Neokyotorphin and Kyotorphin Improve Cardiovascular and Cerebral Resuscitation After Heart Arrest

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The effects of neokyotorphin and kyotorphin, peptides isolated from hibernates, on the rate of postresuscitational restoration and survival after a 12-min heart arrest are examined. Native peptides and peptides stabilized by D-amino acids accelerate restoration of vital functions and neurological status within several days after resuscitation. It is concluded that the use of these peptides is feasible during cardiopulmonary resuscitation.

Key Words: *kyotorphin; neokyotorphin; heart arrest; resuscitation; postresuscitation period*

After analyzing the pharmacodynamics of neokyotorphin (NKT, Thr-Ser-Lys-Tyr-Arg) and kyotorphin (KT, Tyr-Arg) [4] as modifiers of potential-dependent Ca^{2+} currents in the myocardium and the role of Ca^{2+} in pathological processes caused by ischemia-reperfusion [7], we decided to study the effects of these peptides in a rat model of postresuscitation pathology after a 12-min heart arrest.

The idea of using NKT and KT with reanimation has arisen from the concept that numerous biologically active peptides acting as functional modulators exert no appreciable effects under physiological conditions, while in some pathologies and abrupt changes in physiological conditions their effects become well pronounced [1,8]. It was demonstrated that restoration after clinical death can be corrected with the aid of regulatory peptides (thyroliberin, sotamostatin, oxytocin, ACTH 4-10, etc).

The use of NKT as an agonist of Ca^{2+} currents in the beginning of cardiopulmonary resuscitation is dictated first, by the necessity of effective restoration of cardiac activity after arrest (without calcium overload of the myocardium), which largely determines survival and restoration of vital functions [2,3]. Second, taking into account fast metabolism of NKT with possible formation of short peptides (including KT, an antagonist of Ca^{2+} currents), it is necessary to protect the myocardium against calcium overload. Since KT is a potent inhibitor of cardiac activity, it must be applied against the background of effective cardiopulmonary resuscitation in viable animals. All these ideas were tested in experiments with minimal effective doses of L-NKT and L-KT and higher dozes their stabilized analogs: NKT-D-Ser and KT-D-Arg.

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MATERIALS AND METHODS

Albino rats weighing 180-250 g were used. Cardiac arrest (12 min) was performed by clamping the cardiac common vascular bundle under ether anesthesia [5]. The animals were resuscitated by artificial ventilation and closed chest massage. Norepinephrine (0.1 mg/kg) was administered intratracheally from the beginning of resuscitation [9].

Two series of experiments were performed. In series I, the effects of native NKT and KT (L-isomers) were examined. In series II, rats were given NKT and KT stabilized by D-amino acids (NKT-D-

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TABLE 1. Restoration of Vital Functions in the First Series of Experiments

Parameter	Control	L-NKT	L-NKT+L-KT	Control	L-KT
Heart, min	2.42±0.32 (*n*=27)	1.56±0.20 (*n*=17)*		1.23±0.1 (*n*=30)	
Breath, min	6.82±0.17 (*n*=20)	6.87±0.27 (*n*=16)		6.7±0.1 (*n*=30)	
Corneal reflex, min	35.26±1.67 (*n*=20)	36.3±2.9 (*n*=16)		32.5±1.3 (*n*=30)	
Restoration of posture, min	107.5±9.94 (*n*=20)	95.5±5.7 (*n*=11)	99.0±11.6 (*n*=5)	107.5±9.9 (*n*=20)	72.0±6.2 (*n*=10)**
Neurological deficiency:					
1 h	35.1±0.2 (*n*=20)	36.3±0.5 (*n*=11)*	34.0±0 (*n*=5)	35.1±0.2 (*n*=20)	35.0±0.3 (*n*=10).
2 h	32.2±0.5 (*n*=20)	30.8±0.7 (*n*=11)	32.0±0 (*n*=5)	31.7±0.6 (*n*=20)	29.4±0.6 (*n*=10)**
day 1	9.3±0.6 (*n*=19)	11.8±1.2 (*n*=10)*	8.0±1.4 (*n*=5)	9.3±0.6 (*n*=20)	9.4±0.8 (*n*=10)
day 4	3.2±0.2 (*n*=17)	1.8±0.6 (*n*=10)*	1±1 (*n*=4)*	3.2±0.2 (*n*=17)	1.5±0.8 (*n*=9)*

Note: *$p<0.05$, **$p<0.01$ compared with the control. Here and in Table 2: *n* is the number of rats.

Ser and KT-D-Arg) to potentiate and prolong the effects elicited by their metabolites.

The peptides were administered as a single subcutaneous injection: L-NKT (0.1-0.2 mg/kg) in the beginning of resuscitation and L-KT (0.05 mg/kg) when corneal reflex appeared (after 20-30 min of resuscitation). NKT-D-Ser and KT-D-Arg were injected in a dose of 0.5 and 0.05-0.1 mg/kg, respectively, at the same periods of experiment. The peptides were synthesized and characterized at the Institute of Bioorganic Chemistry, Russian Academy of Sciences.

Times required for restoration of cardiac activity, respiration, corneal reflex, and conscience were recorded. The rate of reduction in external neurologic deficiency was estimated during the first days after resuscitation [6], and the effectiveness of resuscitation was evaluated by the number of animals died on days 1-14, the number of animals with restored neurologic status on days 3-4, 5, 6-7, 8-9, and 10-11, and the number of survived animals with incomplete restoration.

