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MAGNESIUM AND THE TREATMENT OF SOME CARDIOVASCULAR DISEASES

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Abstract

In this short article the treatment of some cardiovascular diseases with magnesium is reviewed. Magnesium is one of the most important minerals in almost all physiological systems of the body including the cardiovascular one. The most important functions dependent on magnesium are calcium antagonism, membrane sealing or stabilization, regulation of energy transfer, control of oxidative phosphorylation, glycolysis, production and function of ATP, hormone receptor binding; gating of calcium channels; transmembrane ion flux and regulation of adenylate cyclase; muscle contraction; neuronal activity; control of vasomotor tone; cardiac excitability; and neurotransmitter release. The cardiovascular benefits of magnesium were proved especially in acute myocardial infarction, supraventricular and ventricular cardiac arrhythmias, cardiopulmonary surgery, arterial hypertension, congestive heart failure, antithrombotic therapy, arterial thrombus formation and acute ischaemic stroke. In acute myocardial infarction magnesium administration may provide cellular protection during ischaemia and early i.v. magnesium administration is a useful addition to standard therapy of AMI. In cardiac arrhythmias the both supraventricular and ventricular origin, administration of magnesium reduces frequency of their occurrence but it is ineffective in polymorphic ventricular tachycardia not associated with long QT interval. Many of the actions of magnesium are thought to be caused by calcium antagonism. Magnesium is of significance in the pathomechanisms of reperfusion injury and reduction of malignant arrhythmias in the critical acute phase of myocardial infarction, if applied intravenously. Patients undergoing heart or cardiopulmonary surgery are at risk of magnesium deficiency and hypomagnesaemia may contribute to postoperative arrhythmias. The treatment of cardiovascular diseases with magnesium is useful during magnesium deficiency, in other cases it should be performed with caution.

Key words: Magnesium - Cardiac arrhythmia - Myocardial infarction - Cardiac surgery

1. MAGNESIUM IN THE HUMAN BODY

Magnesium is one of the most important minerals in the body. It is the fourth most common cation in the organism, and the second most common intracellular cation after potassium. It has a fundamental role as a co-factor in more than 300 enzymatic reactions, it is also involved in several processes including: hormone receptor binding; gating of calcium channels; transmembrane ion flux and regulation of adenylate cyclase; muscle contraction; neuronal activity; control of vasomotor tone; cardiac excitability; and neurotransmitter release (1). In many of its actions magnesium functions like a physiological calcium antagonist.

In extracellular fluid there is only 5 % of the total body magnesium, a representative of intracellular level is magnesium in granulocytes. Most of intracellular magnesium content is in the bones (53%), intracellular parts of muscles (27%) and soft tissues (19%), only about 1% of the total body magnesium is in blood serum and red blood cells (2). Approximately 90 % of intracellular magnesium is bound to organic compounds. The reference value of magnesium in the blood serum is 0.7-1.1 mmol/l and 1.7-8.2 mmol/24 h in urine. Biological availability of magnesium is also dependent on anion to which it is bound (sulphate, oxid, chloride, asparaginate, etc.).

In the blood serum it is in 3 forms - ionized, protein bound and in complexes with anions (3). Equilibrium between the tissue pools is reached slowly and the actual serum magnesium level does not inform us on the status of main stores. The daily average requirement is a little higher for males. Rich sources of magnesium in the diet include spring water regularly, cereals and leguminous plants, but their preparation at cooking decreases considerably its content. Its absorp-

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tion is situated in the ileum and colon. Excretion and serum magnesium control occurs predominantly in the kidneys. Most of reabsorption is in the ascending limb of the renal loop of Henle. Magnesium intake is very low in the last decades because of its decreased content in the food sources or incorrect food preparation. The required daily amounts of some minerals are shown in Table 1.

2. PHYSIOLOGICAL IMPORTANCE OF MAGNESIUM

Magnesium is important in almost all physiological systems in the ionic form Mg^{2+}. The most important functions dependent on magnesium are the following: calcium antagonism, membrane sealing or stabilization, regulation of energy transfer, glycolysis, control of oxidative phosphorylation, production and function of ATP (4, 5). Its importance was found in the central and peripheral nervous systems, cardiovascular, respiratory, endocrine and reproductive systems (1). Magnesium has a marked effect on the regulation of transmembrane sodium and potassium movement, a depressant effect on the release of catecholamines, the symptoms of neuromuscular, autonomic and psychological excitability. A more fundamental interaction between magnesium and other ions occurs at the cellular level. (1).

Magnesium deficiency is present in 7-11% of hospitalized patients (e.g. in diabetes, hyperaldosteronism, hyperparathyreoidism or interstitial tubular nephritis) and is found to co-exist in up to 40% of patients with other electrolyte abnormalities. Its deficiency is very frequently connected with cardiovascular diseases and cardiac deaths caused by a diet and drinking water low in magnesium (4) or by some diseases. The causes of magnesium deficiency are shown in Table 2, treatment of magnesium deficiency in Table 3. Beneficial effects of the treatment of cardiovascular diseases by magnesium were noted by many authors in many countries. Three areas of

Table 1: Required daily amounts of minerals according to several authors.

Mineral	Daily required amounts
Sodium (Na)	3.0 g
Potassium (K)	1.0 g
Chlorine (Cl)	3.5 g
Calcium (Ca)	0.8 to 1.2 g
Phosphorus (P)	0.8 to 1.2 g
Iron (Fe)	12 to 18 mg
Iodine (I)	60 to 200 mg
Magnesium (Mg)	300 to 400 mg
Copper (Cu)	1.5 to 4 mg
Zinc (Zn)	10 to 15 mg
Fluor (F)	1.4 to 1.8 mg
Selenium (Se)	25 to 50 µg
Manganese (Mn)	2.5 mg
Chromium (Cr)	25 µg
Molybdenum (Mo)	25 µg
Nickel (Ni)	5 µg
Tin (Sn)	10 µg
Silica (Si)	10 µg
Vanadium (Va)	10 µg

Table 2: Causes of magnesium deficiency development (1).

1. Reduced dietary intake
2. Poor gastrointestinal absorption
3. Increased losses from the gastrointestinal tract (a) Diarrhoea (b) Vomiting (c) Laxative use
4. Increased renal losses (a) Congenital or acquired tubular defects (b) Diabetes mellitus (c) Alcoholism (d) Drug induced losses (diuretics, angiotensin converting enzyme inhibitors, aminoglycosides, amphotericin, cyclosporin and cisplatin)
5. Other causes (a) Increased requirements (growth, pregnancy) (b) Excessive sweating

Table 3: Treatment of magnesium deficiency (1).
Magnesium supplementation

1. Emergency – intravenous route: 8-16 mmol immediately 40 mmol over the next 5 hours
2. Severely ill - intramuscular route 48 mmol on day 1 17-25 mmol on days 2-5
3. Other cases - oral route 15 mmol per day

cardiovascular diseases such as myocardial infarction, cardiac arrhythmias and cardiopulmonary surgery are considered to be proved in management by magnesium.

3. MAGNESIUM AND ACUTE MYOCARDIAL INFARCTION

The role of magnesium in coronary artery disease has been evaluated extensively during the last three decades. The intravenous application of magnesium in acute myocardial infarction (AMI) is of major importance, its beneficial effects have been underlined in several studies. The patients dying suddenly from ischaemic heart disease (IHD) had lower concentrations of myocardial tissue magnesium and potassium than the controls (6) and there were more deaths in the cities with soft water, which is relatively lacking in magnesium and calcium (7). Magnesium has many functions which could be important in AMI, not only in ischaemic-infarcted tissue but also during reperfusion, whether spontaneous, pharmacological or by angioplasty (1). During ischaemia, the aerobic metabolism decreases and intracellular ATP is depleted. As the majority of ATP within the cell is in the form of the magnesium salt, cellular magnesium is also depleted. Moreover, anaerobic metabolism leads to intracellular acidosis and to an increase in mitochondrial uptake of calcium which further inhibits ATP synthesis. Magnesium administration may provide cellular protection during ischaemia.

Magnesium drives calcium into the sarcoplasmic reticulum, reduces mitochondrial calcium overload (8) and competes with calcium for binding to troponin C. Magnesium also inhibits calcium influx into myocytes and thus prevents increases in intracellular concentrations of calcium which are known to be harmful to cellular function. Magnesium helps to conserve cellular ATP as the magnesium salt and therefore preserves energy-dependent cellular activity, particularly in the face of adrenergic overstimulation occurring during ischaemic episodes (1). Other beneficial effects include improvement of the contractile response of stunned myocardium (1, 9) and limitation of infarct size (10). Magnesium increases the excitability threshold of myocardial cells (11), it is a cofactor for sodium-potassium ATPase. Magnesium may also reduce reperfusion injury by inhibition of calcium overload and it may protect cells from free radical damage (12).

Many of the actions of magnesium are thought to be caused by calcium antagonism. When infused, magnesium causes a decrease in peripheral resistance associated with a secondary increase in cardiac index, with little change in arterial pressure or heart rate. Inhibition of the sinus node by magnesium is probably offset by inhibition of acetylcholine release at the vagal nerve terminals (1). Comparing of published data on the haemodynamic effects of magnesium administration in awake subjects have shown that coronary vasodilatation was accompanied by a significant increase in coronary perfusion (13). Intravenously administered magnesium suppressed exercise-induced angina pectoris caused by coronary artery spasm by improving regional myocardial blood flow (14).

Magnesium inhibits basal, myogenic and hormone induced smooth muscle contraction and also has a direct vasodilator effect (15). Magnesium blocks calcium entry into vascular smooth muscle cells via voltage- and receptor-operated channels and it diminishes the reactivity of these cells to a variety of pressor agents. In the same way, magnesium competes with calcium to inhibit the contractility of coronary arteries. In vitro withdrawal of magnesium increases coronary artery tone and potentiates the contractile response to angiotensin, 5-hydroxytryptamine, norepinephrine, acetylcholine and potassium (16). It is known for a long time that magnesium inhibits the release of catecholamines in AMI. It also modulates coagulation by inhibition of platelet function at high concentrations and stimulation of the release of prostacyclin from vascular endothelium (1). Thrombus formation is also modified by administration of magnesium (17). Both acute and chronic depletion of extracellular magnesium are harmful to the myocardium in the setting of AMI. Whether magnesium therapy is merely replacing a deficit or acting as a pharmacological agent is not yet completely solved.

In the large LIMIT-2 study patients with AMI were treated by magnesium 8 mmol over 5 min before thrombolytic therapy, followed by 65 mmol as an infusion over the next 24 hours. Serum magnesium concentrations were doubled for 24 hours after admission but returned to normal

by 48 h. A reduction in fatality rate after 28 days and a lower incidence of left ventricular failure (LVF) was found. There was no difference in the incidence of hypotension, arrhythmias (particularly early ventricular fibrillation) or requirements for anti-arrhythmic agents. The reduction in LVF was associated with a corresponding reduction in fatality rate from IHD (18). The conclusion of this study was that early i.v. magnesium is a useful addition to standard therapy in AMI.

The Slovak Republic is one of the first countries where magnesium (ADALAT, TROMCARDIN, CARDILAN) was used in the treatment of myocardial infarction with positive results already before 1970. The similar positive results were reached also by peroral treatment.

