2. G. G. Gurgenidze, "Immunologic characterization of corticosteroid-dependent clinical form of bronchial asthma," Author's Abstract of Dissertation for the Degree of Candidate [in Russian], Tbilisi (1988).
3. S. P. Orlov and Yu. A. Labas, Biol. Membr., **6**, No. 9, 901 (1989).
4. P. I. Prasolova, E. S. Gershtein, K. D. Smirnova, and T. A. Chervinskaya, Klin. Med., No. 11, 28 (1990).
5. V. I. Pytskii, A New Idea About the Mechanism of Development of Glucocorticoid Dependence: Degree Oration [in Russian], Moscow (1991).
6. G. Grynkiowicz, M. Pocnic, and R. Y. Tsion, J. Biol. Chem., **260**, No. 6, 3440 (1985).
7. R. Y. Tsion, T. J. Rink, and M. Pocnic, Cell Calcium, **6**, No. 1-2, 145 (1985).

SELECTIVE ANALYZERS OF D_2-DOPAMINE RECEPTORS MODULATE SEROTONIN METABOLISM IN THE STRIATUM AND NUCLEUS ACCUMBENS AFTER DOPAMINERGIC NEURON BLOCKADE

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To assess the contribution of the presynaptic component of regulation of dopamine (DA) biosynthesis in the brain, a gamma-butyrolactone model has been suggested [11, 13]. Gamma-butyrolactone (GBL) can selectively block the spike discharge of dopaminergic neurons [3], as a result of which the release of DA from the corresponding nerve endings is reduced [6, 11], as also is the inhibitory effect mediated by terminal DA autoreceptors, belonging to the D_2 subtype. In this way DA biosynthesis is disinhibited on the feedback principle. Replacement of the endogenous ligand by a D_2-receptor agonist reduces the rate of DA biosynthesis and, in turn, this can be reversed by administration of a dopamine receptor antagonist [11, 13].

The state of the serotoninergic systems of the brain under the conditions of this model remains virtually unstudied. Yet this is problem of great interest, bearing in mind the important role of serotoninergic systems in brain functions. Accordingly, the aim of the investigation described below was to assess the possible influence of selective agonists and antagonists of D_2-dopamine receptors on serotonin biosynthesis and metabolism in the striatum and nucleus accumbens of the rat brain, when the dopaminergic spike discharge is blocked, so that effects of pharmacologically modified activity of dopamine neurons can be excluded.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 250-300 g. The state of DA and serotonin biosynthesis in the striatum and nucleus accumbens of the rat brain was assessed by measuring accumulation of L-3,4-dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophan (5-HTP) respectively after inhibition of the decarboxylase of L-aromatic amino acids with 3-hydroxybenzylhydrazine (3-HBH). The schedule of administration of these

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TABLE 1. Concentration (in ng/mg tissue) of Monoamines, Their Precursors, and Metabolites in Rat Brain Structures after Blockade of Spike Discharge of Dopaminergic Neurons and Inhibition of L-Aromatic Amino Acid Decarboxylase (M ± m, n = 5)

Schedule of substances injected	DOPA	DHPAA	VA	DA	5-HTP	Serotonin	5-HIAA
			Striatum				
3-HBH	1,129±0,12	0,337±0,026	0,423±0,034	7,92±0,455	1,963±0,123	0,445±0,018	0,119±0,007
3-HBH + GBL	2,082±0,221*	0,23±0,02*	0,298±0,019*	10,03±0,55*	1,83±0,165	0,453±0,029	0,227±0,016*
			Nucleus accumbens				
3-HBH	0,991±0,058	0,292±0,009	0,193±0,013	4,488±0,334	1,45±0,085	0,752±0,038	0,222±0,022
3-HBH + GBL	1,8±0,104*	0,185±0,023*	0,104±0,015*	6,75±0,325*	1,663±0,082	0,674±0,124	0,389±0,012*

Legend. *$p < 0.05$ (Student's t test). Substances used in doses of 50 and 750 mg/kg for 3-HBH and GBL respectively.

