CORTEXOLONE AS A MODULATOR OF DIAZEPAM ACTIVITY

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KEY WORDS: diazepam; cortexolone; stress hormones.

There is clinical and experimental evidence of a possible connection between a raised cortisol level and the development of dysphoric, depressive, and hyperalgesic states [6, 9, 11].

At the same time, we know that administration of stressor hormones (ACTH, corticosteroids), especially in high doses, reduces the effectiveness of opioid analgesics and antidepressants in experimental animals [8, 11]. This suggests that stressor hormones, during prolonged hypersecretion, may play the role of negative regulators of nociceptive and emotional perception.

It was accordingly decided to study the effect of ACTH and corticosterone on the anxiolytic activity of diazepam and the action of cortexolone (an antagonist of glucocorticoid receptors) on the effectiveness of this tranquilizer in rats.

EXPERIMENTAL METHOD

Experiments were carried out on rats weighing 250-300 g. Anxiolytic activity was determined by motivated intraspecific aggression, a variant of a conflict situation, which is based on the struggle of a pair of rats for a safe place on an electrode floor during painful electrical stimulation of the limbs [1]. If a pair of rats remained on a safe bench for 10 sec, this was interpreted as the appearance of an anxiolytic effect. Diazepam (from Polfa, Poland), cortexolone (from Calbiochem, USA), and corticosterone (from Serva, West Germany) were injected intraperitoneally in the form of a suspension with Tween-80. Cortexolone (20 mg/kg) and corticosterone (20 mg/kg) were injected 60 min before, and $ACTH_{1-24}$ (Serva), in a dose of 200 μg/kg in aqueous solution, 30 min before testing. An equal volume of distilled water was injected into the animals of the control group. The interval between injection of diazepam and of distilled water was 30 min. The dose of cortexolone was determined depending on its ability to block glucocorticoid receptors when the substances are injected in vivo [10]. ACTH was injected in a dose inducing an anxiogenic effect in animals when administered systmeically [5]. The experimental results were computed in alternative form and analyzed by the Litchfield–Wilcoxon method, with calculation of 50% effective doses.

EXPERIMENTAL RESULTS

On the assumption that ACTH and glucocorticoids can induce a state of internal strain [4], the effect of these stressor hormones on the anxiolytic activity of diazepam, manifested as the method of motivated intraspecific aggression, was studied. It will be clear from Fig. 1 that both ACTH and corticosterone reduced the effectiveness of diazepam. Whereas injection of ACTH reduced anxiolytic activity (the effect was manifested in only 14% of animals), corticosterone blocked the anxiolytic action of diazepam virtually completely in a dose of 0.3 mg/kg. This state of affairs suggests that the antianxiolytic action of ACTH may be mediated (perhaps partially) through induction of secretion of glucocorticoid hormones. The effect of parenteral injection of cortexolone on the anxiolytic effect of diazepam within the dose range of 0.3 to 0.1 mg/kg is illustrated in Fig. 2. A dose of diazepam of 0.2 mg/kg corresponds to ED_{50} for the anxiolytic effect of the tranquilizer under the conditions of this particular

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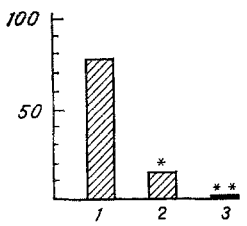

Fig. 1

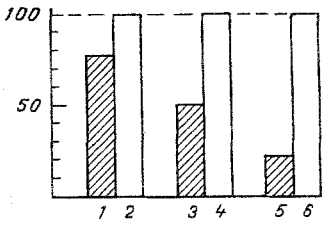

Fig. 2

Fig. 1. Effect of corticosterone and ACTH on the anxiolytic effect of diazepam. 1) Diazepam 0.3 mg/kg; 2) diazepam (0.3 mg/kg) + ACTH (200 μg/kg); 3) diazepam (0.3 mg/kg) + corticosterone (20 mg/kg). Here and in Fig. 2: *p < 0.01, **p < 0.001. Ordinate, anxiolytic effect (in %).

Fig. 2. Effect of cortexolone on the anxiolytic effect of diazepam. Shaded columns — diazepam (20 mg/kg); unshaded — diazepam + cortexolone. 1, 2) 0.3 mg/kg diazepam; 3, 4) 0.2 mg/kg diazepam; 5, 6) 0.1 mg/kg diazepam.

model (Fig. 2). Cortexolone evidently increases the effectiveness of diazepam and an anxiolytic effect is achieved in 100% of animals regardless of the dose of the tranquilizer given within the range studied. Corticosterone and cortexolone themselves caused no changes in the animals' behavior when tested by the method of motivated intraspecific aggression compared with the control.