Clinically important differences have been found between the two big studies (LIMIT 2 and ISIS 4) and in some of earlier studies. They are different in the time of administration of magnesium and its relationship to thrombolytic therapy, variation in the doses administered in the first 24 hours and duration of magnesium therapy, and differences in patient risks in control and treatment groups (1). For the protection of heart magnesium concentrations must be increased before reperfusion occurs. The timing of magnesium administration in relation to either spontaneous reperfusion or after thrombolysis is likely to be critical (1). The therapeutic time for modifying the external concentration of magnesium is probably in the first 12 min of reperfusion; as the beneficial effects of magnesium are diminished when it is administered 1 h after reperfusion (19). That means that magnesium is cardioprotective in AMI, its administration is beneficial before spontaneous reperfusion or thrombolysis and in high-risk patients (1). The latest papers show that short-term mortality is not reduced with early administration of intravenous magnesium in high-risk patients suffering AMI.

4. MAGNESIUM AND CARDIAC ARRHYTHMIAS

While both atrial and ventricular arrhythmias have been associated with hypomagnesaemia (9), this relationship is unclear because of the poor correlation between serum and myocyte magnesium concentrations and the close interaction of magnesium with potassium metabolism. The multiple roles of the magnesium ion in cardiac muscle have difficult interpretation of available data on hypomagnesaemia as a cause or precipitant of arrhythmia (1). Intravenous administration of magnesium is associated with reduction in the incidence of ventricular tachycardia (VT) and ventricular fibrillation (VF) and a smaller non-significant reduction in the incidence of asystole and electromechanical dissociation (1). It is known that administration of magnesium can reduce the frequency of arrhythmias. Side effects of the treatment include an increase in bradyarrhythmias and skin flushing on administration. It is assumed that the antiarrhythmic effect of magnesium is the main mechanism of mortality reduction (1). That mechanism is probably by slow inward calcium current block, which decreases sinus node rate, prolongs the atrioventricular (AV) conduction time and increases the AV node refractivity without major changes in ventricular physiology (20).

Magnesium can be effective in the treatment of both supraventricular and ventricular tachyarrhythmias associated with AMI, long QT syndromes and digoxin toxicity (21). There is no reason to give intravenous magnesium unless patients have other indications for repletion, such as a low magnesium level or arrhythmia responsive to magnesium therapy (22). It has been demonstrated that magnesium increases the threshold for ventricular tachycardia or fibrillation (23). The British Resuscitation Council recommends for management of broad QRS complex tachycardia 10 ml of a 50% solution of magnesium sulphate given over 1 h (24).

However, the use of magnesium in the treatment of polymorphic VT can be associated with marked prolongation of the QT interval and torsades de pointes. Magnesium can be successful in drug-induced torsades de pointes (25) where, despite normal serum potassium and magnesium concentrations, all patients responded to i.v. boluses (1-2 g) of magnesium and no adverse side effects were documented. The mechanism of action of magnesium is not very clear as it has no effect on heart rate or QT interval, which suggests that it does not shorten delayed repolarization of the myocardium (1). Magnesium is ineffective in polymorphic VT not associated with long QT interval (26), as well.

Table 4. Treatment of cardiac arrhythmias with magnesium (1).

1. Emergency treatment of (a) Torsades de pointes (b) Digoxin toxicity (c) Any serious supraventricular or ventricular arrhythmia, especially when hypokalaemia co-exists
2. Dose 2 g over 10-15 min Repeat once if necessary

A short recommendation in treatment of most important cardiac arrhythmias is shown in Table 4. The first positive results in the treatment of cardiac arrhythmias were reached also with prostacyclin. Myocardial infarction and cardiac arrhythmias are pathological conditions which are known to show the biological rhythms (27), circadian, infradian or ultradian ones.

5. MAGNESIUM AND CARDIOPULMONARY SURGERY

Patients undergoing heart or cardiopulmonary surgery are at risk of magnesium deficiency because of pre-existing diuretic therapy and heart failure (28). Hypomagnesaemia is common after cardiopulmonary bypass surgery and may contribute to postoperative arrhythmias (10). In cardiogenic shock developing after cardiopulmonary bypass and unresponsive to therapeutic intervention, magnesium administration was successful (29).

6. CONCLUSIONS

In this short article the treatment of some cardiovascular diseases with magnesium is reviewed. Magnesium is of significance in the pathomechanisms of reperfusion injury and reduction of malignant arrhythmias in the critical acute phase of AMI, if applied intravenously. However, the promising results of the LIMIT-2 study could not be confirmed by the data of the ISIS-4 study. The timing of magnesium therapy is probably the most important key factor. Similar to the guidelines of thrombolytic intervention, magnesium has to be administered as early as possible, at the latest before myocardial reperfusion has started. Nevertheless, because of conflicting results of prior trials doubts on the efficacy of intravenous magnesium in AMI still remain.

Other cardiovascular diseases, where magnesium administration can be beneficial, are arterial hypertension (30), congestive heart failure, antithrombotic therapy, arterial thrombus formation, atherosclerosis and acute ischaemic stroke. Experimentally induced low plasma levels of magnesium accelerate atherogenesis by increasing LDL concentrations and their oxidative modifications, and by promoting inflammation. In vitro studies have shown that low magnesium determines endothelial dysfunction, the initiating event leading to the formation of plaque. Moreover, oral magnesium therapy has been shown to improve endothelial function in patients with coronary artery disease (31). Addition of magnesium to drinking water inhibits atherogenesis (32). Beneficial effects of magnesium supplementation was found also in heart failure (33, 34).

We recommend to use the results of the multinational, multicentric trial MAGIC which has been set up to evaluate the optimal patient cohort as well as the ideal dose regimen for the application of intravenous magnesium sulphate in patients with AMI (35). The role of magnesium in the treatment of chronic diseases, however, is poorly understood. The treatment of cardiovascular diseases with magnesium is useful during its deficiency, in other cases it should be performed with caution.

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INVOLVEMENT OF 5-HT$_{1B}$ RECEPTORS IN THE CENTRAL CARDIOVASCULAR REGULATION IN NORMOTENSION AND DURING CONTROLLED, STEPWISE HAEMORRHAGIC HYPOTENSION IN RATS – STUDIES WITH CGS-12066A

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A b s t r a c t

Background and aim: The serotoninergic system influences various activities of the central nervous system, including cardiovascular regulation. Recent studies demonstrate that centrally acting serotonin may be involved in the initiation of the sympathoihibitory phase of cardiovascular responce in haemorrhagic shock. The study was designed to determine the role of 5-HT$_{1B}$ receptors in cardiovascular regulation in normotension and in a model of blood volume/blood pressure-controlled haemorrhagic hypotension.

Methods: Studies were performed in normovolaemic ethylurethane-anaesthetised male Wistar rats treated intracerebroventricularly (icv) with 5-HT$_{1B}$ receptor agonist CGS-12066A (5, 10 , 15 μg) or saline (10 μl). In separate groups of rats, after pre-treatment with CGS01206A and saline, blood was withdrawn from the right femoral vein to stepwise reduce MAP to 60, 40 and 20 mmHg. Each bleeding period lasted for 5 min, and at the end of that period the bleeding volume was determined.

Results: In normovolaemic animals, CGS-12066A produced dose-dependent (10-15 μg) depressor effect accompanied by bradycardia. The action was associated with a decrease in renal, hindquarters and mesenteric blood flow. The non-active dose of CGS-12066A (5 μg) in normotensive animals evoked an increase in blood volumes necessary to induce hypotension of 40 and 20 mmHg, without the influence on the heart rate.

Conclusion: The study demonstrates that the serotoninergic system, via 5-HT$_{1B}$ receptors, may influence the central cardiovascular regulation in normotension and during pronounced haemorrhagic hypotension, probably as a result of the inhibition compensatory mechanisms.

K e y w o r d s: CGS-12066A, serotoninergic system, cardiovascular regulation, hypotension, rats

INTRODUCTION

The integrated response to acute blood loss consists of two distinct phases of neurohumoral and haemodynamic regulation (1, 2). In the initial sympathoexcitatory phase, reflex reaction from arterial baroreceptors of systemic circulation and/or low-pressure cardiopulmonary receptors causes stimulation of the sympathetic nervous system, which results in an increase in heart rate (HR) and vascular resistance of musculocutaneous and splanchnic vascular beds. In addition to an increase in vascular resistance, blood is moved from the venous reservoirs and diuresis is limited, which together cause the maintenance of blood pressure, despite the fall in cardiac output.

After a loss of 20-35% of the total blood volume and critical reduction of the central blood volume, the second phase, the so-called sympathoinhibitory phase, develops (2). The signal from cardiopulmonary afferents, possibly originating in the left ventricle, initiates the withdrawal of sympathetic vasoconstrictor drive in the whole sympathetic nervous system except for the part innervating the adrenal medulla. The fall in arterial pressure is due to a decrease in total peripheral resistance, especially in the renal, gastrointestinal and muscular vasculature. The decrease in HR with the fall in arterial pressure appears to be vagally mediated (1). Mean arterial pressure (MAP) falls abruptly in this phase, despite the activation of humoral mechanisms, including the increase in catecholamine output by the adrenal medulla, activation of the renin-angiotensin system and the increase in plasma arginine vasopressin (AVP) level (1).

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There is the general agreement that in pre-terminal conditions of haemorrhagic shock endogenous analgesic (opioidergic) and anti-analgesic (melanocortinergic, cholecystokininergic, thyreoliberininergic, histaminergic) systems in the central nervous system become activated (3-5). Studies of recent years reveal the resuscitating effects of many anti-analgesic (non-opioid) neurotransmitters, including ACTH and many ACTH-fragments (6), CCK peptides (7), thyrotropin-releasing hormone (8) and histamine (9-10), at doses which show little or no activity under normal conditions. Moreover, it is suggested that the serotoninergic system may contribute to the development of the sympathoinhibitory phase of regulation in haemorrhagic shock, since methysergide, the 5-HT_1/5-HT_2 receptor antagonist, prevents the sympathoinhibitory phase of the response to simulated haemorrhage in conscious rabbits (11).

The present paper, a continuation of previous studies on cardiovascular regulation in haemorrhagic shock (12-14), demonstrates the involvement of 5-HT_{1B} receptors in the central cardiovascular regulation in normotension and during stepwise haemorrhagic hypotension in ras.

METHODS

Male Wistar rats weighing 230-250 g (5-6 months old) were used in all experiments. The animals were housed five per cage, in controlled conditions of temperature (20-22°C), humidity (60-70%), lighting (12 h light/dark cycle) and provided with food and water *ad libitum*. The Ethical Committee of the Silesian Medical University approved all procedures.

For *icv* treatment, the rats were prepared 5-7 days before the experiment by implantation, under ketamine/xylazine (100 mg/kg/10 mg/kg; *ip*) anaesthesia, of polyethylene cannula into the right brain lateral ventricle (9). Animals were then kept in individual cages until the time of the experiment. All *icv* injections were made in a volume of 10 µl over a period of 60 s, and the correctness of injections was verified as described previously (9).

On the day of the experiment, after induction of general anaesthesia with ethylurethane (1.25 g/kg; *ip*), the rats were implanted with catheters filled with heparinized saline (300 IU/ml) in the right carotid artery and the right jugular vein.

