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ACUTE PANCREATITIS AFFECTS THE METABOLISM OF CATECHOLAMINE NEUROTRANSMITTERS IN RATS

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Abstract—Abnormalities of mental status represent a severe complication and an important cause of death in acute pancreatitis (AP), which is characterized by a pattern of neurological signs and symptoms. As some of the symptoms of AP are also affected by catecholamine neurotransmitters, they cannot be ruled out of the pathophysiology of AP; however, little research has been performed exploring this hypothesis. Our study aimed to elucidate whether AP affects the metabolism of catecholamine neurotransmitters in rats. A total of 300 male SD rats were randomly divided into five groups: control, 6H, 24H, 48H and 72H experimental groups. AP was induced in rats by an injection of a sodium taurocholate solution via a cannulated bile-pancreatic duct. In the striatum, hippocampus and cerebellum, catecholamine neurotransmitters were tested using high performance liquid chromatography equipped with a electrochemical detector, and the activities and protein concentration levels of monoamine oxidase A (MAO-A) and tyrosine hydroxylase (TH) were also evaluated using ELISA and Western blotting analyses. In the hippocampus, the dopamine (DA) concentrations increased in the 48-h and 72-h groups. The 3,4-dihydroxyphenylacetic acid (DOPAC) concentration of the 72-h group also increased. The MAO-A and TH activity of the 6-h and 24-h groups decreased, respectively. The TH activities of the 48-h groups also decreased. The MAO-A and TH protein concentration of the 6-h and 24-h groups decreased. In the corpus striatum, the homovanillic acid concentration of the 72-h group and norepinephrine concentrations of the 24-h and 48-h groups increased, respectively. The MAO-A and TH activities of the 6-h and 24-h groups decreased. The MAO-A and TH protein concentrations of the 6-h and 24-h groups decreased. In the prefrontal cortex (left prefrontal lobe), the DA and DOPAC concentrations of the 48-h group increased. The MAO-A and TH activities of the 6-h, 24-h and 48-h groups decreased. The MAO-A and TH protein concentrations of the 6-h and 24-h groups also decreased. The other catecholamine concentration and enzyme activities fluctuated, but there was no statistically significant difference compared with the control group. Catecholamine neurotransmitter metabolic systems are widely affected in AP, and these fluctuations may play an important role in determining the symptomatology of AP.

Key words: acute pancreatitis, catecholamine neurotransmitter, monoamine oxidase, tyrosine hydroxylase.

INTRODUCTION

Acute pancreatitis (AP) is caused by pancreas exocrine cell damage, and it has an adverse effect on the psychological state (Gupta et al., 2001). Abnormalities of mental status in patients with AP are severely complicated and are also an important cause of death. The mortality of AP patients who had mental and nervous symptoms can reach levels of 67–100% (Menza and Murray, 1989). A few cases and studies have been published, but the pathophysiology of the disease has not yet been completely elucidated.

Abnormalities of mental status in patients with AP were first described by Lowell in 1923, and Rothermich provided more detail on the abnormalities of mental status, such as disorientation, trance, agitation and hallucination (Estrada et al., 1979; Menza and Murray, 1989). The incidence of encephalopathy is not consistent; De Falco et al. reported that in patients with pancreatitis without a history of alcohol abuse, the incidence of abnormalities of mental status ranged from 9% to 35% (De Falco et al., 1980). The neurological signs and symptoms may occur anytime during the first 2 weeks of AP, irrespective of its etiology. Convulsions, paresis of the limbs and dysarthria can arise abruptly, and gradual onset with behavioral changes, such as psychomotor agitation, disorientation, visual and auditory hallucinations, and even coma, can also be observed (Ruggieri et al., 2002). The symptoms can fluctuate over hours or days. Gupta reported that in patients with predicted severe AP, depression first increased and then decreased on day seven (Gupta et al., 2001). The only large-scale, placebo-controlled study showed that aprotinin had no positive effects on

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Abbreviations: ADHD, attention deficit hyperactivity disorder; AP, acute pancreatitis; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; EDTA, ethylenediaminetetraacetic acid; HVA, homovanillic acid; L-DOPA, L-3,4-dihydroxyphenylalanine; MAO, monoamine oxidase; MAO-A, monoamine oxidase A; NE, norepinephrine; TH, tyrosine hydroxylase; THD, TH deficiency.

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the incidence or course of neuropsychiatric symptoms of AP (Anon., 1980). The multiplicity symptomatology of AP suggests that different mechanisms are involved; thus, more data are required to assess this aspect.