TABLE 2. Effects of Selective Agonists and Antagonists on D_2-Dopamine Receptors on Concentration of DA and Serotonin, Their Precursors, and Metabolites in Brain Structures of Rats after Blockade of Spike Discharge of Dopaminergic Neurons and Inhibition of L-Aromatic Amino-Acid Decarboxylase (M ± m, n = 5)

Schedule of substances injected	DOPA	DHPAA	VA	DA	5-HTP	Serotonin	5-HIAA
			Striatum				
3-HBH + GBL + quinpirole (0.1 mg/kg)	53,9±12,8*	130,4±34,8	88,6±11,7	125,7±16,2	111,6±8,8	117,4±8,1	49,8±10,1*
3-HBH + GBL + quinpirole (0.1 mg/kg)	44,5±4,3*	110,9±25,7	79,9±14,4	105,6±5,1	111,5±12,7	117,7±10,4	69,0±6,6*
3-HBH + GBL + quinpirole (0.1 mg/kg)	29,1±10,6*	100,0±16,5	81,9±9,7	119,1±7,5*	97,3±10,4	114,3±6,4	74,0±16,6
3-HBH + GBL + pergolide (0.3 mg/kg)	26,0±4,7*	78,9±9,9	67,7±19,0	80,9±14,1	109,0±6,9	102,3±9,0	84,4±2,6*
3-HBH + GBL + lisuride (0.1 mg/kg)	34,2±4,9*	94,7±10,5	75,5±14,2	88,0±10,1	82,8±5,4*	117,4±3,9*	57,8±4,4*
3-HBH + GBL + quinpirole (0.3 mg/kg) + raclopride (1.2 mg/kg)	75,8±10,4**	223,3± 50,8**	205,0± 24,3**	97,0±7,2	114,8±10,0	82,3±7,7**	78,3±4,4
			Nucleus accumbens				
3-HBH + GBL + quinpirole (0.1 mg/kg)	45,2±8,6*	138,0±40,5	131,7±16,3	121,6±10,4	99,8±10,3	107,5±5,7	73,3±19,8
3-HBH + GBL + quinpirole (0.1 mg/kg)	43,1±2,7*	121,6±18,9	124,0±30,7	106,5±8,5	102,3±11,9	91,5±7,7	67,1±7,0*
3-HBH + GBL + quinpirole (0.1 mg/kg)	41,5±11,6*	104,3±19,5	101,0±13,4	123,1±7,1*	90,6±12,8	87,4±8,4	58,9±7,7*
3-HBH + GBL + quinpirole (0.1 mg/kg)	66,4±8,8*	94,8±5,2	106,1±14,3	100,1±2,0	121,7±12,8	79,0±14,3	88,5±10,9
3-HBH + GBL + lisuride 0.1 mg/kg)	58,8±7,5*	97,1±4,6	74,8±9,5	98,8±6,2	68,5±4,3*	103,7±4,5	60,8±8,5*
3-HBH + GBL + quinpirole (0.3 mg/kg) + raclopride (1.2 mg/kg)	109,7± 13,3**	311,7± 41,1**	335,0± 32,6**	111,7±11,3	98,4±10,1	74,9±2,1**	86,9±5,5**

Legend. Data expressed as percentages of corresponding control, shown in Table 1. *$p < 0.05$ compared with control, **$p < 0.05$ compared with effect of quinpirole (0.3 mg/kg).

