The Effect of FMRFa-like Peptides on Rats Reanimated after Clinical Death

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The effects of the endogenous paraopioid FMRFa and FMRFa-like peptides are compared upon reanimation of rats after clinical death caused by acute hemorrhage. It is found that FMRFa restores cardiohemodynamics and respiratory function more effectively than RFa and RF×2HCl. The nonpeptide opioid antagonist naloxone does not alleviate the effects of acute hemorrhage. It is assumed that the reanimating effect of the studied peptides is realized via mechanisms that are not associated with opiates.

Key Words: *reanimation; paraopioid peptides; naloxone*

When used in shock of various etiologies, opiate receptor blockers (specifically, naloxone) normalize the autonomous functions, particularly in the early stages of shock [2,3,6,8]. In rats exposed to hypoxic shock, the paraopioid tetrapeptide Phe-Met-Arg-Phe-NH_2 (FMRF-amide or FMRFa), which is probably an endogenous opiate antagonist, produces an effect similar to that of naloxone but much more potent [1]. This effect may be due to the pronounced hypertensive and cardiostimulating activity of FMRFa-like peptides [5].

Our objective was to compare the effects of FMRFa-like peptides and naloxone in a model of clinical death brought about by acute hemorrhage.

MATERIALS AND METHODS

Experiments were performed on 33 outbred female albino rats (180-220 g) under Nembutal anesthesia (40 mg/kg intraperitoneally). A tracheotomy tube was inserted in the trachea and hooked up to an assisted ventilation apparatus. Acute hemorrhage was modeled by draining the blood via a catheter in the common carotid artery 2 min after intraarterial injection of 500 U/kg heparin. Blood was collected in a graduated cylinder. FMRFa, RFa, RF×2HCl, or naloxone were added in a dose of 0.5 mg/kg to the collected blood of experimental rats; an equivalent volume of normal saline (0.5 ml) was added to the blood of control rats. Clinical death from acute blood loss lasted 10 min from the cessation of respiration, after which the rats were reanimated. For this purpose blood was delivered via an intraarterial catheter, artificial ventilation was begun, and, if necessary, direct heart massage was performed. The autonomous functions were assessed from the heart rate (HR), respiration rate (RR), and mean blood pressure (MBP) recorded by standard methods throughout the experiment. The survival rate was also taken into account. The results were analyzed using the standard nonparametric tests.

RESULTS

By the 10th min of reanimation, HR and MBP in half of the control rats were 22.5 and 24.5% of the baseline values, respectively (Fig. 1, Table 1). The other rats developed uncontrolled fibrillation in response to reinfusion of heparinized blood with normal saline. Spontaneous respiration was not restored. All control rats died by the 40th min of re-

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animation. In RFa-treated rats, by the 10th min of reanimation spontaneous respiration was restored, HR and MBP being 49.1 and 61.1% of the baseline level, respectively. The survival rate after 40 min of reanimation was 42.9% (3 out of 7 rats). These rats showed positive changes in the RR dynamics. RF×2HCl was more effective: HR and MBP were more than 50% of the baseline values during reanimation. The survival rate by the 40th min was 62.5% (5 out of 8 rats, $p<0.05$ by the cross-tabulation method) and RR was 42% of the baseline value. The reanimation measures proved to be most effective after reinfusion of blood supplemented with FMRFa. The survival rate in this group was maximal (83.3%, 5 out of 6 rats, $p<0.01$), and by the 40th min RR was 50% of the baseline value. HR and MBP normalized rapidly. Naxolone had no effect. There were no significant differences in HR, RR, and MBP compared with the control. All naloxone-treated rats died by the 40th min.

From these results it can be concluded that all tested oligopeptides stimulate cardiovascular and respiratory functions and increase the survival of rats against the background of blood reinfusion after a 10-min clinical death. The effects of RF×2HCl and, particularly, of FMRFa are much more pronounced than the effect of RFa ($p<0.05$ at the 40th min of reanimation).

The differences between the effects of naloxone and the peptides indicate that the endogenous opioid system may play a minimal role in the studied terminal state. This may be due to the rapid development of pathology in acute blood loss. As a result, the reactions typical of various types of shock, such as stepwise activation of the hypothalamus-hypophyseal-adrenal axis and hyperactivation of the endogenous opioid system, do not manage to manifest themselves [2,4]. Consequently, in acute hemorrhage the endogenous opioid system probably loses the "substrate" that provides for its modulating (minimizing) effect, and naloxone blockade of the opiate receptors becomes ineffective. In addition, there is evidence that metabolic acidosis, which is maximal in clinical death, markedly reduces the effectiveness of opiate antagonists [8].

