of the parasympathetic outflow. As yet no data are available relating the activation of sublobule IX-b on cardiac function, and in particularly on inotropic state.

In a previous study, we reported a clinical case of a cerebellar haemorrhage as cause of a neurogenic pulmonar oedema [Gonçalves *et al* 2005]. We suggested that the observed cerebellar vermian and paravermian haemorrhage compressing the cerebellar uvula was the cause of the

initial sympathetic storm that elicited a observed tachycardia and a marked rise in blood pressure that preceded the acute pulmonary oedema. Also, others, in a recently published clinical case [Deininger *at al* 2006] report changes on cardiac function of a patient with no history of cardiac disease who suffered progressive tachycardia, fibrillation and electromechanical decoupling with the rupture of an angioma of the cerebellar vermis. Thus, the purpose of the present work is to study, in the anesthetized rabbit, the changes elicited on cardiac function by the activation of sublobule IX-b of the cerebellum. A preliminary report of this work has been published in abstract form [Gonçalves *et al* 2006].

Methods

Anaesthesia, Surgical Protocol

Nine New-Zealand white rabbits (2.7-3.2kg) were anaesthetized with sodium pentobarbitone (40mg.kg^{-1}; ip) supplemented as required. The depth of the anaesthesia was assessed by pinching a paw before neuro-muscular blockade with pancuronium bromide, (4mg/Kg/h) and by observing changes on arterial blood pressure (BP) and heart rate (HR) after blockade. A tracheotomy was made low in the neck to allow the insertion of a tracheal cannula for artificial ventilation with O_2-enriched air (rate of ventilation- 50 55cpm for an end-tidal CO2 of 4.5-5%). The left femoral vein was cannulated for injecting drugs or saline. Through the femoral artery was introduced a Swan-Ganz catheter (4F), which tip was placed in the abdominal aorta, for measuring BP and also to evoke afterload increases by inflating its balloon. The urinary bladder was cannulated and drained to avoid inhibition of cardiorespiratory reflexes [Daly 1997]. The right common carotid artery was identified, isolated and catheterized at cervical level and through it a Millar micro-tip catheter (Millar, USA) was introduced, under RX control (BV300, Philips), in the left ventricle. The confirmation of the location of its tip was made by the profile of the blood pressure curve observed. The Rectal temperature was maintained at 37.5-39°C by a servo controlled heating blanket (Harvard Apparatus Ltd). The electrocardiogram (ECG) was recorded (Neurolog, Digitimer) with the use of needle electrodes inserted into the limbs and heart rate derived with the use of an instantaneous ratemeter (Neurolog, Digitimer).The animal's head was placed in a stereotaxic frame (Kopf Instruments) and a craniotomy performed to expose the uvula and to allow the insertion of a double-barreled glass microelectrode for electrical stimulation of sublobule IX-b using the barrel filled with Woods metal, (4s train; 50 Hz; 1 ms; 20 μA- submaximal stimulations) and, using the second barrel and for labeling the stimulated sites with pontamine sky blue dye (2%) in sodium acetate 1M.. Arterial blood pressure (BP), left ventricular pressure (LVP) and ECG were monitored (Neurolog, Digitimer). In the end of the experiment, animals were killed with an overdose of

anaesthetic. All the procedures using animals were performed according to national and E.U. laws on animal experimentation and the principles of laboratory animal care.

Experimental protocol

Sublobule IX-b was identified using electrical stimulation to elicit its characteristic cardiovascular depressor responses- hypotension and bradycardia (supramaximal stimulation - 4s train; 50 Hz; 1 ms; >50μA). After the correct placement of the electrode, one submaximal stimulation (≤ 20μA) was performed during which ventilation was suspended. After stimulation and recovery to baseline conditions a prolonged period of electrical stimulation (15s) was performed during which, and as soon as blood pressure begun to fall, the Swan-Ganz balloon that had been placed in the abdominal aorta was inflated in order to simulate an increase in afterload. During this period of 15s the ventilation was suspended. The volume of the inflated balloon was minimized to maintain blood pressure in the same range of values that was before the beginning of electrical stimulation. This balloon was kept inflated during 5s and, after 5s of its disinflation, electrical stimulation was switched-off.

Histology

After the labeling with deposition of pontamine sky blue of the stimulation sites at the end of the experiment, the cerebellum was removed and fixed in a 4% paraformaldehyde saline with 30% sucrose solution for 48 hours. The tissue was sectioned serially (80 μm) and stained with neutral red. Stimulating sites within the cerebellar uvula were identified according to Meesen and Olszewski [1949] rabbit atlas .