MAP and HR were recorded by the pressure transducer RMN-201 (Temed, Zabrze, Poland) and the electrocardiograph Diascope 2 (Unitra Biazet, Białystok, Poland), respectively. Electromagnetic probes (Type 1RB2006, Hugo Sachs Elektronik, March-Hugstetten, Germany) were implanted around the right renal and the superior mesenteric arteries to monitor renal (RBF) and mesenteric (MBF) blood flow, and around the distal abdominal aorta, below the level of the ileocaecal artery, to monitor perfusion of the hindquarters (HBF) using Transit Time Flowmeter Type 700 (Hugo Sachs Elektronik, March-Hugstetten, Germany) (15). All measurements of blood flow were started after a 30 min adaptation period to avoid influences of probe implantation.

To study cardiovascular effects of the central 5-HT_{1B} receptors, a selective agonist CGS-12066A (5, 10, 15 µg; *icv*) was injected in three groups of normotensive rats, and MAP and HR changes were monitored within 1 h after treatment.

To produce standardised haemorrhagic hypotension, according to the modified method of Sándor *et al.* (16), blood was withdrawn from the right femoral vein into the calibrated cannula to stepwise reduce MAP to 60, 40 and 20 mmHg. Each bleeding period lasted for 5 min and MAP was kept constant at the predetermined level by taking more blood away or reinfusing some. At the end of each period the bleeding volume was determined with an accuracy of 0.1 ml. Five minutes before beginning of bleeding, rats were injected with CGS-12066A (5 µg; *icv*) or saline. The dose of 5 µg of CGS-12066A was chosen since it did not produce any changes in MAP in normotensive animals.

Body temperature was monitored by a rectal thermometer and maintained at 36.5-37.5_C using the heating lamp throughout the experiment. All the experiments were performed between 8 a.m. and 2 p.m.

The following drugs were used: CGS-12066A maleate [7-trifluoromethyl-4(4-methyl-1-piperazinyl)-pyrrolo[1,2-a]quinoxaline maleate], xylazine hydrochloride (Research Biochemicals Inc., Natick, MA, USA), ethylurethane (Riedel-de Haën, Seelze, Germany), ketamine (Gedeon Richter,

Budapest, Hungary) and heparin (Polfa, Warszawa, Poland). All drug solutions were prepared fresh on the day of the experiment.

All the data are given as means ± standard error with $P < 0.05$ considered as the level of significance. Statistical evaluation was performed by one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls test.

RESULTS

The pre-haemorrhage MAP and HR values in the control saline-treated group were 89.1 ± 4.8 mmHg and 331 ± 17 beats/min, respectively. There were no differences in initial MAP and HR between all groups.

Cardiovascular effects of CGS-12066A in normotensive rats

The effects of CGS-12066A (5-15 μg; *icv*) on MAP and HR in normovolemic animals are presented in Fig.1A-B. The dose of 5 μg of CGS-12066A did not produce any significant MAP and HR changes, whereas higher doses revealed significant dose-related decreases in MAP and HR which started within 5 min after treatment, reached the maximum within 20-30 min, and lasted up to 50-60 min.

Initial renal RBF, HBF and MBF in the saline-treated control group are 6.55 ± 0.93 ml/min, 8.59 ± 1.43 ml/min and 7.27 ± 1.98 ml/min, respectively. The treatment with of CGS-12066A (15 μg; *icv*) on led to a maximal decrease in RBF, HBF MBF by 18.0%, 16.3% and 19.7%, respectively (Fig. 2A-C).

Influence of CGS-12066A on bleeding volumes necessary for stabilization of graded hypotension of 60, 40 and 20 mmHg

Bleeding volumes necessary to induce hypotension of 60, 40 and 20 mmHg after treatment with CGS-12066A and saline are presented in Table I.

Table I. Effects of CGS-12066A (5 μg; *icv*) and saline (10 μl; *icv*) on the bleeding volumes required to reduce MAP to 60, 40 and 20 mmHg in rats. Data are presented as mean ± SD, six animals per group. * $P < 0.05$, ** $P < 0.01$ *vs.* the saline-treated group

	Blood volume (ml per 100 g body weight)		
Treatment	60 mmHg	40 mmHg	20 mmHg
Saline (10 μl; *icv*)	0.61 ± 0.23	1.13 ± 0.18	2.53 ± 0.31
CGS-12066A (5 μg; *icv*)	0.56 ± 0.18	0.87 ± 0.14*	1.87 ± 0.35**

In the control *icv* saline-treated group, bleeding volumes required to reduce MAP to 60, 40 and 20 mmHg were 0.61 ± 0.23, 1.13 ± 0.18 and 2.53 ± 0.31 ml/100 g body weight, respectively. CGS-12066A at a dose of 5 μg significantly decreased the bleeding volumes necessary to induce hypotension of 40 ($P < 0.05$ *vs.* the control group) and 20 mmHg ($P < 0.01$ *vs.* the control group), while it did not influence the bleeding volume required to reach hypotension of 60 mmHg.

Influence of CGS-12066A on HR changes associated with induction of hypotension of 60, 40 and 20 mmHg

In the control group, the consecutive bleeding periods were associated with the decrease in HR by 15.2 ± 4.9%, 24.6 ± 5.3% and 47.1 ± 11.2%, respectively. Treatment with CGS-12066A did not produce any significant differences in HR in comparison to the control group (data not given).

DISCUSSION

Results of the present study reveal that stimulation of 5-HT_{1B} receptors in anaesthetised normotensive rats leads to a decrease in MAP with bradycardia. Moreover, the studies based on the

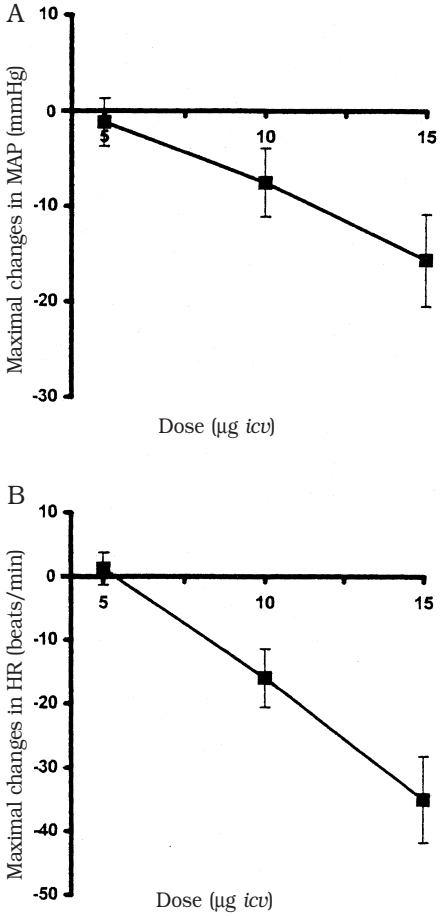

Fig. 1. Maximal changes in MAP (A) and HR (B) induced by CGS-12066A (5-15 µg; *icv*) in normovolemic rats. Data are presented as mean ± SD, six animals per group. Baseline MAP and HR values were 89.1 ± 4.8 mmHg and 321 ± 17 beats/min, respectively. For doses of 10 and 15 µg of CGS-12066A, $P < 0.05$ *vs.* the saline-treated control group

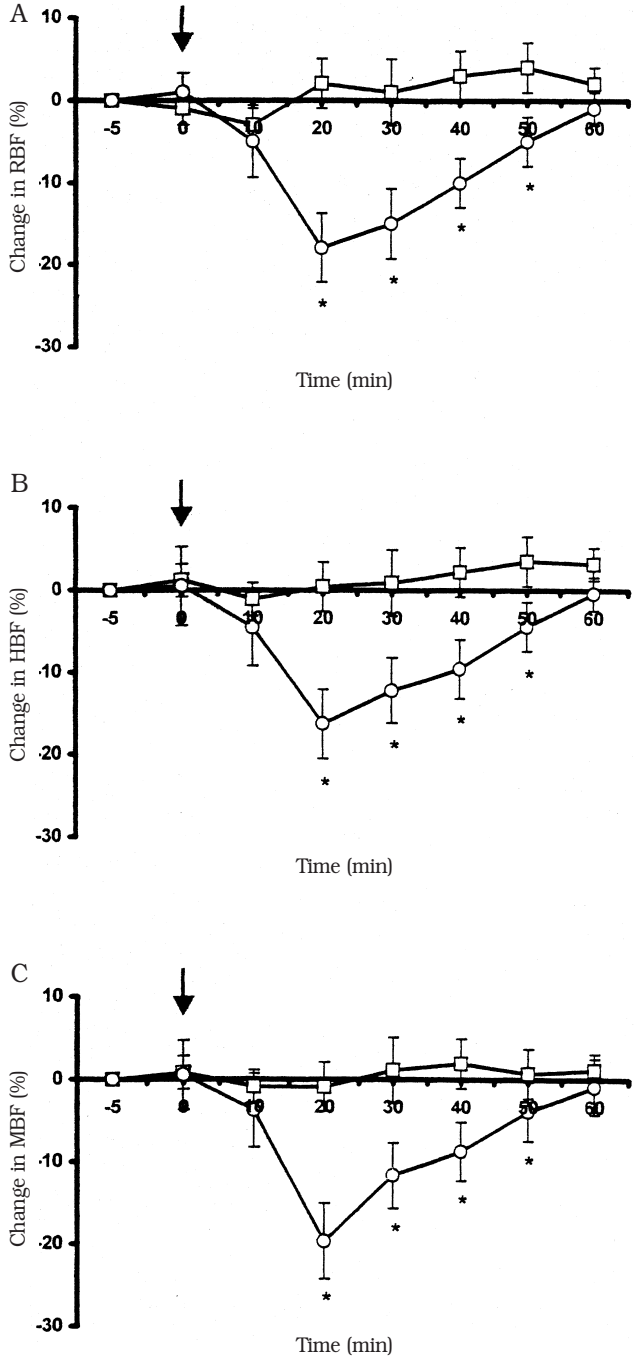

Fig. 2. Changes in renal (RBF; A), hindquarters (HBF; B) and mesenteris blood flow (MBF; C) after *icv* treatment (0 min; arrow) with CGS-12066A (15 µg; ○) and saline (10 µl; ❑) in normotensive rats. Data are presented as mean ± SD, six animals per group. Initial RBF, HBF and MBF in the saline-treated control group are 6.55 ± 0.93 ml/min, 8.59 ± 1.43 ml/min and 7.27 ± 1.98 ml/min, respectively. * $P < 0.05$ *vs.* the saline-treated group

blood volume/blood pressure-controlled model of stepwise haemorrhagic hypotension demonstrate that CGS-12066A produces a decrease in blood volumes required to induce hypotension of 40 and 20 mmHg.

Administration of exogenous serotonin, its precursors, receptor agonists/antagonists and agents depleting brain serotonin are generally accepted pharmacological methods used to study

the biological effects of the serotoninergic system. Serotonin does not cross the blood-brain barrier and, that is why, its brain content depends on the *de novo* synthesis within neurones. The precursor, L-tryptophan, is converted by tryptophan hydroxylase to 5-hydroxytryptophan (5-HTP) which is metabolised to 5-HT by ubiquitous L-aromatic aminoacid decarboxylase. Peripheral administration of either L-tryptophan or 5-HTP produces large increases in cerebral 5-HT content.

