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METABOLISM OF BIOGENIC AMINES IN ACUTE CEREBRAL ISCHEMIA: INFLUENCE OF SYSTEMIC HYPERGLYCEMIA

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Dopamine, norepinephrine and serotonin are biogenic amines which are transmitters of the central nervous system.

The effects of ischemia on the brain parenchyma depends on many factors, such is the mechanism of blood flow interruption, velocity of the occurring blood flow interruption, duration of an ischemic episode, organization of anatomical structures of the brain blood vessels etc., which all influence the final outcome.

During interruption of the brain circulation in experimental or clinical conditions, neurotransmitter metabolism, primarily of biogenic amines, is disturbed. Many researches with various experimental models of complete ischemia reported a decrease in the content of norepinephrine, dopamine and serotonin in the CNS tissue.

It was proven that hyperglycemia can drastically increase cerebral injury followed by short-term cerebral ischemia.

Considering the fact that biogenic amines (dopamine, norepinephrine and serotonin) influence the size of neurologic damage, as well as the fact that in hyperglycemic conditions infarct size (from the morphological aspect) is larger relative to normoglycemic status, the intention was to evaluate the role of biogenic amines in occurrence of damage in conditions of hyperglycemia, i.e. in the case of brain apoplexia in diabetics.

Analysis of biogenic amines metabolism in states of acute hyperglycemia, as well as analysis of the effects of reversible and irreversible brain ischemia on metabolism of serotonin, dopamine and norepinephrine, showed that acute hyperglycemia slows down serotonin, dopamine and norepinephrine metabolism in the cerebral cortex and n. caudatus. Brain ischemia in normoglycemic animals by itself has no influence on biogenic amines metabolism, but the effect of ischemia becomes apparent during reperfusion. In recirculation, which corresponds to the occurrences in penumbra, release of biogenic amines is uncontrolled and increased. Brain ischemia in acute hyperglycemic animals increases the release of biogenic amines

regardless of ischemia duration (5 or 15 minutes). This effect is more apparent during recirculation. Acute hyperglycemia makes brain tissue more sensitive even to ischemia which last shorter, i.e. reversible ischemia

Key words: acute ischaemia, biogenic amines, systemic hyperglycaemia

INTRODUCTION

Catecholamines

Dopamine, norepinephrine and serotonin are biogenic amines which are transmitters of the central nervous system.

Dopamine (DA) and norepinephrine (NE) are synthesized from circulating, tyrosine, through hydroxylation and decarboxylation. In the first phase, tyrosine becomes dihydroxyphenylalanine (DOPA) through hydroxylation, after which dopamine is created through decarboxylation. Tyrosine hydroxylase is the key enzyme of dopamine and norepinephrine synthesis. This enzyme, like DA-betha-hydroxylase (which converts DA into NE), requires oxygen, which means that synthesis of catecholamines is inhibited in anoxia, hypoxia and ischemia. On the other hand, end-products (dopamine and norepinephrine) inhibit tyrosine hydroxilase, which endogenously regulates the quantity of catecholamines. Alpha-methyl-p-tyrosine is used for catecholamine metabolic turnover calculation and measurement due to its kinetic properties.

Catecholamines can be degraded through deamination and O-methylation. Enzymes catalyzing these processes are monoamine oxidase (MAO) in presynaptic mitochondria and catechol-O-methyl-transferase (COMT), an enzyme localized extracellularly, as well as intracellularly. The greatest fraction of catecholamines returns from the synaptic cleft into the presynaptic site before it gets degraded, while a certain quantity is methylated extracellularly under the influence of COMT. Activity of MAO and COMT in endothelial cells of brain capillaries is extremely high, and it quickly degrades catecholamine molecules which appear in the cell. This is the basis of the blood-brain barrier for catecholamines which blocks peripheral dopamine and norepinephrine from entering the brain, as well as their discharge into the periphery in conditions of increased production in the brain. Protection of the brain from peripheral metabolism of catecholamine (hormone) is accomplished in this way, and the periphery is protected from catecholamines (transmitters) metabolism in the brain.

Serotonin (5-hydorxytryptamine) is a biogenic amine which differs from catecholamines by its indolic nucleus. Indolamines are, similarly to catecholamines, widespread in the organism, as well as in the brain. Serotonin is synthesized from the amino acid tryptophan, which under the influence of tryptophan hydroxylase changes into into 5-OH-tryptophan; after that serotonin (5-HT) is created through decarboxylation. MAO-A in the mitochondria is transferred into 5-hydroxyindoleacetic acid (5-HIAA) by deamination of 5-HT.

