ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. LIX, N 1, 71 SECTIO D 2004

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Dopamine activity changes in cerebral cortex in the course of experimental acute pancreatitis

Dopamine (DA) is a compound widely spread in the animal world, belonging to biogenic amines group. Dopamine is a neurotransmitter and neuromediator found in human beings both in peripheral nervous system and in brain surrounding.

In the central nervous system dopaminergic neurones form three main tracts responsible for regulating motor processes (motor drive, coordination and muscular tone), emotional processes and higher psychological activities; they also take part in regulation of hormonal activities (they inhibit secretion of prolactin and growth hormone, regulate secretion of pituitary gonadotropin).

Acute pancreatitis is a disease condition in which as a result of pancreas extrasecretory cells damage, there is the exuding of pancreas enzymes to the surrounding, which leads to self-digestion, shock symptoms, intravascular clotting, brain tissue ischaemia (4, 5). The main symptoms are: oedema, petechia, fat embolism, degeneration changes turning into necrotic ones in ganglionic cells (from which Purkinje's cells of the cerebellum and hypothalamus cells are the most susceptible). Among mechanisms which may take part in the process of encephalopathy in the pancreatitis course, the following should be also enumerated: hypo- and hyperglycaemia, activity of inflammatory mediators malabsorbtion from the alimentary tract (1, 4, 5). Clinical signs of less or more intense encephalopathy are visible after 24 hours in about half of the patients admitted to hospital with advanced stages of the illness. States of sudden excitation, confusion, focal and general convulsions, stupor and even coma, are the most frequent symptoms.

The study aimed at an analysis of changes in the level of dopamine in rats' cerebral cortex in the course of experimental acute pancreatitis.

MATERIAL AND METHODS

The experiment was carried out on 102 male Wistar rats (weight 190–220g). They were divided into three groups: Z – healthy (12), K – control (30), OP – experimental (60). The study was fully approved by the University Ethics Commission. The acute pancreatitis was induced in OP group according to Heinkel and Aho method (1). The procedure was conducted in sterile conditions. Rats were anaesthetised with ketamine. After laparotomy a needle (0.5 x 16 mm) was introduced into the common bile-pancreatic duct through duodenum. Soft forceps near hilus then shut the hepatic duct, a ligature was put on close to the opening of the bile-pancreatic duct and purse-string suture was put on duodenum. 5% sodium taurocholate (Sigma, Chemical Co., St. Louis, Missouri) was injected. The animals in K group had only the needle inserted into the duct to analyse the effects of mechanical damage.

2, 6, 12, 24, 48 hours after acute pancreatitis induction the rats were anaesthetised again and thoracotomy was made to take blood from the left ventricle and determine the activity of pancreatic enzymes and oxygen level. Amylase activity (EC3.2.1.1.) was determined by the enzymatic method using Cormay reagents, and Cobas Mira Plus analyser. Lipase Activity (EC 3.1.1.3) was determined by turbometric method using Roche reagents and Cobas mira Plus analyser. The blood oxygenation was conducted using the Ciba Corning 248 analyser. The brain was sampled so as to determine the biogenic amines level. Hemisphere and the commissural system was taken for biochemical tests. The Brodie method modified by Chang was used (2, 3).

The statistical analysis was carried out. Examining features were characterised by arithmetic mean ± standard deviation. A t-student test was performed to show differences between means. A 5% error was assumed.

RESULTS

The activity of lipase and amylase were determined in serum blood samples as the indicators of severity of acute pancreatitis. In group Z the activity of amylase ranged from 145 to 483 U/dl, mean 292 ± 74.3. Mean values of amylase activity in OP group after 2, 6, 12, 24, 48 hours were: 486.2 U/dl, 115.3 U/dl, 721.7U/dl, 253.8 U/dl, 769.3 U/dl, 276.3 U/dl, 880.7 U/dl, 283.5 U/dl, 950.9 U/dl 281.6 U/dl; in K group: 385.3 U/dl, 166.3 U/dl, 516.4 U/dl, 116.7 U/dl, 784.3 U/dl, 173.8 U/dl, 808.6 U/dl, 148.7 U/dl, 814.2 U/dl, 192.1 U/dl. The differences between values of Z, K and OP groups were always statistically significant ($p < 0.01$).

In group Z activity of lipase ranged 2 –18 u/dl, mean value 9.9 U/dl. Mean values in OP group were (after 2, 6, 12, 24, 48 hours): 18.1 U/dl, 3.36 U/dl, 19.4 U/dl, 5.64 U/dl, 28.9 U/dl, 15.9 U/dl, 140.2 U/dl, 45.2 U/dl, 142.9 U/dl, 55.9 U/dl; in K group: 15.0 U/dl, 5.4 U/dl, 18.1 U/dl, 7.2 U/dl, 23.5 U/dl, 4.8 U/dl, 102.9 U/dl, 31.4 U/dl, 135.0 U/dl, 33.8 U/dl. The differences between values of Z, K and OP were always statistically significant ($p < 0.01$).