Administration of L-tryptophan (25-100 mg/kg; *iv*) produces no changes in MAP in normotensive rats, which can be explained by two mechanisms. Firstly, L-tryptophan evokes a marked inhibition of the firing of the ascending and probably the descending midbrain raphe neuronal tracts, which turns off the presynaptic 5-HT system. Therefore, L-tryptophan loading can increase synaptic serotonin level in the area of the raphe nuclei, but apparently produces a decrease in synaptic 5-HT in the forebrain (17). Secondly, tryptophan loading does not lead to an increase in synaptic 5-HT since the neurotransmitter is rapidly metabolised intracellularly by monoaminooxidase (MAO). On the other hand, 5-HTP administered both in conscious rats and anaesthetised rats produces dose- and time-dependent decreases in MAP (17).

Cardiovascular effects of serotonin administered centrally depend on animal species, concentration and site of drug injection (18). In contrast to 5-HTP, exogenous serotonin administered *icv* or into the anterior hypothalamus/preoptic area in anaesthetised rats produces an increase in MAP and reduction in HR (17). This discrepancy between the cardiovascular effects of 5-HT and 5-HTP is explained by the dependence on the manner in which the transmitter is introduced into the brain – a direct injection produces a pressor effect, whereas 5-HTP loading leads to a depressor action (17). Moreover, the effects of 5-HT may depend on the receptor type activation, since the stimulation of 5-HT_2/5-HT_{1C} receptors evokes the pressor response (19), and 5-HT_{1A} agonist 8-hydroxy-2-(di-n-propylamino)tetralin decreases MAP and HR when injected into the ventromedial hypothalamic area (20).

The present results demonstrate that stimulation of 5-HT_{1B} receptor, after CGS-12066A administered *icv*, produces a dose-dependent decrease in MAP and HR. The mechanism of the depressor effect of CGS-12066A is associated with the decrease in renal hindquarters and mesenteric vascular resistance and increases in RBF, HBF and MBF. The effects of 5-HT_{1B} receptor stimulation are similar to those obtained with 5-HT_{1A} receptor agonists (21) which are suggested to be involved in the initiation of the sympathoinhibitory phase of regulation in hypovolaemia (1). Therefore, further studies are needed to establish the particular peripheral mechanisms activated by the central 5-HT_{1B} receptors.

The recent study by Pelaez *et al.* demonstrates that a severe haemorrhage (40-50% of blood volume) which leads to hypotension and profound bradycardia is associated with the activation of serotonergic neurones within the subependymal parapyramidal nucleus of the medulla oblongata (22). It is suggested that a reduced sympathoexcitatory drive from the rostral ventrolateral medulla may contribute to haemorrhage-induced sympathoihibition. Moreover, haemorrhage leads to an increase in 5-HT and its metabolite 5-hydroxyindoleacetic acid release in the region of the subfornical organ. Thus, the serotonergic pathways from the dorsal raphe nucleus to the subfornical organ may relay activation of the peripheral baroreceptors to the subfornical organ neurones, which results in enhanced excitability, indicating the involvement of the pathways in the regulation of cardiovascular function in hypotension (23). In view of these findings, we have studied the effects of 5-HT_{1B} receptor activation in stepwise haemorrhagic hypotension in a rat model in which the blood pressure – blood volume relationship can be assessed. By using this model, CGS-12066A-evoked influence on the regulatory cardiovascular mechanisms during controlled, stepwise haemorrhagic hypotension can be determined by the measurement of the blood amounts which have to be withdrawn to induce hypotension (16). Our results demonstrate that 5-HT_{1B} receptor activation leads to a decrease in volumes of blood required to induce hypotension of 40 and 20 mmHg. Therefore, it is suggested that serotonin, acting *via* 5-HT_{1B} receptors, decreasing the stability of the cardiovascular system, may participate in the initiation of the sympathoinhibitory phase of the response to blood loss.

Previous studies from our laboratory demonstrate the completely opposite effects resulting

from activation of the histaminergic system in haemorrhagic shock (9-10, 15, 24). It is demonstrated that exogenous (9) and endogenous histamine (10), acting *via* central H_1 receptors, produces a long-lasting pressor effect, with increases in MAP and HR which are significantly higher in haemorrhage-shocked than in normovolaemic rats. The action of histamine is associated with an increase in survival at 2 h, despite critical hypovolaemia (9-10). Moreover, the central histamine-induced reversal of critical haemorrhagic hypotension in rats occurs due to the rise in the cardiac index (15) and mobilisation of blood from venous reservoires (25). Cardiovascular effects are accompanied by a long-lasting rise in respiratory rate and an improvement of blood gas and acid-base balance (26-27).

Our previous study based on blood pressure/blood volume-controlled model of haemorrhagic hypovolaemia demonstrates that histamine N-metyltransferase inhibitor SKF 91488, at a dose which does not influence cardiovascular parameters in normovolaemic rats, produces an increase in blood volumes necessary to induce hypotension of 40 and 20 mmHg, with no influence on blood volumes required to induce less pronounced hypotension (14). Furthermore, pretreatment with histidine decarboxylase (EC 4.1.1.22) inhibitor (S)-_-fluoromethylhistidine, which induces a decrease in central histamine levels, reduces the volumes of blood required to achieve critical MAP 20-25 mmHg (24). Since the histaminergic system interacts with various neuronal systems in the central cardiovascular regulation (28-29), our future studies will be undertaken to explore the interactions between the histaminergic and serotoninergic systems in cardiovascular control in haemorrhagic shock.

In summary, the present results demonstrate that activation of 5-HT_{1B} receptors in normotensive anaesthetised rats produces a decrease in MAP and HR, and the effect is associated with a decrease in regional vascular resistance. Moreover, since CGS-12066A at a dose which does not influence cardiovascular parameters in normotensive animals, decreases blood volumes required to induce hypotension of 40 and 20 mmHg, the results suggest the involvement of 5-HT_{1B} receptors in a decrease in cardiovascular system stability in haemorrhagic shock.

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IN VITRO PROTON MAGNETIC RESONANCE SPECTROSCOPY USED IN DIFFERENTIAL DIAGNOSIS OF MENINGIOMA .

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Abstract

The human brain cells neoplastic transformation leads to specific biochemical changes. Computer tomography (CT) and magnetic resonance imaging (MRI) do not provide all the biological information required for the appropriate management of brain neoplasms. In vitro Magnetic Resonance Spectroscopy (MRS) of human brain tumour specimens provides a wide range of information about the biochemical constitution. Each tumour type is distinguishable corresponding to changes in low molecular metabolites. The spectroscopical characterization based on information obtained from Proton MRS is shown in the meningioma as an example of human brain neoplasm. Meningioma (MEN) are generally slowly growing, benign tumours attached to the dura mater and composed of neoplastic meningothelial cells. We studied 14 specimens obtained by a surgery. We measured samples from different types of MEN tumours graded into World Health Organization (WHO) grade I. We characterized them by the presence of some characteristic low molecular metabolites e.g. N-acetylaspartate, creatine, alanine, inositol, choline-containing metabolites, glutamate and glutamine.

Key words: proton magnetic resonance spectroscopy, human brain tumour, low molecular metabolites, meningothelial and fibroblastic meningioma

INTRODUCTION

Specific biochemical changes occur in cells during cell neoplastic transformation in the human brain These changes include modifications of the energy metabolism of the cells, protein synthesis, etc. Tumour growth also leads to heterogenity in blood flow owing to focal necrosis, changes in metabolic demand, as well as disruption of transport mechanisms of substrates across cell membranes [1]. Conventional imaging methods including CT and MRI are used to solve many diagnostic problems related to brain tumours but they do not provide all the biological information required for the appropriate management of brain neoplasms. Proton MRS is useful in determination of histological type of brain tumour [2,3]. It is noninvasive imaging technique for measuring the biochemical content of living tissues and obtained information about tumour metabolism may be useful in tumour diagnosis [4]. Contrary to *in vivo* analysis, *in vitro* MRS can characterize all the brain metabolites in more details due to longer accumulation time and greater resolution achieved by using high magnetic fields. In Proton MRS, choline-containing compounds (Cho) signal is reported to be predominantly attributed to cellular density [5]. The ratios of Cho and glycine (Gly) to total creatine (Cr) are useful parameters for grading tumours [6]. *In vivo* proton MRS study shows that differences in Cho/Cr and of N-acetylaspartate (NAA)/Cr ratios help to specify the presence or absence of a neoplasm and also in grade solid brain tumours [7,8]. NAA can be used as a neuronal marker as it is found exclusively in neurons in the mature brain [9,10]. Changes in the resonance intensity of Cho probably result mainly

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from increase in the steady-state levels of phosphocholine and glycerophosphocholine. Large change of total Cr concentration can be seen with destructive pathology such as malignant tumours. Lactic acid (Lac) is the end product of glycolysis and it is accumulated when the oxidative metabolism cannot meet energy requirements. Large amount of Lac may be accumulated outside of actively anaerobic tissue (e.g. in necrotic tissue) but wider range of compounds can be studied, for example some key neurotransmitters such as glutamate (Glu) and glutamine (Gln).

A variety of neoplastic lesions develop in the meninges, but most prevalent are those, originating from meningothelial cells. MEN are composed of neoplastic meningothelial (arachnoidal) cells and they typically manifest in middle-aged adults and show predominance among women. On MRI, meningiomas are typically isodense dural masses that may be calcified. They often compress the adjacent brain, but they rarely show invasion of the brain. Meningothelial meningioma tumour cells form lobules, with oval nuclei with occasionally central clearing. Fibroblastic meningioma have spindle-shaped cells in collagen and reticulin [11].

The aim of our work was firstly, to know if the proton MRS like a method provides sufficient information to help to estimate relevant diagnosis of a human intracranial lesion and secondly, to provide information about changes in low molecular metabolites in various types of human brain tumours, especially MEN.

METHODS

In vitro MRS was performed on specimens of human brain tumours obtained from conventional neurosurgical removal. We evaluated 14 specimens from 7 pacients: 8 samples from meningothelial MEN and 6 samples from fibroblastic MEN. The tumours were histologically classified according to WHO classification [11]. The specimens were frozen in liquid nitrogen immediately after surgical removal and extracted with perchloric acid (PCA), according to Klunk et al., 1997. 3-aminopropylphosphoric acid (3-APP) was added to each sample as an internal standard. The PCA extracts were passed through potassium Chelex column and lyophilized. After lyophilisation, 30-40 mg of white powder was dissolved in 1 ml 99,9 % D_2O and pH was adjusted to 5.0-5.2. *In vitro* MR spectra were obtained with a spectrometer Varian VXR 300 (7,5T). Proton spectra were recorded with a repetition time of 2000 ms and an echo time of 136 ms. A total of 200 data points were collected over a spectral width of 3000 Hz. Results were evaluated by computer-software MestRe-C v.2,3 (http://qobre.usc.es). We assigned the resonance of interest: NAA at 2.02 ppm, Cho (including choline, phosphocholine and glycerophosphocholine) at 3.15-3.23 ppm, Glu/Gln at 3.76 ppm, Lac at 1.32 ppm, Ala at 1.48 ppm and the peak of Cr (creatine and phosphocreatine) at 3.93 ppm. Significance of differences between two groups of MEN for each metabolite was tested with the Anova non-parametric analysis of variance. Differences of $p<0,05$ were considered to be statistically significant.

