Cerebral Perfusion Pressure and Behavior Monitoring in Freely Moving Rats

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Summary

Cerebral perfusion pressure (CPP) is the net pressure gradient that drives oxygen delivery to cerebral tissue. It is the difference between the mean arterial pressure (MAP) and the intracranial pressure (ICP). As CPP is a calculated value, MAP and ICP must be measured simultaneously. In research models, anesthetized and acute monitoring is incapable of providing a realistic picture of the relationship between ICP and MAP under physiological and/or pathophysiological conditions. For long-term monitoring of both pressures, the principle of telemetry can be used. The aim of this study was to map changes in CPP and spontaneous behavior using continuous pressure monitoring and video recording for 7 days under physiological conditions (group C – 8 intact rats) and under altered brain microenvironment induced by brain edema (group WI – 8 rats after water intoxication) and neuroprotection with methylprednisolone – MP (group WI+MP – 8 rats with MP 100 mg/kg b.w. applicated intraperitoneally during WI). The mean CPP values in all three groups were in the range of 40-60 mm Hg. For each group of rats, the percentage of time that the rats spent during the 7 days in movement pattern A (standard movement stereotype) or B (atypical movement) was defined. Even at very low CPP values, the standard movement stereotype (A) clearly dominated over the atypical movement (B) in all rats. There was no significant difference between control and experimental groups. Chronic CPP values with correlated behavioral type may possibly answer the question of whether there is a specific, universal, optimal CPP at all.

Key words

Telemetry • Cerebral perfusion pressure • Mean arterial pressure • Intracranial pressure • Behavior pattern

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Introduction

Cerebral perfusion pressure (CPP) is the net pressure gradient that drives oxygen delivery to cerebral tissue. It is the difference between the mean arterial pressure (MAP) and the intracranial pressure (ICP), measured in millimeters of mercury (mm Hg). Normal CPP lies between 60 and 80 mm Hg, but these values can shift to the left or right depending on individual patient physiology. As CPP is a calculated measure, MAP and ICP must be measured simultaneously, most commonly by invasive means. In both preclinical and clinical settings, accurate CPP can only be monitored invasively for a short period of time in unconscious patients or anesthetized animals. In research models, anesthetized and acute monitoring is incapable of providing a realistic picture of the relationship between ICP and MAP under physiological and/or pathophysiological conditions. For long-term monitoring of both pressures, the principle of telemetry can be used. Telemetry has been commonly cited in publications for monitoring both ICP and MAP in rodents since 2014 [1,2]. We utilized dual pressure telemetry technology to monitor both pressures simultaneously in the same animal in 2019 and 2020 [3,4]. The continuous ICP and MAP data gave a first ever look into CPP levels over a 72-hour period in unrestrained and conscious rats. Monitoring CPP over longer periods allowes us to gain new insight on the relationship between ICP and MAP, and, in addition, unrestrained and conscious methods enhance animal welfare, while providing a more translational research

model. The aim of this study is to map changes in CPP and spontaneous behavior using continuous pressure monitoring and video recording for 7 days under physiological conditions and under altered brain microenvironment induced by brain edema and neuroprotection with methylprednisolone in the rat experimental model.

Methods

All experiments were approved by the Ethical Committee of the First Faculty of Medicine (Charles University in Prague) and were in agreement with the Guidelines of the Animal Protection Law of the Czech Republic and Guidelines for the treatment of laboratory animals EU Guidelines 86/609/EEC. For experiments, male rats of the Wistar strain weighing 400-410 g of our own breed were used.

Animals

A total of 24 experimental animals was divided into three groups of 8 rats. Control intact rats formed group C. Animals with cytotoxic cellular brain edema induced by water intoxication were in the WI group, and rats with neuroprotection constituted the WI+MP group.

Induction of edema by water intoxication (WI)

WI consists of fractional administration of distilled water + vasopressin. The modified method of WI is based on intraperitoneal (i.p.) administration of distilled water (DW) in the total amount corresponding to 20 % of body weight in three consecutive doses over 24 h (after eight hours) with a simultaneous administration of desmopressin. Each sub-dose represented one-third of the total dose of 0.032 mg/kg of desmopressin (1-desamino-8-D-arginine vasopressin) (OCTOSTIM®, Ferring). Desmopressin is an antidiuretic hormone, which potentiates the effect of hyperhydration by inducing hyponatremia [5,6].

