Noopept Reduces the Postischemic Functional and Metabolic Disorders in the Brain of Rats with Different Sensitivity to Hypoxia

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 147, No. 3, pp. 311-316, March, 2009
Original article submitted July 24, 2008

Chronic cerebral ischemia was induced by ligation of both common carotid arteries in Wistar rats, divided by sensitivity to hypoxia into highly sensitive and low-sensitive. Noopept (peptide preparation), injected (0.5 mg/kg) during 7 days after occlusion of the carotid arteries, reduced the neurological disorders in rats with high and low sensitivity to hypoxia and improved their survival during the postischemic period. Noopept normalized behavior disordered by cerebral ischemia (according to the open field and elevated plus maze tests), prevented accumulation of LPO products and inhibition of antioxidant systems in the brain of rats with high and low sensitivity to hypoxia. Hence, noopept exhibited a neuroprotective effect in cerebral ischemia.

Key Words: *noopept; brain ischemia; lipid peroxidation; antioxidant systems; neuroprotection*

Chronic cerebrovascular disorders belong to the most prevalent forms of neurological diseases. The severity of clinical status of patients with dyscirculatory encephalopathy determines the progressive limitation of neuropsychological, motor, and cognitive functions and metabolic disorders. Individual features of disease course depend on the severity of previous chronic cerebral ischemia, basal cerebral metabolic status, and reactivity of the neuroendocrine system [3]. The degree of metabolism and higher nervous system functions recovery after cerebral ischemia depend, among other things, on the individual sensitivity to acute ischemia [4,5].

Neurometabolic therapy, aimed at maintenance of the viability of cerebral tissue under conditions of hypoxia and restoration of impaired functions, is an important component of therapy in patients with chronic cerebral ischemia [1]. Great attention is paid to secondary neuroprotection by neuropeptides or drugs based on endogenous bioactive compounds with high bioavailability for brain tissues, which are particularly effective during the postischemic period as reparative drugs. Noopept (N-phenyl-L-prolylglycine ethyl ether) is one of the recently developed neuropeptide agents, exhibiting high efficiency in the treatment of cerebral ischemia. By the level of effective doses, it is by three orders of magnitude superior to piracetam [7,12]. Nootropic and neuroprotective effects of noopept suggest its high efficiency in chronic cerebral ischemia in individuals with different hypoxic sensitivity.

We studied the effects of noopept on the functional and metabolic disorders in the brain in animals with high (HS) and low sensitivity (LS) to hypoxia under conditions of chronic ischemia.

MATERIALS AND METHODS

Experiments were carried out on 112 male Wistar rats (160-180 g) from Rappolovo Breeding Center

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of the Russian Academy of Medical Sciences (Leningrad Region). The study was carried out in accordance with "Guidelines for Experimental and Clinical Studies of New Drugs" (1984), "International Recommendations for Biomedical Studies on Animals" (1985), and "Regulations of Laboratory Practice in the Russian Federation" (Order of Ministry of Health of the Russian Federation, No. 267, 2003).

Before the intervention, all animals were divided into groups by their sensitivity to acute hypoxia by "elevating" them in a pressure chamber to the height of 12,000 m at a velocity of 50 m/sec and exposure at this height till emergence of agonal respiration. Animals enduring hypoxia during 5-10 min were referred to the group of low-sensitive (LS) ones, those enduring the exposure during more than 10 min were considered highly sensitive (HS) [5]. Cerebral ischemia was induced under short-term narcosis by ligation (occlusion) of the common carotid arteries.

The neurological status of animals was studied using McGrow stroke index. Muscle tone was evaluated by the self-pulling-up test with a horizontal beam at the height of 30 cm above the table surface [2].

The physiological reaction of rats was evaluated in a noise-proof room in the open field and elevated plus-maze tests. The orientation and exploratory, emotional, stereotypical, and motor components were evaluated using the rodent behavioral atlas [8]. Cognitive functions were evaluated on day 7 after the operation by the conditioned passive avoidance reflex (CPAR). Antidepressive effects of noopept were evaluated 1 day after CPAR evaluation in the behavioral despair test [13].