RESULTS

The relative levels of low molecular metabolites were obtained from integration of the areas under individual peaks. Relative metabolite ratios were defined as a percentage of signal intensities to a signal corresponding to CH_2–group of the internal standard (3-APP). Our specimens from meningothelial and fibroblastic meningeomas (MEN) were included in grade I due to WHO classification (Fig. 2) [11]. There was no statistically significant difference between these two groups of MEN. Table 1 summarizes the mean and standard deviation obtained from measurements on specimens of MEN. MEN show a characteristic prominent signal from Ala and this low molecular metabolite seemed to be a meningiomal lesions marker. There was practically no signal from NAA. Other findings characteristic of MEN were: decreased levels of Cr, increased levels of Cho and increased levels of Glu/Gln. We also measured a Gly or Ino peak at 3.55 ppm and large amounts of Lac at 1.32 ppm (Fig. 1).

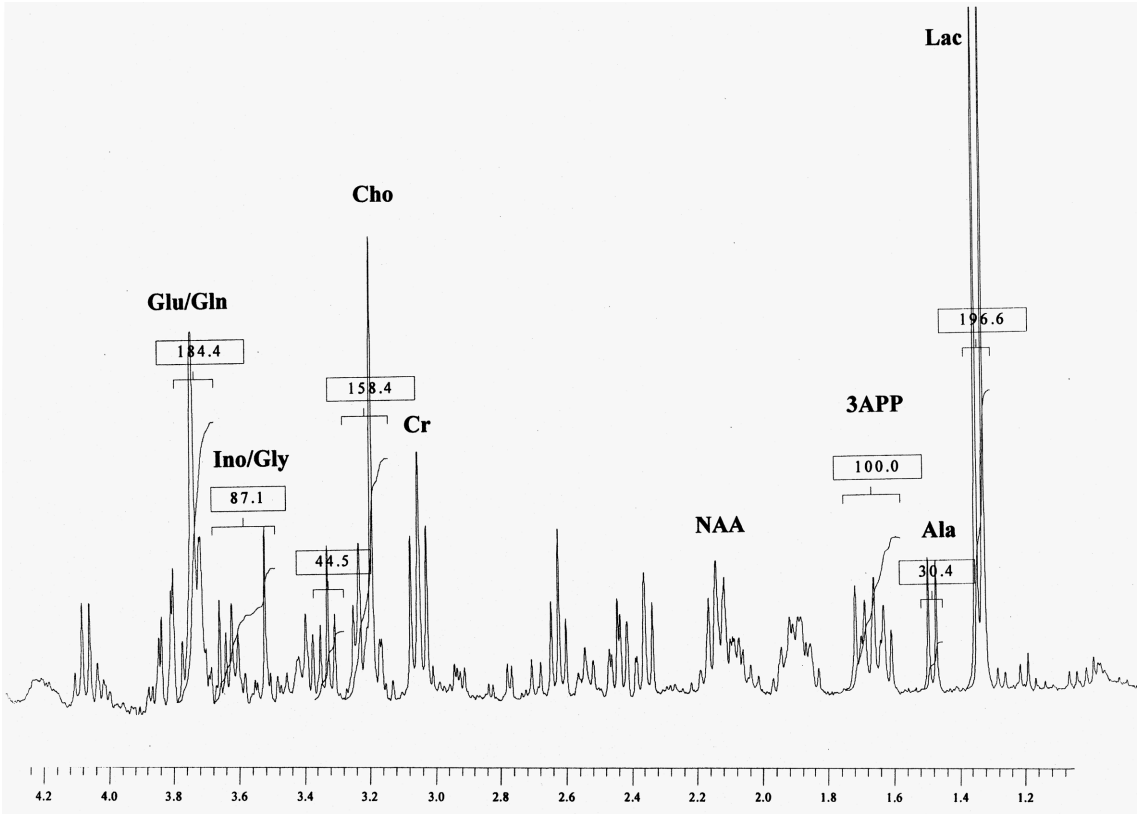

Fig. 1: Representative ^{1}H MRS of brain tumours obtained from meningioma. Spectrum of the tumour shows an Ala doublet centered at 1.48 ppm that is highly suggestive of meningioma. Relative metabolite ratios were defined as a percentage of signal intensities to a signal corresponding to CH_2–group of the internal standard (3-APP: 3-aminopropylphosphoric acid). NAA: N-acetylaspartate, Cr: creatine, Ala: alanine, Ino/Gly: inositol/glycine, Cho: choline-containing metabolites, Glu/Gln: glutamate and glutamine, Lac: lactic acid.

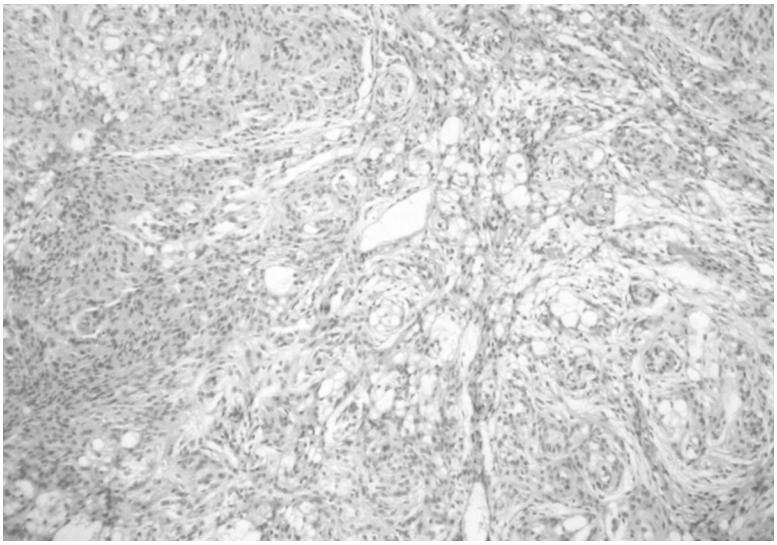

Fig. 2: Meningothelial meningioma cells form lobules which are surrounded by thin collagenous septae. Cells are largely uniform with oval nuclei that on occasion show central clearing.

Table 1: Metabolite resonance in the 14 specimens of meningioma WHO grade I analyzed by *in vitro* ^{1}H MRS. The relative levels of low molecular metabolites were obtained from integration of areas under individual peaks. Values shown are mean and the standard deviation is mean as well. No significant differences were found between meningothelial and fibroblastic meningioma. NAA: N-acetylaspartate, Cr: creatine, Ala: alanine, Ino/Gly: inositol/glycine, Cho: choline-containing metabolites, Glu/Gln: glutamate and glutamine, Lac: lactic acid.

N= 14	Lac	Ala	NAA	Cr	Cho	PC/GPC	Ino/Gly	Glu/Gln	ratio Cho/Cr	ratio NAA/Cr
Mean	205	36	19	23	154	56	101	229	6.6	0.9
SD +/-	56	17	4	9	43	14	51	82	2.3	0.2

DISCUSSION

The results show that the MRS is a possible diagnostic method to characterize some biological factors in MEN. Other most prevalent human brain tumours such as astrocytic neoplasms are characterized according to their grade of differentiation by a high degree of cellular differentiation, slow growth and diffuse infiltration of brain structures. This biological factors lead to different MRS findings as reported by [8,12,13]. According to histopathological analysis, the malignant neoplasms are also characterized by a number of morphological factors [14]. These morphological changes are useful to localize the neoplastic process.

Using information about the level of low-molecular metabolites we can characterize the histopathological type and grade of the brain neoplasm. NAA is located only in neurons and is considered a neuronal marker [9,10]. The signal is practically absent in MEN. Cho metabolites are involved in both the synthesis and breakdown of membrane phospholipids [14]. Choline containing membrane phospholipides are released during active myeline breakdown. Thus, the resonance intensity of Cho increases in acute demyelinating lessions in humans. Cho levels tend to be high in the developing brain and are related to cellular proliferation processes, while Cr derivatives play a major role in energy metabolism. The levels of Cr and phosphocreatine are lower with higher grade of astrocytoma [4,8,15]. We found a strong double peak at 1,47 ppm corresponding to Ala. Spectroscopic findings of MEN were described by Sabatier at al. In accordance with our observations, they found increased ratio of Ala over Cr accompanied by a near absence of myo-inositol [12]. It is well known that different brain tumours feature elevated Cho, which is caused by increased cell membrane turnover [16,17]. Correlation of Cho peak with mitotic and proliferative activity and degree of tumour malignancy has been reported [8,13,15,18].

In our study, there is no comment on preoperative treatment, which might include anti-oedematous drugs, and therefore it remains purely speculative to discuss whether the Gly or Ino peak at 3.55 ppm [5] might be caused by anti-oedematous therapy with hyperosmolar drugs such as mannitol or sorbitol [8].

Contrary to *in vivo* analysis, however, *in vitro* proton MRS can better characterize all the brain metabolites. *In vitro* proton MRS provides a basis for better interpretation of *in vivo* measurements, thus improving their clinical relevance. Our results are in a good agreement with previously reported *in vivo* measurements: MEN are characterized by increased levels of Ala [4] and elevated levels of Cho [12]. For better characterization of MEN the NAA/Cr ratio and Cr/Cho ratio were also used [17].

MRS is a non-invasive method for looking at intracellular pathophysiology. Proton MRS provides information about metabolic aspects of brain tumours. There are no really specific spectroscopic findings of human brain tumours but presently, ^{1}H MRS can be performed with low time requirements on most MR units. This brings the chance to the radiologist to correlate ^{1}H MRS information with anatomical MRI findings in the course of a single MR exam. Clinical state, conventional imaging and ^{1}H MRS taken together might be helpful in diagnosing these tumours correctly.

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ACUTE NORMOVOLEMIC HEMODILUTION DETERIORATES FUNCTIONAL CARDIORESPIRATORY RESERVES IN HYPERTHERMIC RABBITS

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Summary

Cardiorespiratory responses to hyperthermia during an acute normovolemic hemodilution were studied in 16 anesthetized adult rabbits divided into two groups: hemodiluted group (Hct = 18.6 ± 0.4%) and control group (Hct = 41.1 ± 0.9%). In the hemodiluted group, acute normovolemic hemodilution was induced by 60% replacement of the total blood volume with dextran.

The rise in body temperature (BT) to 42°C by a gradual body surface heating caused significant increases in ventilation (VE) and heart rate (HR) in both groups, however, V_E and HR values were significantly ($P<0.05$) higher in the hemodiluted group compared to the controls. Arterial blood O_2 tension (PaO_2) did not show any significant change during overheating in the controls, however, it significantly ($P<0.02$) decreased in the hemodiluted group during hyperthermia. The hemodiluted animals, unlike the controls, were not able to use panting during hyperthermia.

The results indicate a greater activation of the respiratory and cardiovascular systems in the hemodiluted animals during overheating and thus a higher risk of respiratory and circulatory failures.

