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EFFECT OF PSYCHOTROPIC DRUGS OF THE PHENOTHIAZINE SERIES ON THYMOCYTE DNA TEMPLATE ACTIVITY AND GLUCOCORTICOID-RECEPTOR INTERACTION

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Specific cytoplasmic glucocorticoid receptors, participating in the mechanism of the effect of glucocorticoids at the level of the genetic apparatus of the cell have been found in various organs and tissues of man and animals [6, 7]. Glucocorticoid receptors, like adrenoreceptors [1], are evidently targets for pharmacological intervention [8].

Many effects of drugs of the phenothiazine series, such as anti-inflammatory [4], antihistamine [9], antiserotonin [5], and inhibiting cell metabolism [3, 7] are characteristic of both natural and synthetic glucocorticoids.

It was accordingly decided to study the effect of chlorpromazine and tisercin on thymocyte DNA template activity and glucocorticoid-receptor interaction.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 80-100 g adrenalectomized 7 days before the experiments began. The effect of chlorpromazine and tizercine on thymocyte DNA template activity was evaluated on the basis of their ability to reduce incorporation of ^{3}H-uridine into the acid-insoluble fraction of thymocyte mRNA.

The antiglucocorticoid effect of chlorpromazine and tisercin was judged from the degree of weakening of the inhibitory action of triamcinolone acetonide (10^{-8} M) on incorporation of ^{3}H-uridine into the acid-insoluble fraction of thymocyte mRNA [8]. For this purppse a suspension of thymocytes (2×10^6 cells in 1 ml) was prepared in medium No. 199. The next stages of the investigation followed the description of the method in [6]. Chlorpromazine and tisercin were used in concentrations of 3.5×10^{-7}, 3.5×10^{-6}, and 3.5×10^{-5} M. To determine the

*Levomepromazine

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TABLE 1. Effect of Drugs of Phenothiazine Series on Binding of ^{3}H-Triamcinolone Acetonide with Glucocorticoid Receptors of Rat Liver Cytosol

Experimental conditions	Number of rats	Concentration of total ^{3}H-triamcinolone acetonide: 0,25·10 M^{-8} M		0,5·10^{-8} M		1·10^{-8} M		2·10^{-8} M		4·10^{-8} M	
		concentration of ^{3}H-triamcinolone acetonide bound with cytosol									
		·10^{-10} M	%	·10^{-10} M	%	·10^{-10} M	%	·10^{-10} M	%	·10^{-10} M	%
Control	3	10,70±2,02	100	12,73±1,91	100	14,35±1,64	100	16,71±1,51	100	19,86±1,91	100
Unlabeled triamcinolone acetonide (8×10^{-6} M)	3	0,59±0,01*	5	1,13±0,14*	9	1,85±0,64*	13	3,83±0,38*	23	7,49±1,41*	38
Chlorpromazine											
3,5·10^{-4} M	3	1,89±0,69*	17	3,37±1,29*	26	5,40±2,03*	37	9,19±2,94	55	14,71±3,06	74
7·10^{-4} M	3	1,15±0,34*	10	1,83±0,37*	14	3,70±0,99*	26	6,08±0,88*	36	11,48±1,29*	58
Tisercin											
3,5·10^{-4}	3	1,69±0,55*	16	3,23±1,06*	25	5,14±1,65*	36	9,07±2,34*	54	15,61±3,31	79
7·10^{-4} M	3	1,02±0,11*	9	1,79±0,22*	14	3,37±0,44*	23	6,58±0,85*	39	12,62±1,09*	63

Legend. Here and in Tables 2 and 3: *p<0.05 compared with control.

TABLE 2. Effect of Drugs of Phenothiazine Series on Binding of ^{3}H-Cortisol by Glucocorticoid Receptors of Rat Liver Cytosol

Experimental conditions	Number of rats	Concentration of total ^{3}H-cortisol: 0,25·10^{-8}		0,5·10^{-8} M		1·10^{-8} M		2·10^{-8} M		4·10^{-8} M	
		concentration of ^{3}H-cortisol bound with cytosol									
		·10^{-10} M	%	·10^{-10} M	%	·10^{-10} M	%	·10^{-10} M	%	·10^{-10} M	%
Control	3	0,92±0,05	100	1,77±0,08	100	2,88±0,12	100	5,22±0,22	100	9,70±0,76	100
Unlabeled cortisol (5×10^{-6} M)	3	0,52±0,12	57	0,94±0,07*	53	1,78±0,13*	62	3,44±0,18*	66	7,38±0,55*	76
Chlorpromazine											
3,5·10^{-4} M	3	0,84±0,16	91	1,56±0,12	88	2,76±0,22	96	5,12±0,43	98	9,76±0,87	101
7·10^{-4} M	3	0,86±0,20	94	1,58±0,35	89	2,98±0,39	103	5,74±0,71	110	10,76±1,35	111
Tisercin											
3,5·10^{-4} M	3	1,04±0,39	113	1,86±0,37	105	3,19±0,46	111	5,83±0,83	112	10,47±0,99	108
7·10^{-4} M	3	1,18±0,30	128	2,27±0,14*	128	3,71±0,21*	129	6,75±0,58*	129	12,83±0,96*	132

