CHANGES IN ADRENOCORTICAL FUNCTION AND LIPID METABOLISM DURING PERIPHERAL ELECTRICAL NOCICEPTIVE STIMULATION AS A MEANS OF STUDYING THE ANTISTRESS ACTION OF DIAZEPAM AND ACTIVATION OF THE POSITIVE REINFORCEMENT SYSTEM

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Experiments on rats showed that the self-stimulation (SS) response reduces the increase in the 11-hydroxycorticosteroid (11-HCS) concentration in blood after electrical nociceptive stimulation (ENS) of the limbs. Diazepam (1 mg/kg) reduced the liberation of 11-HCS and the β-lipoprotein concentration in response to SS and ENS and raised the phospholipid concentration. Diazepam and preliminary ENS evoked activation of SS. It is concluded that diazepam and activation of the positive reinforcement system act in the same direction with respect to 11-HCS excretion and the changes in lipid metabolism during ENS.

KEY WORDS: diazepam; 11-hydroxycorticosteroids; lipid metabolism; self-stimulation.

In small doses (1 mg/kg) diazepam is known to prevent the increase in the synthesis of corticosterone and its liberation into the blood stream in rats induced by emotional stress [4]. The effect of diazepam on lipid metabolism during positive and negative emotional responses has not been investigated. It is not yet clear whether positive emotions are the opposite of negative in the context of hormonal responses or whether positive emotional excitation can cancel out metabolic and endocrine changes arising in connection with negative emotions. The object of this investigation was to study changes in adrenocortical function and lipid metabolism during positive and negative emotional responses and the effect of diazepam on these parameters.

EXPERIMENTAL METHOD

Experiments were carried out on 45 noninbred male rats weighing 200-300 g with monopolar electrodes implanted into the region of the lateral hypothalamus and medial forebrain bundle in accordance with coordinates from a stereotaxic atlas [6]. The self-stimulation (SS) response of the rats in a Skinner box under constant reinforcement conditions was used as the model of a positive emotional state. In response to each pressure on the pedal the animal received electrical stimulation of its brain for 0.25 sec (frequency 100 Hz, pulse duration 1 msec). The strength of current used was that with which the frequency of pressing on the pedal was 400-600 times in the course of a 10-min session. A negative emotional state was induced in the rats by electrical nociceptive stimulation (ENS) of the limbs in a box with an electrode floor (voltage 60 V, stimulus duration 0.5 sec, frequency 100 Hz, interval between stimuli 0.5 sec, duration of exposure 20 min). Behavioral manifestations and changes in lipid metabolism and adrenocortical function during positive and negative emotional responses were studied during isolated stimulation (SS or ENS) and combined stimulation (SS immediately after ENS). The same variants of the experiments were carried out after preliminary injection of diazepam (1 mg/kg, intraperitoneally) 30 min before the beginning of the experiment. Blood was taken from the caudal vein immediately after the end of the experiment for determination of the concentrations of 11-hydroxycorticosteroids (11-HCS) [2], β-lipoproteins [3], total cholesterol (by the Liebermann–Burchardt color reaction), triglycerides [7], and phospholipids [5]. The rats were used twice in the experiments after an interval of 1 week. The mean values were calculated and the significance of results assessed by Student's t test.

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TABLE 1. Changes in Blood Levels of Total 11-HCS and Lipids during Emotional Responses and after Administration of Diazepam (M ± m)