The intensity of free-radical processes in the brain was evaluated by the content of unsaturated fatty acids conjugated dienes and ketotrienes and MDA. Antioxidant system of the brain was evaluated by the activity of SOD, content of reduced glutathion, protein SH groups, and fat-soluble antioxidants [9,11].

Directly after occlusion of the carotid arteries and then daily during 7 days the animals were intraperitoneally injected with the noopept substance (Masterfarm; 0.5 mg/kg) dissolved in saline.

The data were statistically processed using Student's *t* test.

RESULTS

Intact rats phenotypically differing by sensitivity to hypoxia exhibited different behavioral reactions. Testing in the open field and elevated plus-maze showed steady behavior of LS animals, while HS rats exhibited more pronounced locomotor and exploratory activities and anxiety. The behavioral reactions of sham-operated animals were similar to that of intact ones.

Spontaneous motor activity reduced during the postischemic period in rats of both groups. Highly sensitive rats were "inhibited" and lost their capacity to exploratory activity, this indicating disintegration of some components of the integral behavioral reaction. Rats with low sensitivity retained such elements of behavior as sniffing, movements on the spot, excursions to the field center. Autonomic manifestations of emotions reduced in HS rats, which manifested by reduced number and duration of grooming acts, reduced number of boluses and defecation acts. By contrast, these characteristics increased in LS rats, indicating increase of their emotional status. Animals of both groups exhibited high anxiety, manifesting by shorter time spent in the open sleeves of the maze, peeping out from closed sleeves, ventures to the center, and peeping down from the maze edges; these characteristics were more pronounced in HS animals. Porsolt's test showed a longer duration of immobilization in LS and even more so in HS animals, while active swimming reduced, indicating the formation of depression. Inhibition of the orientation and exploratory activity and reduced mobility of animals, paralleled by increased emotional reactivity and anxiety are characteristic of stress situations [4].

Reproduction of CPAR 24 h after training (painful stimulation in the dark section of the cage) showed that 80% intact LS and HS rats remembered the electric current stroke and did not enter the dark "dangerous" chamber during the entire period of observation. The remaining 20% rats entered the dark section after a long latent period. No changes in this parameter were observed in sham-operated rats during the same periods. The training capacity of LS and HS rats was disordered after occlusion of both carotid arteries. This manifested by a significant reduction of the CPAR latent period (125±16 sec in LS and 114±11 sec in HS rats) in comparison with the values in sham-operated animals (165±13 sec in LS and 152±17 sec in HS rats). The disorders in the cognitive functions were more pronounced in animals highly sensitive to hypoxia.

Neurological symptoms of different severity were registered during 7 days after occlusion of the common carotid arteries: slight (0.5-2.5 points), medium (2.5-5.5 points), and severe (5.5-12 points). Severe and medium-severe course of experimental