It is logical to suggest that the modulating action of cortexolone is connected with its ability to antagonize the action of glucocorticoid hormones at the receptor level. However, the possibility that cortexolone may act directly on the function of central neurotransmitter systems cannot be ruled out.

The data described above are in agreement with the observations of Clarke and File [5], according to which ACTH, in high doses (over 100 μg/kg) gives an anxiogenic effect, which can be estimated from the reduction of the latent period of avoidance during electrical stimulation of the periaqueductal gray matter of the brain.

The biological nature of the antianxiolytic effect of stressor hormones can be easily explained in the light of the many concepts of stress, according to which states of internal anxiety and emotional tension are essential components of the complex of adaptive reactions of the body to stressor agents [2]. Meanwhile chronic exposure to stress factors leads in some cases to permanent hyperactivity of the pituitary-adrenal system, with the development of resistance to the effect of natural negative regulators of these hormones. This state can be revealed by the dexamethasone test (absence of the suppressive response of ACTH and glucocorticoids to injection of dexamethasone). As a rule, a positive dexamethasone test accompanies a depressive state and is normalized parallel with improvement of the clinical picture [6]. It will be evident that under stress conditions, especially if chronic, it will be necessary to block the action of stressor hormones in order to increase the effectiveness of psychotropic drugs (evidently of tranquilizers, antidepressants, and analgesics).

The ability of glucocorticoid hormones to reduce the action of such a wide range of preparations with different chemical structure and direction of action can be explained by the presence of a serotoninergic component in the mechanisms of action of the psychotropic drugs belonging to the classes mentioned above. Corticosteroids are distinguished by their high affinity for the serotoninergic system [7]. Blockade of the effect of these hormones on the activity of serotoninergic processes by means of cortexolone may be responsible for the modulating effect of this drug.

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ANTIWITHDRAWAL ACTION OF FENIBUT AND BACLOFEN ON EXPERIMENTAL WITHDRAWAL INDUCED BY CGS 8216, A BENZODIAZEPINE RECEPTOR ANTAGONIST, IN RATS RECEIVING DIAZEPAM

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Fenibut (FB, a phenyl derivative of GABA) was originally introduced into clinical practice as a tranquilizer and sedative [3, 7]. However, animal experiments have shown that FB is virtually without properties characteristic of the benzodiazepine tranquilizers [8]. In clinical practice FB, unlike diazepam, had little effect on fear, phobias, and anxiety, but was effective against symptoms of asthenic neurosis [4]. This particular feature of FB can most probably be explained by the presence of a nootropic component of its action. The question accordingly arose of whether it is justifiable to regard FB as a tranquilizer [2, 4]. The absence of activity of FB in the conflict situation test or in the antimetrazol effect is most probably evidence that FB differs from the tranquilizers of the benzodiazepine series, but it does not disprove that it has tranquilizing properties.

This paper gives data to show that FB and its chlorophenyl analog baclofen are highly effective in abolishing withdrawal symptoms induced by CGS 8216, a benzodiazepine receptor antagonist, in rats chronically receiving diazepam.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 220-250 g. For 20-30 days the animals were given diazepam by intraperitoneal injection in a dose of 10 mg/kg, after which it was withdrawn and the benzodiazepine receptor antagonist CGS 8216 (generously provided by the firm of Ciba Geigy, Switzerland) was given after 24-72 h in a dose of 2.5 mg/kg which induced well-defined features of withdrawal for 1-1.5 h after its administration in 90-95% of the animals. The behavioral signs characterizing this syndrome were recorded for 1 h. The test substances – diazepam (5-20 mg/kg), FB (10-100 mg/kg), baclofen (1.25-10 mg/kg), and TGIP, an agonist of $GABA_A$-receptors (5-20 mg/kg, from Lundbeck Denmark) were injected 5 min before the CGS 8216.

The experimental results were subjected to statistical analysis by the Mann–Whitney U-test and by methods of variance analysis.

EXPERIMENTAL RESULTS

Injection of CGS 8216 caused a behavioral syndrome in 95% of the animals chronically receiving diazepam, which included shaking of the head, attacks of myoclonus of the forelimbs, turning of the body, intensive chewing movements and sniffing, increased emotional reactivity to external tactile or acoustic stimuli, and tension of the tail muscles (Table 1). The high in-

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