Index	Control (n=10)	Diazepam (n=6)	ENS (n = 10)	ENS + diazepam (n=10)	SS (n=10)	SS + diazepam (n=10)	ENS + SS (n=9)	ENS + SS + diazepam (n=9)
Total 11-HCS, μg%	13,9±1,5	20,7±2,8	48,0±1,6 $P_1<0,05$	20,0±2,1 $P_1<0,05$ $P_2<0,001$	31,3±2,7 $P_1<0,001$ $P_3<0,001$	17,1±1,0 $P_1=0,05$ $P_2<0,01$	29,3±5,1 $P_1<0,01$ $P_3<0,001$	15,9±1,2 $P_2<0,02$
β-Lipoproteins, mg%	238,4±17,4	255,0±27,0	332,0±19,5 $P_1<0,001$	162,0±16,6 $P_1<0,01$ $P_2<0,001$	385,0±29,0 $P_1<0,001$	364,0±27,5 $P_1<0,01$	407,0±38,0 $P_1<0,01$	316,0±31,0 $P_1<0,05$
Cholesterol, mg%	65,2±4,0	82,1±9,0	69,1±5,9	57,6±3,7	70,5±9,5	69,1±9,3	60,6±5,9	62,9±4,2
Triglycerides, mg%	59,7±6,0	60,3±3,6	58,8±5,4	44,9±2,9 $P_1<0,05$	44,5±3,2 $P_1<0,05$ $P_3<0,05$	47,5±2,2 $P_1<0,05$	46,9±2,2 $P_3=0,05$	46,5±3,8
Phospholipids, mg%	64,7±5,0	80,5±12,6	114,3±17,8 $P_1<0,02$	155,1±16,6 $P_1<0,01$	66,0±7,1 $P_3<0,02$	82,4±7,0 $P_1=0,05$	69,6±5,5 $P_3<0,02$	84,5±7,2 $P_1<0,05$
Frequency of self-stimulation in 10 min	—	—	—	—	512±36,9	743,2±81,2 $P_2<0,05$	718,6±74,9	828,4±50,5 $P_2<0,05$

Legend. P_1) comparison with control; P_2) comparison of experimental groups before and after diazepam; P_3) comparison of ENS and SS groups. Values of P are given only if significant.

EXPERIMENTAL RESULTS

The experimental results are given in Table 1. Negative emotional excitation induced by ENS was characterized by marked motor excitation, increased aggressiveness of the animals, and a vocal response. The blood 11-HCS level was raised by 244% compared with the control and mobilization lipemia developed, as reflected in an increase in the β-lipoprotein and phospholipid concentrations (by 39.5 and 76.7% respectively). The cholesterol and triglyceride concentrations did not change significantly under these circumstances (Table 1). The negative emotional response after preliminary administration of diazepam was accompanied by a fall in the β-lipoprotein and triglyceride levels to the control value, but in this case the phospholipid and cholesterol concentrations were unchanged. Administration of diazepam led to a significant fall, by 58.3%, in the 11-HCS level.

During the SS response the 11-HCS concentration rose by 125% compared with the control, but the increase was much less marked than that of ENS. Activation of adrenocortical function during SS is evidently associated with provision for the motor activity of the SS process. At the same time the β-lipoprotein concentration was increased and the triglyceride concentration reduced compared with the control. The response to SS after preliminary diazepam was characterized by an increase in the frequency of pressing on the pedal by 44.9% compared with the control. Just as in the case of ENS, diazepam prevented the rise in the 11-HCS level during SS, but the lipid metabolism indices were unchanged in this case.

The increase in the frequency of SS after diazepam may be due to potentiation of the positive reinforcing properties of central stimulation or to a decrease in the activity of the negative reinforcement system. In the present experiments a distinct inverse relationship was observed between the increase in the frequency of SS under the influence of diazepam and the 11-HCS level. Considering that during ENS the rise in the 11-HCS level was much less marked than during SS, it can be postulated that diazepam modulates primarily the activity of the negative reinforcement system, and this may lead to reciprocal activation of the "reward" system.

The SS response after ENS led to a decrease in the liberation of 11-HCS to the level characteristic of SS alone. In this case, however, the β-lipoprotein concentration was higher than in the experiments with ENS, whereas the other parameters of lipid metabolism were unchanged. ENS led to an increase in the frequency of SS by 40.1% compared with the control.