Pathomorphological and neuroischemic changes in the brain caused by ischemia

Effect of ischemia on brain parenchyma depends on many factors, such as the mechanism of blood flow interruption, velocity of the occurring blood flow interruption, duration of an ischemic episode, organization of anatomical structures of brain blood vessels etc., which all influence the final outcome (Harukuni and Bhardwai, 2006). Normal symmetrical Willis polygon is observed in 20% of persons. Apart from congenital anomalies in blood vessel anatomy at the base of the brain, there can be several sites where collaterals or arterio-arterial connections occur, which can influence the final outcome of an ischemic episode, as well.

Parenchyma lesions can vary depending on the interruption of blood flow under the anastomosis site of collateral arteries or behind main collateral arteries - occlusion. Ischemia causes cell death, but it is not sudden. It is a long process of cell death, consisting of necrobiosis, which is to some degree reversible, after which it goes into the irreversible phase, i.e. the cell dies off even if blood flow through the tissue is reestablished. Necrobiosis can be morphotropic, in which immediate and momentary tissue destruction occurs, as well as morphostatic, in which the structures are preserved for a certain period of time. Depending on the degree of cerebral blood flow reduction and presence or absence of postischemic circulation, various types of morphological cell injury can occur.

Necrobiotic changes are influenced by biochemical events in ischemia, the ones of "energy crisis", as well as accumulation of products that can damage brain tissue. These changes are of hydrophilous character. They appear early, and they are characterized by swelling of astrocyte and dendrite extensions. Apart from these changes at the cell level, some changes at the subcellular level can occur, too, such as swelling of mitochodria and endoplasmatic reticulum.

The initial phase of ischaemic encephalomalacia is characterized by neuropil vacuolation, increased neuron size and microvacuolation of the cytoplasm. Swelling of astrocyte extensions appears around blood vessels. Astrocyte swelling can be intense enough to reduce the capillary lumen at the fissure. This intracellular astrocytic oedema is not a specific response to ischemia or hypoxia because similar reactions of astrocytes appear after various pathological processes. Apart from astrocytes, neurons are endangered in infraction, too. Microvacuoles, or empty circular spaces within the cytoplasm, can be observed. Morphological analysis of the brain tissue distinguishes two basic types of response to ischemia: "contracting" and "swelling". All the stages of neuronal injury can be observed in the affected zone. Swelling – the increase in volume, is recognized by lower nucleus and cytoplasm staining – the cell is like a "balloon". Contraction, i.e. the decrease in neuron body, appears as an irregular cell shape, and nucleoplasmic and cytoplasmic condensation. There is still no clear answer to the question why decompensation process prevails in one neuron, while in the other one edema prevails regarding passive loss of K^+ ions and water, as well as influx of Na^+ into cells.

The infarction zone is a closed system in which all the metabolic processes depend exclusively on endogenous substrate reserves (glucose and ATP) and

oxygen, while metabolite elimination is disabled, unlike in the case of permanent supply of substrates and discharge of degradation products, which is a status of balanced metabolism.

In the early phase of ischemia, oxygen and glucose reserve in CNS are small and sufficient for 30 seconds, but aerobic metabolism has to be maintained for the brain to function normally, and exclusively with aerobic glucose as long as there are oxygen reserves (Siesjo and Ljunggren, 1973). In the first minutes of ischemia energy crises is acute (Klatzo, 1975) and it can cause a series of disorders in energy production.

In experimental ischemia caused by rat embolization or occlusion of carotid arteries in gerbils, acute energy crises is followed by decreased ATP content, phosphocreatinine, glucose and glycogen, while the content of lactate, piruvate and cAMP increases (Weinberger J, Nieves-Rosa J, 1987, Watanabe and Passonneau, 1976; Lust et al., 1975; Lust et al., 1977; Mršulja et al., 1976; Kobayashi et al., 1977; Murakami et al., 1979). However, unlike anoxia and acute hypoxia in which there is complete exhaustion of energy reserves (Siesjo, 1978), these deposits are not completely depleted in ischemia. It was demonstrated experimentally that after 6 hours of ischemia in the cerebral cortex of a gerbil a small concentration of ATP, phosphocreatinine, glucose and glycogen is still present, but the damaged tissue most probably cannot use them due to metabolic insufficiency (Lust et al., 1975). In the early phase of ischemia lactic acidosis and hypercapnia protect the ischemic tissue by causing dilatation of inactive collateral capillaries (Meyer and Denny-Brown, 1975). Acidosis prevents further lactate production (Williamson et al., 1967), so Bohr effect appears, while hypercapnia reduces energy consumption (Kogure et al., 1975). The main cause of brain injury in hypoxia, anoxia and ischemia is lactate accumulation. It was proven (Myers and Yamaguchi, 1976) that tissue lesions along the circulation pathways in animals are larger if the animals previously received glucose, and that they correspond to the degree of lactate accumulation. Tricarbon acid cycle appears in later phase disruption, unlike with previous phases of ischemia: content of citrate, alpha-ketoglutarate and oxaloacetate is reduced, while the content of malate is significantly increased (Duffy et al., 1972; Ljunggren et al., 1974). Apart from this, the activity of this cycle's dehydrogenase is reduced (isocitrate-, succinate- and glutamate-), and there are changes in the content of free amino acids as a consequence of the increased transamination, due to which the content of ammonia increases (Mršulja et al., 1976; Duffy et al., 1972; Đuričić and Mršulja, 1979).