Administration of MP

Methylprednisolone (Solu-Medrol®, Pfizer) was applied intraperitoneally. The total amount of MP 100 mg/kg bw was divided into three subdoses and administered during edema induction with each dose of both the distilled water and vasopressin.

Telemetry

The DSI™ telemetry system (Data Sciences International) was used to monitor ICP and MAP. Its implantable components were the transducer and two pressure sensors.

Spontaneously breathing rat under the inhalation anesthetic isoflurane (Forane ®, AbbVie Ltd.) in concentration of 2 volume percentage underwent in the prone position the longitudinal incision of the skin and subcutaneous tissue in the midline of the head, free galea aponeurotica was dissected, and the skull was trephined 3 mm lateral to the midline at right and 3 mm frontally to bregma and the ICP pressure sensor was inserted into the extradural space. Transducer was placed in a subcutaneous pocket formed on the back at the level of cervicothoracic transition.

The incisura was closed with a continuous suture and the rat was then turned to the supine position. The microsurgical approach exposed the common carotid artery (CCA) on the right. From the arteriotomy the MAP pressure sensor was placed into the CCA lumen with the sensor tip located as close as possible to the cranial base. The wound was closed by continuous suture and inhalation anesthesia was terminated. The awakened and freely moving animal was placed in a cage on a receiver that transmitted signals from the transducer to the PC hardware. The recorded and stored data were evaluated by the software as average pressure values and in the form of a pressure curve during the entire 7-day reference period for each group of study rats.

Video recording

During the entire 7-day monitoring period, the rats were under constant camera control (Amcrest system) with the possibility of recording and retrospective analysis. During the experiment, rats moved freely in their natural environment with free access to food and water. Cages were placed on receiving plates in a 22 °C room with a natural light/day and dark/night cycle.

Video recording was triggered by the movement of the animal to avoid unnecessary registration during sleep, which in rats significantly outweighs wakefulness during the 24 h cycle [7,8]. To analyze the behavior of awake animals, we defined two fundamentally different movement patterns: A (standard movement stereotype) – the animals presented themselves with all basic movement patterns, i.e. locomotion ("front-wheel drive" – dominant forelimbs), rearing (exploratory behavior) and grooming (comfort behavior); B (atypical movement) – the animals did not show any of the classical movement

patterns, they moved chaotically. Classification into each movement category was performed by minute-long visual analysis.

For each group of rats, the percentage of time that the rats spent during the 7 days in movement pattern A or B was defined. The total number of minutes during which the rats were awake and moving in each group corresponded to 100 %.

The video recording time of awake, moving rats for behavioral analysis was 3,210 min (100 %) for the C group, 2,950 min (100 %) for the WI group, and 3,180 min (100 %) for the WI+MP group. The camera system software divided the entire recording into minute time intervals that exactly corresponded to the equal minute time interval of the ICP and MAP pressure curves. For a given moment, it was thus possible to accurately determine the corresponding current value of the pressure to the movement pattern (A or B).

Termination of the experiment

After seven days, each rat was reanesthetized with isoflurane, the transducer and both sensors were removed, and the animal was sacrificed by an overdose of anesthetic.

Statistical analysis

The results were statistically evaluated using the GraphPad Prism program (parametric ANOVA and nonparametric Kruskal-Wallis test), the statistical significance was set at 5 %.

Results

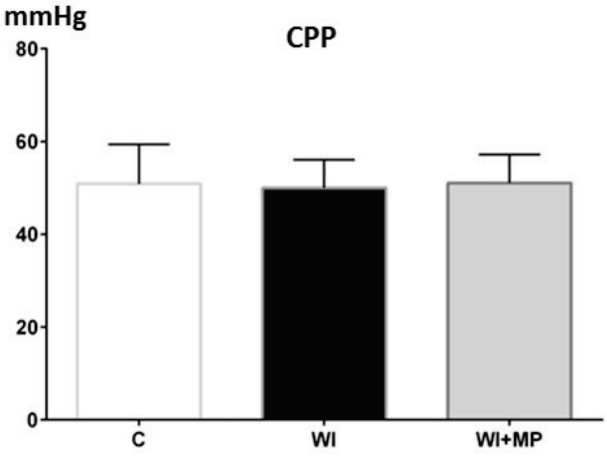

Fig. 1. Values of CCP. The bars represent values of CPP from 8 animals in each group during 7 days, mean ± SEM. x-axis: CPP values (mm Hg), y-axis: bars with mean value ± SEM, C=control group (intact animals); WI (WI group – the edema induction by the water intoxication method); WI+MP (WI+MP group – the intraperitoneal administration of MP together with distilled water during the edema induction).