Catecholamine neurotransmitters are an important neurotransmitter and neuron mediator, which are not only responsible for regulating motor and emotional processes, psychological activities and hormone secretion but are also associated with most of the neuropsychiatric symptoms of AP, as previously described. The initial reaction of L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) catalyzed by tyrosine hydroxylase (TH) has been confirmed to be the rate limiting step of catecholamine production (Nagatsu et al., 1964). L-DOPA is the precursor for dopamine (DA), which in turn is the precursor for norepinephrine (NE). Monoamine oxidase A (MAO-A) catalyzes the oxidative deamination of catecholamines, of which DA mainly converts into 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). NE is mainly degraded into 3,4-dihydroxymandelic acid, 3-methoxy-4-hydroxyphenylglycol and various other metabolites via MAO-A.

In our opinion, because some of the neuropsychiatric symptoms of AP are also affected by catecholamine neurotransmitters, they cannot be ruled out as the pathophysiology of AP in the brain. However, few studies have been performed investigating this hypothesis. Kopieniak et al. performed a study investigating DA activity changes over the course of experimental AP but only in the cerebral cortex (Kopieniak et al., 2004). We assayed catecholamine neurotransmitters in SD rats and the key enzymatic activities and protein concentration levels of TH and MAO-A in the corpus striatum, left prefrontal cortex and hippocampus to confirm our hypothesis.

EXPERIMENTAL PROCEDURES

Sample preparation

Three hundred and seventy-five male Sprague–Dawley rats (180–220 g) were used for this study. The rats were maintained in groups of five or less in polycarbonate cages under standard animal laboratory conditions (22 ± 1 °C, 12-h light/dark cycles, lights on at 8:00 AM). The committee for animal experimentation at the Capital Medical University of China approved all of the experimental protocols. The animals were given standard rat food and fasted overnight before the experiment in which AP was induced.

The rats were divided into five groups: C – control, 6H – experimental, 24H – experimental, 48H – experimental and 72H – experimental groups. AP was induced by an injection of 0.3 mL of a 0.5% sodium taurocholate solution via a cannulated bile-pancreatic duct (Abe et al., 2002). In the C group, the needle was only inserted into the common bile-pancreatic duct, which is analogous to mechanical damage. The survival rate of the control, 6H, 24H, 48H and 72H groups was approximately 95%, 91.67%, 71.67%, 63.33% and 53.33%, leading to the harvesting of 57 rats in the control group, 55 rats in the 6H group, 43 rats in the 24H group, 38 rats in the 48H group and 32 rats in the 72H group.

Preparation of brain samples

The animals were sacrificed by decapitation. The brain samples were rapidly dissected on ice, frozen in liquid nitrogen, and then stored at −80 °C until further analyses (HPLC analysis, enzyme function tests and Western blotting tests) (Glowinski and Iversen, 1966).

To observe the histological changes in the pancreas in the AP groups, every pancreatic tissue sample was immediately fixed in neutral buffer formalin, embedded in paraffin and stained with hematoxylin and eosin. Every pancreatic sample from the AP group was confirmed as AP by a pathologist before the brain samples were used in catecholamine and enzyme analyses.

Neurochemical assay

The levels of catecholamines (DA, DOPAC, HVA, and NE) were determined blindly using high-performance liquid chromatography combined with electrochemical detection (HPLC-ECD) using the method by Beyer (Beyer et al., 2002). Brain sample tissues were sonicated in 0.5 ml of pre-cold 0.4-N $HClO_4$. The resulting homogenates were centrifuged for 25 min at 12,000*g* at 4 °C. The mobile phase consisted of 10% methanol (pH 4.7), 0.09 mol/L without water–sodium acetate, 0.035 mol/L anhydrous citric acid, 0.23 mmol/L sodium alkane sulfonate, and 0.13 mmol/L EDTA. The electrochemical condition during the experiment was +0.50 V. The flow rate of the separations was 1.0 ml/min. The samples (20 μl) were self-acting injected and quantified by comparing the area under the curve (AUC) with reference standards. Concentrations of the samples were expressed in nanograms of neurotransmitter per gram of wet weight of the brain tissue (Sun et al., 2010).

Enzymatic activities

The MAO-A activities were determined using a rat monoamine oxidase (MAO) ELISA kit (CUSABIO BIOTECH, Wuhan, China). Each sample was homogenized in 10 volumes of ice-cold 0.2 M potassium phosphate. The resulting homogenates were centrifuged at 5000*g* for 5 min at 4 °C. The fluorescence was tested using a fluorescence microplate reader with a filter set for excitation and emission at 450 nm. The protein contents were tested using the Lowry method.

TH activities were measured using the GenMed kit (GenMed Scientifics, Arlington, USA), according to the manufacturer's instructions. Tissues were sonicated in 10 volumes of 50-mm Hepes buffer, 10% glycerol, 5 μg/ml leupeptin, and 5 μg/ml aprotinin at 4 °C. After centrifugation, 2 μl of the supernatant was added to 0.5 ml assays containing 40-mm Hepes, 100-μm tyrosine, 100 μg/ml catalase, 10-μm ferrous ammonium sulfate, and 1-mm dithiothreitol, pH 7.0. The reaction was initiated by the addition of 400-μm