During interruption of brain circulation in experimental or clinical conditions, neurotransmitter metabolism, primarily the one of biogenic amines, is disturbed. Many researches with various experimental models of complete ischemia indicate a decrease in the content of norepinephrine, dopamine and serotonin in the CNS (Weinberger and Nieves-Rosa, 1988). The content of biogenic amines changes in patients with acute cerebral infarction, old cerebral infarcts or hemorrhagia (Meyer and Denny-Brown, 1975; Meyer et al., 1973).

It was proven that hyperglycemia can drastically increase cerebral injury followed by short-term cerebral ischemia (Myers and Yzmzguchi, 1977). However,

effects of hyperglycemia on changes in focal ischemia are controversial (Song, 2003). One study (de Coupten-Myens et al., 1988) showed that hyperglycemia caused by glucose infusion increases infarct size. Constant occlusion of middle carotid artery (MCA) does not significantly increase infarct size in acute diabetic rats. The reason for this variable response of the brain to hyperglycemia is not known. There were assumptions that while hyperglycemia is dangerous to brain regions with elaborate collateral circulation, ischemic regions with a weak collateral flow are not dangerous and may even be more useful than hyperglycemia (Ginsberg et al., 1987; Prado et al., 1988). Insulin reduces brain injury and neurological deficit followed by a temporary cerebral ischemia (LeMay et al., 1988; Voll et al., 1989). It is also possible that when the blood-brain barrier is broken, as it happens in focal ischemia, brain parenchyma can be exposed to abnormally high levels of blood hormones. Such exposure can change metabolic responses of cells to brain ischemia. Since blood-brain barrier is broken – damaged by blood proteins, it is supposed that plasma insulin can get into the brain, change glucose metabolism, and thus change the metabolic response of tissue in focal ischemia, as well as in hyperglycemia conditions (Combs et al., 1990).

Experimental hypothesis

The experiments were conducted on experimental models of desert mice-gerbils (*Meriones unguiculatus*).

It is a fact that metabolism of biogenic amines is significantly disturbed in brain ischemia, and that these changes can be the immediate cause of ischemic damage progression (Mšulja et al., 1978; Cvejić et al., 1980; Spatz and Mršulja, 1990). Considering the fact that biogenic amines (dopamine, norepinephrine and serotonin) influence the size of neurologic damage, as well as the fact that in hyperglycemic conditions infarct size (from the morphological aspect) is larger relative to the normoglycemic status, the intention was to evaluate the role of biogenic amines in the occurrence of damage in conditions of hyperglycemia, i.e. in the case of brain apoplexia in diabetics.

MATERIAL AND METHODS

Experimental model

Adult desert mice (50-60 grams of weight) were kept in standard laboratory conditions during the experiment: daily light-dark cycle, room temperature 22-25^{o}C, fed *ad libitum*.

Hyperglycemia was induced by intraperitoneal administration of 50% glucose solution (2.78M), 5 g/kg of body weight.

Brain ischemia was induced by occlusion of both carotid arteries through a sagittal incision in neck region. After separating from the surrounding tissue, arteries were occluded with Heifetz clips. After expiration of ischemic time (5 or 15 minutes), recirculation was accomplished by atraumatic removal of clips. Animals were sacrificed by decapitation and their heads kept at -25^{o}C until the beginning of brain dissection.

Dissection of brain tissue was performed at 0°C and the following brain structures were separated: cerebral cortex (Cx), hippocampus (Hippo) and nucleus caudatus (NCd), according to Javoy and Glowinsky method (1971).

Experimental design and groups of animals

1. Control group (normoglycemic)
2. Control group (hyperglycemic)
   (a) 15-minute hyperglycemia (acute hyperglycemia)
3. Ischemic (normoglycemic)
   (a) Sacrificed after 5 minutes of ischemia
   (b) Sacrificed after 15 minutes of ischemia
4. Ischemic (hyperglycemic)
   (a) Sacrificed after 5 minutes of ischemia
   (b) Sacrificed after 15 minutes of ischemia
5. Ischemic – postischemic (normoglycemic)
   I – 5 - minutes ischemia
   (a) 15-minute recirculation
   (b) 30-minute recirculation
   (c) 60-minute recirculation
   II – 15-minute ischemia
   (a) 15-minute recirculation
   (b) 30-minute recirculation
   (c) 60-minute recirculation
6. Ischemic-postischemic (hyperglycemia)
   I – 5-minute ischemia
   (a) 15-minute recirculation
   (b) 30-minute recirculation
   (c) 60-minute recirculation
   II – 15-minute ischemia
   (a) 15-minute recirculation
   (b) 30-minute recirculation
   (c) 60-minute recirculation

In each group of animals there were 6 to 18 animals.

