EFFECT OF EMOTIONAL STRESS ON CONTRACTILITY AND SENSITIVITY OF PORTAL VEIN SMOOTH MUSCLES TO NORADRENALIN AND CALCIUM IN SPONTANEOUSLY HYPERTENSIVE RATS

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Changes in the functional state of vascular smooth muscles are of great importance in the regulation of vascular tone and the resistance of the vessel to the blood flow in arterial hypertension [8]. However, most attention in the majority of investigations has been devoted to the arterial component of the circulation, and venous smooth muscle has received much less study, and the available data are highly contradictory [6, 9, 10, 12]. The writers showed previously [5] that the contractility (spontaneous activity) of the portal vein of spontaneously hypertensive rats depended on the stage of the disease: in the early hypertensive stage contractility of the portal vein and its response to noradrenalin were enhanced, in the stage of persistent hypertension the parameters were reduced and increased permeability of the membranes of the smooth-muscle cells to calcium and weakened dependence of spontaneous activity of the smooth muscles on exogenous calcium also were discovered.

The aim of this investigation was to study the functional state of smooth muscles of the portal vein in spontaneously hypertensive and normotensive rats at rest and during emotional (immobilization) stress, taking into consideration the important role of the latter in the etiology and pathogenesis of essential hypertension [4, 8].

EXPERIMENTAL METHOD

Experiments were carried out on 20 spontaneously hypertensive Kyoto-Wistar rats — SHR — aged 20 weeks (body weight 314 ± 8 g; BP 186 ± 6 mm Hg). The control consisted of 20 normotensive Wistar-Kyoto rats (WKY) of the same age (body weight 326 ± 14 g; BP 122 ± 5 mm Hg). A stressed situation was created in half of the animals of the experimental and control groups, with predominance of the psychoemotional component, by immobilizing the animals in the supine position in special frames for 6 h, which led to the appearance of ulcers in the gastric mucosa and enabled the strength of the stress factor to be monitored. The rats were killed by decapitation 1 h after the end of exposure to stress. The portal vein was then immediately removed and transferred to a constant-temperature chamber, filled with oxygenated Krebs' solution at 35°C, and subjected to a load of 400 mg. The preparation remained under these conditions for 1 h before the beginning of the experiment, in order to stabilize spontaneous contractile activity, which was recorded on a two-channel (control—experiment) system under isometric contraction conditions. In each experiment the response of the portal vein to a change in the calcium concentration in the external solution and the effects of various doses of noradrenalin (NA) were studied. When the response of the vein to a change in the exogenous Ca^{++} concentration was investigated the Krebs' solution was replaced by a solution not containing calcium; after cessation of spontaneous activity it was successively replaced by solutions containing 0.35–8.1 mm Ca^{++}. To determine the response to noradrenalin the maximal tonic response to each dose of the mediator was recorded. The following parameters of contractile function were calculated from the traces of spontaneous contractions: the developed phasic contraction, the frequency of spontaneous contractions per minute, the intensity of functioning of structures (IFS), equal to the product of the developed phasic contraction and the frequency of contractions, calculated per unit mass of the portal vein, and the max-

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TABLE 1. Parameters of Contractility and Response to Noradrenalin of Portal Vein Smooth Muscles of Normotensive and Spontaneously Hypertensive Rats Subjected to Emotional Stress

Group of animals	Developed contraction, mg force	Frequency of contractions per minute	IFS, mg force/min·mg	Velocity of contraction, mg force/sec	Velocity of relaxation, mg force/sec	Response to noradrenalin, ng/ml NA
WKY ($n = 10$)	105±15	7,0±2,6	259±54	71±15	112±22	700±130
WKY + stress (n = 10)	40±8,1*	6,5±1,2	100±24*	26±5,8*	43±10*	2200±560*
SHR ($n = 10$)	42±14**	15±1,3**	201±42	43±10	58±19**	210±10
SHR + stress (n = 10)	58±20	14±2,1***	309±87***	62±9,0	66±25	540±130

Legend. Here and in Table 2: *P < 0.05 between WKY and WKY + stress; **P < 0.05 between WKY and SHR; ***P < 0.05 between WKY + stress and SHR + stress.