The mean values of pO_2 are presented in Table 1. The differences between values of Z, K, OP were statistically significant $p < 0.001$, with the exception of result in K group after 2 hours.

The mean values of dopamine in the successive hours of the experiment in each group and standard deviation are presented in Table 2. The levels of importance for each group in successive hours of experiment are presented in Table 3.

Table 1. The values of mean partial pressure of O_2 in blood in the course of experiment (mmHg)

	Group Z	Group OP	Group K
Time	mean ± SD	mean ± SD	mean ±SD
2 h	89.42 ± 4.12	60.48 ± 1.87	79.03 ± 3.15
6 h	85.37 ± 4.41	53.12 ± 2.38	74.80 ± 2.76
12 h	87.65 ± 3.83	51.77 ± 2.15	73.30 ± 1.57
24 h	87.54 ± 4.07	41.18 ± 2.47	67.97 ± 1.11
48 h	88.6 ± 3.78	39.90 ± 2.97	66.13 ± 3.45

Table 2. The level of dopamine (U/dl) and standard deviation in the course of experiment

	Group Z		Group OP		Group K	
Time	mean ± SD	range	mean ± SD	range	mean ±SD	range
2 h	446.9 ± 61.6	346.4–543.1	456.3 ± 34.1	401.5–505.5	495.5 ± 12.8	480.1–508.1
6 h	445.4 ± 63.6	333.4–543.1	494.7 ± 15.7	476.5–518.5	504.4 ± 30.5	476.1–534.1
12 h	456.9 ± 56.2	365.4–562.1	510.2 ± 32.8	482.5–563.5	495.6 ± 13.9	482.1–513.1
24 h	447.4 ± 62.5	346.4–549.1	478.1 ± 33.5	429.5–516.5	498.8 ± 1.5	497.1–500.1
48 h	446.7 ± 63.1	340.1–547.1	491.5 ± 29.5	458.5–524.5	494.4 ± 18.9	481.1–516.1

Table 3. The levels of importance for each group in successive hours of experiment

	2-h	6-h	12-h	24-h	48-h
	DA	DA	DA	DA	DA
K vs Z	$p < 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
OP vs Z	$p > 0.05$	$p < 0.05$	$p < 0.05$	$p > 0.05$	$p > 0.05$
K vs OP	$p < 0.05$	$p > 0.05$	$p < 0.05$	$p > 0.05$	$p > 0.05$

DISCUSSION

Shock, DIC, rapid changes of glucose level in blood, inflammatory mediators, fat embolism, digestion and absorption outcome are responsible for pancreatic encephalopathy.

Excitement, confusion, convulsions, dementia, coma are the signs and symptoms of acute pancreatic encephalopathy: CNS hypoxia and ischemia (which can take place during acute pancreatitis) cause energetic crisis first. The most active cells are affected in that way: reticular system GABA-ergic cells, brain neurones, cerebellum cortex Purkinje's cells, and hippocamp cortex pyramid cells. Damage of the GABA-ergic cells, which form a kind of filter impairing convulsive stimulation, causes convulsions. GABA-ergic cells have also inhibitory influence on DA neurones. Their damage may manifest itself in the DA level increase, which in turn may intensify motor agitation/hiperkinesia and convulsions both focal and general, which are found in the pancreatic course (4, 5). In experiment with rats, submitted to chronic stress that developed reduced fighting behaviour, the brain level of DA was unchanged, but in rats, treated chronically with an antidepressant, the brain concentration level of DA was reduced. This group of animals restored the intensity of fighting behaviour to control value, which may indicate that DA neurotransmission is connected with regulation of aggressive behaviour (6, 10).

Hypoxia and ischemia in acute pancreatitis damage brain vessel epithelium, which may increase the permeability of brain-blood barrier the more serious and longer hypoxia, the earlier and the more severe damage of brain-blood barrier (5). Due to that neurotoxic substances can penetrate from vessels to brain tissue. Inflammatory mediators such as: TNF, PAF, leukotriens, phospholipase A2, cytokines, prostaglandines should be mentioned here. These substances can also be produced by epithelium. It has been established that the synthesis of interleukines begins in the early hours of the disease and grows till 5th day (5, 9). Interleukins cause the increase of neurones oedema and their malfunction. It has been proved that IL–1 causes the increase of release P substance. P substance boosts the release of DA in *nucleus caudatus*. Substance P is a neuropeptide, which functions as both neurotransmitter and

neuromediator, particularly in the course of pain sensation. The research carried out on mice by Bhatio M. showed that the level of substance P in experimental acute pancreatitis increases. (Bhatio M. 1998) Final effect of that process is intensification of pain sensation. However, the influence of inflammatory state on the nervous system is generally reflected in the decrease of its activity and the presence of oedema, which is protection activity.