Signal Acquisition and Data analysis

All recorded variables were digitised (Instrutech VR100B, Digitimer Ltd) and recorded on video-tape. Off-line analysis was done using a PowerLab system computer and analysis software (PowerLab).

For the variables recorded (BP, LVP and heart rate), baseline values were taken immediately before the beginning of the stimulation [control]. These values were compared with those obtained at the peak of the response evoked by the stimulation [Stim]. From LVP values dP/dtmax, Vpm, $dP/dt/DP_{40}$ and τ were calculated. The index τ was calculated by the derivative method [Weisfeldt et al 1978, Weiss et al 1976]. Briefly, this method is based on the mathematical principles that a derivative of an exponential function is also exponential and when an exponential function is expressed by other exponential function a linear relation is obtained. That is, the fall in ventricular pressure which is described by an exponential function is transformed in an equation of a line and τ will correspond to the symmetrical of the inverse of the slope of the calculated line equation.

For statistical analysis the *t*-Student test for paired observations was used and values of *t* were considered significant when $p<0.05$. All data are expressed as mean±SD.

Results

Before any electrical stimulation, the baseline values of mean blood pressure [BPm], heart rate [HR] and maximum left ventricular pressure [LVPmax] were 101±9.3 mmHg, 229±12 bpm and 133±11.5 mmHg, respectively. Electrical stimulation of the uvula (4s train; 50Hz; 1ms; 20μA) elicited the characteristic cardiovascular response – bradycardia and hypotension - showing a significant decrease of BPm, HR and LVPmax to 79±7.0 mmHg, 204±8.0 bpm and 108±13.8mmHg respectively ($n=9$, $p<0.05$), as is shown on Figure 1.

The computation of dP/dtmax showed a statistically significant decrease, during-systole on stimulation of sublobule IX-b as dP/dtmax decreased from 2038±81.7 to 1675±118.8 $mmHgs^{-1}$ (Figure 1 and Table 1) while τ increased significantly from 10.9±1.50 to 13.1±1.70 ms (Figure 2 and Table 1) which indicates that IX-b stimulation evokes a decrease of the rate of isovolumic relaxation ($n=9$, $p<0.05$). The values of Vpm and $dP/dt/DP_{40}$ also decreased from 37±3.3 to 30±4.9 s^{-1} and from 34±1.0 to 29±0.6 s^{-1}, respectively ($n=9$, $p<0.05$, Table 1). In relation to end-diastolic pressure no significant modifications were observed as values changed from 7.4±1.51 [basal period] to 8.1±2.28 mmHg,[on stimulation] ($n=9$, $p=0.06$).

Furthermore, during the inflation of the indewlling Swan-Ganz balloon placed in the abdominal aorta which increased afterload and brought LVP on stimulation to values similar to those observed during basal condition, the calculation of dP/dt showed a significant decrease to 1840±70.5$mmHgs^{-1}$ ($n=9$, $p<0.05$). In this condition, heart rate was 200±91 bpm and end-diastolic pressure was 8.3±2.5 mmHg.

Discussion

The primary result of this study is to show that the activation of the sub-lobule IX-b of the cerebellar uvula evokes changes in cardiac function. Furthermore, these results provide indications of the putative origin of the cardiovascular signs observed in patients that had a vermian hemorrhage with compression of the cerebellar uvula.

End-diastolic fibre length, myocardial contractility (inotropism) and relaxation (lusitropism) are the determinants of cardiac ejection and filling and their assessment could be made by parameters that describe contractile function and relaxation.

Most of the parameters that described the contractile function are based on the analyse of pressure measurements obtained during isovolumic contraction and have, as a major advantage, that data are collected before the opening of the aortic valve (Katz, 2001). The majority of these parameters is dependent on the load but dP/dtmax that represents the peak rate of the rise of left ventricular pressure is mainly influenced by contractility [Braunwald 1988, Little, 1987] but regional abnormalities in left ventricular function and the size and thickness of the left ventricle can also affect this parameter (Katz, 2001). Changes in dP/dtmax are known to be sensitive to acute changes in contractility so together with end-diastolic volume and filling pressure, dP/dtmax can be used to evaluate the directional changes in contractility when performing an intervention [Leite-Moreira 1997]. Other indices of cardiac function that are based on events that occur in the isovolumetric phase of the cardiac cycle are $dP/dt/DP_{40}$ where DP is the developed left ventricular pressure (i.e. left ventricular pressure minus end-diastolic pressure) computed at a DP of 40 mmHg, the peak dP/dt/TP, also termed as Vpm, where TP refers to the total pressure development. Other index is Vmax, which corresponds to the maximum velocity of shortening of the unloaded contractile elements, but controversy still exist in relation to its calculation both in isolated myocardial fibres and in the intact heart and despite being independent from pre and afterload appear to have little advantage over the maximum dP/dtmax or over $dP/dt/DP_{40}$. Conversely, $dP/dt/DP_{40}$ is relatively simple to obtain and has advantage over maximum dP/dt