compounds was as follows: raclopride ("Astra") 1.2 mg/kg or 0.85% NaCl in a volume of 2 ml/kg body weight 40 min, agonists of D_2-dopamine receptors: quinpirole ("Eli Lilly") 0.1, 0.3, and 1 mg/kg, pergolide ("Eli Lilly") 0.3 mg/kg, or lisuride ("Schering") 0.1 mg/kg 60 min, and 3-HBH 50 mg/kg 30 min before decapitation [1]. All substances were injected intraperitoneally in a volume of 2 ml/kg. The brain was removed, the striatum and nucleus accumbens were isolated in the cold, homogenized in 0.1 M $HClO_4$, and centrifuged at 10,000*g* for 10 min. Concentrations of DOPA, DA, 3,4-hydroxyphenylacetic acid (DHPAA), homovanillic acid (VA), 5-HTP, serotonin, and 5-hydroxyindoleacetic acid (5-HIAA) in the samples were determined by HPLC with electrochemical detection. The determination was carried out on a reverse phase column (3 × 150 mm, C_{18}, 5 μ "Diagnostikum" Research-Production Cornbine), with the aid of 0.1 M citrate-phosphate buffer, containing 0.5 mM sodium octanesulfonate, 0.1 mM EDTA, and 8% acetonitrile (pH 3.9). Detection was carried out on a glass-carbon electrode at +0.8 V (LC-4B, "BAS," USA). The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Table 1 gives data on the concentration of monoamines and their precursors and metabolites in the rat striatum-and nucleus accumbens, when the spike discharge from dopaminergic neurons was blocked by GBL. As the results show, against the background of inhibition of the decarboxylase of L-aromatic amino acids high concentrations of L-dopa and 5-HTP were detected, although under ordinary conditions they are present only in traces. Injection of GBL was accompanied by a marked increase in the DOPA concentration and a moderate in the DA concentration in the basal ganglia, evidence of acceleration of DA biosynthesis [13] under conditions of disinhibition of terminal autoreceptors, which regulate this process. At the same time there was a certain fall of the level of metabolites DHPAA and VA in both brain structures (Table 1). Meanwhile GBL did not change the concentrations of serotonin and precursor 5-HTP in the striatum and nucleus accumbens. The increase in concentration of the serotonin metabolite 5-HIAA was an interesting fact. Considering the suggestion that 5-HIAA is of mainly intraneuronal origin (due to metabolism of unreleased serotonin [5, 8], and also bearing in mind the absence of changes in serotonin biosynthesis under the influence of GBL, it is logical to regard this effect as the result of a decline of serotonin release. This could be the result either of the direct effect of GBL on serotoninergic mechanisms in these two brain structures, or it could be secondary, mediated by interruption of dopaminergic neurotransmission. Incidentally, no data on the effect of GBL on serotoninergic mechanisms could be found.

Effects of selective analyzers of D_2 dopamine receptors on DA and serotonin biosynthesis and metabolism during blocking of the spike discharge of dopaminergic neurons in the striatum and nucleus accumbens are shown in Table 2. Agonists of D_2 dopamine receptors depress DOPA accumulation when enhanced as a result of blockade of the dopaminergic spike discharge. This effect, in turn, can be reversed by preliminary injection of a D_2 receptor antagonist [11, 13]. As Table 2 shows, quinpirole in doses of 0.1, 0.3, and 1 mg/kg, pergolide (0.3 mg/kg), and lisuride (0.1 mg/kg) depress DOPA accumulation in brain structures. The effect of quinpirole (0.3 mg/kg) in turn is reversed by the selective D_2 receptor antagonist raclopride (1.2 mg/kg). No significant changes were found in metabolite levels in a study of effects of agonists, whereas after injection of raclopride there was a marked increase in concentrations of DHPAA and HVA in the structures studied. No significant changes in the DA content likewise were found, aside from a small increase in concentration of the monoamine after injection of quinpirole in a large dose (1 mg/kg). These changes in the parameters of activity of the dopaminergic systems are in good agreement with data in the literature [11].

