COMPARISON OF NEUROCHEMICAL ACTIVITY PROFILES OF REMOXIPRIDE, RACLOPRIDE, AND METOCLOPRAMIDE

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The search for new antipsychotic drugs among the group of substituted benzamides is one of the most promising ways of obtaining new therapeutic agents. Substances of this group, belonging to the class of dopamine D_2 receptor antagonists, are heterogeneous and possess a varied spectrum of neuroleptic activity. For instance, sulpiride is an effective antipsychotic, giving rise to virtually no extrapyramidal disorders, whereas metoclopramide exhibits antipsychotic activity only in large doses, which at the same time give rise to marked dystonic reactions [15]. New members of this group of substances, namely raclopride and remoxipride, produced by the Swedish firm "Astra," have recently attracted attention. Raclopride is the most selective antagonist of dopamine D_2-receptors today, exhibiting antipsychotic activity in doses not giving rise to extrapyramidal reactions [4, 6]. Remoxipride is regarded as a powerful antipsychotic, equal in efficacy to haloperidol, whereas by the absence of extrapyramidal effects it is comparable with clozapine [14]. Neurochemical mechanisms lying at the basis of these differences in the pharmacological spectrum of activity of these drugs have let much unexplained.

The aim of this investigation was to compare effects of remoxipride, raclopride, and metoclopramide on different neurochemical models, in order to evaluate parameters such as levels of monoamines and their metabolites in brain structures, the rate of dopamine (DA) and serotonin biosynthesis in the basal ganglia, and DA release in the striatum, recorded in experiments in vitro and in vivo.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 250-300 g. The content of noradrenalin (NA), DA, 3,4-dihydroxyphenylacetic acid (DHPAA), homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA), and serotonin was determined in the frontal cortex, hypothalamus, nucleus accumbens, and striatum of the rat brain 30 min after intraperitoneal injection of the substances by high performance liquid chromatography with electrochemical detection (HPLC ED) [1]. The rate of biosynthesis of DA and serotonin in the striatum and nucleus accumbens of the rat brain was estimated from accumulation of L-3,4-dihydroxyphenylalanine (dopa) and 5-hydroxytryptophan (5-HTP) after inhibition of L-aromatic amino acid decarboxylase (AADC) by 3-hydroxybenzylhydrazine (3-HBH). 3-HBH in a dose of 50 mg/kg was injected 30 min before decapitation, and the test substances were given 15 min before injection of 3-HBH. Concentrations of dopa and 5-HTP were determined by HPLC ED on a reversed-phase column (3 × 150 mm, C_{18}, 5 μ, from the "Diagnostikum" Research-Production Combine), with the use of 0.1 M citrate-phosphate buffer, containing 0.5 mM sodium octanesulfonate, 0.1 mM EDTA, and 8% acetonitrile (pH 3.9). DA release in vitro was studied on a model of the isolated striatum [9]. The isolated striatum was placed in carbogenized buffer in a separate chamber. The first incubation lasted 1 h, after which the buffer was replaced by fresh.

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TABLE 1. Effect of Remoxipride, Raclopride, and Metoclopramide on Concentrations of Monoamines and Their Metabolites in Rat Brain Structures

Substances	NA	DHPAA	DA	5-HIAA	HVA	Serotonin
			Frontal cortex			
0,9 % NaCl	0,272±0,026	0,087±0,006	0,056±0,002	0,194±0,012	—	0,564±0,021
Remoxipride	0,254±0,013	0,077±0,003	0,059±0,011	0,168±0,013	—	0,530±0,097
Raclopride	0,249±0,015	0,090±0,007	0,064±0,009	0,186±0,011	—	0,577±0,051
Metoclopramide	0,270±0,016	0,136±0,015*	0,062±0,015	0,212±0,008	—	0,557±0,026
			Hypothalamus			
0,9 % NaCl	1,419±0,071	0,079±0,003	0,219±0,019	0,528±0,045	—	0,696±0,022
Remoxipride	1,076±0,036***	0,082±0,003	0,191±0,015	0,437±0,050	—	0,742±0,043
Raclopride	1,065±0,083***	0,076±0,002	0,189±0,022	0,481±0,031	—	0,663±0,049
Metoclopramide	1,630±0,088	0,092±0,004*	0,246±0,011	0,422±0,023*	—	0,746±0,040
			Striatum			
0,9 % NaCl	0,156±0,008	1,212±0,140	11,608±0,912	0,972±0,068	0,732±0,092	0,708±0,016
Remoxipride	0,228±0,028	1,488±0,132	11,360±0,844	1,004±0,044	0,932±0,096	0,756±0,036
Raclopride	0,164±0,012	3,500±0,116***	11,672±0,620	1,032±0,006	2,112±0,088***	0,628±0,028*
Metoclopramide	0,130±0,014	3,459±0,207***	10,970±0,065	0,891±0,038	1,665±0,060***	0,636±0,014*
			Nucleus accumbens			
0,9 % NaCl	0,904±0,075	1,080±0,072	7,220±0,316	0,767±0,041	0,504±0,060	1,004±0,064
Remoxipride	0,904±0,104	1,348±0,064**	7,040±0,504	0,804±0,040	0,516±0,064	1,032±0,040
Raclopride	0,600±0,072***	2,332±0,148***	5,804±0,356**	0,732±0,048	1,124±0,072***	0,804±0,052*
Metoclopramide	0,683±0,080*	2,642±0,098***	6,758±0,361	0,644±0,022*	1,549±0,056***	0,792±0,85*