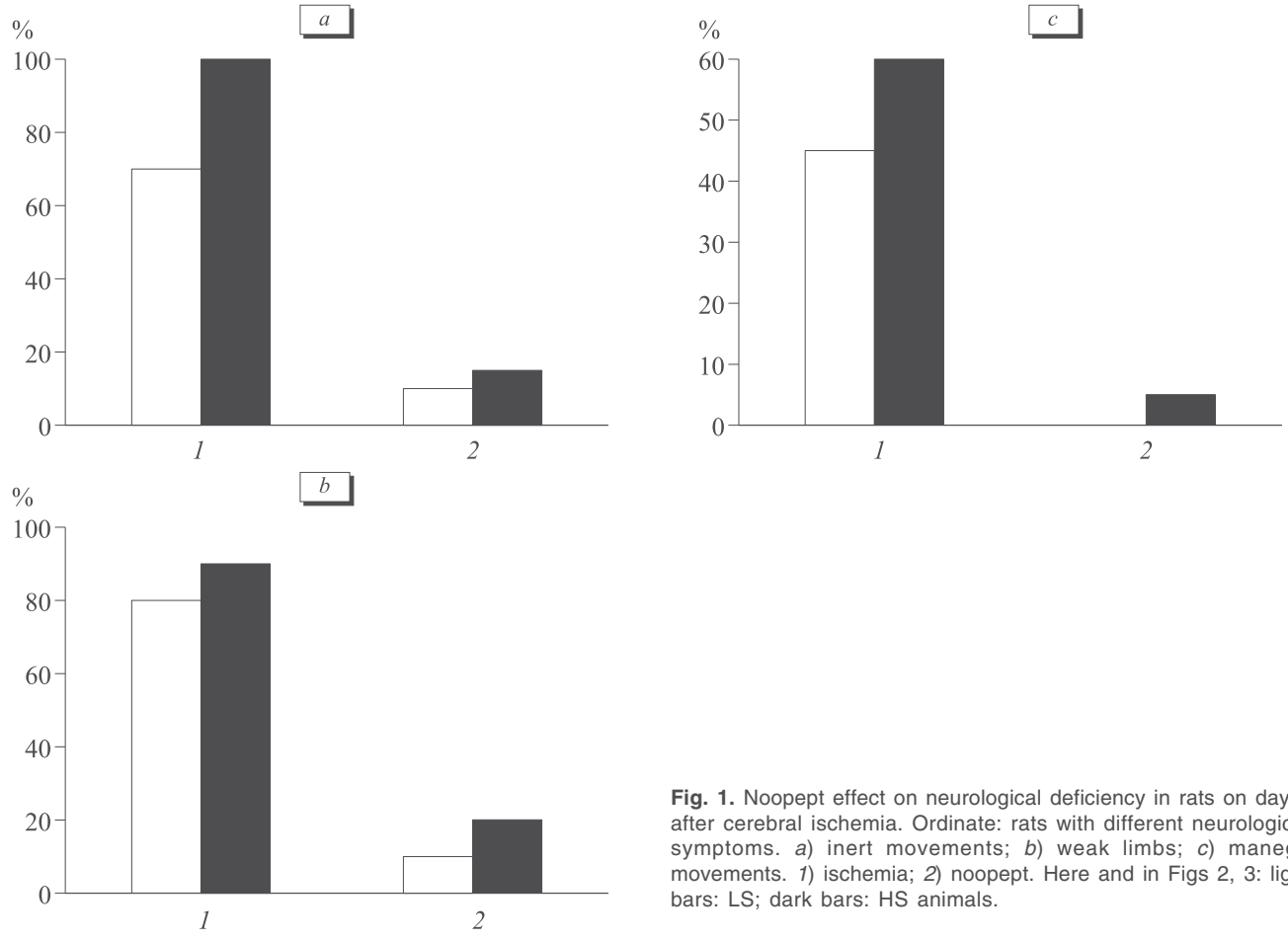

Fig. 1. Noopept effect on neurological deficiency in rats on day 3 after cerebral ischemia. Ordinate: rats with different neurological symptoms. *a*) inert movements; *b*) weak limbs; *c*) manege movements. *1*) ischemia; *2*) noopept. Here and in Figs 2, 3: light bars: LS; dark bars: HS animals.

cerebral ischemia was observed in 95% control rats with occlusion of the common carotid arteries. Neurological disorders (inertness and slow movements) were observed on day 3 after occlusion of the common carotid arteries in 70-100% rats with different sensitivity to hypoxia. These disorders were observed in 10% LS and 20% HS sham-operated rats. No severe neurological disorders, manifesting by manege movements in a circle and limb palsy, were noted in the group of sham-operated animals. Manege movements were observed in 45% LS and 60% HS animals with cerebral ischemia, pareses in 30 and 40%, palsy in 10 and 20% animals, respectively. On day 3 after occlusion of the common carotid arteries the muscle tone reduced in 50% LS and 70% HS rats, on day 7 in 40 and 50% animals, respectively. The severity of neurological disorders was higher in the HS group. A total of 45% LS and 30% HS survived by day 3 after occlusion of the common carotid arteries. Studies of the neurological status and conditioned reflex activity of animals after occlusion of the common carotid arteries demonstrated the significance of individual sensitivity to hypoxia for the course of the postischemic period.

