administration of bemitil may be associated with a change in the emotional level under its influence. Thus bemitil modifies several processes connected with behavior formation. Meanwhile analysis of the internal structure of states arising under the influence of bemitil requires the use of additional experimental methods and also of methods of analysis of experimental data.

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CUCURBITACIN R GLUCOSIDE AS A REGULATOR OF STEROID AND PROSTAGLANDIN E_2 PRODUCTION AND SPECIFIC MODULATOR OF THE HYPOTHALAMO–HYPOPHYSEO–ADRENOCORTICAL SYSTEM

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KEY WORDS: adaptogens; corticosteroids; cucurbitacin; prostaglandins; stress.

The writers showed previously that one of the most active components of an extract of the roots of *Bryonia alba* L., exhibiting adaptogenic properties, is the glucoside cucurbitacin R [2β-25-di(O-β-D-glucopyranosyloxy)-16α,22-dihydroxycucurbit-5-ene-3,11,22-trione] (CRG) [3], which increases the working capacity of mice during physical exertion [5]. Endurance is known to be increased as a result of the adaptive response of the hypophyseo-adrenocortical system, resulting in increased formation and secretion of corticosteroids by the adrenal cortex [1]. The possibility of enhancing endurance and resistance of the body when there is insufficiency of this system, by inducing the synthesis of endogenous corticosteroids through the use of adaptogens of plant origin, notably CRG, as inducers, is of great interest. The biochemical targets for factors influencing this process are probably icosanoids and, in particular, prostaglandin E_2 (PGE_2), which acts as specific modulator of activation of the hypothalamo-hypophyseo-adrenocortical system at all its levels [6-12].

The aim of this investigation was to study the effect of CRG on steroid production and on the formation of PGE_2 and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in the adrenal cortex during stress.

EXPERIMENTAL METHOD

Noninbred albino rats weighing 160-180 g were used. Daily for 14 days the animals were given an intramuscular injection of 0.2 ml of a 0.1 mM solution of CRG in a 0.15 M NaCl. Control animals received the same volume of physiological saline. Stress was induced by immobilization once for 2.5 h. Blood containing EDTA (10 mg/ml) was centrifuged at 800g for 15 min, plasma was separated, and hydrocortisone was added (0.8 μg to 1 ml of plasma). To one volume

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TABLE 1. Corticosterone Levels in Blood Plasma (in μg/ml) and Adrenals (in μg/g) of Rats during Stress and under the Influence of CRG (M ± m)

Tissue	Experimental conditions			
	control	immobilization	CRG (10^{-6} moles/kg)	CRG + immobilization
Blood plasma	0,187±0,030	0,783±0,052*	0,337±0,022*	0,510±0,030*
Adrenals	60,2±4,7	128,0±2,5*	113,7±10,3**	122,7±1,03*

Legend. *p < 0.001, **p < 0.025 compared with control.

TABLE 2. Effect of CRG on PGE_2 and $PGF_{2\alpha}$ Levels in Adrenal Cortex (in pg/mg tissue) during Stress (M ± m)

PG	Experimental conditions			
	control	immobilization	CRG (10^{-6} moles/kg)	CRG + immobilization
E_2	18,1±1,63	28,5±1,37*	49,6±3,58*	37,8±2,07*
$F_{2\alpha}$	27,4±1,57	33,27±1,02**	32,9±2,01**	34,37±1,32***

Legend. *p < 0.001, **p < 0.025, ***p < 0.01 compared with control.

of plasma six volumes of acetone were added, the mixture was vigorously shaken for 5 min, and then extracted with an equal volume of diethyl ether. The ethereal layer was separated, the solvent was evaporated to dryness, and the residue was dissolved in 100 μl of methanol and analyzed by high-performance liquid chromatography ("LKB-Bromma") on a column (4 × 250 mm) with "Lichrosorb-RP8, 10 μ, using a solvent system of methanol—water (1:1.1 ml/min) as the mobile phase. Substances were detected with the aid of a variable wavelength UV-detector at 245 nm. The retention volume of hydrocortisone was 11.7 ml and of corticosterone 19.3 ml. Corticosterone was assayed by measuring the areas of the peaks of corticosterone and of hydrocortisone, added as an internal standard.

The adrenals were removed, freed in the cold from connective and adipose tissue, the adrenal cortex was weighed and homogenized in 1 ml of 0.04 M Tris-HCl buffer, pH 7.4, the internal standard (hydrocortisone, 10 μg to 1 ml of suspension) was added, and the sample was extracted as described above.

PGE_2 and $PGF_{2\alpha}$ levels in the rats' adrenal cortex were determined by radioimmunoassay using kits from "Izotop" (Hungary) and "Clinical Assays" (USA).

The adrenal cortex of the rats was homogenized in a mixture containing 1 ml 0.1 M phosphate buffer (pH 7.4) and 3 ml of the upper phase of an ethyl acetate—isopropanol—0.2 M HCl (3:3:1) solution. Next, 2 ml of ethyl acetate and 3 ml of distilled water were added to the samples, which were vigorously shaken, and the organic phase was withdrawn and evaporated to dryness. The dry residue was dissolved in 3 ml of 0.04 M Tris-HCl buffer (pH 7.4), and equal portions were taken from this solution for quantitative determination of prostaglandins.

To allow for losses of the substances during extraction $^3H_8$-PGE_2 and $^3H_8$-$PGF_{2\alpha}$ were used. The yield of the substances was 70 and 85%, respectively.

The radioactivity of the samples was measured by means of an SL-4221 scintillation spectrophotometer ("Roche Bioélectronique," France), using Bray's mixture. The counting efficiency was 60% for ^3H.

EXPERIMENTAL RESULTS

Injection of CRG caused an increase in the corticosterone concentration both in the blood and in the adrenals. PGE_2 formation in the adrenal cortex was simultaneously increased (Tables 1 and 2). Changes of the same type were observed in stress.

If after receiving CRG the rats were subjected to stress, their adrenocortical activity did not increase by the same degree as that of animals not receiving CRG. Changes in the PGE_2 concentration were similar.

The causes of the "cytoprotective" action of CRG, which we observed previously, must also be noted; the PGE_2 level in the rats' blood plasma fell sharply during stress (this may also have given rise to ulcers in the stomach), and injection of CRG prevented this change [2]. The writers showed previously that CRG inhibits arachidonic acid release and leukotriene biosynthesis [4]. The results of the present investigation indicate that CRG can also affect activity of PGG_2-PGE_2-isomerase.

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EFFECT OF ISOLATION STRESS ON BLOOD ETHANOL PHARMACOKINETICS DURING ALCOHOL MOTIVATION FORMATION AND PHYSICAL DEPENDENCE ON ALCOHOL IN RATS

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KEY WORDS: alcohol intoxication; motivation.

Isolation stress has been shown to be one factor conditioning the induction of ethanol-oxidizing enzyme systems [1]. Activity of these systems, induced by isolation of animals, may lead to a marked increase in their ethanol consumption, for there is evidence [2] that alcohol can abolish the consequences or prevent the development of emotional stress in rats associated with painful electrical stimulation of the limbs, in a similar way to what happens under the influence of tranquilizers, such as diazepam.

Considering the facts described above and the conditions for production of models of experimental alcoholism (keeping animals in individual cages), in the investigation described below the effect of isolation stress on the pharmacokinetics of the blood ethanol level were studied in rats with different levels of alcohol motivation and in rats with physical dependence on alcohol.

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