EFFECT OF PSYCHOTROPIC DRUGS ON DOPAMINE UPTAKE BY SYNAPTOSOMES AND GLIAL CELLS

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Uptake of dopamine-^{3}H by synaptosomes and glial cells of the rabbit cerebral cortex and synaptosomes of the rat cerebral cortex was investigated. The value of K_m for dopamine-^{3}H uptake was found to be the same ($0.075 \pm 0.01\ \mu M$) for both synaptosomes and glial cells. Rabbit cortical synaptosomes took up dopamine-^{3}H twice as fast as glial cells (the rate of uptake was compared in terms of protein). Among the psychotropic drugs studied, the most active inhibitors of synaptosomal uptake were amphetamine and cocaine, but they were less active against glial uptake. Neuroleptics and antidepressants inhibited both types of uptake equally. The results are in agreement with the view that the mechanism of action of psychostimulants include a striatal dopaminergic component.

KEY WORDS: synaptosomes; glia; dopamine uptake; psychotropic drugs.

Recent investigations have shown that various classes of psychotropic drugs (neuroleptics, antidepressants, psychostimulants) inhibit dopamine uptake by brain slices and synaptosomes [1, 6, 8, 9, 10, 13], evidence of the important role of the presynaptic component in the mechanism of action of these substances on central processes.

With these data and also the possibility that the glia may play a role in dopamine inactivation it was decided to study dopamine uptake by isolated nerve endings and glial cells under the influence of various psychotropic drugs from a comparative aspect.

EXPERIMENTAL METHOD

Fractions enriched with glial cells were obtained from the rabbit cerebral cortex by Rose's method [12] in the modification of Aleksidze et al. [2].

The cerebral hemispheres were minced in 25-30 ml of medium containing 2% Ficoll, 0.32 M sucrose, 0.05 mM Tris-HCl, and 1 mM EDTA (pH 7.4). The suspension was filtered with slight suction through a plastic syringe with pores 1500 μ in diameter, and then centrifuged for 20 min at 150g. The residue was resuspended in 20% Ficoll and then passed through the syringe with pores 1500 μ in diameter and through nylon sieves with pores 1000, 500, 100, 75, and 50μ in diameter. The resulting suspension was centrifuged in a density gradient (40% sucrose–30% Ficoll–suspension in 20% Ficoll–15% Ficoll–10% Ficoll; all ingredients contained 100 mM NaCl and 0.1 M Na-phosphate buffer, pH 7.4) for 120 min at 54,000g on the MSE-65 ultracentrifuge. After centrifugation the fraction of glial cells was collected from the top layer of 15% Ficoll, diluted with 0.32 M sucrose, and sedimented by centrifugation at 1500g (30 min). Expressed as protein, the yield of glia was 20% and the purity of the fraction, as shown by phase-contrast microscopy, was 90%.

The total fraction of synaptosomes was obtained by the method of Gray and Whittaker [7] in the modification of Shevtsov et al. [3].

Dopamine-^{3}H uptake by glial cells or synaptosomes (0.25 mg protein/ml) was determined in incubation medium containing (in mM): NaCl 100, KCl 6, $CaCl_2$ 2, $MgCl_2$ 3, glucose 10, sucrose 100, Tris-phosphate buffer, pH 6.4, 30, with continuous agitation for 20 min at 37°C. Dopamine-^{3}H (Radiochemical Centre, Amersham) with a specific activity of 5.0 Ci/mmole and nonradioactive dopamine (from Sigma), in molar proportions of 1:1000, were used in the experiments. The reaction was stopped by cooling the samples to 0-4°C. After centrifugation

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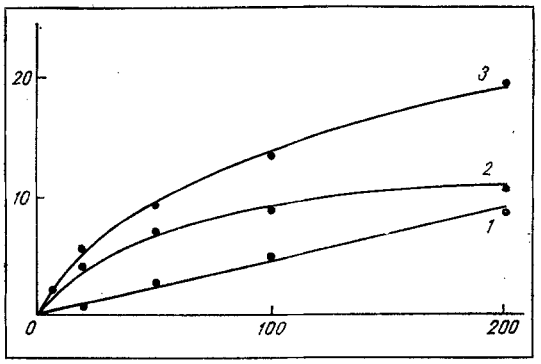

Fig. 1. Uptake of dopamine-^{3}H by synaptosomes from rat cerebral cortex. Abscissa, dopamine-^{3}H concentration (in nm); ordinate, quantity of dopamine-^{3}H bound (in moles · 10^{-12} · mg^{-1} synaptosomal protein in 20 min). 1) Adsorption of dopamine at 0° C; 2) true dopamine uptake at 37° C in 20 min; 3) total binding of dopamine by synaptosomes at 37° C (results of five or six experiments).

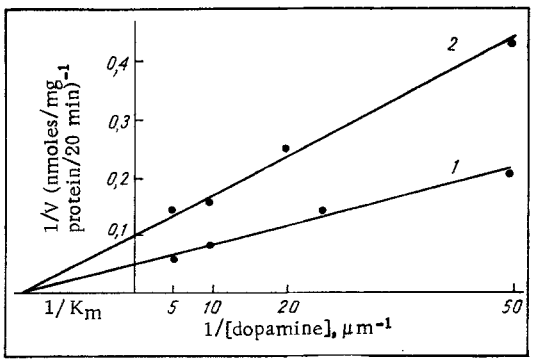

Fig. 2. Kinetics of dopamine-^{3}H uptake by glial cells and synaptosomes from rabbit cortex (Lineweaver–Burk graph, results of five or six experiments). 1) Dopamine-^{3}H uptake by synaptosomes; 2) by glial cells.

