THE ROLE OF THE AUTONOMIC NERVOUS SYSTEM IN THE REFLEX HUMORAL REGULATION OF THE PHYSIOLOGICAL ANTICLOTTING SYSTEM IN THE FROG

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In a paper published earlier [1] it was shown that the physiological anticlotting system [2, 3] exists not only in mammals, but also in amphibians (Rana temporaria). The rapid injection of a certain dose of thrombin into the cavity of the ventricle of a frog's heart not merely does not cause thrombosis but, on the contrary, it leads to complete absence of clotting of the blood in vitro in the presence of thromboplastin obtained from the lung tissues of the same species. It has also been observed that after destruction of the spinal cord and part of the medulla in frogs (by inserting a sound through the rhomboid fossa), a completely opposite effect is produced: generalized clotting of the blood takes place in the vascular system soon after injection of a moderate dose of thrombin into the heart. In our subsequent investigations, however, we found that destruction of the spinal cord alone does not have this effect. Destruction of part of the medulla – in the region of the floor of the fourth ventricle – deprives the animal of its protective reaction of the physiological anticlotting system, and if thrombin is injected into the blood stream it causes generalized thrombosis of the vessels. This protective reaction is completely preserved in frogs after destruction of the remaining parts of the brain. There is experimental evidence that the activity of the physiological anticlotting system is directly dependent on the functional state of the region of the medulla. The performance of the reflex activity of this system in frogs, as in mammals [4-7], is associated with the activity of the autonomic nervous system, especially with the cranial division of the parasympathetic nervous system.

EXPERIMENTAL METHOD

Experiments were carried out on frogs (Rana temporaria) kept in laboratory conditions during the winter. Blood was taken from the cavity of the ventricle by means of a syringe without the addition of any anticoagulant. The clotting time of the blood (in seconds) was measured at 37° in Sahli tubes with the addition of thromboplastin, obtained from the frog's lungs, to the blood. In these experiments we used a solution of a dried preparation of thrombin obtained from horse plasma. The thrombin activity was determined from the clotting time (in seconds) of horse blood when 0.1 ml of blood and 0.1 ml of thrombin solution were mixed in a tube (at 37°). The surgical operations on the experimental animals were performed without general or local anesthesia.

EXPERIMENTAL RESULTS

Injection of thrombin into the blood stream causes a protective reaction in frogs and the blood loses its ability to clot in the presence of thromboplastin (Table 1). Destruction of the spinal cord and the adjacent portion of the medulla by means of a sound leads in all cases to the abolition of the protective function of the anticlotting system and to the development of a prethrombotic condition in the body, changing to a thrombotic state if the animal receives an injection of a moderate dose of thrombin. These animals died from generalized thrombosis, in contrast to the animals undergoing the operation and receiving an injection of inactive thrombin. Removal of the brain with preservation of part of the medulla, and also total destruction of the spinal cord (see Table 1), did not interfere with the protective reaction of the anticlotting system. Only if the medulla was destroyed in the region of the fourth ventricle did the animal lose this reaction.

Experiments on mammals (rats, rabbits) showed that the activity of the anticlotting system is related to the

TABLE 1. Changes in the Reaction of the Anticlotting System in Frogs Depending on the Functional State of the Brain and Spinal Cord (mean values). Dose of Thrombin 0.3-0.4 ml, Thrombin Activity 7-9 sec

Experimental conditions	No. of animals	Clotting time of blood (in seconds)	
		before injection of thrombin	4 min after injection of thrombin
Control (animals with intact nervous system)	48	11	>1800. Blood did not clot
Destruction of spinal cord and part of medulla	22	10	Blood clotted in blood stream after a few seconds
Destruction of spinal cord, brain intact	16	12	>1800. Blood did not clot
Destruction of brain, medulla intact	25	12	>1800. Blood did not clot
Destruction of medulla (through floor of fourth ventricle)	32	12	Blood clotted in blood stream after a few seconds

