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EFFECT OF ACETYLCHOLINE AND ATROPINE ON THE SECRETION OF BLOOD CLOTTING COMPOUNDS INTO THE BLOOD STREAM BY THE KIDNEYS

N. V. Sokratov and V. P. Skipetrov

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Experiments with perfusion of the kidneys of cats in situ showed that the secretion of clotting factors and fibrinolytic substances by the kidneys into the blood stream is a controlled process. Acetylcholine reduces the supply of blood clotting substances and of antiheparin compounds into the blood stream but increases the liberation of plasminogen activators from the kidneys to some extent. Atropine stimulates the liberation of thromboplastic substances and antiheparin components from the kidneys but reduces the secretion of antithrombin compounds. Atropine slightly increases the fibrinolytic activity of the perfusion fluid.

KEY WORDS: *kidney*; *blood clotting*; *fibrinolysis*; *acetylcholine*; *atropine*.

Adrenalin and choline chloride have been shown to stimulate the discharge of thromboplastin and fibrinolysis activators into the blood stream [4, 6-10]. It has also been shown that the kidney is one of the organs which participates actively in the regulation of blood clotting and fibrinolysis [3, 5, 11, 13-15]. However, the role of the kidneys in the modifications of blood clotting observed during changes in the functional state of the autonomic nervous system has received insufficient study.

An attempt was accordingly made to determine whether the kidneys secrete blood clotting compounds into the blood stream and also to examine the effect of acetylcholine and atropine on this process.

EXPERIMENTAL METHOD

Experiments were carried out on 27 cats. Under thiopental anesthesia (50 mg/kg) the renal vessels of the animals were cannulated and the kidney was perfused through the artery with warm Ringer–Locke solution. For a period of 2 h samples of perfusion fluid were collected every 20 min. In special series of experiments, after the first two samples of perfusion fluid had been taken, acetylcholine (0.1 mg/kg) or atropine (0.1 mg/kg) was added to the perfusion fluid, and samples were then again taken in accordance with the scheme above. The

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TABLE 1. Effect of Kidney Perfusion Fluid on Blood Clotting Indices (M ± m)

Index studied	Control	Samples				
		1	2	3	4	5
Degree of thrombotest	I—II	VII—VI	VI	VI	V—IV	IV—III
Recalcification time, sec	155	54,2±8	64,4±5,5	96,6±5,7	103,7±5,4	114±5,6
P		<0,001	<0,001	<0,001	<0,001	<0,001
Prothrombin consumption, sec	47	79±11,7	71,1±9,1	67,3±7,6	63,4±6,7	61±4,7
P		<0,05	<0,05	<0,05	<0,05	<0,05
Plasma heparin tolerance, sec	295,4	130,3±40,7	163,6±21,7	171,2±21	185,3±19,6	191±20,3
P		<0,01	<0,001	<0,001	<0,001	<0,001
Antiheparin activity, sec	94	72,1±8,8	75±7,3	80,7±8,4	74±2,5	89,3±2,5
P		<0,02	<0,02	<0,05	<0,02	<0,02
Prothrombin time of ordinary plasma, sec	32,2	24,2±1,1	25,1±1,2	27,5±1,3	28,1±1,2	29,4±1
P		<0,001	<0,001	<0,01	<0,01	<0,01
Prothrombin time of plasma without factor V, sec	73,3	45,2±1,4	47,5±0,6	50,6±0,5	58,1±1,6	60,1±1,4
P		<0,001	<0,001	<0,001	<0,001	<0,001
Plasma thrombin time, sec	27,2	60,4±5,1	48,1±2,2	47,1±2,2	46,1±2,4	44±2
P		<0,001	<0,001	<0,001	<0,001	<0,001
Fibrinolysis, sec	84,4	115,2±1,3	100±1,7	98,7±1,3	90,5±1	8,8±0,5
P		<0,001	<0,001	<0,01	<0,01	<0,05
Fibrinolysis, min	155	95,3±10,1	116,4±13	123,1±13,7	128±15	135,5±16
P		<0,01	<0,05	<0,05	<0,05	<0,05