Concentrations of serotonin, norepinephrine and dopamine were measured according to the modified Laverty and Taylor method (1968). Measured tissue (samples) (30-50 μg) were homogenized with 6 mL acidified n-butanol and centrifuged at 3.000 rpm for 10 minutes. Five mL of supernatant were mixed with 5 mL n-heptane and 1 ml 0.1 M HCL in order to extract serotonin, norepinephrine and dopamine from the organic phase tissue extract. The mixture was shaken for 15 minutes and centrifuged at 3.000 rpm for 10 minutes. Upper layer – organic phase – was discarded, and some quantity was taken from the acidic residue for fluorometric estimation of serotonin, norepinephrine and dopamine.

Serotonin fluorescence was obtained using l-cystine and o-phthalaldehyde with 10 minutes warming at 100°C and was determined after cooling at 470/360 nm.

Norepinephrine and dopamine fluorescence was determined at 385/485 nm, i.e. 325/375 nm, respectively, after extract reaction with elementary iodine solution which produces fluorescent oxidation products. Fluorescence of these products was detected by "Perkin Elmer" spectrophotofluorometer, model 204.

Blood glucose level was measured by fluorometric procedure.

RESULTS

Intraperitoneal administration of 50% glucose very quickly induces systemic hyperglycemia. In the presented experimental model, 15 minutes after administration is considered to be acute hyperglycemia time.

Hyperglycemia itself can influence biogenic amines level (Tables 1 and 2). Dopamine, norepinephrine and serotonin levels are significantly increased ($p<0.01$) in the cerebral cortex (Table 1) as soon as 15 minutes after hyperglycemic stress; level of biogenic amines remains unchanged relative to the previous status as hyperglycemia time is prolonged. In *n. caudatus* increased level of biogenic amines after intraperitoneal administration of 50% glucose (Table 2) is in the range of 50-60% as soon as 15 minutes later, and it remains at those levels. These findings indicate slowed metabolism of biogenic amines in acute hyperglycemia.

Table 1. Effect of hyperglycemia on cerebral cortex biogenic amines level (*$p<0.01$ compared to controls)

Time after administering glucose	Biogenic amines, μg/g, M±S.E.M. (n)		
	5-HT	DA	NE
Controls (normoglycemia)	813±57 (17)	1056±180 (16)	250±25 (17)
15 minutes (acute hyperglycemia)	1239±114* (14)	1949±160* (14)	376±35* (14)

Table 2. Effect of hyperglycemia on n. caudatus biogenic amines levels (*$p<0.01$ compared to controls)

Time after administering glucose	Biogenic amines, μg/g, M±S.E.M. (n)		
	5-HT	DA	NE
Controls (normoglycemia)	1274±187 (18)	12416±1449 (18)	774±157 (18)
15 minutes (acute hyperglycemia)	2054±150** (14)	13541±1145* (14)	821±148* (14)

Reversible (5 minutes) and irreversible (15 minutes) ischemia during normoglycemia show opposite effects on biogenic amines levels in cerebral cortex (Table 3). Level of 5-HT is significantly increased ($p<0.01$) after reversible, and it is reduced after irreversible ischemia. Contrary to that, dopamine level is

decreased ($p<0.01$) after reversible and increased after irreversible cerebral cortex ischemia. Norepinephrine is unchanged after 5-minute, and it is increased ($p>0.01$) after 15-minute cerebral cortex ischemia in normoglycemic animals.

Table 3. Effect of reversible (5 minutes) and irreversible (15 minutes) ischemia on cerebral cortex biogenic amines level during normoglycemia (*$p<0.05$ compared to controls, **$p<0.01$ compared to controls)

Time after administering glucose	Biogenic amines, μg/g, M±S.E.M. (n)		
	5-HT	DA	NE
Controls (normoglycemia)	813±57 (17)	1056±180 (16)	250±25 (17)
5 minutes	1019±73* (6)	563±77** (6)	197±30 (6)
15 minutes	652±93* (5)	2392±221** (11)	523±47** (6)

In acute hyperglycemia (15 minutes), reversible cerebral cortex ischemia induces a decrease ($p<0.01$) in 5-HT and DA, and increase in NE. The decrease in 5-HT can be observed after 15-minute (irreversible) ischemia, too, as well as the decrease in DA and increase in norepinephrine, but these changes are smaller compared to reversible (5-minute) ischemia (Table 4).

