ROLE OF GABA-ERGIC AND DOPAMINERGIC MECHANISMS IN THE DEVELOPMENT OF THE "REBOUND" SYNDROME AFTER CESSATION OF LONG-TERM ADMINISTRATION OF PHENAZEPAM

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The action of drugs of the benzodiazepine series is connected with different neuromediator systems of the brain. In particular, benzodiazepines, after administration of a single dose, are known to potentiate the inhibitory action of endogenous GABA [2, 7], they are able to modify serotonin metabolism [15], they reduce the circulation of noradrenalin and dopamine [11], and they block the increase in noradrenalin turnover caused by stress [6]. Metabolism of the neuromediators during prolonged administration of benzodiazepines has received much less study and the results so far obtained are contradictory. For instance, after long-term administration of diazepam to rats some workers [3, 13] observed increased tryptophan hydroxylase activity, intensification of serotonin metabolism, and a reduction in the turnover and increase in the concentration of noradrenalin and dopamine, whereas other investigators [14] found no change in the concentration of these mediators. The dynamics of changes in the functions of the GABA-ergic system during long-term administration of the benzodiazepines and after their withdrawal likewise has not yet been explained.

TABLE 1. Effect of Drugs on "Rebound" Syndrome observed 24 h after Cessation of Long-Term Administration of Phenazepam (2 mg/kg daily for 30 days)

Drug	Dose, mg/kg	Interval between administration of drugs and experiment	Duration of maze reflex, sec
Suspension of Tween-80	—	30 min	104,2 (96,1÷112,3) — "rebound" syndrome
Phenazepam	2	30 min	2,4 (1,6÷3,2)
n-dipropyl acetate	200	1 h	45,3 (37,1÷53,5)
α-methyldopa	200	24 h	62,9 (50,3÷75,5)
5-hydroxytryptophan	100	4 h	93,7 (81,6÷105,8)
3,4-dopa	200	30 min	>120
Thiosemicarbazide	4	20 min	>120
Disulfiram	230	20 h	101,8 (89,9÷113,7)
Phenazepam + bicuculline	2 1	30 min 10 min	79,3 (54,2÷94,1)
Phenazepam + thiosemicarbazide	2 4	30 min 50 min	90,0 (71,1÷108,9)
Control (administration of suspension of Tween-80 for 30 days)	—	30 min	3,6 (2,9÷4,3)
Phenazepam (single dose)	2	30 min	16,3 (9,2÷23,4)

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TABLE 2. Changes in Thresholds of Aggressiveness of Rats during Long-Term Administration of Phenazepam (2 mg/kg) and after Its Withdrawal

Drug	Duration of administration, days	Threshold of aggressiveness, μA
Control	1	0,88 (0,71÷1,05)
Control	40	1,0 (0,83÷1,17)
Phenazepam	1	2,18 (1,78÷2,58)
Phenazepam	40	0,98 (0,81÷1,07)
Depakine (300 mg/kg)	1	1,2 (1,12÷1,32)
48 h after withdrawal of phenazepam	40	0,29 (0,24÷0,34)
Depakine (300 mg/kg), 48 h after withdrawal of phenazepam	40	1,3 (1,02÷1,58)

The object of the present investigation was a neuropharmacological analysis of the mechanisms of development of the "withdrawal" ("ricochet") syndrome during long-term administration of the new benzodiazepine drug phenazepam, in an attempt to elucidate the role of the various mediator systems in the formation of this phenomenon.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 150-220 g. Tolerance and the manifestations of the "withdrawal" syndrome were assessed by relation to disturbance of the animal's conditioned-reflex activity and the antiaggressive action. A conditioned maze reflex with positive reinforcement (water) was first formed in the rats in a T-shaped maze, after which the same animals were given phenazepam (2 mg/kg daily, intraperitoneally) for 30 days. During the period of administration of phenazepam, the correctness and speed of performance of the reflex by the rats continued to be tested daily. The antiaggressive action was assessed from the change in the thresholds of aggressiveness (in μA), determined by counting the number of fights developing between pairs of rats receiving a painful electric shock of gradually increasing strength through the electrode floor. The appearance of a "withdrawal" syndrome was recorded 24 and 48 h after withdrawal of the drugs. Substances affecting synthesis and conversion of different neuromediators were injected against the background of a developed "withdrawal" syndrome in doses producing optimal changes in the mediator concentration of the brain.