The behavioral and hormonal (11-HCS level) manifestations during interaction between positive and negative emotional responses indicate reciprocal relations between positive and negative emotions. Activation of SS after ENS evidently arises as a "rebound" phenomenon and has a compensatory mechanism. Preliminary administration of diazepam under these experimental conditions caused normalization of the 11-HCS level and a decrease in the β-lipoprotein concentration but did not affect the other indices of lipid metabolism. It is a very interesting fact that diazepam, in the experiment with a combination of ENS and SS, also caused activation of SS.

Under all experimental conditions administration of diazepam was followed by normalization of the 11-HCS level, a decrease in the B-lipoprotein concentration, and an increase in the concentration of phospholipids, which play the role of stabilizer of lipoprotein complexes. The experimental results show that the action of diazepam and activation of the positive reinforcement system act in certain respects in the same direction on 11-HCS excretion and indices of lipid metabolism during ENS. The medial forebrain bundle, one of the most important pathways of the "reward" system, is known to conduct forebrain influences on ACTH secretion [1], and this may perhaps explain the fall in the 11-HCS level during combined ENS and SS compared with ENS alone. Activation of the positive reinforcement system thus has antistress properties. Considering the characteristic changes in lipid metabolism during emotional responses corrected by administration of diazepam, tranquilizers of the benzodiazepine series can be recommended for the prevention of neurogenic atherosclerosis.

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INTERACTION BETWEEN ACTIN-LIKE BRAIN PROTEIN AND ISOLATED SYNAPTIC VESICLES

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Mg-ATP was shown to increase the light diffusion of a suspension of rat brain synaptic vesicles (SV) in the presence of rat brain actin-like protein (ALP) (superprecipitation reaction). ALP increases the Mg-ATPase activity of SV and also the liberation of endogenous noradrenalin from SV of bovine hypothalamus, which is abolished by cytochalasin B. Glycolipids (gangliosides and cerebrosides) inhibit the superprecipitation reaction. The results are examined from the standpoint of the contractile hypothesis of mediator secretion.

KEY WORDS: brain actin-like protein; synaptic vesicles; secretion of mediators.

Interest in the study of contractile proteins of nonmuscular origin, especially proteins of brain tissue, has recently strengthened. For instance, an actin-like protein (ALP) has been localized in brain nerve endings mainly in presynaptic membranes (pre-SM); a myosin-like protein (MLP) mainly in synaptic vesicles (SV); and troponin- and tropomyosin-like protein in free SM and to some extent also in SV [5, 6]. In an enriched rat brain synaptic membrane fraction 11% of the total protein has been found to consist of ALP [11].

According to the contractile hypothesis of mediator secretion [2, 5], the act of secretion of mediators by nerve endings is the result of interaction between the MLP of the SV membranes with ALP which is a structural component of pre-SM. The formation of an actomyosin-like protein complex (AMLP) is initiated by an increase in the Ca^{++} concentration in nerve endings during their depolarization. The act of secretion also involves transport of SV to the active zone of pre-SM, possibly on account of interaction of the vesicles with microtubules and neurofilaments. Data on the blocking of secretion of various mediators by mitotic alkaloids [1, 2] can be regarded as confirmation of the contractile hypothesis. The effect of potentiation of liberation of exogenous mediator into the incubation medium from isolated SV, previously loaded with [^{14}C]-glutamate, on interaction between SV and brain ALP preparation or actin from skeletal muscles, containing Ca-sensitive components of the contractile system – in these experiments the formation of an AMLP complex was tested by finding increased Mg-ATPase activity of the SV fraction [13].

In the present investigation an attempt was made to test the contractile hypothesis on a new model: liberation of endogenous noradrenalin (NA) mediator from SV during the formation of an AMLP complex, monitored by measuring Mg-ATPase activity and changes in light diffusion of the SV suspension in the presence of ALP. The role of glycolipids as possible regulators of formation of the AMLP complex also was studied.

EXPERIMENTAL METHOD

A preparation of ALP containing Ca-sensitive proteins also was isolated from bovine cerebral cortex [12]. Tests showed that the ALP preparation did not possess ATPase activity and did not contain lipid components. The SV fraction was isolated from bovine hypothalamus or from whole rat brain (without the cerebel-

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