EFFECT OF EMOTIONAL-PAINFUL STRESS ON SENSITIVITY OF PORTAL VEIN SMOOTH MUSCLE TO NORADRENALIN AND ACETYLCHOLINE

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Severe emotional-painful stress (EPS) is known to lead to marked disturbances of contractility of smooth muscle of the portal vein and to depress its responses to noradrenalin (NA); this may play a role in the development of arterial hypovolemia and states of collapse. However, there are as yet no data on quantitative characteristics of changes in sensitivity of the adrenoreceptors and acetylcholine receptors of the portal vein in EPS.

Accordingly in the investigation described below changes in sensitivity of adrenoreceptors and acetylcholine receptors in the portal vein were compared in animals exposed to stress and the changes taking place were estimated quantitatively.

EXPERIMENTAL METHOD

Male Wistar rats weighing 180-200 g were divided into two groups: 1) control and 2) exposed to EPS. EPS was produced in the form of an "anxiety neurosis" by Desiderato's method [7] for a period of 6 h. Its effectiveness was judged by the presence of gastric ulcers, which were not observed in the control rats. The control and experimental animals were decapitated simultaneously 2 h after the end of EPS and the portal veins were removed and placed in thermostatically controlled working chambers perfused with oxygenated Krebs' solution. Spontaneous contractions of the portal vein preparations were recorded on a two-channel instrument ("Ugo Basile," Italy), capable of recording contractions of the portal vein of a control animal and of an animal exposed to stress simultaneously.

The following optimal conditions for contractile activity of the preparations were determined beforehand: 30°C, stretching with a load of 400 mg. The control and experimental preparations were kept under these conditions for 1 h before the investigation began to stabilize spontaneous contractions. The effect of successively increasing concentrations on contractility of the preparations was studied: NA 1×10^{-7}, 3×10^{-7}, 6×10^{-7}, and 1×10^{-6} g/ml; acetylcholine (ACh) 1×10^{-8}, 3×10^{-8}, 6×10^{-8}, and 1×10^{-7} g/ml.

The following parameters of contractile activity of the portal vein were calculated: the developed phasic and tonic tension of smooth muscle (in mg), the frequency of spontaneous contractions per minute, and the intensity of functioning of structures (IFS), the product of the developed tension and frequency of contractions divided by the weight of the portal vein (in mg/min/kg weight).

To assess the sensitivity of adrenoreceptors and acetylcholine receptors of the portal vein smooth muscle, concentration—effect graphs were plotted in a Lineweaver—Burk system of double reciprocal coordinates [10], from which the apparent dissociation constants (K) of the NA—adrenoreceptor and ACh—ACh receptor complexes were calculated. Numerically speaking K is equal to the concentration of substance inducing a response equal to half the maximal response [1].

EXPERIMENTAL RESULTS

Under normal conditions the mean developed tension was 122 ± 24 mg, the frequency of spontaneous contractions 4.4 ± 0.4/min, and IFS was 192 ± 15 mg/min/mg weight (Table 1). As a result of exposure to EPS marked depression of spontaneous contractile activity was observed:

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TABLE 1. Effect of EPS on Contractile Activity of Rat Portal Vein (M ± m; n = 8)

Parameter	Control	EPS
Developed tension, mg	122±24	15±2,9*
Frequency of contractions per minute	4,4±0,4	5,1±0,5
IPS, mg/min/mg weight	192±15	33±9*

*P < 0.001 compared with control.

The developed tension was reduced approximately eightfold and IFS sixfold, possibly due to stress injury to the membranes of the portal vein myocytes, disturbing Ca^{++} transport to the contractile system, or to a disturbance of the energy supply to the smooth-muscle cells.

All three dose-dependent components of the response studied previously were found as a result of the action of NA on the isolated portal vein preparation: an increase in the frequency of spontaneous contractions and a decrease in the developed tension against the background of increased tone [11]. All these three components were considerably reduced as a result of exposure to EPS (Table 2). This was accompanied by an increase in the threshold concentration of NA: The response of the vein, in animals exposed to stress, to NA in a concentration of 1×10^{-7} g/ml did not appear, whereas this dose in the control gave rise to an appreciable response.

To assess the sensitivity of the portal vein smooth muscle to noradrenalin quantitatively, the constant K was calculated for each parameter: the developed phasic tension, the frequency of contractions, and the increase in tonic tension. EPS was found to cause a marked increase in K for all the parameters studied (Table 3). For the developed tension the value of K increased more than sixfold compared with the control, for frequency of contractions it increased about 17 times, and for the increase in tone it increased 21 times. These figures are evidence of a marked decrease in the response of the portal vein smooth muscle to noradrenalin, due to a decrease in the sensitivity of its receptors to NA.