TABLE 2. Effect of Acetylcholine on Blood Clotting and Fibrinolytic Properties of Kidney Perfusion Fluid (M ± m)

Index studied	Control	Samples					
		1	2	3	4	5	6
Recalcification time, sec	137,3	103,7	105,8	135,2±4,1	143,7±7,5	148,5±14,6	150,6±18,7
P				<0,01	<0,001	<0,001	<0,001
Prothrombin consumption, sec	42,7	71,9	73	65±3,5	58,8±2,7	56,2±2,1	57,9±3,2
P				<0,05	<0,01	<0,01	<0,01
Antiheparin activity, sec	101,6	66,5	66,3	79,8±3,5	82,9±3,8	86,2±3,9	79,6±4,4
P				<0,05	<0,01	<0,01	<0,05
Plasma heparin tolerance, sec	275,3	148,5	150,7	151,6±22,2	153,9±22	155,7±17	153,8±20
P				<0,05	<0,05	<0,05	<0,05
Prothrombin time of ordinary plasma, sec	38,6	36,3	34,5	40±0,9	43±1,2	44,4±0,3	44,5±0,9
P				<0,05	<0,05	<0,01	<0,01
Prothrombin time of plasma without factor V, sec	72,9	67,6	67,3	72,1±1,6	77,9±1,3	82,1±1,8	79,2±1,4
P				<0,05	<0,01	<0,001	<0,01
Thrombin time of ordinary plasma	31,9	41,1	43,2	49,1±1,6	50,6±1,3	54,3±1,6	55,7±1,2
P				<0,05	<0,01	<0,01	<0,01
Free heparin time, sec	5,7	10,1	10,3	16,6±0,6	20,5±0,8	22,8±1,9	20±2,2
P				<0,05	<0,01	<0,01	<0,01
Fibrinolysis, min	153,2	125,2	128,7	120±9,9	116,2±9,6	114±7,4	115±8
P				<0,05	<0,05	<0,01	<0,05

effect of different samples of perfusion fluid on the plasma recalcification time [16], prothrombin consumption [2], antiheparin activity and plasma heparin tolerance [1], plasma prothrombin time, measured by the Leningrad Blood Transfusion Institute method, plasma thrombin time and free heparin time [12], and fibrinolytic activity, studied by the euglobulin method [17], was determined on donors' plasma. In the experiments with acetylcholine and atropine the properties of the 3rd to the 6th samples of perfusion fluid were compared with the properties of the 2nd sample.

EXPERIMENTAL RESULTS AND DISCUSSION

Fluid obtained on perfusion of the kidneys with Ringer—Locke solution increased the readings in the thrombotest to the VI-VII degree (Table 1). At the same time the perfusion fluid reduced the recalcification time of platelet-free plasma, increased the plasma prothrombin consumption, and shortened the recalcification time of heparinized plasma. These results indicate the secretion of thromboplastic substances by the kidneys into the blood stream. The shortening of the prothrombin time of ordinary plasma and of plasma without

TABLE 3. Effect of Atropine on Blood Clotting and Fibrinolytic Properties of Kidney Perfusion Fluid (M ± m)