Table 4. Effect of reversible and irreversible ischemia on cerebral cortex biogenic amines level during acute hyperglycemia (*$p<0.05$ compared to controls, **$p<0.01$ compared to controls)

Time after administering glucose	Biogenic amines, μg/g, M±S.E.M. (n)		
	5-HT	DA	NE
Controls (normoglycemia)	1239±114 (14)	1949±160 (14)	376±35 (14)
5 minutes	639±65** (8)	1188±193** (6)	714±50** (8)
15 minutes	564±96** (6)	1436±132* (6)	558±46* (6)

In *n. caudatus* during normoglycemia (Table 5), 5-minute ischemia changes only 5-HT level, while DA and NE levels are changed after the same intensity ischemia in acute hyperglycemia (Table 6). The remarkable fact is that 15-minute ischemia changes 5-HT, DA and NE levels more significantly in acute hyperglycemia than in normoglycemia. Decrease in 5-HT and DA and increase in NOR were observed in acute hyperglycemia (Table 6).

Cerebral cortex serotonin level in normoglycemic animals in periods of recirculation after reversible ischemia (Table 7) is significantly higher compared to control values (Table 1). In reversible ischemia (Table 7), level of 5-HT is decreased by approximately 20% after 60 minutes of recirculation compared to control values in normoglycemic animals.

Cerebral cortex serotonin level in acute hyperglycemia is decreased ($p<0.01$) in shorter recirculation times (Table 7) after reversible ischemia, and increased ($p<0.01$) after irreversible ischemia (Table 7).

Table 5. Effect of reversible and irreversible ischemia on *n. caudatus* biogenic amines level during normoglycemia (*p<0.05 compared to controls, **p<0.01 compared to controls)

Time after administering glucose	Biogenic amines, μg/g, M±S.E.M. (n)		
	5-HT	DA	NE
	acute hyperglycemia		
Controls (normoglycemia)	1247±187 (18)	12416±1449 (18)	774±157 (18)
5 minutes	1689±66* (6)	11343±1683 (6)	783±143 (6)
15 minutes	926±96* (8)	2838±202** (6)	538±59* (5)

Table 6. Effect of reversible and irreversible ischemia on n. caudatus biogenic amines level during acute hyperglycemia (*p<0.05 compared to controls, **p<0.01 compared to controls)

Time after administering glucose	Biogenic amines, μg/g, M±S.E.M. (n)		
	5-HT	DA	NE
Controls (normoglycemia)	2054±150 (14)	13541±1145 (14)	821±148 (14)
5 minutes	1044±144** (6)	2816±322** (8)	1052±141* (6)
15 minutes	1302±85** (6)	9402±555* (6)	1106±160* (6)

Table 7. Postischemic changes in cerebral cortex serotonin level after reversible and irreversible ischemia (*p<0.05 compared to normoglycemic values, **p<0.01 compared to normoglycemic values)

Recirculation time	μg/g, M±S.E.M. (n)			
	normoglycemia		acute hyperglycemia	
	reversible	irreversible	reversible	irreversible
15 minutes	1136±73 (6)	1100±120 (6)	830±139* (8)	1949±262* (5)
30 minutes	1161±128 (6)	1258±134 (6)	840±72 (10)	1014±90 (6)
60 minutes	2723±227 (6)	608±52 (6)	1139±229 (6)	1090±107 (6)

N. caudatus serotonin level after reversible ischemia in normoglycemic animals (Table 8) is increased (p<0.01). On the other hand, serotonin level after irreversible ischemia is decreased (p<0.01) only after 60-minute recirculation (Table 8).

Serotonin level shows no significant postischemic changes after reversible *n. caudatus* ischemia (Table 8), while after irreversible ischemia an increase in serotonin level by over 50% is observed only 15 minutes after facilitating recirculation (Table 8).

Table 8. Postischemic changes in n. caudatus serotonin level after reversible and irreversible ischemia (*p<0.05 compared to normoglycemic values, **p<0.01 compared to normoglycemic values)

Recirculation time	μg/g, M±S.E.M. (n)			
	normoglycemia		acute hyperglycemia	
	reversible	irreversible	reversible	irreversible
15 minutes	1582±310 (6)	1575±111 (6)	939±77** (10)	3138±334* (5)
30 minutes	1913±276 (6)	1302±76 (6)	1418±107* (10)	1296±89 (6)
60 minutes	2728±266 (6)	548±43 (6)	918±105** (6)	1752±145** (6)

Cerebral cortex dopamine level changes in normoglycemic animals in periods of recirculation after reversible ischemia (Table 9) are significantly (p<0.01) higher compared to control values (Table 1). In irreversible ischemia (Table 9), dopamine level is significantly (p<0.01) increased after 30 and 60 minutes of recirculation relative to control values in normoglycemic animals.

Cerebral cortex dopamine level in acute hyperglycemia is significantly (p<0.01) increased after 30-minute recirculation (Table 9) after reversible ischemia, while in irreversible ischemia significant increase (p<0.01) can be observed after 15-minute recirculation.