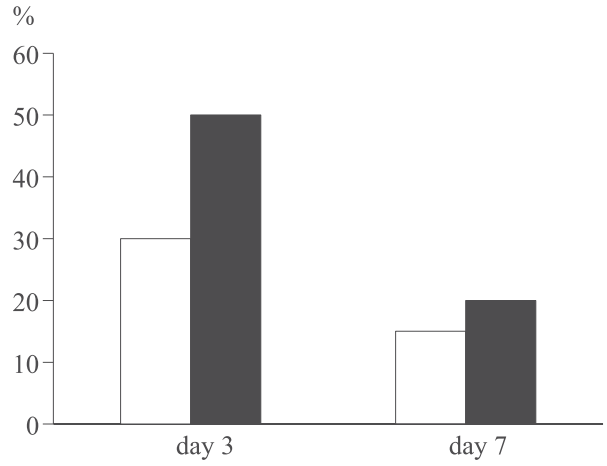

Fig. 2. Noopept effect on muscle tone of animals after cerebral ischemia. Ordinate: rats which failed to pull themselves up to the horizontal beam.

After noopept treatment the survival of LS rats reached 84%, of HS ones 76%. Noopept reduced the manifestations of neurological deficiency (Fig. 1). The rats developed no palsy and pareses of the limbs, the number of manege movements reduced