Index studied	Control	Samples					
		1	2	3	4	5	6
Recalcification time, sec	128,6	92	93	84,8±1,6	78,4±3,6	68,6±3,8	72,4±4,3
P				<0,01	<0,001	<0,001	<0,001
Prothrombin consumption, sec	47,4	68,8	69,4	76,4±1,5	80,6±1,6	89,6±2,1	81,4±1,3
P				<0,001	<0,001	<0,001	<0,001
Antiheparin activity, sec	113	71,2	72,1	48,8±6,4	41,8±7,4	41,6±7,1	52±8,3
P				<0,001	<0,001	<0,001	<0,001
Plasma heparin tolerance, sec	245	150,6	148	131,4±7,2	122,4±6,2	112±5,7	116,2±6,4
P				<0,01	<0,001	<0,001	<0,001
Prothrombin time of ordinary plasma, sec	41	34,2	35	30,8±1,8	26,8±2,1	29±1,1	30,2±1,6
P				<0,05	<0,05	<0,01	<0,05
Thrombin time of ordinary plasma, sec	36,6	43	41	35,2±1,8	31,4±2,7	29,4±1,9	31,4±1,9
P				<0,05	<0,05	<0,01	<0,05
Free heparin time, sec	5,4	8,8	9,6	6±0,9	5,4±0,8	4,4±1,2	4,8±0,9
P				<0,05	<0,05	<0,05	<0,05
Fibrinolysis, min	146,4	128,6	128	120,4±1,7	115±2,7	112±3,2	119±1,3
P				<0,05	<0,05	<0,01	<0,05

factor V under the influence of the kidney perfusion fluid indicates that the kidneys can liberate tissue Ac-globulin.

On the other hand, kidney perfusion fluid prolonged the thrombin time of plasma and a 1% solution of fibrinogen, evidence that the kidney also liberates anticoagulants into the blood stream. A special series of experiments showed that the antithrombin activity of the perfusion fluid was due to heparin. The perfusion fluid increased the resistance of fibrin to plasmin, indicating that the kidneys liberate tissue fibrinase. Fibrinolytic substances also were contained in the kidney perfusion fluid, especially the first portions of it.

In the next experiments the effect of acetylcholine and atropine on the secretion of clotting factors and fibrinolytic agents by the kidneys was assessed.

The first and second samples of perfusion fluid (obtained before the addition of acetylcholine), just as in the previous experiments, shortened the recalcification time of platelet-free plasma (Table 2). After the addition of acetylcholine to the solution the perfusion fluid began to lengthen this time. Under the influence of the third to the sixth samples of perfusion fluid, far less prothrombin was used than under the influence of the first and second samples. These findings indicate that acetylcholine reduces the secretion of thromboplastic compounds from the kidneys. The action of the perfusion fluid on the plasma heparin tolerance was unchanged after the addition of acetylcholine.

The first batches of perfusion fluid shortened the prothrombin time of ordinary plasma and of plasma with a deficiency of factor V as a result of the presence of compounds catalyzing the second phase of blood clotting. Acetylcholine weakened the action of the perfusion fluid on the conversion of prothrombin into thrombin in ordinary and accelerin-free plasma.

Under the influence of acetylcholine the perfusion fluid lengthened the thrombin time of plasma by a greater degree (by 50-68% more than in the control) through stimulating the liberation of natural anticoagulants from the kidneys. This was confirmed by investigation of free heparin. The free heparin time in the control plasma was 5.7 sec, but on the addition of perfusion fluid from the first samples to it it was increased to 10 sec, and from the third to the sixth samples, to 16.6-22.8 sec.

Atropine strengthened the coagulation properties of the perfusion fluid (Table 3). Perfusion fluid obtained after the addition of atropine shortened the recalcification time of platelet-free plasma by 7.5-25% and increased its prothrombin consumption by 10-30.4% more than the first portions. Atropine stimulated the liberation of antiheparin compounds into the blood stream also. During perfusion of the kidneys with pure Ringer–Locke solution, the secretion of thromboplastin and of heparin inhibitors decreased gradually from sample to sample. Meanwhile the addition of atropine to the perfusion fluid increased its powers of raising the plasma heparin tolerance, on account of an increase in the secretion of thromboplastic and antiheparin compounds by the kidneys. Perfusion fluid obtained after the addi-

tion of atropine reduced the prothrombin time of ordinary plasma by 11.4-23% more than the first samples.

This perfusion fluid reduced the thrombin time on account of a decrease in the secretion of natural anticoagulants by the kidneys. This is also confirmed by the shortening of the free heparin time in the third to the sixth samples.

Both acetylcholine and atropine increased the liberation of activators of fibrinolysis from the kidneys.

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