because is relatively independent of the time and level of arterial pressure in the instant of aortic valve opening; but, despite being insensitive to changes in afterload it increases slightly with larges changes in preload. Vpm is an index that is relatively independent of changes in both after and pre-load [Leite-Moreira 1997] but according to others (Katz, 2001) was found to be relatively insensitive to changes of afterload but is influenced by pre-load, decreasing with the increase of left ventricular end-diastolic pressure.

To evaluate relaxation, the maximum rate of fall of left ventricular pressure during isovolumic relaxation -dP/dtmax is reasonable but this index is highly dependent on aortic pressure. Since the decline in left ventricular pressure is often assumed to be exponential with time it allows to express -dP/dtmax as an exponential function thus making possible to calculate a time constant [τ] based on the time required for ventricular pressure to decline to half or 1/e of its peak pressure, beginning at the time of aortic valve closure [Little, 1987].

Accordingly, the three indices of contractile function [dP/dtmax, $dP/dt/DP_{40}$ and Vpm] and the index τ, for analysis of lusitropism were applied in our study. Our results show a significant decrease in cardiac performance as the three indices of systolic function [dP/dtmax, $dP/dt/DP_{40}$ and Vpm] decreased on stimulation and the τ index increased significantly during the activation of sublobule IX-b without significant changes on end-diastolic pressure. Great care was used to minimize the effect of afterload during balloon inflation in the abdominal aorta as the purpose of the inflation during stimulation was to compensate the decrease of pressure evoked by IX-b stimulation. In this condition dP/dtmax values decreased significantly during sublobule IX-b stimulation confirming the decrease on the rate of contraction of myocardial fibres during the isovolumic period of systole.

Several animal studies have shown that the sublobule IX-b of the cerebellar vermis is involved in cardiovascular control [Bradley *et a,* 1987a, Bradley *et al* 1987b, Gonçalves *et al* 2002, Paton and Spyer 1992]. Animal experiments have provide evidence for the co-existence of two functionally distinct pathways from the cardiovascular region of lobule IX –b to the lateral

parabracheal nucleus (PBN) of the pons, which is one of the relay stations of the central autonomic network (Paton and Spyer 1990). In particular, IX-b activation evokes a depressor response, hypotension accompanied by bradycardia, a decrease of respiratory rate and a transient inhibition of the renal sympathetic activity [Bradley *et al* 1987a, Bradley *et al* 1987b] in the decerebrate anaesthetized animal and this depressor response is mediated by inhibitory Purkinje cells that project from the sublobule IX-b to the rostral lateral PBN (Paton and Spyer, 1990). In the decerebrate non-anaesthetized animal, a tachycardia together with a pressor response are observed. These cardiovascular responses appear to be mediated by a different neuronal circuit that includes the caudal part of the parabracheal nucleus and its neuronal connexions [thought the nucleus tractus solitarius of the medulla (NTS)] with the rostroventrolateral medulla (RVLM) which is the origin of sympathetic outflow to the cardiovascular system [Paton *et al* 1990, Paton and Spyer 1992, Silva-Carvalho *et al* 1991]. We should stress that the pressor/ depressor response was reversible in its nature, from the decerebrate to the anaesthetized decerebrate animal, when anaesthetic was given to the animal (Paton and Spyer, 1990) which made authors to suggest that the neuronal circuit through the NTS was more sensitive to anaesthetic action. In conclusion, at the present, the cardiovascular responses from the sublobule IX-b of the cerebellar vermis are presumed to be mediated by two neuronal pathways- one sympatho-inhibitory and, the other, sympatho-excitatory- that relay at the rostral and caudal PBN, respectively, which allow IX-b to access the cardiovascular control network and thereby permitting the modulation of peripheral inputs of cardiovascular neurons within both the NTS and RVLM(Paton, 1997)

In a previous study [Gonçalves *et al* 2005], we reported a case of neurogenic pulmonary edema in a 27 years-old woman caused by a cerebellar haemorrhage due to a vermian and paravermian arteriovenous malformation rupture and we emphasised the involvement of sublobule IX-b, due to its compression by the accumulation of blood, in the increase of sympathetic activity (hypertension and tachycardia) and the observed neurogenic pulmonary oedema. Recently [Deininger *et al* 2006] have reported changes in cardiac function, with

modifications of ventricular kinetics similar to those observed on tako-tsubo cardiomyopathy, in a 23 years-old healthy male subject who suffered a four ventricular haemorrhage due to an angioma of the cerebellar vermis.

