EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Effect of Peptidergic Correction of Hemorrhagic Shock on the Behavior of Rats in the Posthemorrhagic Period

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Hemorrhagic shock is shown to disturb the behavior of rats in the open field test. Infusion of a mixture of thyrotropin releasing hormone and FMRFa (Phe-Met-Arg-Phe amide) in subthreshold doses led to a partial recovery of the neurological status.

Key Words: *hemorrhagic shock; thyrotropin releasing hormone; FMRFa; naloxone; neurological status*

Administration of opioid antagonists such as naloxone is one way to correct hemorrhagic shock [7]. However, some claim that naloxone is ineffective and even exerts negative effects in hemorrhagic shock [2,6]. Hence, we decided to investigate the mode of action of endogenous antiopioids.

Previously we showed that some peptides (thyrotropin releasing hormone - TRH and Phe-Met-Arg-Phe amide - FMRFa) suppressing the effects of opioids can exert a pronounced protective effect on the cardio- and hemodynamics in narcotized and awake rats with hemorrhagic shock, although in many cases it is associated with side effects. Therefore, we attempted to investigate the effects of these peptides as components of mixtures with their doses reduced to subthreshold. Simultaneous intraarterial infusion of FMRFa and TRH in subthreshold doses (0.01 mg/kg/min) improved the recovery of arterial pressure after hemorrhagic shock.

It is especially interesting to analyze the effects of such a mixture on the behavioral reactions in the posthemorrhagic period, when persistent changes develop in the metabolic and ultrastructural characteristics of biosynthetic processes in the brain [8]. Hence, we analyzed the aftereffect of this peptide mixture on the neurological status.

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MATERIALS AND METHODS

Hemorrhagic shock was induced in awake rats (outbred females) by letting 40% of the blood through a catheter implanted in the carotid artery under Nembutal (40 mg/kg) narcosis. Immediately after bloodletting, a 40-min intraarterial infusion of a mixture of FMRFa and TRH (Sigma) in subthreshold doses (0.01 mg/kg/min) was started. Equivalent volumes of normal saline were infused to control animals.

The orienting-exploratory activity and emotional status of the animals were assessed on days 2, 4, and 7 after blood loss in the open field test (a stress-free modification) [5]. Horizontal motor activity (HMA), vertical activity, grooming movements, and the number of radial transpositions into the center of the field were visually recorded for 2 min.

For additional control, in order to single out the effects of the operation and hemorrhagic shock on behavior, two groups of animals were examined: intact and sham-hemorrhagic. An operation without bloodletting was performed in sham-hemorrhagic rats.

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The effect of the peptide mixture was compared to the effect of naloxone (Du Pont) infused in a dose of 0.1 mg/kg/min for 40 min directly after blood loss. The dose was selected as described elsewhere [4].

In addition, we investigated the effect of the peptide mixture on intact animals. In order to simulate the method of administration (infusion) of the peptide mixture after hemorrhagic shock as closely as possible, the mixture was injected intraperitoneally 4 times over 40 min in a dose of 0.1 mg/kg, this corresponding to the total dose during infusion.

The samplings were compared using the nonparametric Wilcoxon-Mann-Whitney U test [3].

RESULTS

Figure 1 shows that a high HMA (906 cm) is typical of intact animals during the first examination. Upon repeated examinations in the open field it decreased ($p<0.05$), indicating a weakening of the orienting-exploratory reaction (in other words, the animals remember the experimental situation well). The operation led to an almost twofold reduction of HMA ($p<0.01$), but the time course of HMA was the same during repeated examinations.

Comparison of the control and sham-hemorrhagic groups showed that hemorrhagic shock brought about serious disorders in behavior. HMA was still more reduced in the animals subjected to hemorrhagic shock, and this parameter did not change in the course of the follow-up, i.e., hemorrhagic shock impedes the perception and memorization of an experimental setting. Infusion of a peptide mixture led to a reliable increase of HMA in comparison with the control group on day 2 ($p<0.05$), no differences being observed between the experimental and sham-hemorrhagic groups on days 2 and 4. Hence, a mixture of peptides partially reinstates the orienting-exploratory reaction. However, the time course of HMA is the same as in the control group, indicating that a mixture of FMRFa and TRH does not restore the animals' perception and memorization of the experimental situation.

The time course of movements to the center is further evidence of memory impairments. The mixture of peptides did not influence this parameter.

The vertical activity changed similarly as HMA in all the groups, this confirming our conclusion about the influence of the peptide mixture on the orienting-exploratory reaction after hemorrhagic shock.

It is widely believed that the emotional status of animals can be assessed from the intensity of grooming [5]. Figure 2 shows that the number of grooming movements in intact rats dropped from 5-6 to 3 during repeated examinations, this indicating a reduction of emotional strain. The intensity of grooming in sham-hemorrhagic rats was reliably lower. Since the number of groomings reflects the extent of activity shifts which results from competition between two motivations - exploration and fear - we assume that in this case the reduced intensity of grooming was due to a decrease of the orienting-exploratory reaction. Hemorrhagic shock caused an increase of groomings on day 7 in comparison with sham-hemorrhagic animals, this apparently being due to increased emotional strain during the late posthemorrhagic period. Injection of the peptide mixture did not alter the intensity of grooming in comparison with the control. Hence, infusion of a mixture of peptides did not affect the emotional status.