Legend: results expressed in ng/mg tissue (M ± SEM, n = 5). *p < 0.05; **p < 0.01; ***p < 0.001. Substances were injected intraperitoneally in doses of: remoxipride 2.4 mg/kg, raclopride 1.2 mg/kg, metoclopramide 5 mg/kg.

TABLE 2. Effect of Remoxipride, Raclopride, and Metoclopramide on Extracellular Content of DA, DHPAA, HVA, and 5-HIAA in Striatum of Unrestrained Rats

Substances	Time before injection			Time after injection of substance (min)					
	60—40	40—20	20—0	0—20	20—40	40—60	60—80	80—100	100—120
					DA				
0,9 % NaCl	105±4	97±4	98±3	98±2	100±7	103±6	100±12	95±6	104±3
Remoxipride	108±12	98±13	96±8	115±11*	165±11***	181±20***	179±37*	166±29*	149±27
Raclopride	105±8	91±3	102±3	128±10	195±30***	203±34***	172±15***	180±21***	124±23
Metoclopramide	98±6	101±9	98±6	124±24	133±17	167±33*	157±7***	173±20***	143±19*
					DHPAA				
0,9 % NaCl	100±2	98±3	102±1	100±2	99±3	100±4	98±5	98±4	95±4
Remoxipride	103±2	99±3	99±1	105±2	136±10***	168±7***	177±12***	180±16***	181±22***
Raclopride	97±3	102±3	103±2	123±8**	172±8***	219±13***	208±10***	204±9***	193±10***
Metoclopramide	101±3	101±3	102±3	110±2	161±6***	166±8***	221±15***	219±16***	225±16***
					HVA				
0,9 % NaCl	98±2	97±2	103±2	104±3	105±3	108±4	102±5	108±5	100±5
Remoxipride	105±9	96±4	98±2	102±3	121±8**	150±7***	178±8***	182±10***	198±18***
raclopride	98±2	101±3	102±1	115±7	152±4***	219±27***	230±27***	238±30***	250±32***
Metoclopramide	100±5	102±3	103±5	110±3	134±7***	187±10***	219±23***	242±22***	257±23***
					5-HIAA				
0,9 % NaCl	102±2	93±4	105±2	101±3	106±3	106±3	102±3	105±2	105±2
Remoxipride	105±3	99±4	100±1	100±3	103±6	104±4	102±5	96±6	96±7
Raclopride	99±3	101±1	98±4	114±7	108±4	118±5	99±6	96±6	86±6**
Metoclopramide	101±3	100±2	99±4	100±2	108±3	104±4	108±5	100±7	105±7

Legend: dialysates were collected every 20 min (M ± SEM, n = 5). *p < 0.05; **p < 0.01; ***p < 0.001. Mean content in three basal specimens, found to be 0.181 ± 0.002 for DA. 24.2 ± 1.7 for DHPAA, 15.7 ± 1.18 for HVA, and 7.9 ± 0.86 for 5-HIAA (pmoles/20 μl perfusate) taken as 100%. Substances were injected intraperitoneally in the following doses: remoxipride 2.4 mg/kg, raclopride 1.2 mg/kg, metoclopramide 5 mg/kg.