(20,000g, 15 min, 0-4° C) the residues were twice washed with cold incubation medium (without the isotope) and dissolved in 1 ml 10% Triton X-100. To 0.2 ml of the resulting solutions was added 10 ml of scintillation fluid containing 3 ml ethanol, 7 ml toluene, 0.5% 2,5-diphenyloxazole (PPO) and 0.01% 1,4-bis(2,5-phenyloxazolyl)-benzene (POPOP). Radioactivity was measured with a Mark 1 (Nuclear Chicago) scintillation counter. Protein was determined by Lowry's method [11].

Adsorption of dopamine in 0.32 M sucrose at 0° C was studied without incubation in a parallel series of control samples with both synaptosomes and glial cells.

EXPERIMENTAL RESULTS

Binding of dopamine by synaptosomes is an enzymic process of active transport which obeys the Michaelis–Menten kinetics (Fig. 1). Adsorption of dopamine was directly proportional to its concentration at 0° C. By subtracting this value from the total quantity of dopamine adsorbed at 37° C the true curve of dopamine uptake by the synaptosomes at 37° C in the course of 20 min was obtained and, on that basis, a Lineweaver–Burk graph was plotted and the Michaelis constant (K_m) calculated – its value was $(0.6 \pm 0.1) \times 10^{-7}$ M. This value was in good agreement with those obtained by Snyder and Coyle [14] (0.8×10^{-7} M) and Alpers et al. [4] (0.96×10^{-7} M) with rat brain synaptosomes. According to Garey [5], K_m for cat brain synaptosomes is 2.0×10^{-7} M and depends on the method

TABLE 1. Effect of Psychotropic Drugs on Uptake of Dopamine-^{3}H by Synaptosomes and Glial Cells from Rat and Rabbit Cerebral Cortex (M±m)

Drug	Concn. of drug, μM	Dopamine-^{3}H uptake, % of control		
		rat synaptosomes	rabbit synaptosomes	rabbit glial cells
Control		100±10	100±10	100±10
Fluphenazine	50	39±4	—	—
	500	20±4	—	—
Chlorpromazine	50	—	48±8	48±6
Trifluperidol	50	45±6	58±6	57±7
	500	17±4	17±4	—
Carbidine	50	78±8	—	—
	500	65±6	—	—
Azabutyron	50	101±12	—	—
	500	65±7	—	—
Imipramine	50	57±9	59±7	57±6
	500	21±2	21±2	—
Fluacizine	50	62±10	—	—
	500	21±3	—	—
Amphetamine	50	21±3	43±8	81±9
	500	21±2	25±3	54±7
Cocaine	50	36±4	37±4	54±6
	500	24±3	20±3	44±5
Apomorphine	50	67±7	70±7	59±6
	500	24±3	34±4	43±5

Legend. Mean values of uptake and their confidences intervals (P=0.05) relative to control (results of 5-7 measurements) given; 100% control corresponds to dopamine binding of 14.14 and $7 \cdot 10^{-12}$ moles/20 min/mg protein for rat synaptosomes, rabbit synaptosomes, and rabbit cortical glial cells respectively.

used to isolate the synaptosomes. Following the technique of Snyder and Coyle [14] in detail, Garey [5] found no difference in the values of K_m for dopamine uptake by synaptosomes of the rat, cat, and man. The results of a comparative study of dopamine-^{3}H uptake by rabbit synaptosomes and glial cells are illustrated in Fig. 2 (in Lineweaver–Burk coordinates). As Fig. 2 shows, the uptake of dopamine for both glial cells and synaptosomes was 0.075±0.01 μM. No species-specific differences in the values of the Michaelis constant for dopamine uptake by rat and rabbit synaptosomes were found. Dopamine was taken up twice as quickly by synaptosomes of the rabbit cerebral cortex as by glial cells if the rates of uptake were compared as protein. No significant differences were found between the synaptosomal and glial uptake of dopamine in the present experiments. This is confirmed by data in Table 1 showing the effect of psychotropic drugs on the dopamine uptake by synaptosomes and glial cells when a standard concentration of the amine (0.1 μM, the approximate value of K_m) was used.

As Table 1 shows, the most active inhibitors of synaptosomal dopamine uptake were the psychostimulants amphetamine and cocaine, and the neuroleptics fluphenazine, chlorpromazine, and trifluperidol had a somewhat weaker action. Apomorphine and the tricyclic antidepressants in a concentration of 50 μM inhibited dopamine uptake by 30-40%. The neuroleptics carbidine and azabutyron gave a similar effect, but in a concentration of 500 μM.

Comparison of the synaptosomal and glial uptake indicates that psychostimulants are more selective inhibitors of synaptosomal uptake and less active against glial uptake. Neuroleptics and antidepressants inhibit both types of uptake about equally.

The results of the present investigation are in agreement with others showing that chlorpromazine and haloperidol in concentrations of 10-100 μM [13], desimipramine (50 μM), and apomorphine (36 μM) [1] inhibit synaptosomal dopamine uptake by 50%. The effect of psychotropic drugs on glial dopamine uptake was being studied by the writers for the first time.

Of all the drugs tested the most active inhibitors of dopamine uptake were the psychostimulants amphetamine and cocaine. The physiological significance of this phenomenon in all probability is that inhibition of reuptake is the result of an increase in mediator (dopamine) concentration in the synaptic space and potentiation of its postsynaptic effect. This hypothesis is in agreement with modern views regarding the role of striatal dopamine in the mechanism of the stimulant effect of amphetamine.

An increase in the dopamine concentration in the synaptic space can also play a definite role in the genesis of the phenomenon of potentiation by antidepressants of a motor stereotype induced in rats by dopaminomimetic agents (amphetamine, apomorphine).

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