Table 9. Postischemic changes in cerebral cortex dopamine level after reversible and irreversible ischemia (*p<0.05 compared to normoglycemic values, **p<0.01 compared to normoglycemic values)

Recirculation time	μg/g, M±S.E.M. (n)			
	normoglycemia		acute hyperglycemia	
	reversible	irreversible	reversible	irreversible
15 minutes	1714±195 (6)	1031±109 (5)	2065±230 (8)	5127±427** (5)
30 minutes	1619±268 (6)	1882±137 (6)	3829±655* (8)	2137±397 (5)
60 minutes	2129±348 (6)	2770±212 (6)	1515±222 (5)	2153±249 (6)

N. caudatus dopamine level after reversible ischemia is significantly decreased (p<0.01) after 60-minute recirculation (Table 10) relative to control values (Table 1). After irreversible ischemia, a very significant decrease (p<0.01) in dopamine level can be observed (Table 10).

Cerebral cortex norepinephrine level in normoglycemic animals in periods of recirculation after reversible ischemia (Table 11) shows no significant changes relative to control values (Table 1). In irreversible ischemia (Table 11), norepinephrine level is significantly (p<0.01) increased relative to control values.

Table 10. Postischemic changes in n. caudatus dopamine level after reversible and irreversible ischemia (*p<0.05 compared to normoglycemic values, **p<0.01 compared to normoglycemic values)

Recirculation time	μg/g, M±S.E.M. (n)			
	normoglycemia		acute hyperglycemia	
	reversible	irreversible	reversible	irreversible
15 minutes	11696±1248 (6)	2237±140 (7)	11159±862 (10)	17752±287* (6)
30 minutes	15737±1441 (6)	4449±413 (6)	13890±126 (10)	24872±1833* (7)
60 minutes	3110±245 (6)	4147±426 (6)	12267±958** (8)	17042±881* (6)

In acute hyperglycemia after reversible cerebral cortex ischemia, there are no differences in norepinephrine level in the postischemic period (Table 11) relative to control values (Table 1). Norpeinephrine level is significantly (p<0.01) increased relative to irreversible ischemia after 15-minute recirculation (Table 11).

Table 11. Postischemic changes in cerebral cortex norepinephrine level after reversible and irreversible ischemia (*p.05 compared to normoglycemic values)

Recirculation time	μg/g, M±S.E.M. (n)			
	normoglycemia		acute hyperglycemia	
	reversible	irreversible	reversible	irreversible
15 minutes	401±75 (6)	1051±106 (6)	428±71 (7)	5072±1219* (4)
30 minutes	356±56 (6)	1342±116 (5)	540±49 (7)	396±128* (5)
60 minutes	287±36 (6)	654±86 (6)	476±60 (8)	273±39* (6)

Cerebral cortex norepinephrine level after reversible ischemia shows a significant (p<0.01) decrease only 30 minutes after recirculation (Table 11), and at all recirculation times in irreversible ischemia (Table 11) decrease in level (p<0.05) relative to control values (Table 1).

Table 12. Postischemic changes in n. caudatus norepinephrine level after reversible and irreversible ischemia (*p<0.05 compared to normoglycemic values, **p<0.01 compared to normoglycemic values)

Recirculation time	μg/g, M±S.E.M. (n)			
	normoglycemia		acute hyperglycemia	
	reversible	irreversible	reversible	irreversible
15 minutes	778±66 (6)	1041±118 (6)	885±70 (10)	7858±899** (4)
30 minutes	877±125 (6)	1353±99 (6)	1016±88 (9)	826±103* (6)
60 minutes	291±38 (6)	748±147 (5)	1048±77* (8)	614±35 (6)

N. caudatus norepinephrine level in normoglycemic animals is significantly ($p<0.01$) decreased in irreversible ischemia after 60-minute recirculation (Table 12) relative to control values (Table 2). In irreversible ischemia in normoglycemic animals, norepinephrine level is increased only after 15- and 30-minute recirculation (Table 12).

N. caudatus norepinephrine level after reversible ischemia in acute hyperglycemia shows no significant changes in the investigated recirculation periods (Table 2), while an increase in irreversible ischemia ($p<0.01$) after 15-minute recirculation (Table 12) relative to control values (Table 2) can be observed.

DISCUSSION

The role of catecholamine and serotonin in the pathogenesis and progression of cerebral ischemia has been drawing attention for more than 15 years (Spatz and Mršulja, 1990). Changes in content and metabolic turnover of catecholamines (norepinephrine and dopamine) and their metabolites during and after ischemia in gerbils were extensively investigated in the 70s (Mršulja et al., 1975; 1976; 1978; Welch et al., 1976; Moskovitz and Wurtman, 1976; Cvejić et al., 1980). Subsequent studies (Cvejić et al., 1984; Spatz et al., 1986; Kumami et al, 1989; Mršulja et al., 1989; Spatz and Mršulja, 1990) indicate that there is a decrease in catecholamine and serotonin levels and lack of correlation between ischemic changes of energy metabolism and catecholamines during and after ischemia. Disturbance of catecholamines metabolism in postischemic brain is contradictory to previous indications that energy metabolism in the postischemic brain is restored to preischemic condition. However, the observed reduction in catecholamines after long-term ischemia was not observed after short-term ischemia (Mršulja et al., 1989), i.e. the development of "ischemic profile of monoamines" was not stopped by recirculation.