Key words: hemodilution, hyperthermia, physical treatment of hyperthermia, ventilation, heart rate

INTRODUCTION

Hyperthermia evokes a marked respiratory and cardiovascular changes: a special pattern of rapid shallow breathing with thermoregulatory effects - panting , a rise in heart rate, the increases in cardiac output and skin blood flow, the decreases in splanchnic and renal blood flows and a reduction in muscle blood flow (1, 2). Cardiorespiratory responses to hyperthermia appear to be well described at normal hematocrit (Hct). Although several authors have shown changed cardiorespiratory responses to hyperthermia/fever during dehydration (3, 4, 5), there is no available information on cardiorespiratory responses to heat stress under the conditions of acute hemodilution - anemia.

Therefore, the present study was designed to study how acute normovolemic hemodilution affects the cardiorespiratory responses to hyperthermia in rabbits.

MATERIALS AND METHODS

Sixteen adult rabbits (chinchilla) of both sexes, weighing 2.1 ± 0.08 kg ($\bar{x}$ ± SEM), were anesthetized with a mixture of 20 mg/kg ketamine and 5 mg/kg xylazine i.m. in an introductory dose and the maintenance doses of 20 mg/kg/h ketamine by intravenous infusion. The animals were tracheotomized and they breathed room air spontaneously through a tracheal cannula connected with the Fleisch head of a pneumotachograph (ÚMMT SAV, Bratislava) to record tidal volume (V_T). The frequency of breathing (f) was determined from the tidal volume record. Ventilation (V_E) was calculated as a product of V_T and f (V_T x f).

The polyethylene catheters were placed bilaterally in the femoral arteries for blood with-

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drawal and arterial blood pressure (ABP) monitoring with the electromanometer LDP 102 (Tesla, Czech Republic). The femoral venous catheter was used for a continuous administration of the anesthetic by the injection pump IPA 2050 (COMPACT Co., Czech Republic).Via a catheter surgically placed in the internal jugular vein and advanced into the vena cava superior, the central venous pressure (CVP) was continuously recorded by electromanometer LDP 165 (Tesla, Czech Republic). Heart rate (HR) was evaluated from the ECG record – R-R intervals obtained by means of subcutaneous needle electrodes. Signals of the V_T, ABP, CVP and ECG were recorded simultaneously by a multi-channel recorder 6 NEK 4 (RFT, Germany).

After taking blood samples from the femoral artery, the arterial P_{O2}, P_{CO2}, pH and hemoglobin (Hb) were monitored using a blood gas analyzer Rapidlab™ 348 (Bayer Diagnostics, England). Blood gases and pH were corrected for actual BT. A microhematocrit centrifuge (type 316, Mechanika precyzyjna, Poland) was used for Hct determination. Rectal temperature was measured by a mercury thermometer at a depth of 6-7 cm.

Induction of acute normovolemic hemodilution: Normovolemic hemodilution (NH) was induced using dextran for blood exchange. 6% dextran solution in 0.9% saline was warmed to 38°C before infusion. NH was then achieved by simultaneous and repeated blood withdrawals (via femoral arterial catheter) and infusions (via jugular venous catheter) of an equal volume (5 ml) of dextran until 60% of the total blood volume (70 ml/kg, 6) was replaced. This process took 51.9 ± 3 min on average. Stabilization period of 20 min was provided after induction of NH.

Experimental protocol: The animals were divided into two groups. In one group (control group, n=8) cardiorespiratory responses to hyperthermia were studied at normal Hct (41.1 ± 0.9%) and in the second group of rabbits (hemodiluted group, n=8) cardiorespiratory responses to hyperthermia were studied after the induction of acute normovolemic hemodilution (Hct=18.6 ± 0.4%). In both groups, the animal body temperature (BT) was gradually elevated to 42°C by body surface heating using a heating pad and radiant heat from an infrared lamp. This process took 48 ± 2 min on average. Cardiorespiratory variables were recorded and evaluated at each 1°C change in BT during the body surface heating.

The rabbits were killed by overdosing with the anesthetic drug at the end of the experiment. Experiments were performed according to the Helsinki Declaration.

Statistical analysis: Statistical analysis was performed using a Wilcoxon test and Mann-Whitney U test. The results are expressed as means ± SEM. Differences were considered significant when $P < 0.05$.

RESULTS

Acute normovolemic hemodilution produced significant increases in V_E ($P<0.02$), HR ($P<0.02$) and CVP ($P<0.02$), but there was no significant change in ABP.

Hyperthermia caused a significant increase in V_E in both groups, however, V_E values were significantly higher in the hemodiluted group compared to the controls (at 40°C: 1192.5 ± 91.5 ml/min vs. 923.6 ± 67.8 ml/min, $P<0.05$; at 41°C: 1303.3 ± 105.1 ml/min vs.1001.8 ± 58.6 ml/min, $P<0.05$; at 42°C: 1420.6 ± 119.5 ml/min vs. 997.7 ± 51.3 ml/min, $P<0.01$; Fig. 1). Pa_{O2} did not show any significant change during overheating in the control group, however, it significantly ($P<0.02$) decreased in the hemodiluted group during hyperthermia. Pa_{CO2} decreased ($P<0.05$) during hyperthermia in both groups, but significantly deeper decrease in this variable was found in the hemodiluted group compared to the controls. In both groups pH did not significantly change during overheating. The hemodiluted animals, unlike the controls, were not able to use panting during hyperthermia.

Hyperthermia elicited a significant increase in HR in both groups. However, significantly higher HR values were found in the hemodiluted group compared to the control group (at 41°C: 327 ± 9 beats/min vs. 282 ± 16 beats/min, $P<0.05$; at 42°C: 346 ± 15 beats/min vs. 306 ± 13 beats/min, $P<0.05$; Fig. 2). CVP decreased ($P<0.05$) during hyperthermia only in the controls. ABP did not significantly change during overheating in controls, but it rose significantly ($P<0.02$) in the hemodiluted group.

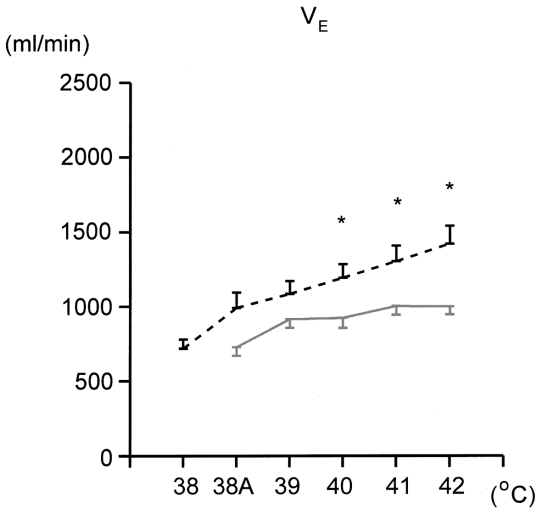

Fig. 1 Changes in minute ventilation (V_E) during hyperthermia in the control (solid line) and hemodiluted (dashed line) groups. Each point represents mean ± SEM. * P< 0.05 significantly different from the control group at the same body temperature. 38A – initial level (38°C) of animal body temperature after induction of acute normovolemic hemodilution/anemia.

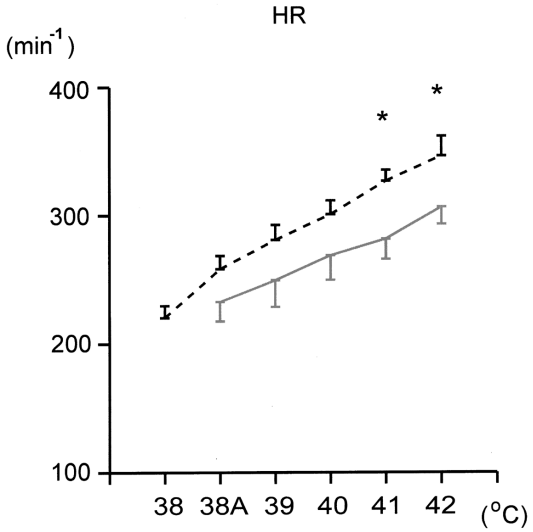

Fig. 2 Changes in heart rate (HR) during overheating to 42°C in the control (solid line) and hemodiluted (dashed line) groups. Each point represents mean ± SEM. * P< 0.05 significantly different from the control group at the same body temperature. 38A – initial level (38°C) of animal body temperature after induction of acute normovolemic hemodilution/anemia.

DISCUSSION

In our experiments the cardiorespiratory responses to hyperthermia were studied at normal Hct and following the induction of acute normovolemic hemodilution.

Hyperthermia in rabbits with normal Hct led to the increase in V_E. At the body temperature of 41 - 42°C panting and an increase of the breathing frequency with a marked decrease in V_T appeared in this group. We found no marked change in Pa_{O2} during panting. Pa_{CO2} decreased significantly during panting.

With the rise in BT a significantly higher increase of V_E was observed in the hemodiluted animals compared to the controls. Thus, the decrease in Pa_{CO2} was deepened in anemic rabbits compared to those with normal Hct. Despite the greater ventilatory response to hyperthermia under acute anemic conditions, the anemic animals, unlike those with normal Hct, were not able to pant and to hinder the significant decrease in Pa_{O2} during hyperthermia. We suggest that the higher V_E values in the hemodiluted animals during hyperthermia may be reponsible for respiratory failure at low Hct values through the exhaustion of functional reserves, indicating a higher risk in the hemodiluted group at high temperatures.

Hyperthermia elicited a significant increase in HR in both groups. However, significantly higher HR values were found in the hemodiluted group compared to the controls. Despite the tachycardia existing in hyperthermia, ABP did not significantly change by heating of controls, but it significantly rose in the hemodiluted animals during hyperthermia.

In our study, the ventilatory and cardiovascular responses to hyperthermia were found to be much stronger in the hemodiluted animals compared to the controls. We assume that these results could be attributed to the co-existence of such severe stress conditions as acute anemia and hot environment.

It is known that cardiovascular system plays an important role in the development of heat stroke (7). However, physiological mechanism(s) producing circulatory failure associated with

heat stroke are not well known. In our study, we observed significantly higher values of HR in the hemodiluted group compared to the controls. Therefore, we suggest that acute normovolemic hemodilution/anemia intensifies the cardiovascular effects of hyperthermia and can precipitate the development of circulatory failure during severe hyperthermia at low Hct values.

In conclusion, in hemodiluted animals hyperthermia was accompanied by the higher V_E and HR values, indicating more intensive activation of the respiratory and cardiovascular systems, the diminished functional cardiorespiratory reserves and the higher risk of respiratory and circulatory failures in anemic animals during hyperthermia.

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THE URINARY CALCIUM AND PHOSPHATE EXCRETION IN PATIENTS WITH PRIMARY HYPERPARATHYROIDISM BEFORE AND AFTER PARATHYROID SURGERY

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Abstract

We have compared urinary calcium and phosphate excretion before and after the parathyreidectomy in patients with primary hyperparathyroidism. In samples of 24-hour and the second morning urine several indices were assessed: the urinary calcium excretion/24 h, the urinary phosphate excretion/24 h, the Nordin`s index (=urinary calcium/creatinine), the excretion of calcium/GFR, the renal calcium threshold, and the renal phosphate threshold. In all 36 patients successful parathyroid surgery resulted in a return of the values of observed indices to normal. The typical changes we have observed can be useful in follow-up of patients with primary hyperparathyroidism.