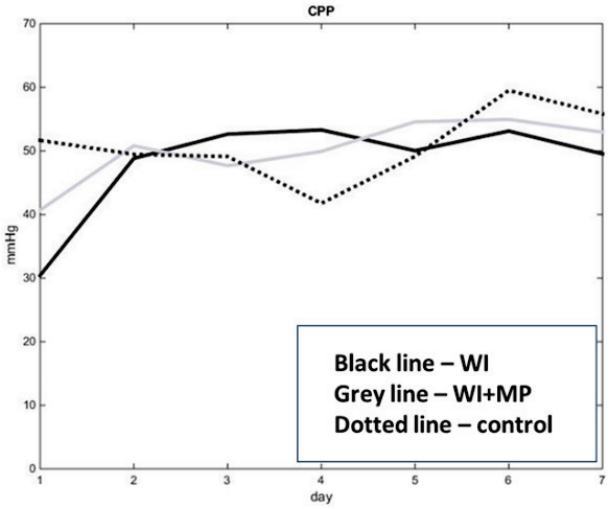

Fig. 2. Fluctuations in CCP values (pressure curves) over a period of 7 days. x-axis: CPP values (mm Hg), y-axis: days. Dotted line: control group (intact animals); black line: WI group (the edema induction by the water intoxication method); grey line: WI+MP group (the intraperitoneal administration of MP together with distilled water during the edema induction).

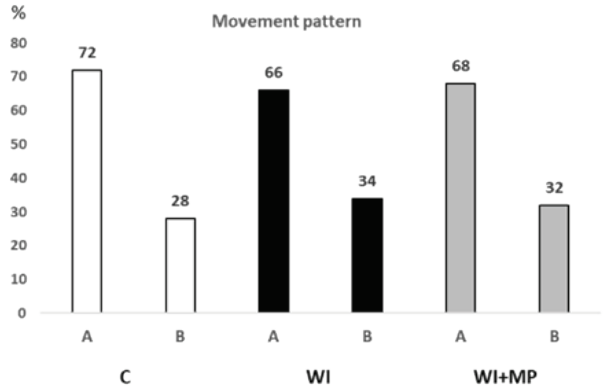

Fig. 3. Types of movement patterns over a period of 7 days. x-axis: the percentage of time that the rats spent during the 7 days in movement pattern A or B (%), y-axis: white bars: control group (intact animals); black bars: WI group – the edema induction by the water intoxication method; grey bars: WI+MP group – the intraperitoneal administration of MP together with distilled water during the edema induction. A bars: standard movement stereotype (the rats presented themselves with all basic movement patterns, i.e. locomotion, rearing and grooming; B bars: atypical movement (the rats did not show any of the classical movement patterns, they moved chaotically).

The mean CPP values in each group are shown in Figure 1. The values in all three groups were in the range of 40-60 mm Hg (C=50.89±3.462; I=49.98±2.719; WI+MP=51.06±2.496) and no significant statistical difference was found between them.

The pressure curves (Fig. 2) in the individual groups differed greatly over the course of 7 days. The pressure curve in the control group (intact animals, dotted line) started and ended at values around 50 mm Hg, its

course was not linear and between the 4th and 6th day it showed a difference in measured pressures of 20 mm Hg. The course of the pressure curves in the WI (black line) and WI+MP (grey line) groups was similar – a sharp rise during the first two days was followed by a gradual linear increase with the resulting pressure value 10 mm Hg and 20 mm Hg higher than the initial pressure, respectively.

Figure 3 shows the percentage representation of the time interval of standard movement stereotype (A) and atypical movement (B) in individual groups. The A/B ratio was 72 %/28 % in the C group (white bars), 66 %/34 % in the WI group (black bars), and 68 %/32 % in the WI+MP group (grey bars). No statistically significant difference was found between the groups.

Discussion

Today the implantable radiotelemetry devices allow to continuously monitor MAP and ICP of laboratory animals without the stress artefacts associated with restraint.