Quinpirole and pergolide did not change serotonin biosynthesis, whereas lisuride lowered the 5-HTP concentration in the striatum and nucleus accumbens, although the decrease was smaller than in the case of DOPA. Quinpirole and pergolide are known to be selective agonists of D_2 dopamine receptors, but are inactive against serotonin receptors [4, 12]. Lisuride, also a powerful agonist of dopamine D_2 receptors, also possesses a marked stimulating effect on serotonin receptors [7]. A decrease in 5-HTP accumulation was found in the striatum and neocortex of the rat brain after administration of terguride, a lisuride derivative, under conditions of monoamine depletion induced by reserpide [2]. The same investigations show that quinpirole can increase serotonin biosynthesis in the rat striatum and cerebral cortex. The absence of this kind of effect of quinpirole in our experiments can be explained both by the very small increase in the rate of 5-HTP accumulation found in [2] and differences in the experimental set up. Raclopride had no effect on 5-HTP accumulation in the striatum and nucleus accumbens, but caused a significant decrease in the serotonin concentration in both structures. It is difficult to explain this effect of raclopride, for we know that it is the most selective of all known antagonists of D_2 receptors currently known, and it does not change the neurochemical parameters of activity of the serotoninergic systems of the brain [9]. Quinpirole and pergolide did not affect the serotonin concentration in the striatum and nucleus accumbens, whereas lisuride raised its level in the striatum a little, possible confirmation that this substance is active against serotonin receptors.

The most interesting changes were observed in relation to the concentration of the serotonin metabolite 5-HIAA in the two rat brain structures studied.

All agonists of D_2 receptors used in the study caused a regular decrease in the 5-HIAA concentration in both brain structures. Raclopride significantly reversed this effect in the nucleus accumbens. In the striatum, raclopride similarly increased the 5-HIAA concentration when depressed through the influence of quinpirole (0.3 mg/kg), but this increase was less marked. If it is assumed that correlation is possible between the 5-HIAA concentration under the conditions of this model and serotonin release, it is logical to conclude that agonists of dopamine D_2

receptors cause an increase, whereas raclopride causes a decrease in serotonin release in the striatum and nucleus accumbens. The view was expressed previously that DA heteroreceptors, localized on serotoninergic neurons and capable of modulating their activity, may exist [10]. Modulation of serotonin metabolism with the aid of selective analyzers of D_2 receptors when dopaminergic transmission in the striatum and nucleus accumbens is blocked, as our experiments demonstrated, may provide confirmation of this point of view.

It can thus be concluded from the results of this investigation that blocking the spike discharge from dopaminergic neurons with the aid of GBL leads to marked changes in the neurochemical parameters of activity, not only of dopaminergic, but also of serotoninergic systems of the rat brain, manifested by an increase in the 5-HIAA concentration in the striatum and nucleus accumbens, without any corresponding change in levels of serotonin and its precursor 5-HTP. Selective agonists/antagonists of dopamine D_2 receptors can modulate metabolism of serotonin without affecting its synthesis.

LITERATURE CITED

1. V. S. Kudrin, I. I. Miroshnichenko, and K. S. Raevskii, Neirokhimiya, **7**, No. 1, 3 (1988).
2. S. Ahlenius, V. Hillegaart, and A. Wijkstrom, Neurosci. Lett., **126**, 57 (1991).
3. G. L. Gessa, F. Crabai, L. Vargiu, et al., J. Neurochem., **15**, 377 (1968).
4. M. Goldstein, A. Lieberman, J. Y. Lew, et al., Proc. Nat. Acad. Sci. USA, **77**, 3725 (1980).
5. D. G. Grahame-Smith, Advanc. Biochem. Psychopharmacol., **10**, 83 (1974).
6. A. Imperato and G. Di Chiara, J. Neurosci., **5**, 297 (1985).
7. W. Kehr, Eur. J. Pharmacol., **41**, 261 (1977).
8. D. M. Kuhn, W. A. Wolf, and M. B. H. Youdim, Neurochem. Int., **8**, 141 (1986).
9. O. Magnusson and B. Mohringe, J. Neurochem., **57**, Suppl.-P. S-130 (1991).
10. N. T. Maidment and C. A. Marsden, Neuropharmacology, **26**, 187 (1987).
11. R. H. Roth, in: Neuroleptics, Neurochemical, Behavioral and Clinical Perceptives, ed. by J. T. Coyle and S. J. Enna, New York (1983), p. 119.
12. K. Tsuruta, E. A. Frey, C. W. Grewe, et al., Nature, **292**, 463 (1981).
13. J. R. Walters and R. H. Roth, J. Pharmacol. Exp. Ther., **191**, 82 (1974).