During the action of ACh on portal vein smooth muscle the same three components of the response were found as in the case of its response to NA: an increase in the frequency of contractions and a decrease in phasic tension against the background of increased tone [8]. However, no significant differences were found when the principal parameters of contractility of the preparation from control and stressed animals were measured. Table 3 gives the mean values of K for these parameters. These values for the portal vein of animals exposed to EPS likewise did not differ significantly from the control, evidence of any marked differences in sensitivity of the smooth muscle receptors to ACh.

One of the most important characteristics of the response to stress is activation followed by exhaustion of the adrenergic system [4]. Under these circumstances the NA concentration falls significantly in various tissues and organs [2], including in the portal vein [3]. It will be clear from Tables 2 and 3 that this process is accompanied by a considerable decrease in sensitivity of the smooth muscle adrenoreceptors to NA. The question arises why in this case, contrary to the Cannon—Rosenblueth principle [6], sensitivity of the smooth muscle receptors of the vessel wall to noradrenalin is reduced. One result of exposure to severe stress is known to be activation of the sympathetic nervous system with release of catecholamines [4]. It is evidently because of the action of high concentrations of endogenous NA that desensitization of adrenoreceptors in vascular smooth muscle takes place. It has been shown that the harmful action of an excess of catecholamines in stress is associated with the appearance of what is called the lipid triad of membrane injury, made up of activation of lipid peroxidation, activation of phospholipases, and the detergent action of increasing concentrations of fatty acids, in the lipid bilayer of myocyte membranes, leading to considerable changes in the lipid environment of the membrane proteins [5]. Meanwhile we know that the state of the lipid bilayer of the membranes has a considerable influence on function of adenylate cyclase complex and, in particular, on transmission of the signal from the receptor to adenylate cyclase [9].

TABLE 2. Action of Different Concentrations of NA on Contractile Activity of Portal Vein from Normal Rats and Rats with EPS (M ± m)

Parameter	Experimental conditions	NA concentration, g/ml				
			$1 \cdot 10^{-7}$	$3 \cdot 10^{-7}$	$6 \cdot 10^{-7}$	$1 \cdot 10^{-6}$
Developed tension, mg	Control	140±20	87±19	70±20	48±13	—
	EPS	19±3,1*	19±3,1*	14±2,6*	11±1,4*	8,5±1,4
Frequency of contractions per minute	Control	3,4±0,4	9,5±0,9	12±1,5	19±3,8	—
	EPS	4,4±0,6	4,4±0,6*	8,4±0,6*	11±0,5*	14±0,7
IFS, mg/min/mg weight	Control	170±16	286±38	293±54	272±58	—
	EPS	35±8*	35±7,6*	46±9*	48±9*	46±8,8
Increase in tone	Control	0	52±17	75±23	146±32	—
	EPS	0	0	9,5±4,0*	14±4,4*	24±2,9

*P < 0.05 compared with control.

TABLE 3. Apparent Dissociation Constants (in g/ml) of NA—Adrenoreceptor and ACh—ACh Receptor Complexes for Different Parameters of Contractile Activity of Portal Vein from Normal Rats and Rats with EPS (M ± m)

Parameter	Experimental conditions	NA	ACh
Developed tension, mg	Control	$1{,}6 \cdot 10^{-7} \pm 0{,}5 \cdot 10^{-7}$	$1{,}6 \cdot 10^{-8} \pm 0{,}4 \cdot 10^{-8}$
	EPS	$10 \cdot 10^{-7} \pm 1{,}5 \cdot 10^{-7}$*	$2{,}9 \cdot 10^{-8} \pm 0{,}8 \cdot 10^{-8}$
Frequency of contractions per minute	Control	$2{,}4 \cdot 10^{-7} \pm 0{,}6 \cdot 10^{-7}$	$1{,}8 \cdot 10^{-8} \pm 0{,}2 \cdot 10^{-8}$
	EPS	$40 \cdot 10^{-7} \pm 8{,}3 \cdot 10^{-7}$*	$3{,}2 \cdot 10^{-8} \pm 1{,}3 \cdot 10^{-8}$
Increase in tone	Control	$2{,}9 \cdot 10^{-7} \pm 0{,}5 \cdot 10^{-7}$	$4{,}1 \cdot 10^{-8} \pm 1{,}0 \cdot 10^{-8}$
	EPS	$62 \cdot 10^{-7} \pm 10{,}6 \cdot 10^{-7}$*	$3{,}9 \cdot 10^{-8} \pm 0{,}7 \cdot 10^{-8}$

*P < 0.05 compared with control.

It can accordingly be postulated that the observed decrease in sensitivity to noradrenalin is due to a poststress decline in the efficiency of function of the adenylate cyclase complex.

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