Data were processed using Student's *t* test, Kholmogorov—Smirnov test, and exact method of Fischer.

RESULTS

The dynamics of restoration of vital functions of rats treated with L-NKT and L-KT and the results of resuscitation are presented in Tables 1 and 2.

L-KT significantly shortened posthypoxic coma and improved neurological status during the first days of the postresuscitation period and had no effect on lethality (Tables 1 and 2).

Neurological status (visual estimation) was restored in 60% of rats on day 3-4 after heart arrest, when neurological deficiency was observed in all control animals. In 30% control rats neurological status was restored on day 5 and in 45% of the rats on days 6-7 (Table 2).

These data indicate that in survived rats (effective resuscitation), L-KT accelerated restoration of neurological status in comparison with the control.

Ten out of forty intact rats died during resuscitation. L-NKT injected in the beginning of resuscitation potentiated the effect of epinephrine and accelerated restoration of cardiac activity in 92% rats (74% in the control, $p<0.06$). Further restoration of other vital functions did not differ significantly from the control. However, L-NKT significantly improved neurological status: on days 3-5 it was completely restored in 58.4% rats (22% in the control).

Simultaneous administration of L-KT and L-NKT significantly accelerated restoration of neurological status, although the changes were insignificant in comparison with those observed in L-NKT-treated group (Table 2). This may be due to the fact that L-KT is a fragment of L-NKT, i.e., the ability of the peptide to abolish the primary effect of heart arrest is associated with its primary structure. Therefore, administration of L-KT was unnecessary. Thus, L-NKT not only facilitates cardiopulmonary resuscitation but also accelerates restoration of neurological status.

The use of KT-D-Arg in the second series of experiments had no significant effect on the rate of restoration. Presumably, D-isomer of KT has a lower affinity for biological receptors [10] and, consequently, cannot produce the same effect as L-isomers. However, KT-D-Arg significantly accelerated the restoration of neurological status compared with the control and NKT-D-Ser-treated rats (Table 3).

In should be noted that there were no rats with incomplete restoration of neurological status among those treated with KT-D-Arg. Thus, our results indicate that KT produces a positive effect on the restoration of neurological status.

Stabilization with D-serine prolongs circulation of NKT in the body and presumably preserves its positive effects on the basic vital systems, as evidenced by a significant decrease in lethality on day

TABLE 2. Restoration of Neurological Status in the First Series of Experiments

Group	Time after resuscitation, days				Not resuscitated	Died on days 1-14
	3-4	5	6-7	8-9		
Control	0 (*n*=0)	22 (*n*=6)	33 (*n*=9)	4 (*n*=1)	26 (*n*=7)	14.7 (*n*=4)
NKT*	41.7 (*n*=5)	16.7 (*n*=2)	25 (*n*=3)	0 (*n*=0)	8.3 (*n*=1)	8.3 (*n*=1)
NKT+KT*	60 (*n*=3)	0 (*n*=0)	20 (*n*=1)	0 (*n*=0)	0	20 (*n*=1)
Control	0 (*n*=0)	30 (*n*=6)	45 (*n*=9)	5 (*n*=1)	0	20 (*n*=4)
KT*	60 (*n*=6)	10 (*n*=1)	20 (*n*=2)	0 (*n*=0)	0	10 (*n*=1)

Note. Here and in Table 3: *$p<0.05$ compared with the control (Kholmogorov—Smirnov test).

TABLE 3. Rats with Restored Neurological Status Structure in the Second Series of Experiments

Group	Time after resuscitation, days				Incomplete restoration	Died (1-14 days)
	4	6-7	8-9	10-11		
Control	0	12.5 (*n*=2)	18.75 (*n*=3)	0	18.75 (*n*=3)	50 (*n*=8)
NKT-D-Ser*	0	13.3 (*n*=2)	20 (*n*=3)	26.7 (*n*=4)	20 (*n*=3)	20 (*n*=3)
KT-D-Arg*	60 (*n*=3)	0	0	20 (*n*=1)	0	20 (*n*=1)

1 (up to 6.7% *vs.* 50% in the control, $p<0.01$). In contrast to L-NKT, NKT-D-Ser did not accelerate restoration of neurological status, which confirms the hypothesis on a double positive effect of NKT owing to its splitting in the body and formation of KT. However, since lethality during the first 2 weeks after resuscitation was lower (20% *vs.* 50% in the control), in some rats neurological deficiency disappeared only on days 10-11 (26.7% *vs.* 0% in the control, $p=0.05$).

Thus, stabilization of NKT with D-serine, which prolongs its ability to increase potential-dependent Ca^{2+} currents in cardiomyocytes, significantly reduced lethality on day 1 after resuscitation and did not accelerate restoration of neurological status. By contrast, in KT-D-Arg-treated rats the rate of neurological status restoration was significantly higher than in control and NKT-D-Ser-treated rats, although lethality was practically the same (20%). Presumably, stabilization of NKT by D-arginine provides predominant cleavage of KT from NKT during NKT hydrolysis, thus promoting postresuscitation restoration of vital functions.

From our findings it can be concluded that NKT and KT elicit positive effects during both cardiopulmonary resuscitation and postresuscitation period by reducing lethality and improving neurological status, which increases survival.

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