After 10 min the buffer was removed and repeated incubation for 10 min carried out, using medium with a high K^+ concentration (28 mM) and with a corresponding equimolar reduction of the Na^+ concentration. Both 10-min

samples, reflecting basal and K^+-stimulated DA release, after precipitation on alumina, were used for DA determination by HPLC ED [1]. To study DA release in vivo the method of intracerebral microdialysis of the striatum of unrestrained rats [5] was used. Under general anesthesia, concentric dialyzers were implanted stereotactically into the striatum. Perfusion with Ringer's solution at the rate of 0.7 μl/min was carried out 24-48 h after the operation. Samples of dialysate were collected every 20 min and analyzed by HPLC ED. All the substances and physiological saline were injected intraperitoneally into the animals in a volume of 2 ml/kg. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

As Table 1 shows, raclopride, remoxipride, and metoclopramide, in doses equally effective in the apomorphine stereotypy test [11, 15], increased the concentrations of the dopamine metabolites DHPAA and HVA in the striatum and nucleus accumbens of the rat brain. This type of acceleration of DA turnover is a common property of substances with D_2-antagonism in their receptor spectrum [12] and was described previously for benzamide derivatives [3, 12]. Absence of an increase in the concentration of DA metabolites in the frontal cortex and hypothalamus in response to injection of raclopride and remoxipride can be explained by the early period of observation (the animals were decapitated 30 min after injection of the substances). In confirmation of results communicated previously [11] raclopride in our experiments caused a significant lowering of the DA content in the nucleus accumbens. Interesting changes were observed in relation to neurochemical parameters, characterizing activity of the serotoninergic and noradrenergic systems of the brain (Table 1). The effect of metoclopramide on concentrations of 5-HIAA and serotonin in the brain structures could be explained by antagonism of this substance relative to 5-HT^3 serotonin receptors [2], but it was shown previously that blockade of 5-HT^3 receptors has no effect on the content and metabolism of serotonin in brain structures [10]. Considering the high selectivity of raclopride and remoxipride relative to dopamine D_2 receptors [6], and also bearing in mind the fact that the neuroleptics chlorpromazine, haloperidol, spiperone, and flupenthixol, which differ in their activity toward serotonin receptors, also caused a decrease in the serotonin concentration in the striatum [8], it can be tentatively suggested that the changes observed in the 5-HIAA and serotonin concentrations were the result of changes in activity of the dopaminergic systems of the brain induced by the neuroleptics. The same conclusion can be drawn as regards the decrease in the NA content in the brain structures under the influence of the test substances.

Raclopride, remoxipride, and metoclopramide caused a marked increase in the rate of biosynthesis of DA, but not of serotonin in the striatum and nucleus accumbens of the rat brain (Fig. 1). This effect is regarded as the result of the increase in neuronal activity of the mesolimbic and nigrostriatal dopaminergic systems, induced by D_2-receptor blockade [12]. Atypical neuroleptics are known to have a greater influence on neurons of the mesolimbic than of the nigrostriatal system of the brain, whereas the opposite is the case for classical antipsychotics [12]. Our results show that acceleration of DA biosynthesis in response to injection of raclopride and remoxipride has a more marked effect in the nucleus accumbens, whereas the effect of metoclopramide is manifested more strongly in the striatum. However, no such selectivity as regards dopaminergic neurons of the mesolimbic and nigrostriatal systems of the brain could be found by determining the rate of DA turnover (Table 1).

Raclopride and remoxipride increased both spontaneous release of DA in the striatum of the unrestrained rats (Table 2) and also K^+-stimulated release from the isolated striatum (Fig. 2), whereas metoclopramide caused the least increase in DA release in vivo, and did not affect DA release in vitro. It is claimed that the increase in DA release in the striatum induced by neuroleptics is mediated mainly by D_2-autoreceptors, located on neuron terminals [12]. It is difficult to explain why metoclopramide had no effect on the K^+-induced increase in DA release, for it is known to be highly active against D_2 receptors and, moreover, metoclopramide has been shown to enhance electrically stimulated DA release in vivo in the striatum [13]. Meanwhile, attention is drawn to the fact that in our experiments, under the conditions of the model of in vitro release, haloperidol and trifluoperazine, which are cataleptogenic neuropeptides, likewise were inactive, whereas atypical neuroleptics caused a marked increase in DA release. Raclopride and remoxipride, which give rise to extrapyramidal symptoms only in large doses [11], increased the extracellular concentration of DA about equally with the increase in concentration of its metabolites (Table 2). A similar neurochemical profile in vivo has been described for the atypical neuroleptics clozapine, fluperlapine, and