The miniaturized, biocompatible radio telemetry devices are surgically implanted and the live physiologic data can be automatically collected by sophisticated, easy-to-use electronic data collection systems. Implantable radiotelemetry has improved significantly over the last 10-15 years and is now considered the state of the art for collecting a wide variety of physiologic parameters from freely moving animals [9].

The principle of telemetry in freely moving rats for long-term monitoring of both MAP and ICP was established in 2014 [1,2], and we published the first experience with telemetric monitoring of both pressures simultaneously in the same animal for 72 h five years later [3,4].

Although the implantation of components is a standard procedure, a few notes should be attached. The positions of the ICP pressure sensor and transducer during telemetry monitoring are uniform. The sensor is inserted into the extradural space through a borehole 3 mm lateral to the right midline and 3 mm frontal to bregma, and the transducer is placed in a subcutaneous pocket created on the back at the level of the cervicothoracic junction.

For blood pressure monitoring (MAP) the transducer is in rats usually positioned in the peritoneal cavity with the pressure sensor advanced upstream into the abdominal aorta. When monitoring both pressures simultaneously, the location of the blood sensor should be different. In our studies, the MAP sensor was always inserted into the CCA because the original definition of CPP is based on MAP measurement at head level [10]. In clinical practice, MAP is usually measured from the level of right atrium where the pressure sensor is implanted *via* the venous pathway (v. subclavia, v. jugularis). With a standard head elevation of 30 degrees in patients with brain edema, due to the height difference between the location of the ICP sensor and the MAP sensor, the CPP value is up to 11 mm Hg higher than that with the MAP sensor at the cranial base level [11]. In the existing experimental models of telemetric monitoring of both pressures, abdominal aorta [2] or femoral artery [1] were used to store the pressure MAP sensor. During vertical motor activities (rearing or grooming), a certain discrepancy is possible in the telemetric measurement of the monitored pressures due to the height difference of the sensors. During rearing, the height difference between the brain and the abdominal aorta region in a rat with an average weight of 400 g is about 4 cm. To minimize potential errors, we chose the CCA for MAP pressure sensor placement as the closest arterial region relative to the ICP sensor placement.

CPP plays a vital role in maintaining the physiological function of neurons, as it ensures a continuous and adequate supply of energy sources – oxygen and glucose. CPP is a calculated value derived from the difference between MAP and ICP [12,13]. There is still no consensus on what the optimal value of CPP is because the existence of a single ideal CPP for pathological conditions of different etiologies is unlikely. Among these conditions, each of which affects CPP in its own way, are the following: head injury, cerebrovascular disorders, hydrocephalus, brain tumor, brain edema, 'benign' intracranial hypertension, CNS infection, and metabolic encephalopathy [14]. Also CPP resulting from any given MAP varies from individual to individual due to a number of variable factors. Cerebral tissue oxygenation represents the balance between oxygen supply and consumption, largely reflecting the adequacy of cerebral perfusion. Multiple physiological parameters determine the oxygen delivered to the brain, including blood pressure, hemoglobin level, systemic oxygenation, microcirculation and many factors are involved in the delivery of oxygen to its final recipient, through the respiratory chain [15]. The cerebrovascular system, unlike other systems, exhibits autoregulation, a physiological process that refers to the specific ability to

maintain a constant cerebral blood flow (CBF) regardless of changing systemic blood pressure: CBF=CPP/CRV (CBF = cerebral blood flow, CPP = cerebral perfusion pressure, CRV = cerebrovascular resistance). Autoregulation is mediated by a myogenic mechanism – higher MAP increases vascular tone and vasoconstriction occurs, and conversely, vasodilation occurs when MAP and vascular tone decrease. It is an almost immediate process (vessel tone reacts within 1-10 s from a change in pressure), it only works at MAP values in the range from 50 to 150 mm Hg, and its main mediators are endothelium-derived relaxing factor (EDRF) and nitric oxide (NO). Above and below these MAP values, CBF is dependent on changes in systemic blood pressure. The arbitrary value of "normal" CPP is now considered to be the range between 70-90 mm Hg, and CPP values <50 mm Hg lead to irreversible damage to brain functions through brain ischemia [12,13].