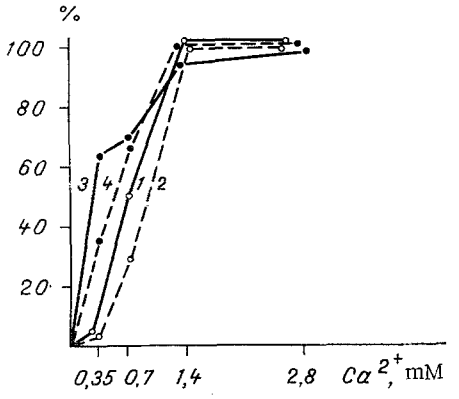

Fig. 1. Effect of different calcium concentrations in external medium of portal vein on number of contracting veins (percentage of total number) of normotensive and spontaneously hypertensive rats exposed to emotional stress. Abscissa, Ca^{++} concentration, mM; ordinate, number of contracting veins, % of total number); 1) intact WKY; 2) WKY + stress; 3) intact SHR; 4) SHR + stress.

imal rates of development of contraction and relaxation. The response of the portal vein to noradrenalin was calculated as the value of the dissociation constant (K_d) of the NA–adrenoreceptor complex, which was determined graphically in a system of double reciprocal coordinates. The significance of the results obtained after analysis of paired experiments was estimated by Student's t test.

EXPERIMENTAL RESULTS

Data on the effect of stress on the contractile function of the portal vein are given in Table 1. Clearly stress in WKY rats led to a marked decrease of all the original parameters of portal vein smooth muscle function by more than half. This is in agreement with results obtained by the writers previously [2, 3]. Those few discrepancies which were found in the present investigation in the magnitude of the effect (it was weaker) were evidently the result of using different models of exposure to stress. The developed contraction in SHR before stress was significantly lower than in intact WKY rats. However, the frequency of contractions, characterizing the intrinsic automatism of smooth muscle, was more than twice as high in SHR as in WKY rats. Despite the reduced amplitude of contractions, IFS was very slightly lower in SHR ($P > 0.5$). Exposure of SHR to stress did not cause depression of the contractile function of the vein. On the contrary, a tendency toward a poststress increase in all the parameters of contractile activity was clearly observed.

The data in Table 1 show that stress led to a decrease in the response of the portal vein smooth muscle of WKY rats to noradrenalin by more than two-thirds. Meanwhile in SHR, the response to noradrenalin, which was increased before stress (K_d was reduced by 3.5 times), was reduced after stress by a lesser degree and it remained higher than in WKY rats before and after stress. This may indicate that even before exposure to emotional stress the SHR were already in a state of some emotional stress (possibility hereditarily determined), or neurogenic stress, evidence of which has been obtained by other workers in the writer's laboratory [4].

TABLE 2. Effect of Changes in Ca^{++} Concentration in External Solution in Contractile Activity of Portal Vein of Normotensive and Spontaneously Hypertensive Rats in Initial State and during Emotional Stress

Group of animals and experimental conditions	Parameter	Calcium concentration, mM							
		0,35	0,7	1,4	2,8	3,5	4,9	6,7	8,1
WKY (n=10)	Developed contraction, mg	0	3,8±1,1	13±3,4	63±9,4	105±19	166±27	216±27	218±53
	Frequency of contractions per minute	0	7,2±3,4	18±1,0	9,1±1,4	6,0±15	3,7±0,5	2,8±0,4	2,4±0,3
	IFS, mg force/(mg·min)	0	15±6,4	92±23	218±57	235±62	229±48	236±32	227±36
WKY + stress (n = 10)	Developed contraction, mg	0	1,3±0,4	9,6±1,8	26±5,8	43±10	70±16	94±16*	104±18*
	Frequency of contractions per minute	0	4,3±0,8	13±1,0	9,4±1,0	5,8±1,2	3,5±0,9	2,7±0,5	2,5±0,4
	IFS, mg force/(mg· min)	0	7,3±1,0	55±9,2*	84±10*	102±25*	84±20*	93±22*	83±18*
SHR (n=10)	Developed contraction, mg	5,8±2,7	12±5,1	22±11	49±17	83±39	129±34	153±47	156±43
	Frequency of contractions per minute	17±4,2	17±2,6	15±1,0	17±3,9	14±3,8**	9,3±3,7**	7,5±3,5	6,3±3,0
	IFS, mg force/(mg·min)	63±25**	92±35**	140±49	184±64	191±52	158±31	171±3,6**	154±29**
SHR + stress (n =10)	Developed contraction, mg	4,0±0,5***	9,2±1,9***	31±12***	68±25***	86±28	108±32	134±26***	146±24
	Frequency of contractions per minute	6,0±0,9	12±1,7	15±3,7	12±2,0	9,0±2,1	6,8±2,1	4,8±2,1	4,1±1,6
	IFS, mg force/(mg·min)	28±17	74±16	202±62***	286±77***	221±56***	171±56	170±40	154±45