EXPERIMENTAL RESULTS

Phenazepam (2 mg/kg), given in a single dose, had a distinct antiaggressive effect and depressed the performance of the conditioned maze reflexes. After long-term (30 days) administration of phenazepam the animals became accustomed to it, their activity was reduced, but withdrawal of the drug led to the development of a "rebound" ("ricochet") syndrome, characterized by reversal of the antiaggressive and sedative effects of the drug (Tables 1 and 2). A state of generalized inhibition was observed in the animals 24 or 48 h after the last dose of phenazepam, the adequacy of their response to test stimuli and the performance of the conditioned reflex, were disturbed (the maze transit time was increased 20-fold). When placed in the maze the animal assumed strained posture, squeaked, and developed tachycardia and tachypnea. At the same time, the thresholds of the rats' aggressive response were sharply reduced (below control values), and 80% of the animals showed the appearance of spontaneous aggressiveness. Injection of phenazepam into the abstinent animals completely abolished the motor and emotional manifestations of the "ricochet" syndrome (Table 1).

During analysis of the role of GABA-ergic mediation in the development of the "rebound" syndrome arising out of withdrawal of phenazepam the following test substances were used: Depakine (the calcium salt of dipropylacetic acid), a GABA agonist, raising its concentration in the brain [10], thiosemicarbazide, an inhibitor of GABA synthesis [9], and bicuculline, a drug blocking GABA-ergic receptors [8]. It has been shown that Depakine abolishes behavioral disturbances due to cessation of long-term administration of phenazepam.

In most animals (60%) under the influence of Depakine the stuporose state disappeared, performance of the conditioned reflex was restored, the spontaneous aggression was abolished, and the normal thresholds of the aggressive response were restored. Improvement of passage through the maze was not connected with the stimulant effect, for in the dose used Depakine had no significant effect on the performance of the reflex by control animals. By contrast to this, GABA-negative substances such as thiosemicarbazide and bicuculline aggravated the manifestations of the "withdrawal" syndrome and prevented the action of phenazepam on the "ricochet" syndrome (Table 1).

The serotonin precursor 5-hydroxytryptophan caused no significant change in the picture of the "ricochet" syndrome arising after cessation of long-term administration of phenazepam. Administration of α-methyldopa, which inhibits catecholamine synthesis through inhibition of dopa-decarboxylase [1], abolished to a large degree manifestations of the "withdrawal" syndrome and, like phenazepam and Depakine, restored the disturbed equilibrium. However, the effect of α-methyldopa was somewhat weaker than that of the GABA-positive drug 3, 4-dihydroxyphenylalanine (dopa) which, as the precursor of dopamine and noradrenalin, increases the concentration of the catecholamines in the brain and aggravates the "rebound" syndrome associated with withdrawal of phenazepam. Disulfiram, a powerful inhibitor of dopamine-hydroxylase, which reduces or slightly modifies the noradrenalin level and raises the dopamine level in the brain [12], also had a similar effect on this syndrome.

The results thus suggest a role for GABA-ergic and dopaminergic mechanisms in the development of tolerance and of the "withdrawal" syndrome after long-term administration of benzodiazepine. Abolition of the manifestations of the "withdrawal" syndrome after administration of a drug potentiating the inhibitory action of endogenous GABA and, conversely, the aggravation of this syndrome by GABA-negative drugs, were shown to be a regular feature. Abolition of the syndrome also was observed when the brain dopamine level was lowered, and to a lesser degree, when its noradrenalin level was lowered, whereas an increase in the dopamine concentration intensified the "ricochet" syndrome. It can be concluded from these results and from information that during long-term administration of benzodiazepines, dopamine and noradrenalin accumulate in different regions of the rat brain, and the levels of the metabolites 4-hydroxy-2-methoxyphenylglycol and homovanillic acid fall [13], that after discontinuation of long-term administration of benzodiazepines compensatory mechanisms are brought into action which cause an increase in the dispersal or a disturbance of the readmission of monoamines.

Considering the positive effect of the GABA-positive drug on the "rebound" syndrome, and data in the literature indicating the effect of GABA on dopaminergic mediation [4, 5], the GABA-ergic and dopaminergic mechanisms can be assumed to be interlinked in the realization of the "rebound" syndrome after the cessation of long-term administration of benzodiazepines.

From the practical point of view the results enable Depakine to be recommended as a promising drug for abolishing manifestations of the "rebound" syndrome arising in patients when drugs of the benzodiazepine series are withdrawn after long-term administration.

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