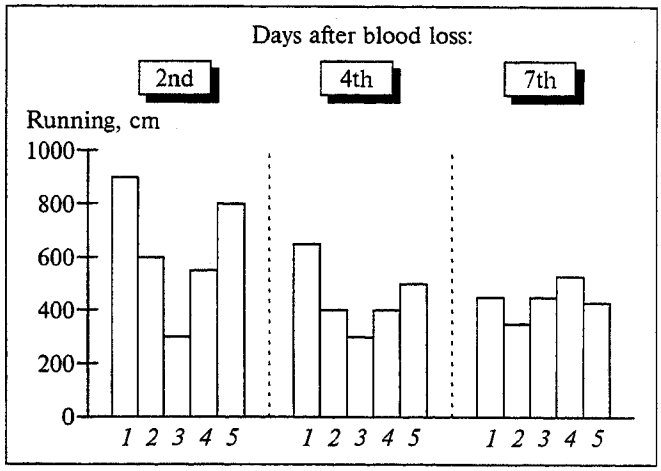

Fig. 1. HMA in the open field test in the late posthemorrhagic period. Here and in Fig. 2: *1*) intact, *2*) sham−hemorrhagic, *3*) control rats, *4*) hemorrhage+peptide mixture, *5*) hemorrhage+naloxone. Time of observation 2 min. Differences between the groups: day 2: *1* and *2*) $p<0.01$; *2* and *3*, *3* and *4*) $p<0.05$; *3* and *5*) $p<0.0005$; day 4: *1* and *2*, *1* and *3*) $p<0.05$; *3* and *5*) $p<0.01$.

The influence of a mixture of FMRFa and TRH on intact animals not exposed to any invasions or

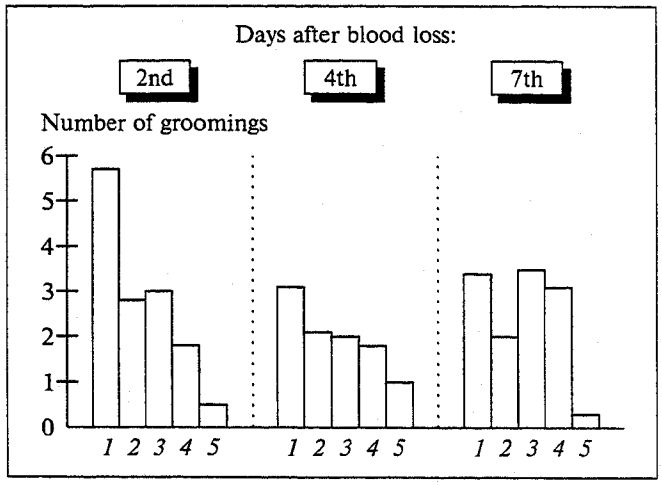

Fig. 2. Grooming in the open field test in the late posthemorrhagic period. Differences between the groups: day 2: *1* and *2*, *1* and *3*, *1* and *4*) $p<0.005$; *3* and *5*) $p<0.05$; day 4: *1* and *2*, *2* and *3*, *3* and *5*) $p<0.05$.

hemorrhagic shock was studied in a special series of experiments with intraperitoneal injections of the mixture. No differences were observed between the experimental and control groups.

Naloxone in a dose of 0.1 mg/kg/min did not affect the hemodynamic parameters. In spite of this, the recovery of the neurological status in the naloxone group was much more pronounced. HMA reliably increased in comparison with the control group on days 2 ($p<0.005$) and 4 ($p<0.01$) and did not differ from the HMA of intact rats. The vertical activity increased in comparison with the control. Moreover, injection of naloxone restored the pattern of changes in HMA and vertical activity observed in intact and sham-hemorrhagic animals. The number of groomings fell in comparison with the control ($p<0.05$), and movements to the center reliably increased in number on days 2 and 4 ($p<0.05$). The changes in these parameters indicate that naloxone suppressed the emotional strain and reduced the fear motivation.

The positive effect of naloxone on the recovery of neurological status evidently results from blocking of the opioid receptors in the course of infusion. FMRFa and TRH are rather rapidly broken down by peptidases, and their effects on the neurological status may be due either to a positive action during infusion or to triggering of the "cascade" reaction typical of peptides [1].

Hence, our studies demonstrate that a mixture of FMRFa and TRH in subthreshold doses abolishes the negative effects of hemorrhage on the orienting-exploratory reaction of rats but does not repair the animals' perception and memorization of the experimental situation and does not influence their emotional status. The corrective effect of the peptide mixture is specific, as this mixture does not affect intact animals. Because the effects of the studied mixture and naloxone on the neurological status following hemorrhagic shock are similarly directed as regards the orienting-exploratory reaction, we speculate that a "naloxone-like" component is present in the effect of the peptide mixture. Our study revealed the possibility of correcting behavioral disturbances following hemorrhagic shock by administering a mixture of peptides in subthreshold doses.

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