We know that stress is accompanied by a sharp increase in activity of the sympathetic nervous system and by release of large quantities of catecholamines [1], leading to intensification of lipid peroxidation, of lipase action, and of the detergent action of an increasing concentration of fatty acids in the cells (in this case myocytes), which gives rise to considerable changes in the energy supply for the smooth muscle cells and to metabolic changes in them [1, 2], with the result that contractile activity is inhibited (Table 1). Meanwhile disturbances of the lipid environment of the membrane proteins and, in particular, of adenylate cyclase [1, 2], may cause a decrease in the response to noradrenalin, which was observed in the writers' investigations, both present and previous [2, 3]. Finally, through a change in the activity of the corresponding enzymes, this lipid triad found in stress may cause disturbance of calcium transport [1, 3], which lies at the basis of the mechanism of "contraction–relaxation" coupling. The results of the present investigation provides further evidence in support of this view. In the case of WKY, as will be clear from Fig. 1, with a Ca^{++} concentration of 0.7 mM the number of contracting veins reached 50% of the number of contracting veins in a Ca^{++} concentration of 2.8 mM, whereas after stress it was only 28%. With an increase in the calcium concentration in the external medium from 2.8 to 8.1 mM (Table 2) the developed contraction in the initial state increased threefold, and after stress it increased more than fourfold. In other words, stress led to an increase in dependence of the contractile function of the small muscles of the vein in WKY rats on the extracellular calcium concentration, possibly as a result of a decrease in the ability of the cell membranes to take up and accumulate Ca^{++}, and also of a disturbance of function of the membrane mechanisms aimed at actively removing Ca^{++} from the cell [3].

Different relations between Ca^{++} and contractility of the portal vein were obtained in SHR. In SHR in the intact state 63% of veins contracted in an external medium containing a very low Ca^{++} concentration (Fig. 1). This indicates, it is considered [7], increased membrane permeability of vascular smooth muscle cells to Ca^{++}. A high percentage of contracting veins in a low Ca^{++} concentration also was observed in SHR during stress. Veins of SHR, unlike those of WKY rats, also were less dependent on the extracellular Ca^{++} concentration. In fact, with an increase in the Ca^{++} concentration in the external solution from 2.8 to 8.1 mM, the developed contraction of SHR veins increased about threefold, whereas in the veins of hypertensive animals exposed to stress the increase was only by 1.7 times (Table 2). Perhaps as a result of the mechanism mentioned above (increased permeability) the processes of "rebound" of Ca^{++} from the cell in SHR not only were not depressed but, evidently, they were actually stimulated.

The changed response to noradrenalin discovered by the writers in SHR, together with increased membrane permeability of the smooth-muscle cells to Ca^{++} and reduced reactivity to exogenous Ca^{++} gives the smooth muscle cells of the portal vein greater resistance to stress, which is manifested as a tendency toward an increase in contractile activity after stress.

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ZONAL CORTICOSTEROID HORMONE BIOSYNTHESIS IN THE ADRENAL CORTEX IN RATS EXPOSED TO EMOTIONAL STRESS COMBINED WITH SALT LOADING

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The adrenal cortex is a complex endocrine organ which specifically changes the secretion of corticosteroid hormones under the influence of stress and water-salt environmental factors [1]. Nevertheless, the combined action of these factors on corticosteroid biosynthesis in the adrenals remains virtually unstudied. Investigation of salt loading which, like emotional stress, has a definite pathogenic influence on the state of the cardiovascular system [2, 4], is particularly interesting.

The aim of this investigation was to study the pattern of biosynthesis of corticosteroid hormones in the zona glomerulosa (ZG) and the combined zona fasciculata + zona reticularis (ZF-ZR) of the adrenals, which are responsible for the mineralocorticoid and glucocorticoid function of the glands, during simultaneous exposure of animals to salt loading and emotional stress.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 230-260 g. For 8 days the animals took a normal or excessive amount of salt with their food: 2.5 or 50 meq sodium per rat per day. In the latter case the rats received a semisolid diet with a 2% concentration of salt and (to drink) 1.8% NaCl solution. A state of emotional stress was induced in the animals 30 min before decapitation (at 10-11 a.m.) on account of group conflict arising after 15-18 rats were put together in a cage measuring 46 × 30 × 15 cm, each rat having a rubber ring fitted on its hind limb, to compress it and cause irritation. The adrenals were divided into capsular and decapsulated parts and parallel samples were incubated in vitro with the addition of ^{3}H-progesterone (53 Ci/mmole) to each sample in a dose of $3 \cdot 10^5$ cpm. The technique of incubation, of chromatographic isolation of the ^{3}H-corticosteroids, and their quantitative assay were described previously [5]. The numerical data were subjected to statistical analysis by Student's t test.

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