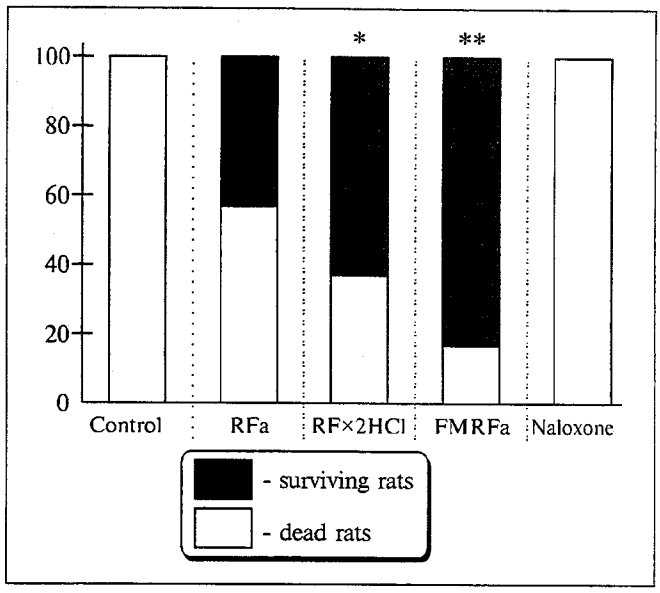

Fig. 1. Effects of FMRFa and FMRFa-like peptides on survival of rats after a 10-min clinical death caused by acute hemorrhage. Ordinate: percentage of rats dying (white bars) and surviving (black bars) by the 40th min of reanimation. $^{*}p<0.05$, $^{**}p<0.01$ compared with the control.

Thus, the positive effect of FMRFa-like compounds during reanimation may be mediated by nonopiate mechanisms. It cannot be ruled out that the physiological effects of these peptides stem from interaction with specific receptors located on FMRFa-immunoreactive neurons [6,9], which are present in considerable amounts in rat brain and pituitary and probably form a specific peptidergic sys-

TABLE 1. Changes in Heart Rate (HR), Respiration Rate (RR), and Mean Blood Pressure (MBP) Occurring in Rats during Reanimation after Clinical Death Caused by Acute Hemorrhage (*M±m*)

Parameter		Group of animals				
		control (n=6)	RFa (n=7)	RF32HCl (n=8)	FMRFa (n=6)	naloxone (n=6)
Baseline values	HR, beats/min	340.0±9.6	348.0±18.9	387.5±33.9	314.8±24.0	362.0±18.9
	RR, breaths/min	37.5±2.9	29.2±4.0	69.2±5.7	46.2±12.4	39.5±6.7
	MBP, mm Hg	102.5±7.3	108.0±9.6	131.7±17.2	112.0±7.7	108.0±5.7
10 min of reanimation	HR, beats/min	76.6±11.6	171.0±10.5*	212.5±24.0*	180.6±17.2*	53.0±11.6
	RR, breaths/min	0	0.4±0.3*	2.0±0.9*	5.8±4.7*	0
	MBP, mm Hg	25.0±3.2	66.0±11.5*	68.3±13.4*	96.0±17.2*	20.0±3.2
40 min of reanimation	HR, beats/min	-	105.0±24.0	209.2±33.9	197.4±33.9	-
	RR, breaths/min	-	4.8±2.7	25.3±12.3	23.8±9.9	-
	MBP, mm Hg	-	38.0±15.3	68.3±18.9	64.0±5.7	-

Note. n is the number of animals; $^{*}p<0.05$ compared with the control (Mann—Whitney test).

tem [10]. We believe that the administration of FMRFa-like peptides stimulates this system and activates both the sympatho-adrenal axis and, probably, an as yet unstudied reflex component responsible for positive shifts in respiratory and cardiovascular functions during reanimation.

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Effect of Histidine-Containing Dipeptides on Brain Tyrosine Hydroxylase

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Histidine-containing dipeptides inhibit highly purified tyrosine hydroxylase. The degree of inhibition is dependent on the peptide structure and decreases according to the following sequence: carnosine>anserine>homocarnosine. This effect is not associated with the buffer and antioxidant properties of the dipeptide.

Key Words: *tyrosine hydroxylase; antioxidants; carnosine; anserine; homocarnosine*

The histidine-containing dipeptides carnosine and its analogs display a diverse physiological and biochemical activity [5,8]. However, the molecular mechanisms of the effects of these compounds are not fully understood. Histidine-containing peptides fulfill two major functions in the cell: they act as a buffer, facilitating the stabilization of the intracellular pH [6], and as an endogenous antioxidant, protecting membranes against the damaging effect of lipid peroxidation products [1].

However, the recently reported localization of both tyrosine hydroxylase (TH) and carnosine in neurons [7] cannot be explained solely by these functions.

Tyrosine hydroxylase (tyrosine-3-monoxygenase, EC 1.14.16.2) catalyzes the first step of catecholamine biosynthesis: the hydroxylation of L-tyrosine to form L-DOPA. In animals, L-DOPA is probably not involved in plastic processes and fulfills a specific function, being a catecholamine precursor. The TH reaction is the limiting stage of catecholamine biosynthesis [10], which determines its importance for normal functioning of the nervous system. A complex and rapid regulation of TH enables this functionally important enzyme to adapt to the rapidly changing intracellular environment. Allosteric regulation is a means of rapid modulation of the enzyme activity [9]. Allosteric properties of TH have been demonstrated with pterin co-

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