Key words: primary hyperparathyroidism, parathyroid hormone, calcium, urinary excretion

INTRODUCTION

Primary hyperparathyroidism is overwhelmingly the most common cause of hypercalcemia in outpatients. It is a generalized disorder of calcium, phosphate, and bone metabolism due to an increased secretion of parathyroid hormone (1,2). It is now recognized to be quite common, particularly in the elderly (most patients are postmenopausal women). The disease is caused by a benign, solitary adenoma in 80% of the cases (1-6). Surgery is the only therapy; primary hyperparathyroidism is cured when the abnormal parathyroid tissue is removed (7-8). Many reliable diagnostic tests are now available to establish the diagnosis. The most important are measurements of serum intact parathyroid hormone and ionized calcium.

We think that also urine samples can give us valuable information. In our study we have compared urinary excretion of calcium before and after surgical removal of parathyroid adenoma. We think that the typical changes of the indices of urinary calcium and phosphate excretion can help us in diagnosis and in follow-up of patients with primary hyperparathyroidism.

METHODS

Thirty-six patients with primary hyperparathyroidism indicated for surgical treatment were enrolled in this study. There were 29 women and 7 men, aged 27-67 years (average age 50.3 ± 10.2). Samples of 24-hour and the second morning fasting urine were taken before and 2 months after parathyroidectomy. The following indices were assessed in these samples:

The urinary calcium excretion/24h
The urinary phosphate excretion/24h
The Nordin`s index = urinary calcium/creatinine
The excretion of calcium/GFR
The renal calcium threshold
The renal phosphate threshold

Statistical analysis: The data are presented as means ± standard deviation. Comparison of the data was performed using t-test.

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RESULTS

Successful parathyroid surgery resulted in a return of the observed values to normal. Excretion of calcium/24h decreased from 10.22 ± 3.77 mmol/24h to 4.44 ± 1.74 mmol/24h (**Fig. 1**). Excretion of phosphate/24h decreased from 30.75 ± 9.45 mmol/24h to 23.61 ± 7.16 mmol/24h (**Fig. 1**). In the second morning urine there was a decrease in elevated values of Nordin`s index (from 0.724 ± 0.41 to 0.271 ± 0.20 mmol/mmol) (**Fig. 2**) and excretion of calcium/GFR (from 53.5 ± 25.6 to 20.4 ± 14.6 μmol/l.1.73 m^2) (**Fig. 3**). The renal calcium threshold decreased from 2.13 ± 0.22 to 1.94 ± 0.25 mmol/l (**Fig. 4**). The renal phosphate threshold increased from 0.648±0.217 mmol/l to 0.947 ± 0.270 mmol/l (**Fig. 4**). All of these results were statistically significant ($p<0.001$).

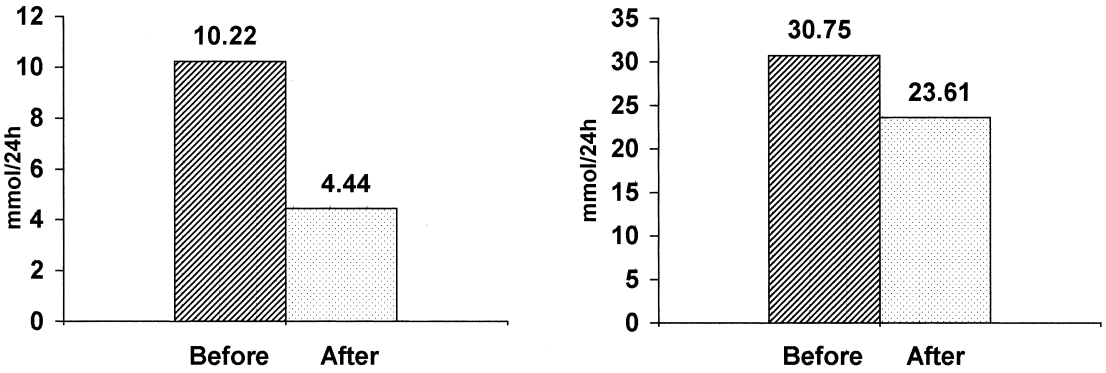

Fig.1 The renal excretion of calcium (left) and phosphate (right) before and after parathyroidectomy

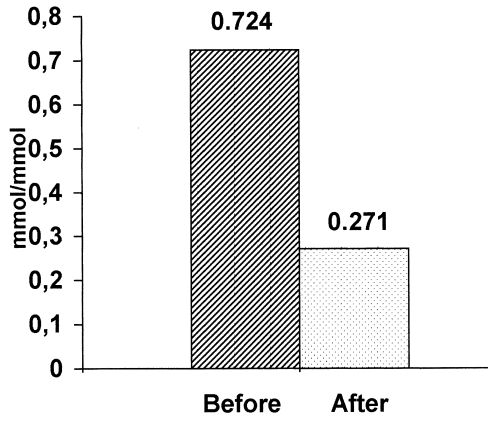

Fig.2 The Nordin's index before and after parathyroidectomy

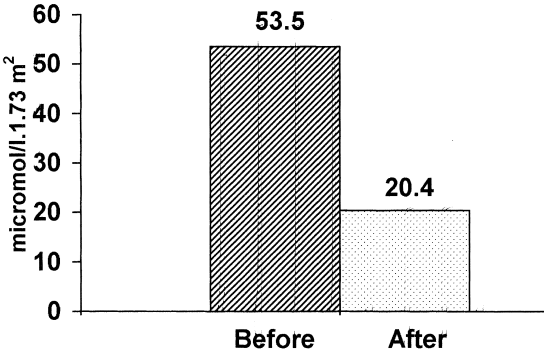

Fig.3 The excretion of calcium/GFR before and after parathyroidectomy

DISCUSSION

Primary hyperparathyroidism is a disease characterized by elevated levels intact PTH and serum ionized calcium and low serum phosphorus level. These findings are accompanied by hypercalciuria (with normal or elevated renal calcium threshold) and hyperphosphaturia (with

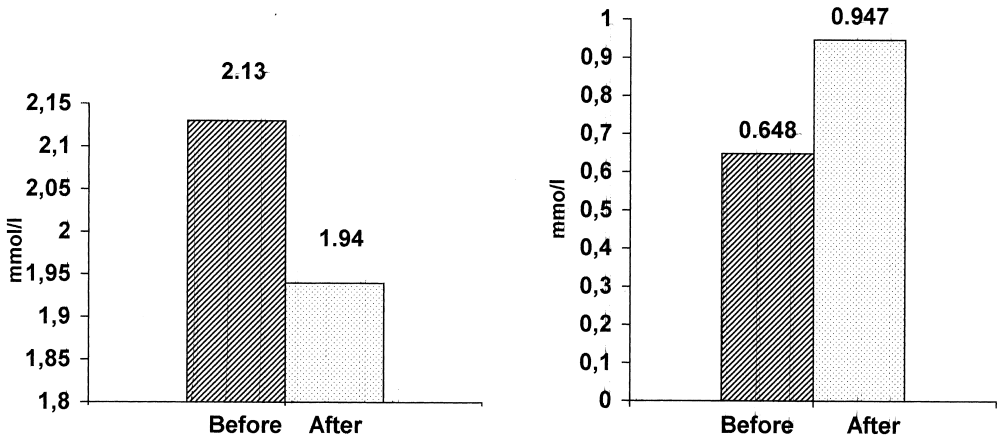

Fig.4 The renal calcium (left) and phosphate (right) threshold before and after parathyroidectomy

very low renal phosphate threshold) (1, 3, 8). There is no doubt that elevated serum ionized calcium level with increased intact PTH are essential for the diagnosis of primary hyperparathyroidism. The assessment of urinary calcium and phosphate excretion is usually not included in the evaluation of patients with primary hyperparathyroidism. But the indices of urinary calcium and phosphate excretion can provide information, which is useful not only in the diagnosis but also in the clinical management of these patients (degree of urinary calcium and phosphate losses, prediction of "hungry bone disease", intake and output balance of calcium and phosphate after surgical removal of adenoma) (9,10).

From the results of this study it can be concluded that evaluation of urinary calcium and phosphate excretion is important not only for diagnosis and differential diagnosis of primary hyperparathyroidism, but also in follow-up of patients with this disease after parathyroidectomy. It has its place among the other diagnostic procedures and should be done routinely.

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QUALITY OF LIFE OF PATIENTS WITH STOMAS

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Abstract

Ostomy is one of the basic surgical procedures. 34 stomas were performed in the Clinic of Surgery in Martin in 2002. Quality of life (QoL) expresses the effect of treatment and patient's satisfaction. The central purpose of care of patients with stomas is to improve their QoL. We made a survey among ostomates from all over Slovakia, using questionnaires.

The QoL is evaluated by a five point scale: 1 (very good) – 5 (very poor). The sample of 50 ostomates represents patients with stomas who are active members of ILCO clubs. Their mean global QoL is good/2. We measured subjective and objective QoL of the patients. The quality of the psychological sphere of life of the ostomates is good/2, their physical QoL and social QoL is mostly neither good nor poor/3 or good/2. Most of the patients are not able to return to work, to their social life, sexual life, travel activities, and sport activities are limited. 74% of the subjects consider the stoma bearable.

To improve QoL, ostomates themselves recommend especially stoma clubs and high quality appliances. In post-surgery adaptation the role of ILCO clubs is crucial.

Key words: stoma, ostomates, quality of life (QoL)

INTRODUCTION

Quality of life (QoL) and quality of health are not identical. The World Health Organisation (WHO) defines health as a state of complete physical, mental, and social well-being. On the other hand WHO defines the QoL as an individual's perception of the position in life in the context of value systems and in relation to their expectations. The importance of QoL is crucial to an individual (1). Interest in QoL of patients has been recognized as one of the important aspects of humanization of medicine (2). QoL instruments should form a part of the evaluation of treatments and health services, on the other hand they provide information about areas in which a patient is most affected, and thus increase the physician's understanding of how disease or disability affects the patient's life. At the same time we have to admit that most of the judged aspects of QoL are subjective, and they are perceived differently due to patient's personality and his value system and expectations (3).

Stoma construction has a serious impact on a patient's life. Ostomy still remains one of the basic surgical procedures. The purpose of the stoma construction is to prolong patient's life (quantity). But the important criterion of expressing the effect of treatment and patient's satisfaction, is quality of life (QoL). Therefore the central purpose of care of patients with stomas is to achieve satisfactory quality of their lives (4).

There are 1 500 new stoma patients in Slovakia (5.5 mil. inhabitants) yearly (year 2000). The assumed prevalence of ostomates in our country is up to 10 000 (5, 6). In administration they are classified as disabled, because their physical QoL is seriously impaired. There are 30 patients' self-help organizations called ILCO clubs, the central Slovak stoma organization is called Slovilco. 20% of ostomates in Slovakia are members of stoma organizations recently. Because we assume improvement of care of patients with stoma (7), we were interested whether and in what aspects the achieved improvement of care have reflected in their lives. We made a survey among ostomates from all over Slovakia, using questionnaires.