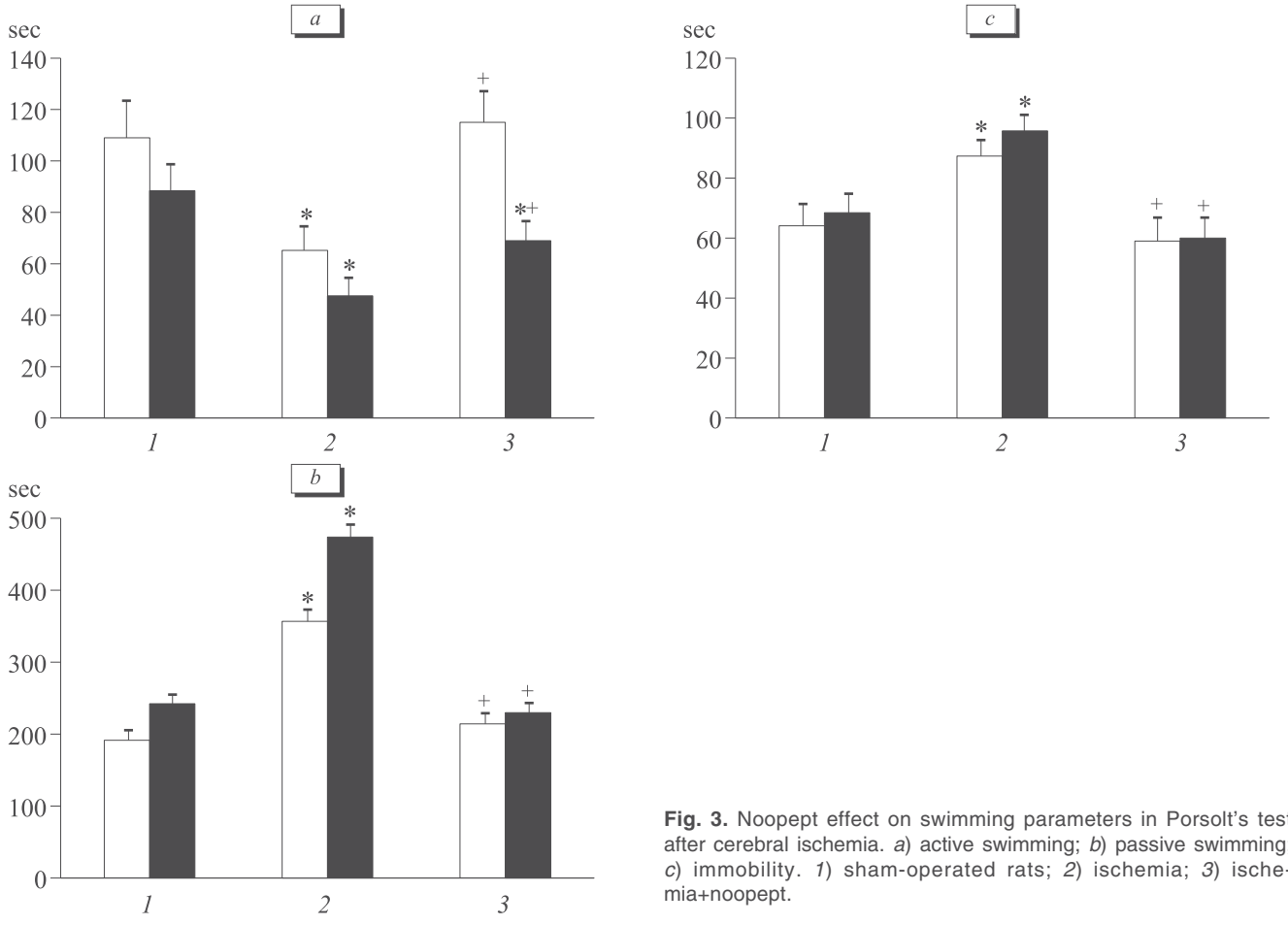

Fig. 3. Noopept effect on swimming parameters in Porsolt's test after cerebral ischemia. *a*) active swimming; *b*) passive swimming; *c*) immobility. *1*) sham-operated rats; *2*) ischemia; *3*) ischemia+noopept.

to just 5%, muscle tone increased (Fig. 2). Noopept treatment of animals with high and low sensitivity to hypoxia led to similar increase of their locomotor and orientation and exploratory activities, this indicating recovery of the integral behavioral reaction of animals. Noopept notably corrected the emotional status of animals, and by day 7 it approached the values in sham-operated rats. Anxiety was reduced, which fact indicated the anxiolytic effects of the drug. Noopept therapy restored the cognitive functions of animals by improving their training capacity, lost as a result of cerebral ischemia, and preserving the memory trace. Seven days after noopept injection the animals subjected to ischemia remembered painful stimulation in the dark section of the cage and reproduced the CPAR. In comparison with untreated rats, the latent time of entry into the dark dangerous chamber increased to 157±17 sec in LS and to 148±15 sec in HS rats. On day 3 of testing the latent period was 168±15 sec in LS and 154±16 sec in HS rats. The number of excursions during all stages of CPAR reproduction correlated with the latent period before entry into the dangerous chamber. Noopept exhibited a pronounced antidepressive effect in LS and HS animals, restoring the swimming and immobilization duration to the values in sham-operated animals (Fig. 3).

Individual typological characteristics of animal behavior correlate with their metabolic status, including the intensity of free-radical processes [6,10]. Pathogenetic significance of changes in the levels of lipid peroxidation and endogenous antioxidants in cerebral ischemia prompted study of noopept effect on these processes under conditions of occlusion of the common carotid arteries in rats with different individual sensitivity to hypoxia. Intact animals with different sensitivity to hypoxia differed by metabolic characteristics. The level of LPO products in the brain of HS animals was higher and the activity of antioxidant systems lower than in the brain of LS animals. These differences can play the key role under conditions of cerebral ischemia, as its outcome is largely determined by the previous status of lipid peroxidation processes in normal brain. Occlusion of both carotid arteries was associated with manifest accumulation of first and second LPO products in brain tissues and by

TABLE 1. Noopept Effect on the Content of LPO Products in the Brain of Rats after Ischemia ($M\pm m$, n=10)

Group	Conjugated dienes, μmol/g tissue		Conjugated ketotrienes, OD_{275}		MDA, nmol/g tissue	
	day 3	day 7	day 3	day 7	day 3	day 7
Sham-operated						
LS	25.00±0.75	24.1±1.2	0.039±0.003	0.041±0.004	10.7±0.5	10.4±0.5
HS	35.2±1.2	33.9±1.4	0.052±0.005	0.054±0.002	12.7±0.5	12.8±0.5
Ischemia						
LS	75.5±1.13*	65.2±0.2*	0.090±0.004*	0.075±0.004*	52.8±0.7*	44.2±0.4*
HS	88.2±1.2*	76.2±0.2*	0.099±0.004*	0.087±0.004*	72.2±1.2*	65.2±0.2*
Ischemia+noopept						
LS	64.4±1.2*	57.6±1.2*+	0.072±0.004*+	0.054±0.004*+	42.9±1.2*+	39.5±1.2*+
HS	76.6±1.2*+	45.2±0.2*+	0.084±0.004*+	0.062±0.004*+	59.7±1.2*+	52.4±0.2*+

Note. Here and in Tables 2, 3: $p<0.05$ compared to *sham-operated animals, +ischemia.

reduction of antioxidant systems activity. These changes were the most pronounced on day 3 after occlusion of the common carotid arteries, persisted during 7 days of observation, and were more manifest in HS animals.