glucocorticoid effect of chlorpromazine and tisercin samples without the addition of unlabeled triamcinolone acetonide were used as the control, whereas samples with the addition of unlabeled triamcinolone acetonide were used as the control, whereas samples with the addition of unlabeled triamcinolone acetonide (10^{-8} M) were used as the control for detection of antiglucocorticoid activity. Template activity of thymocyte DNA was assessed by the quantity of ^{3}H-uridine incorporated into the acid-insoluble fraction of thymocyte mRNA, expressed in moles/liter of thymocyte suspension.

The effect of chlorpromazine and tisercin on glucocorticoid-receptor interaction was estimated by the method in [10] with very slight modifications [2]. The liver was homogenized and centrifuged at 110,000g for 1 h in a BAK-601 centrifuge (East Germany). Type II glucocorticoid receptors in the liver cytosol were detected by binding with the synthetic glucocorticoid ^{3}H-triamcinolone acetonide (0.25×10^{-8}, 0.5×10^{-8}, 10^{-8}, 2×10^{-8}, and 4×10^{-8} M) for 18 h at 0°C, and type III glucocorticoid receptors were detected by binding with the natural glucocorticoid ^{3}H-cortisol (0.25×10^{-8}, 0.5×10^{-8}, 10^{-8}, 2×10^{-8}, and 4×10^{-8} M) for 15 min at 0°C. To discover the effect of the psychotropic drugs on glucocorticoid-receptor interaction, near-therapeutic concentrations of the drugs were used (3.5×10^{-4} and 7.0×10^{-4} M). To determine nonspecific binding of type II or type III glucocorticoid receptors, unlabeled triamcinolone acetonide (8×10^{-6} M) or cortisol (5×10^{-6} M) respectively was added. Samples without addition of the unlabeled steroid or without addition of the neurotropic drugs were used as the control. The level of binding of labeled glucocorticoids by type II or type III receptors was expressed in moles per liter of incubation medium.

EXPERIMENTAL RESULTS

The study of competition between ^{3}H-triamcinolone acetonide, on the one hand, and unlabeled triamcinolone acetonide or drugs of the phenothiazine series, on the other hand, for binding sites of type II glucocorticoid receptors in the liver cytosol showed (Table 1) that unlabeled triamcinolone acetonide, chlorpromazine, and tisercin actively displaced labeled tri-

TABLE 3. Effect of Drugs of Phenothiazine Series on Template Activity of Thymocyte DNA of Adrenalectomized Rats

Experimental conditions	Number of rats	Incorporation of ^{3}H-uridine into acid-insoluble fraction of thymocyte mRNA			
		$\cdot 10^{-10}$ M	%	$\cdot 10^{-10}$ M	%
		without addition of triamcinolone acetonide		with addition of triamcinolone acetonide	
Control	18	18,04±0,13	100	13,04±0,40	100
Chlorpromazine					
$3{,}5 \cdot 10^{-7}$ M	3	15,44±0,65*	85	11,35±0,22*	87
$3{,}5 \cdot 10^{-6}$ M	3	10,35±0,15*	57	7,16±0,27*	55
$3{,}5 \cdot 10^{-5}$ M	3	0,18±0,01*	1	0,27±0,06*	2
Tisercin					
$3{,}5 \cdot 10^{-7}$ M	3	16,14±0,26*	89	11,39±1,01*	87
$3{,}5 \cdot 10^{-6}$ M	3	12,03±0,86*	66	8,70±0,48*	66
$3{,}5 \cdot 10^{-5}$ M	3	0,12±0,03*	0,7	0,23±0,12*	1,8

amcinolone acetonide from the steroid-receptor complex. Higher concentrations of chlorpromazine and tisercin displaced ^{3}H-triamcinolone acetonide more strongly from the steroid-receptor complex.

The study of competition between ^{3}H-cortisol and unlabled cortisol or drugs of the phenothiazine series for binding sites of type III glucocorticoid receptors of the liver cytosol showed (Table 2) that unlabeled cortisol actively displaces ^{3}H-cortisol from the steroid-receptor complex. However, neither chlorpromazine nor tisercin weakened cortisol-receptor interaction. On the contrary, tisercin in a concentration of 7.0×10^{-4} M significantly increased binding of ^{3}H-cortisol with type III glucocorticoid receptors of liver cytosol.