Disbalanced metabolism of catecholamines is manifested by slow development of lesions described as "maturations", and it coincides with morphological neuron changes. Progression of brain damage during reperfusion of previously ischemic brain shows clearly that transmission rather than energy is responsible for the functional deficit appearing after ischemia (Spatz and Mršulja, 1990).

The role of catecholamines and serotonin in the pathology of cerebrovascular injuries is not clear, but there are indications of their importance. We can establish three significant factors participating in the ischemic decrease of brain functions:

(1) disturbance of high-energy phosphate pool;

(2) disturbance of ATP-dependent sodium-potassium-calcium gradient; and

(3) release of neurotransmitters such as glutamate and monoamines.

This leads to the conclusion that the period after ischemia is not a simple restoration of metabolism to preischemic condition as it has been suggested (Mršulja et al., 1977). Generally speaking, there are two postischemic phases: a period immediately after ischemia, which is characterized by brain

hypermetabolism, and after that a period of reduced metabolic activity. Immediately after re-establishing circulation, increase in metabolic turnover and decrease in metabolic rate were found for norepinephrine and dopamine in the cerebral cortex; in the *striatum* the metabolic turnover for dopamine is increased, but it is reduced for norepinephrine (Cvejić et al., 1981). These observations are the first biochemical proofs of existence of regional selective sensitivity to catecholaminergic pathways (Kumami et al., 1989).

The mechanism causing the increase in catecholamine levels in the extracellular space in ischemia may be the result of increased discharge of dopamine into extracellular space and decreased elimination of dopamine from the same region. Synaptic release of catecholamines can be caused by an increase in extracellular potassium level, or level of intracellular calcium, or an disorder of both electrolytes homeostasis during ischemia, i.e. increase in extracellular potassium and increase in intracellular calcium cause the disturbance of Na-K-Ca-ATPase, which depends on ATP. On the other hand, elimination of catecholamine from extracellular space depends on neural reuptake, which is also an ATP dependent process. Impairment of these mechanisms can be responsible for the impaired catecholamine release during ischemia, but the question of participation of other mechanisms in secondary (postischemic) release of monoamines is still not answered. Biphasic striatal dopamine release during transient ischemic attack and reperfusion in gerbils was proven by Ahn et al. (1991). The first increase in extracellular dopamine was observed after 2 minutes of ischemia, and the second, significantly larger, during recirculation. These findings support earlier observations that decrease in ATP and decrease in catecholamine are not synergic processes since striatal ATP is used in the course of one minute and it is renewed during 15 minutes of recirculation after 5-minute ischemia (Mršulja et al., 1986; Lust et al., 1986; Mršulja et al., 1989).

Diabetes is a significant metabolic risk factor for the development of brain atherosclerosis, and also a significant factor in the occurrence and development of ischemic cerebrovascular disorders since disturbed glucoregulation leads to metabolic and hemorheological damages.

Hyperglycemia existing before brain ischemia significantly influences the outcome of injury (Li Pa et al, 1994; Kozuka et al., 1989). This can primarily be observed in the decreased capability of the brain to utilize glucose and the appearance of clinical signs of irritability and convulsions. It is believed that greater brain damage is caused by disturbed circulation, while in hyperglycemic conditions it is caused by the occurrence of intracellular and extracellular acidosis (Smith et al., 1986). Acidosis impairs metabolism in brain mitochondria, and this impairment is more noticeable during ischemia (Hillered et al., 1985).

After 15 minutes of hyperglycemic status, which is considered to be the acute hyperglycemic status, level of all biogenic amines: serotonin, dopamine and norepinephrine, is increased. This implies increased synthesis and decreased release of monoamines. This indicates that hyperglycemia slows down metabolism of biogenic amines in the brain, which means that tissue does not utilize biogenic amines within physiological limits regardless of their increased

content, so it can be considered that, for example, there is insufficient dopamine in the basal ganglia, which occurs in Parkinson's disease. It should be pointed out that hyperglycemia does more damage to dopaminegric and serotonergic than to noradrenergic mechanisms in the *nucleus caudatus*, as well as in the cerebral cortex.

Ischemia (reversible and irreversible) during the very onset of attack has almost no effect on the content of biogenic amines, but these effects present themselves after reestablishing circulation. This confirms the earlier opinion that recirculation is a new attack, i.e. a new pathophysiological status (Mršulja, 1979). During recirculation there is an increased release of biogenic amines in early postischemic periods (Cvejić et al., 1970).