TABLE 2. Effect of Unilateral and Bilateral Division of 3 Roots of the Vagus Nerves on the Physiological State of the Anticlotting System in Frogs (mean values)

Experimental conditions	Clotting time (in seconds)			No. of animals		
	before division	30 min after division	4 min after injection of thrombin	total	surviving	dying
Control						
Bilateral division of 3 roots of the vagus nerves followed by injection of inactive thrombin	56	84	87	6	6	0
Trephining the skull without division of roots, followed by injection of active thrombin	53	75	>1800. Blood did not clot	6	6	0
Experimental						
Bilateral division of 3 roots of the vagus nerves followed by injection of inactive thrombin	63	85	Blood clotted in blood vessels	24	0	24
Unilateral division of 3 roots of the vagus nerves followed by injection of active thrombin	65	75	>1800. Blood did not clot	6	6	0

activity of the autonomic nervous system. Experiments using autonomic poisons (atropine, ergot, chlorpromazine) and also experiments involving bilateral division of the sympathetic chain or bilateral vagotomy in the neck [6, 7] showed that the parasympathetic division of the autonomic nervous system plays a direct part in the reflex reaction of the anticlotting system.

The results of the present experiments, conducted on frogs, were as follows. Bilateral division of the roots of the vagus nerve emerging from the medulla, in contrast to unilateral disturbance of this innervation, led to death of the animals from thrombosis after intravenous injection of corresponding doses of thrombin (Table 2). Thrombin was injected at different times after division of the nerves: after 30, 60, and 120 min, and 24 h. The results of the experiments carried out at different times after division of the nerves were similar in their trend to those obtained 30 min after division (see Table 2). Control animals were subjected to a similar operation and received an intravenous injection of the same dose of inactive thrombin. No thrombosis took place in these animals. If, however, the vagus nerves remained intact and the 2nd and 3rd sympathetic ganglia were removed (Table 3), we did not observe the prethrombotic state, and if these animals received injections of the same doses of active thrombin, no thrombosis took place. The blood of these animals lost the ability to clot to the same degree as did the blood of the frogs of the control group.

TABLE 3. Effect of Division of the Sympathetic Chain on the Physiological State of the Anticlotting System in Frogs (mean values). Dose of Thrombin 0.3-0.4 ml, activity 7-9 sec

Experimental conditions	Clotting time (in seconds)			No. of animals		
	before division	30 min after division	4 min after injection of thrombin	total	surviving	dying
Control						
Bilateral extirpation of 2nd and 3rd sympathetic ganglia followed by injection of active thrombin	34	72	>1800. Blood did not clot	27	27	0
Experimental						
Bilateral extirpation of 2nd and 3rd sympathetic ganglia followed by injection of inactive thrombin	30	54	59	10	10	0
Injection of active thrombin into animals with intact sympathetic ganglia	40	–	>1800. Blood did not clot	5	5	0

Hence, analysis of the experimental results demonstrates that in amphibians, as in mammals (rats, rabbits), the performance of the reflex activity of the physiological anticlotting system is particularly associated with the activity of the cranial parasympathetic division of the autonomic nervous system. The central link for the reflex arc of the anticlotting system in frogs, as in mammals, is situated in the region of the medulla.

SUMMARY

In the previously published studies a statement was made that the physiological anticoagulating system was present not only in the mammals, but also in amphibians. The present paper deals with experiments on frogs showing that after bilateral division of the vagus roots arising from medulla oblongata, the animals died of thrombosis resulting from intravenous injection of thrombin (moderate doses – 0.3-0.4 ml).

However, following bilateral extirpation of sympathetic ganglia II and III no thrombosis occurred upon injection of similar doses of thrombin. Therefore, closure of the reflex arc of the physiological anticoagulating system is located in amphibians (as in mammals) in the sphere of medulla oblongata–vagus nuclei; protective response of the anticoagulating system is controlled by the cranial part of the parasympathetic nervous system.

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