These novel findings on cardiac function elicited by sublobule IX-b of the cerebellum together with data from previous work [Gonçalves *et al* 2006, Rocha *et al*, unpublished observations] [where we showed that during IX-b activation the observed cardiovascular changes are due to a decrease of sympathetic activity rather that an increase in the parasympathetic outflow] could, at least partially, be used to speculate on the origin of the cardiac changes observed in the two patients, with a condition comparable with the decerebrate non-anaesthetized animal, where clinical reports showed a vermian haemorrhage and–resulting in changes in cardiac function that were, in nature, reversible after the decompression of the lower part of the brainstem.

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Figure Legends

Figure 1. Effect of electrical stimulation of sub-lobule IX-b of the cerebellar vermis showing the characteristic depressor response- hypotension and bradycardia- accompanied by a decrease in maximum dP/dt which indicates a decrease in the rate of the myocardial fibers during systole (BPm: mean blood pressure; LVP: left ventricular pressure; HR: heart rate)

Figure 2. The rate of isovolumic relaxation decreases during the stimulation of sublobule IX-b as is shown by the rise of the slope of the lines representing basal [τBasal] and stimulation conditions [τStim] taken from one representative animal.

Figure 3. On this figure are depicted the stimulation sites (n=9) showing that they are restricted to the sublobule IX-b of the vermis [ML – molecular layer; GL – granular layer].

Table 1

Parameters	Control values	Values on stimulation
dP/dtmax ($mmHgs^{-1}$)	**2038±81.7**	**1675±118.8**
Vpm (s^{-1})	**37±3.3**	**30±4.9**
dP/dt/DP (s^{-1})	**34±1.0**	**29±0.6**
τ (ms)	**10.9±1.50**	**13.1±1.70**
End-diastolic pressure (mmHg)	**7.4±1.51**	**8.1±2.28 [NS]**

Table Legend

The values of the computed indexes of ventricular function are depicted in this table. The significant decrease of dP/dtmax, Vpm and dP/dt/TP indicate a decrease in the speed of contraction of the myocardial fibres during the isolvolumic phase of systole while τ, by its significant increase, shows a decrease in the speed of relaxation during the isovolumic phase of diastole (n=9; $p<0.05$; data expressed as mean±SD). The previous changes occurred without significant changes of end-diastolic pressure (n=9, p=0.06, [NS- non-significant]).

Figure 1

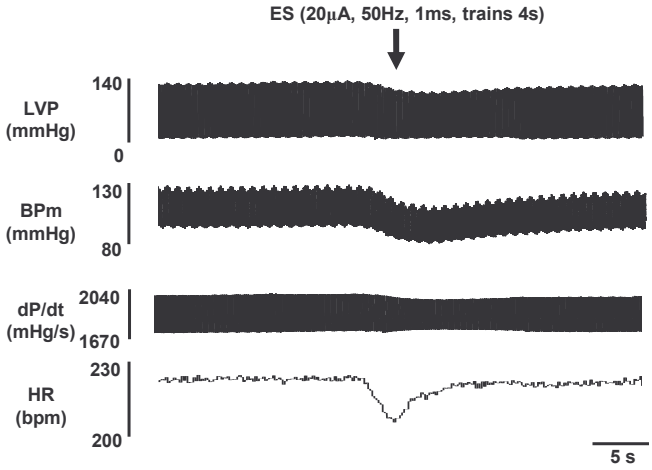

Figure 2

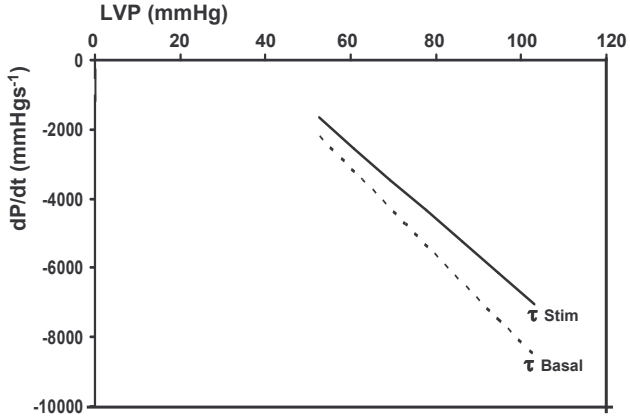

Figure 3