Content of biogenic amines in states of acute hyperglycemia is drastically decreased, which implies an increased, uncontrolled monoamine release. In normoglycemic animals this occurs only in the phase of recirculation. There is no doubt that acute hyperglycemia gets worse, i.e. it decreases the metabolic turnover of biogenic amines, and that way influences central nervous system during ischemia. A longer period of recirculation is necessary for pathological metabolism of biogenic amines to occur. These findings once more confirm the earlier know phenomenon of ischemic lesion maturation (Mršulja, 1979; Mršulja and Mršulja, 1991). If we allow that status during ischemia according to its chemodynamic parameters responds to the "central zone", and the period of recirculation to the occurrences in "penumbra" (Mršulja et al., 1989), it is obvious that biogenic amines metabolism is more sensitive, and thereby more vulnerable in "penumbra". In addition to that, if metabolism in "penumbra" is responsible for neurologic deficit in cerebrovascular attack (Mršulja et al., 1989), we can assume that the very clinical signs of neurological insufficiency in brain apoplexia are the result of disturbed metabolism of biogenic amines in "penumbra". During the period of acute hyperglycemia, damaged metabolism of biogenic amines in "penumbra" region is more apparent than in normoglycemia, which is another proof of negative influence of hyperglycemia on the final outcome of cerebrovascular attack.

CONCLUSIONS

Analysis of biogenic amines metabolism in states of acute hyperglycemia, as well as analysis of the effect of reversible and irreversible brain ischemia on metabolism of serotonin, dopamine and norepinephrine, showed that:

1. Acute hyperglycemia slows down serotonin, dopamine and norepinephrine metabolism in cerebral cortex and n. caudatus.
2. Brain ischemia in normoglycemic animals by itself has no influence on biogenic amines metabolism, but the effect of ischemia becomes apparent during recirculation.
3. In recirculation, which corresponds to the occurrences in penumbra, release of biogenic amines is uncontrolled and increased.

4. Brain ischemia in acute hyperglycemic animals increases the release of biogenic amines regardless of ischemia duration (5 or 15 minutes). This effect is more apparent during recirculation.

5. Acute hyperglycemia makes brain tissue more sensitive even to ischemia which last shorter – reversible ischemia.

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METABOLIZAM BIOGENIH AMINA U AKUTNOJ ISHEMIJI MOZGA: UTICAJ SISTEMSKE HIPERGLIKEMIJE

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SADRŽAJ

Efekat ishemije na parenhim mozga zavisi od mnogih faktora, kao što su mehanizam nastalog prekida protoka krvi, brzina nastalog prekida protoka krvi, dužina trajanja ishemične epizode, organizacija anatomskih struktura krvnih sudova mozga itd., što sve utiče na krajnji ishod. Dopamin, noradrenalin i serotonin su biogenic amini koji se ubrajaju u transmitere centralnog nervnog sistema.

Pri prekidu moždane cirkulacije u eksperimentalnim ili kliničkim uslovima metabolizam neurotransmitera, u prvom redu biogenih amina, je poremećen. Mnoga istraživanja na raznim eksperimentalnim modelima kompletne ishemije ukazuju na smanjenje sadržaja noradrenalina, dopamina i serotonina u centralnim nervnim tkivima.

Dokazano je da hiperglikemija može drastično da poveća cerebralno oštećenje praćeno kratkotrajnom cerebralnom ishemijom.

Imajući u vidu činjenicu da biogeni amini (dopamin, noradrenalin i serotonin) neposredno učestvuju u određivanju veličine neurološkog oštećenja i činjenicu da je u stanjima hiperglikemije veličina infarkta (sa morfološkog aspekta) veća u odnosu na normoglikemičnu situaciju, hteli smo da proverimo kolika je uloga biogenih amina u nastajanju oštećenja u stanjima hiperglikemije, odnosno u situacijama pojave apopleksije mozga u dijabetičara.

Analizom metabolizma biogenih amina u stanjima akutne i hronične hiperglikemije i efekat reberzibilne i ireverzibilne ishemije mozga na metabolizam serotonina, dopamina i noradrenalina, ustanovljeno je da akutna hipeglikemija usporava metabolizam serotinana, dopamina i noradrenalina u kori mozga i *n. caudatus*-u. Ishemija mozga u normoglikemičnih životinja po sebi je bez uticaja na metabolizam biogenih amina, ali se efekat ishemije ispoljava u toku recirkulacije. U recirkulaciji, što odgovara događajima u penumbri, oslobađanje biogenih amina je nekontrolisano i pojačano. Ishemija mozga u akutno-hipeglikemičnih životinja povećava oslobađanje biogenih amina nezavisno od dužine trajanja ishemije (5 ili 15 minuta). Ovaj efekat je još više izražen tokom recirkulacije. Akutna hiperglikemija čini moždano tkivo osetljivim već na ishemiju kraćeg trajanja – reverzibilnu ishemiju.