Noopept treatment led to a significant reduction in the cerebral levels of conjugated dienes and ketotrienes, MDA, which could be due to reduced generation of lipid peroxides as a result of antioxidant system activation (Table 1). Noopept led to an increase of SOD activity in the brain in both groups (Table 2). A lesser formation of lipid radicals after drug therapy was also seen from an increase in the levels of fat-soluble antioxidants. Increase in the level of reduced glutathion, protecting the thiol protein groups from oxidation, was paralleled by an increase in the content of SH groups (Table 3).

After treatment by the metabolic action antihypoxants the parameters of LPO and antioxidant system activity in the brain of HS animals virtually did not differ from those in LS animals [4,5]. Noopept exhibited no effect of this kind on the metabolic parameters in the brain of HS rats. After noopept treatment the levels of LPO products and activity of antioxidant systems differed significantly from the values in sham-operated animals with respective individual sensitivities to hypoxia.

Hence, noopept treatment during 7 days of the postischemic period after occlusion of the common carotid arteries in rats resulted in a reduction of the levels of lipid peroxidation products and increase of antioxidant defense values. Individual sensitivity to acute hypoxia largely determined the functional and metabolic disorders in the brain, caused by chronic ischemia, and the probability of their repair after drug therapy.

Use of noopept in a dose of 0.5 mg/kg during 7 days after occlusion of the common carotid arteries reduced the severity of neurological disorders in rats with high and low sensitivity to hypoxia and prolonged their survival during the postischemic

TABLE 2. Noopept Effect on SOD Activity and Content of Fat-Soluble Antioxidants in the Brain of Rats after Ischemia ($M\pm m$; n=10)

Group	SOD, U/mg protein		Fat-soluble antioxidants, μequivalent	
	day 3	day 7	day 3	day 7
Sham-operated				
LS	3.15±0.15	3.18±0.14	0.227±0.014	0.229±0.015
HS	2.17±0.13	2.14±0.12	0.117±0.012	0.119±0.013
Ischemia LS	0.73±0.12*	1.59±0.20*	0.077±0.016*	0.111±0.014*
HS	0.32±0.20*	0.65±0.20*	0.021±0.014*	0.034±0.012*
HSIschemia+noopept				
LS	0.95±1.20*+	1.98±1.20*+	0.098±0.014*+	0.125±0.014*+
HS	0.57±1.20*+	0.94±0.20*+	0.035±0.014*+	0.053±0.014*+

TABLE 3. Noopept Effect on the Content of Reduced Glutathion and SH Groups in the Brain of Rats after Ischemia (*M*±*m*, *n*=10)

Group		Reduced glutathion, μmol/g tissue		SH groups, μmol/g tissue	
		day 3	day 7	day 3	day 7
Sham-operated					
	LS	40.11±0.88	40.54±0.86	3.60±0.15	3.55±0.12
	HS	25.22±0.88	25.34±0.88	2.15±0.14	2.12±0.13
Ischemia	LS	18.73±0.77*	20.22±0.42*	1.12±0.15*	1.42±0.12*
	HS	7.12±0.88*	12.12±0.22*	0.25±0.15*	0.73±0.14*
Ischemia+noopept					
	LS	30.9±1.2*+	21.15±0.22*+	1.35±0.13*+	1.99±0.15*+
	HS	39.7±1.2*+	15.84±0.32*+	0.34±0.12*+	0.94±0.16*+

period. Noopept increased the locomotor, orientation and exploratory activities, restored the emotional status of animals with different sensitivity to hypoxia, reduced the level of their anxiety and depression and improved training capacity, prevented accumulation of LPO products and suppression of the antioxidant systems in the brain of rats with different sensitivity to hypoxia after occlusion of the common carotid arteries.

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