ROLE OF MEDIUM MOLECULAR WEIGHT BLOOD PEPTIDES IN THE DEVELOPMENT OF CARDIODEPRESSION AFTER THERMAL BURNS

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The role of peptides in the development of the endogenous intoxication syndrome is currently under wide discussion [7, 11]. In particular, data demonstrating the high biological activity of medium molecular weight peptides (MMWP) in the blood of burned patients have been obtained in the writers' laboratory [3, 6]. The diversity of the effects of these compounds, manifested in various experimental models, suggests that they are involved in the pathological process at the principal levels of regulation of the bodily functions. Disturbance of cardiac function in thermal trauma is known to be brought about both by humoral agents of burn toxemia [2, 14] and by the influence of the extracardiac nervous sytem [9]. There is evidence that the blood serum of burned patients contains dialyzable negative ionotropic factors, whose action has been demonstrated on preparations of the isolated myocardium [2, 10]. It is not clear what is the nature of these compounds or whether they affect central mechanisms of regulation of cardiac functions.

The aim of this investigation was to study the cardiodepressor action of blood MMWP from burned animals on the isolated myocardium and also on the intact organism when injected beyond the blood-brain barrier (BBB). The probability of a central action of these compounds under pathological conditions was assessed from their effect on permeability of the BBB on intravenous injection.

EXPERIMENTAL METHODS

MMWP were obtained from the blood plasma of four normal and four burned dogs (12 h after a burn of the IIIA-IIIB degree involving 25-30% of the body surface) by the technique described previously [3]. After the identity of the elution profiles had been established in both cases five fractions were isolated, and numbered in order of elution from the chromatography column. The peptide concentrations in the fractions were determined by Lowry's method. Contractions of the papillary muscles of the left ventricle of the normal rats, close to isometric in character were recorded with a 6MKh 1S mechanotron. Krebs' solution saturated with carbogen, at 30-31°C, was used for perfusion. After adaptation of the preparation and reocrding of the force of the contractions at different frequencies, perfusion with Krebs' solution containing the test peptide fractions in concentrations equal to those in the blood of burned dogs began. After 45 min the frequency—force dependence was again determined. The central action of the MMWP was tested on anesthetized albino rats after injection of solution of the peptide fractions into the subarachnoid space by suboccipital puncture. Fractions of MMWP were dissolved in physiological saline in a volume of 0.1 ml in a standard dose of about 12 μg of Lowry-positive substances for injection. The ECG was recorded in standard lead II for 25 min after suboccipital injection. Changes in the heart rate (HR) were expressed as percentages of HR before injection of the material. The damaging action of MMWP on the BBB was assessed by a study of changes in permeability of BBB for trypan blue [15] and subsequent determination of doses of the active fractions causing the maximal increase in permeability of BBB after intravenous injection into mice.

The numerical results were subjected to statistical analysis by Student's t test [1] and by the Wilcoxon—Mann—Whitney test [5]. The significance of the dose-dependent action of the peptides on BBB permeability was estimated by calculation of the correlation ratio η [8].

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TABLE 1. Changes in Force of Contractions of Papillary Muscle during Perfusion with Test Solutions (in % of force of contractions of muscle in Krebs' solution at a frequency of 0.1 Hz)

Experimental conditions	Frequency of stimulation, Hz				
	0,1	0,2	0,5	1	2
Control Krebs' solution	100	80,63±1,065	64,42±2,34	64,11±3,17	102,04±6,38
Normal solution of fraction 3 (1,38 μg/ml)	106,6±5,57	98,85±6,6	77,6±5,88	76,05±6,68	104,2±9,225
Burns solution of fraction 3 (1,38 μg/ml)	94,67±13,5	75,25±14,3	47,43±6,66[a,b]	35,52±6,54[a,b]	41,49±0,38[a,b]

Legend. a) $P \leq 0.05$ compared with control, b) $P \leq 0.05$ compared with normal.

TABLE 2. Changes in HR of Rats after Suboccipital Injection of Test Solutions (in % of HR before suboccipital injection)

Time after injection, min	Test solution		
	physiological saline (control)	fraction 3	
		normal	burns
5	96,04±3,51	89,89±7,18	78,56±0,95[a,b]
15	101,03±4,78	85,73±3,82[a]	74,59±1,64[a,b]
25	100,57±4,17	94,03±2,75	79,43±5,16[a,b]

Legend. a) $P \leq 0.05$ compared with control, b) $P \leq 0.05$ compared with normal.

TABLE 3. Basic Characteristics of Action of Fractions with BBB-Damaging Activity

No. of fraction	Maximally active dose, μg	Maximal damage to BBB, μg/g tissue	$\eta \pm m\eta$
2(normal)	20,36	607,08±19,38*	0,98±0,045
2(burns)	5,4	578,02±43,28*	0,706±0,196
4(burns)	4,166	753,46±57,08*	0,935±0,099

Legend. *$P < 0.05$ compared with control. Uptake of trypan blue by brain of control mice was 300.3 ± 39.33 μg/g tissue.

EXPERIMENTAL RESULTS

Screening the cardiotropic activity of all the fractions of MMWP tested showed that only material of fraction 3 from the blood of the burned animals (burns) had a negative ionotropic action in a concentration corresponding to that in the blood of the burned animals. As Table 1 shows, after perfusion for 45 min with a solution of this fraction the force of contraction of the papillary muscle at frequencies of stimulation of 0.5, 1, and 2 Hz was significantly lower than after perfusion with Krebs' solution and with a solution of fraction 3 from the blood of intact animals. The fact will be noted that the negative ionotropic agent contained in fraction 3 (burns) did not change the frequency–force relationshp, which was triphasic in character in the control.

Material of fraction 3 from the blood of the burned dogs had a marked cardiodepressor action when injected into the subarachnoid space also; as Table 2 shows, this was manifested as a marked fall of HR throughout the period of recording. It is important to note that of all the other fractions tested, this effect was observed, although weaker and for a shorter duration, in tests of fraction 3 from the blood of intact animals. Peptides are known to pass with difficulty through BBB [12]. A fundamental problem was accordingly the reality of the central action of MMWP of fractions 3 from blood of intact animals (normal) and fractions

3 from the blood of burned dogs, when circulating in the blood stream. Screening the BBB-damaging action showed that fraction 2 (normal), fraction 2 (burns), and fraction 4 (burns) had a marked effect on BBB permeability after intravenous injection. The basic characteristics of their action, obtained by a study of dose–effect relationships, are given in Table 3. It was found that in order to induce effects of approximately equal magnitude when fraction 2 (normal) was used, the dose required was 4-5 times greater than when active fractions from the blood of burned dogs.was tested.

It can be tentatively suggested that during accumulation of concomitant fractions of blood MMWP with a BBB-damaging action, peptides of fraction 3 (burns) in the early periods after thermal trauma not only directly affect myocardial contractility, but also exert an influence on cardiac function.

Experimental and clinical data on the phenomenon of bradycardia in the torpid phase of burn shock, which later, admittedly in the period of toxemia, changes into tachycardia [4], can be cited as confirmation of arguments on the pathogenetic role of blood MMWP in the development of myocardial insufficiency in the early stages after burns. However, if it is considered that the inhibitory effect of the extracardiac nervous system may be accompanied by a decrease in myocardial contractility even at a constant and experimentally assigned frequency [13], it can be postulated that the depression of cardiac function, mediated centrally by peptides, in thermal trauma is probably realized by this mechanism also.

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