DIC in the course of acute pancreatitis brings out eventually in the central nervous system changes similar to those, which occur in ischaemia hypoxia. Disseminated focuses of necrosis are more probable to occur in better supplied with blood parts of brain, which manifests itself in the changes of the patient's behaviour (higher psychic processes disturbances). In the course of acute pancreatitis glycaemia fluctuations are caused by pancreatic system damage and hypoinsulinaemia. Changes in the content of monoamines such as dopamine and serotonin in the insulin granules are known to influence insulin release. They may lead to periodic lactic acidosis, which may cause cerebral oedema and GABA-ergic neurones damage (7, 8).

CONCLUSIONS

1. The most significant changes of DA level during acute experimental pancreatitis were noticed during the first 24 hours of experiment.

2. DA level changes were in a statistically significant way correlated with the level of amylase and lipase in blood serum.

3. Peak DA concentration was detected between 6^{th} and 12^{th} hour of experiment.

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SUMMARY

Dopamine (DA) is biogenic amine that in the central (CNS) and peripheral nervous system plays an important role as a neurotransmitter and neuromediator. Dopamine neurones in CNS are responsible for regulating motor and emotional processes, higher psychological activities and hormone secretion. Acute pancreatitis (AP) is a general disease condition caused by pancreas extrasecretory cell damage which leads to self-digestion. One of its results is encephalopathy in consequence of shock symptoms, intravascular clotting, brain tissue ischaemia etc. The study has aimed at analysis of changes in the level of DA in rats' cerebral cortex in the course of experimental AP. The experiment was carried out on 102 rats. They were divided into three groups: Z – healthy (12), K – control (30), OP – experimental (60). The AP was induced in OP group according to Heinkel and Aho method. In K group the needle was only inserted into the common bile-pancreatic duct to analyse the effects of mechanical damage. The activity of pancreatic enzymes was determined to estimate the intensity of pancreatitis. The dopamine level was measured in rats« brain samples and to do so, the Brodie method modified by Chang was used. The statistical analysis was carried out. Conclusions: The greatest changes of DA level during experimental AP were noticed during the first 24 hours of experiment and they were in statistically significant way correlated with the level of lipase and amylase in blood serum. Peak DA concentration was detected between 6^{th} and 12^{th} hour of experiment.

Aktywność dopaminy w korze mózgu w przebiegu doświadczalnego ostrego zapalenia trzustki

Dopamina (DA) jest związkiem z grupy amin biogennych. Jest ona neuroprzekaźnikiem i neuromediatorem, występującym u ludzi zarówno w obwodowym układzie nerwowym, jak i w mózgowiu. W CUN neurony dopaminergiczne tworzą trzy główne szlaki, które odpowiedzialne są za regulację procesów ruchowych, emocjonalnych, wyższych czynności psychicznych oraz mają wpływ na wydzielanie hormonów. Ostre zapalenie trzustki (OZT) jest stanem chorobowym, w którym na skutek uszkodzenia komórek zewnątrzwydzielniczych dochodzi do wydostania się do środowiska enzymów trzustkowych, co prowadzi do samostrawienia, objawów wstrząsu, wykrzepiania wewnątrznaczyniowego, niedokrwienia tkanki mózgowej i w konsekwencji bardzo często do encefalopatii. Celem pracy była analiza zmian poziomów DA w korze mózgu szczurów w przebiegu doświadczalnego OZT u tych zwierząt. Doświadczenie przeprowadzono na 102 szczurach, samcach rasy Wistar. Wyodrębniono 3 grupy zwierząt: K – kontrolne (30), Z – zdrowe (12), OP – operowane (60). Zwierzętom z grupy OP wywołano OZT metodą Heinekela i Aho, natomiast w grupie K wprowadzano jedynie igłę iniekcyjną w celu prześledzenia skutków mechanicznego uszkodzenia narządów. Do oceny nasilenia zmian zapalnych w trzustce posłużono się analizą aktywności amylazy i lipazy. W celu oznaczania poziomu amin biogennych pobierano mózgowie zwierzęcia. Poziom DA oznaczono przy użyciu metody Chang, zmodyfikowanej przez Brodiego. Uzyskane wyniki poddano analizie statystycznej. Wnioski: Najbardziej istotne zmiany poziomu stężenia DA w mózgu szczurów w przebiegu doświadczalnego OZT zanotowano w pierwszej dobie choroby, były one w sposób istotnie statystyczny skorelowane z poziomem aktywności amylazy i lipazy w surowicy krwi. Szczytowe stężenia DA obserwowano między 6 a 12 godziną doświadczenia.