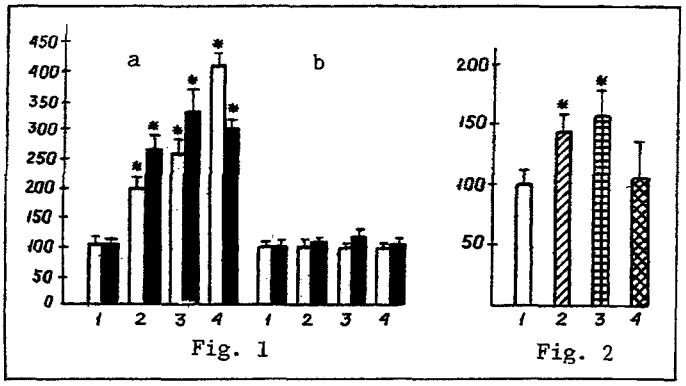

Fig. 1. Effect of remoxipride, raclopride, and metoclopramide on accumulation of dopa (a) and 5-HTP (b) in striatum (unshaded columns) and nucleus accumbens (black columns) of the rat brain during AADC blockade. 1) Control 2) remoxipride 2.4 mg/kg; 3) raclopride 1.2 mg/kg; 4) metoclopramide 5 mg/kg. Asterisks indicate significant differences from control ($p < 0.05$). Data shown as percentages of control (M ± SEM, n = 5), which was for dopa: in the striatum 1.393 ± 0.158 nq/mg tissue; in the nucleus accumbens 1.075 ± 0.12 ng/mg tissue; for HTP: in the striatum 0.335 ± 0.012 ng/mg tissue; in the nucleus accumbens 0.56 ± 0.034 ng/mg tissue. Substances and physiological saline were injected 45 min, and 3-HBH 30 min before decapitation.

Fig. 2. Effect of remoxipride, raclopride, and metoclopramide on K^+-stimulated DA release from isolated striatum. 1) Control; 2) remoxipride 10^{-6} M; 3) raclopride 10^{-6} M; 4) metoclopramide 10^{-6} M. *$p < 0.05$: significant differences from control. Data shown as percentages of control (M ± SEM, n = 5). Basal DA release in control samples was 60 ± 7 pmoles/min·mg tissue, K^+-stimulated release 228 ± 22 pmoles/min·mg tissue.

carbidine isomers, whereas classical neuroleptics are known to have a more marked effect in relation to intensification of metabolism of DA rather than its release [5, 7]. Thus these observations, together with data given above on DA release in vivo and in vitro, help to explain the manifestations of atypical behavior in the spectrum of action of remoxipride and raclopride by a greater degree of intensification of DA release, which may compete in this case with the neuroleptic molecules for binding of postsynaptic DA receptors. The possible result of this interaction could be unblocking of these receptors followed by a decrease in the probability of development of extrapyramidal symptoms. At the same time, the results of the present investigation do not give an unambiguous explanation of the differences in strength of the antipsychotic activity of the benzamines studied by it.

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EFFECT OF PROPHYLACTIC BENZONAL ON HEPATIC CYTOCHROME P-450 LEVEL IN IRRADIATED RATS

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Besides disturbance of the functions of such vitally important systems as the hematopoietic, endocrine, and immune systems, the irradiated organism develops a "hepatic syndrome" [16], which, in turn, makes a substantial contribution to the aggravation of postradiation changes in these systems. In the investigation described below, from a wide spectrum of liver functions we chose its detoxicating function, which is effected by monooxygenases, located in membranes of the endoplasmic reticulum; their terminal constituent is cytochrome P-450, which is marked by high inducibility and broad substrate specificity. However, in postradiation toxicosis the inducibility of the hemoprotein is blocked and its activity considerably reduced, a condition associated with qualitative and quantitative changes in cytochrome P-450 [1, 2, 9, 14, 17, 19]. On the basis of modern views regarding the development of the hepatic syndrome, aggravating the course and complicating the treatment of radiation sickness, the search for preparations with a marked hepatoprotective effect is fully justified. Some anticonvulsants, including benzonal, are known to be inducers of microsomal oxidation, and to have a beneficial effect on the general functional state of the liver [4, 7, 12].

The aim of this investigation was to study the effect of prophylactic benzonal on the content of the hepatic microsomal hemoprotein P-450 and, on the rat liver as a whole, during the 1st, 2nd, and 4th days of development of acute radiation sickness.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 160-190 g, kept on the standard animal house diet. The rats were deprived of food for 20-24 h before the experiment but were given water ad lib. The animals were divided into three groups. Rats of one group received a suspension of benzonal in starch gel per os in a dose of 100 mg/kg body weight daily for 3 days before irradiation. Rats of the second group received starch gel alone before irradiation, by the same schedule. The induced and noninduced rats were irradiated in a dose of 12 Gy

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