Thus psychotropic drugs of the phenothiazine series (chlorpromazine, tisercin) weaken interaction of type II glucocorticoid receptors with triamcinolone acetonide and strengthen, to a lesser degree, interaction of type III glucocorticoid receptors with cortisol.

The possibility cannot be ruled out that chlorpromazine and tisercin do not so much compete with triamcinolone acetonide for binding sites on type II glucocorticoid receptors, as destroy them. This idea was studied in experiments in which tisercin was added to type II glucocorticoid receptors of the liver cytosol in concentrations of 10^{-4} or 10^{-3} M, and the tisercin was removed from the cytosol 3 h later by solid-phase adsorption. It was found that before addition of tisercin in a concentration of 10^{-4} M to the cytosol the content of type II glucocorticoid receptors in the liver cytosol was 59.2 ± 1.1 fmoles/mg protein, whereas after removal of tisercin by solid-phase adsorption it was 54.3 ± 0.8 fmoles/mg protein. Consequently, tisercin in a concentration of 10^{-4} M inhibited the level of type II glucocorticoid receptors of the liver cytosol, although not strongly. Tisercin in a concentration of 10^{-3} M had a more marked inhibitory action on the level of type II glucocorticoid receptors in the liver cytosol.

The inhibitory effect of tisercin on the level of type II glucocorticoid receptors thus revealed was due to the direct action of drugs of the phenothiazine series on the state of the type II glucocorticoid receptors. The increase in the content of type III glucocorticoid receptors in the liver cytosol may perhaps be attributable to the fact that type II glucocorticoid receptors of the liver cytosol inhibited by drugs of the phenothiazine series acquire the properties of type III glucocorticoid receptors of the liver cytosol.

Investigation of the effect of chlorpromazine and tisercin on template activity of thymocyte DNA showed (Table 3) that both drugs inhibit ^{3}H-uridine incorporation into the acid-insoluble fraction of thymocyte mRNA. The effect of the drugs was dose-dependent. Combined exposure to unlabeled triamcinolone acetonide (10^{-8} M) and different concentrations of chlorpromazine or tisercin potentiated additively the inhibitory effect of incorporation of ^{3}H-uridine into the acid-insoluble fraction of thymocyte mRNA. These data are evidence that chlorpromazine and tisercin can not only inhibit template activity of thymocyte DNA independently, but can also potentiate this effect additively when used in conjunction with glucocorticoids.

The results of the present investigations thus provide some evidence that chlorpromazine and tisercin realize their inhibitory effect on template activity of thymocyte DNA through their effect on type II glucocorticoid receptors, which play an important role in DNA template activity and biosynthesis of the acid-insoluble fraction of thymocyte RNA.

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EFFECT OF REPEATED ACUPUNCTURE ON NOCICEPTION AND ON β-ENDORPHIN LEVELS IN THE RAT HYPOTHALAMUS AND MIDBRAIN

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Many investigations have shown that acupuncture (AP) is accompanied by the development of marked analgesia in man and animals [1-6, 9]. The development of this phenomenon has been explained by activation of the opioid systems of the brain, as shown by indirect pharmacological and direct biochemical evidence. It has been shown, for instance, that the opiate antagonist naloxone inhibits the development of the analgesic effect of AP [1, 3, 4, 6, 9]. Weakening of nociception after AP is accompanied by changes in opioid substances in the brain and cerebrospinal fluid [1, 6]. All these data were obtained in experiments with single AP. The problem of the effect of repeated AP on regulation of nociception and on the opioid peptide level in various brain formations has not yet been studied.

It was accordingly decided to study the background level of nociception, the development of analgesia, and the β-endorphin (βE) concentration in the midbrain and hypothalamus of rats subjected to repeated AP.

EXPERIMENTAL METHOD

Experiments were carried out on 59 albino rats weighing 200-250 g. Animals of the experimental group (23 rats) underwent a daily session of AP lasting 15 min for 15 days. AP was given by insertion of needles into the acupuncture zone in the region of the Ho-Ku point on both forelimbs. Rats of the control group were not subjected to AP sessions. Nociception was evaluated before the experiment began and on the 1st, 3rd, 5th and 15th days before and after AP. The parameters tested in the control rats were measured at the same time intervals. Nociception was determined as the latent periods (LP) of the paw licking response (PLR) to placing the rats on a hot plate at 55°C and the tail withdrawal response (TWR) to focusing a beam of light from a 150-W lamp on it.

After the experiments the animals of both groups were decapitated and the hypothalamus and midbrain were removed by the method in [7]. βE was extracted from the tissue with 0.2 N hydrochloric acid (w/v = 1/10), during boiling on a water bath for 10 min, followed by homogenization of the samples. The homogenates were centrifuged at 10,000g for 15 min. The supernatant was frozen and lyophilized, and the dry extract was kept at −30°C. The βE concentra-

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