The results of this study reflect the current inconsistency in the view of optimal CPP values. Our first telemetric monitoring of ICP and MAP over 72 h confirmed mean CPP values above 70 mm Hg in both intact rats and rats with brain edema, which was explained by MAP values in the autoregulatory range. Several episodes of CPP that fell below 70 mm Hg were registered, but no correlate of behavioral change was available due to the absence of continuous behavioral monitoring of the rats [3,4]. In this study the mean values of CPP in all three groups were in the range of 40-60 mm Hg with no significant statistical difference between them, but pressure curves for 7 days in each group recorded CPP fluctuations of up to 20 mm Hg. Thus, the average CPP values hovered around the lower limit of autoregulation.

The 7-day continuous recording of the rats' behavior is of great importance for evaluating the results of this study. Normal rat behavior is characterized by three basic movement stereotypes. The first pattern of behavior is locomotion, representing a horizontal movement activity. The other two activities are vertical: rearing – which in addition to motor activity represents also the exploratory behavior, and grooming – which is included into the category of comfort behavior [16].

In the case of locomotion, it is necessary to take into account that the rat is primarily "front-driven" – its forelimbs are dominant for spontaneous locomotion [17]. A corticospinal pathway supported by extrapyramidal motor systems is essential for normal forelimb function [18]. Rearing is classified as a vertical movement activity, but unlike locomotion, the movement is performed in a position on the hind legs with the forelimbs resting on the walls of the cage. The goal of the movement is to get to know the environment and, as a rule, to search for a food source [19]. In addition to the role of motor pathways, rearing is generated from the medial prefrontal cortex, amygdala, and hippocampus [20]. Grooming is a motor activity performed exclusively with the front limbs in a resting position on the hind limbs (comfort behavior) [19]. Grooming in a rat consists of 4-5 sequences routinely, up to 100× repeated movements coordinated into a craniocaudal syntactic pattern. This "non-stressful and comfortable grooming" is an innate pattern and constitutes the main activity of the rat when it is awake. A completely different type of grooming is "displacement grooming", in which the syntactic pattern of movement is completely broken down and the rat produces chaotic, uncoordinated movements of the upper limbs. The anatomical structure generating syntactic grooming is in the striatum along with a number of connections with the brainstem [21].

In this study, rats were divided into groups A and B according to behavior. In group A, the rats presented themselves with all three basic movement stereotypes. In group B, the rats did not show any of the basic movement stereotypes, their behavior was uncoordinated and chaotic.

It is a known fact that rats are able to sleep up to 15 h a day, in other words, they spend only about 24 % of the 24-hour cycle awake [7,8]. In this study, rats were awake and moving during the 24-hour cycle in the control group in 31 %, in the group of animals with induced brain edema (WI) in 29 %, and in the group with neuroprotection (WI+MP) in 31.5 %. For each group of rats, the percentage of time that the rats spent during the 7 days in movement pattern A or B was defined. The total number of minutes during which the rats were awake and moving in each group equalled 100 % and from this the A/B ratio, i.e. the predominant behavior, could be determined: 72 %/28 % in group C, 66 %/34 % in group WI, and 68 %/32 % in the WI+MP group. It is clear from the results that even at very low CPP values, the standard movement stereotype (A) dominated over the atypical movement (B) in all rats. Because the camera system software divided the entire recording into minute time intervals that exactly corresponded to the same minute time interval of the ICP and MAP pressure waveforms, it was possible to accurately determine the matching actual pressure value with the movement pattern (A or B) for

the corresponding moment. For standard movement stereotype A, current CPP values were higher than 40 mm Hg, for atypical movement B they were around 20 mm Hg.

Thus, it appears that only knowledge of long-term and chronic CPP values can provide objective insight into the interrelationships between ICP and MAP under physiological and pathological conditions. The fact that there was no significant difference between the individual groups in the average values of CPP or in the behavior of the rats can be explained by the gradual absorption of cerebral edema [22], as clearly shown by the changed course of the pressure curves after the 48th hour of registration (Fig. 2).

It is the translational research using telemetry to determine chronic CPP values with correlated behavioral type that may answer the question of whether there is a specific, universal, optimal CPP at all. The answer to this question is of fundamental physiological importance, considering that a constant CBF of ~50-60 ml/100 g/min [23] has never been challenged, although it depends almost exclusively on the value of CPP: CBF=CPP/CRV [12,13].

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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