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METHODS

We created a questionnaire consisting of 45 questions, as a QoL instrument. The first six questions inquire about stoma type, gender, age, age at the time of stoma construction, educational, and marital status. According to the WHO definition of health, we accept 3 main aspects of QoL: physical well-being, psychological and spiritual well-being, and social well-being. We created questions for stoma patients after identifying components of each of 3 large domains (8). Six questions inquire about physical aspects, ten questions about psychological and spiritual aspects, and fifteen questions about social apects of life. In addition there is one question that is global, namely „How would you rate your overall quality of life ?“ (9). Thus we are able to compare objective QoL which includes physical, psychological and social aspects of QoL, and subjective QoL. We also added 7 multiple-choice questions which inquire about the very special issues of ostomate's life, such as stoma appliances or aspects in which ostomates feel inconvenience from stoma. These questions are not divided among the categories, and are not used to measure QoL. We tried to address any problems the patients identify. We also asked what and who improves their QoL.

For each of the 31 questions divided among the domains, people circled a number on the five point scale that gave the best answer for them. The response categories range from the best possible [1] to the worst possible [5]. Thus each answer produces a score. We calculated a mean of answer scores in each domain (physical, psychological and spiritual, social) for each patient. Because we have presumed that the three domains of life are equal, by integrating the scores of three domains we assess a value, which we have called the objective QoL. Using the five point scale we express the value of the objective QoL as very good/1, good/2, neither poor nor good/3, poor/4, or very poor/5 for each patient (9,10). Global values are expressed as mean ± SD for the study sample..

We got back 100% of the anonymous questionnaires. The study sample consists of 50 patients with stomas from all over Slovakia. All of them are members of stoma organizations. There are 44 subjects with colostomies, 4 (2 male and 2 female) with ileostomies, 2 (male) with urostomies. Out of the total 50 patients, there are 32 men and 18 women. Their mean age is 61 years, their mean age at the time of stoma construction was 50 years. The highest received education is primary school in 13 (26%), secondary school in 28 (56%), and tertiary in 9 (18%) ostomates. 41 (82%) of the subjects are married, 6 (12%) are widowed, 2 (4%) are divorced, 1 (2%) is single.

RESULTS

Thirty-four patients underwent surgery outcome of which was a stoma in the Clinic of Surgery in Martin in 2002. There were performed 29 (85%) colostomies and 5 (15%) ileostomies. 25 (74%) out of the created stomas were permanent, 9 (26%) were temporary. Only 9 (26%) procedures were planned in advance. 3 (9%) stomas were closed. 27 (79%) procedures were performed for intestinal cancer, the others mainly for diverticulitis perforans, perianal abscess, trauma or iatrogenic lesion.

We made the questionnaire survey among 50 ostomates from all over Slovakia, not only among those who underwent surgery in our Clinic. Two (4%) of the ostomates assess their QoL as very good, 31 (62%) of them as good, 14 (28%) as neither good nor poor, 3 (6%) as poor, none as very poor. Thus their mean subjective QoL is 2.36 ± 0.66 (median=2), that means good QoL.

We evaluated global objective QoL and its three components - physical, psychological, social (Figure 1.). The mean overall QoL of the study sample is 2.25 ± 1.19, that means good QoL. The best mean partial QoL (1.85 ± 0.90) is achieved in the psychological component, the worst (2.48 ± 1.37) in the social domain. We assess the overall objective QoL as very good in 3 (6%), as good in 31 (62%), as neither good nor poor in 16 (32%) subjects. There is none with objective QoL assessed as either poor or very poor (Figure 2.). There are 31 (62%) ostomates, whose objective and subjective overall QoL were equal. The subjective QoL of 7 (14%) subjects was a point better

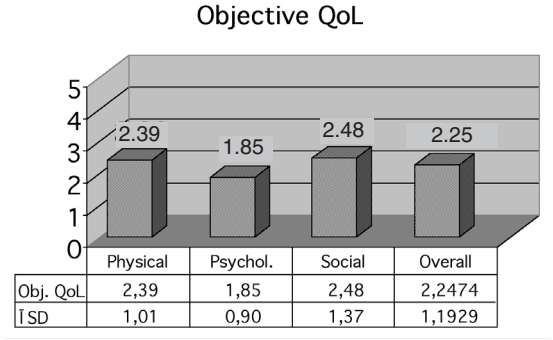

Figure 1. Values of objective global QoL and of QoL in 3 main spheres of life, expressed as mean ± SD.

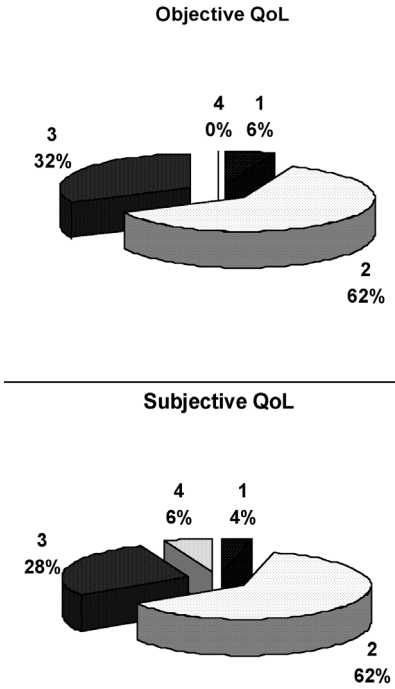

Figure 2. Variation of subjective and objective overall QoL values among the ostomates in the study sample.

than their objective QoL, and the subjective QoL of 12 (24%) was a point worse than their objective QoL. The best (mostly good/2) QoL is achieved in the psychological domain – there is none with poor/4 or very poor/5 QoL in psychological and spiritual sphere of life. QoL in social and in physical aspects of life was assessed in most of the subjects as neither poor nor good [3 or good [2] (Table 1.).

We have not identified any differences in QoL of colostomates, which would depend on their gender or age (Table 2.). Because the number of ileostomates and urostomates in the study sample is not significant, the possible correlation between age or gender and their QoL is uncertain. We assess QoL of ileostomates in the study sample as good [2], and QoL of two urostomates as neither poor nor good [3] and poor [4].

Because ostomates have unique problems in the social sphere of their lives, we were interested in some special aspects of post-surgery life and in difficulties in post-surgery adaptations. 57% of the patients in productive age were not able to return to their jobs, 4% did not want to,

Table 1. Objective qualities of physical, psychological, and social sphere of life, and in how many subjects they were assessed.

QoL	Physical	Psychological	Social
1/very good	3 (6%)	8 (16%)	2 (4%)
2/good	20 (40%)	35 (70%)	22 (44%)
3/neither poor nor good	24 (48%)	7 (14%)	24 (48%)
4/poor	3 (6%)	0	2 (4%)
5/very poor	0	0	0

Table 2. QoL of colo-, ileo-, and urostomates.

Colostomy						
Age	Gender	Subj. QoL	Obj. QoL	Phys.	Psych.	Soc.
41-50	M (3)	2	2	3	2	2
	F (3)	3	2	3	2	3
51-60	M (9)	2	2	2	2	3
	F (5)	2	2	3	2	2
61-70	M (9)	2	2	2	2	3
	F (6)	2-3	2	3	2	2
71-80	M (6)	2	2	3	2	2
	F (2)	2	2	3	2	2
81-90	M (1)	2	2	3	2	2
	F (0)					
Ileostomy						
31-60	M (2)	2	2	2	2	3
	F (2)					
Urostomy						
61-80	M (2)	3-4	3	3	3	3
	F (0)					

only 15% returned to their jobs, 11% returned to part-time jobs. Sexual life was interrupted in 31%, was changed in 57%, was not influenced at all in 12% of the subjects. Social life of only 22% of the ostomates was not changed at all, of 69.5% was affected by stoma, only 8.5% of the stoma patients stay at home. Only 1 (2%) ostomate does sports as much as he did before the surgery, 22% of those asked do sports little bit less than they did before. 6% of the ostomates do not travel anymore. In 77% stomas limit traveling, in 16.6% stomas do not limit travel activities at all. Financial situation of 14% of the patients with stomas was not changed, of 86% was affected by stoma. Everbody in the study sample is a member of stoma organization, 96% of them visit ILCO clubs regularly, 4% often.

Answers on the additional multiple choice questions which were not used to measure QoL, enlighten unique problems and needs of ostomates. Ostomates need help in their post-surgery adaptation. Family helps in 74%, ILCO club in 60% of the ostomates. The others recognize help of physician, stoma nurse, him/herself, friends. 41% of the patients received enough information during perioperative period, 38% got only partial information in hospital, 18% did not even know how to take care of their stomas when being discharged from hospital. A family member of 26% of the ostomates has learned how to take care of stoma. The most frequent troubles with stomas are flatulence and bad smell and feelings of weakness. 32% of the patients have no serious troubles, 16% complain of painful skin over stoma, 14% suffer from pain. Most of the patients use pouching systems, only few of them still use colostomic belt.

DISCUSSION

The quality of life of ostomates who are members of ILCO clubs, is good [2]. A well-performed stoma is crucial for an ostomate's QoL. That is why there are 32% of the patients, who have no serious health troubles, although troubles associated with stoma are in general common among ostomates. In post-surgery adaptation medical advice and psychological guidance is necessary. That's why counseling services and stoma organizations with one-to-one mentorship are irre-

placeable in improving QoL of patients with stomas. Family understanding and public awareness are also very important.

In general, the subjective and objective QoL of the patients do not differ much. Physical QoL is seriously affected by incontinence, wearing of stoma appliances, and troubles associated with stoma. High quality pouching systems and irrigation systems are necessary for ostomates' good QoL. In fact the physical QoL of ileo- and urostomates is worse than of colostomates. Although it is difficult to adapt to living with a stoma and these people are mindful of having a stoma, their QoL in psychological and spiritual aspect is good. Patients' self-help organizations and one-to-one mentorship contribute a lot to successful post-surgery adaptation. Still there is often insufficient information about future life with stoma during the perioperative period. That's why nurse specialist training is also needed. Lack of care of stoma patients still involves problems in the social sphere of their lives. We evaluate QoL in the social domain of the most of the ostomates as neither poor nor good, or good.

To many ostomates stoma still means a limit. Most of ostomates are not able to return to work, stomas limit their sexual life, social life, travelling and sport activities. Although stoma patients get financial aid monthly, the financial situation of most of them is impaired due to stoma.

For psychological well-being it is important how an ostomate considers his/her situation (11). 74% of the subjects consider the stoma bearable, 26% unpleasant, and only 2% (one woman) repulsive. These results are very similar to those from a survey made in Czech republic in 1995 (7).

90% of the stoma patients advise other ostomates to visit stoma club in order to improve their QoL, 86% recommend to use high quality appliances. They also recommend rehabilitation services, change of dietary habits, literature, and counseling services.

Most of the patients underwent surgery for cancer. We did not scrutinize the impact of life-threatening illness on their QoL, but it certainly reflects in their objective QoL (12).

Our study sample represents ostomates, who are active members of stoma clubs. But 80% of patients with stomas in Slovakia are not members of ILCO clubs, they are not involved in any stoma organization. We assume that their lives are even more seriously affected by stomas. The role of stoma organizations in improving QoL of ostomates, especially in psychological and social spheres of their lives, is irreplaceable.

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