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EFFECT OF β-PHENYLETHYLAMINE ON SYNAPTOSOMAL AND GLIAL TRANSPORT OF LABELED NEUROTRANSMITTERS

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β-Phenylethylamine (β-PEA), an endogenous [5] sympathomimetic amine, has a powerful stimulating action on the CNS [7, 8, 12]. There are grounds for considering that β-PEA may participate in the regulation of monoaminergic synaptic transmission in certain brain structures, including the caudate nucleus [4, 6, 8]. For instance, *in vitro* experiments on synaptosomes of the caudate nucleus have shown that β-PEA moderately inhibits reverse transport of dopamine-^{3}H and stimulates its liberation [4]. The object of the present investigation was to study the role of β-PEA in the regulation of reverse transport into nerve endings of the caudate nucleus of other hypothetical mediators responsible for the activity of this structure (serotonin, glutamate, and GABA). For comparison, the effect of β-PEA was studied on synaptosomal and glial serotonin transport.

EXPERIMENTAL METHOD

Experiments were carried out on male albino mice weighing 180-250 g. The intensity of synaptosomal and glial transport processes was judged from the uptake of labeled mediators. The fraction of glial cells was isolated from the cerebral cortex of rabbits by Rose's method [10] with certain modifications described previously [2]. Total synaptosomal fractions from the caudate nucleus of the rat brain and from the rabbit cerebral cortex were obtained by Whittaker's method in the modification of Shevtsov et al. [3]. Uptake of GABA-^{3}H, glutamate-^{14}C, and serotonin-^{14}C was determined by incubating synaptosomes or glial cells (0.25 mg protein/ml) in medium containing 100 mM NaCl, 6 mM KCl, 10 mM glucose, 100 mM sucrose, 0.54 mM EDTA, 0.125 mM pargyline (a monoamine oxidase inhibitor), 1.14 mM ascorbic acid, and 30 mM Tris-phosphate buffer, pH 7.4, with continuous agitation (20 min, 37°C). β-Phenylethylamine (from Serva, West Germany) was converted into the hydrochloride and purified by repeated recrystallization. The following materials were used in the experiments: β-PEA hydrochloride

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TABLE 1. Effect of β-PEA on Uptake of Labeled Serotonin, Glutamate, and GABA by Synaptosomes and Glial Cells from Various Animal Brain Structures

Mediator	β-PEA concentration, μM	Uptake of mediator, % of control		
		rat caudate nucleus, synaptosomes	rabbit cerebral cortex	
			synaptosomes	glial cells
Control	—	100±10*	100±10 †	100±10 ‡
Serotonin-^{14}C	50	57±7	65±8	55±6
	100	27±4		
	500	3±1	28±4	26±3
	50	126±13		
Glutamate-^{14}C	100	166±11		
	500	107±11	—	—
GABA-^{3}H	50	108±11		
	100	100±10	—	—
		118±12		

Legend. *) 100% denotes 30 nmoles GABA-^{3}H, 22 nmoles serotonin-^{14}C, and 14 nmoles glutamate-^{14}C respectively bound by 1 mg synaptosomal protein in 20 min; †100% denotes 50 nmoles serotonin-^{14}C bound by 1 mg synaptosomal protein in 20 min; ‡100% denotes 25 nmoles serotonin-^{14}C bound by 1 mg glial cell protein in 20 min.

in concentrations of 50, 100, and 500 μM; a mixture of GABA-^{3}H (from New England Nuclear, USA), with specific radioactivity of 10 Ci/mole, with nonradioactive GABA in molar proportion of 1:1000; a mixture of glutamate-^{14}C (Prague, Czechoslovakia), specific radioactivity 0.175 Ci/nmole, with nonradioactive glutamate in a molar radio of 1:1; serotonin-^{14}C (from the Radiochemical Centre, Amersham, England) with specific radioactivity of 58 Ci/mole. The reaction was stopped by cooling the samples to 0-4°C. After centrifugation (20,000g, 15 min, 0-4°C) the residues were twice washed with cold incubation medium (without isotope) and dissolved in 1 ml of Triton X-100. A 0.2-ml sample was taken from the resulting solution and added to 10 ml of scintillation fluid, containing 3 ml ethanol and 7 ml toluene with 0.5%, 2,5-diphenyloxazole and 0.1% 1,4-bis-2-(5-phenyl)-oxazolylbenzene. Radioactivity was measured on a Mark-1 counter (Nuclear Chicago, USA). Protein was determined by Lowry's method. The results were subjected to statistical analysis with calculation of means and their confidence intervals at P = 0.05.

EXPERIMENTAL RESULTS

The effect of β-PEA on uptake of serotonin-^{14}C, glutamate-^{14}C, and GABA-^{3}H by brain synaptosomes of animals was studied by the use of mediators in concentrations close to the Michaelis constants (K_m). K_m for glutamate uptake by rat cortical synaptosomes is 10 μM [14] and K_m for GABA is also 10 μM [2]. In the present experiments K_m for serotonin uptake by synaptosomes and K_m for serotonin uptake by glial cells were 0.083 μM, in agreement with the findings of other workers [13, 15]. Accordingly, the following standard concentrations of mediators were used: glutamate 10 μM, GABA 10 μM, and serotonin 0.1 μM. The experimental results are given in Table 1. They show that β-PEA had practically no effect on uptake of GABA-^{3}H and glutamate-^{14}C by synaptosomes of the rat caudate nucleus. However, with β-PEA used in a concentration of 50 μM a tendency toward stimulation of glutamate uptake was found. It is impossible at present to explain this fact and further experiments are evidently needed to clarify the effect of β-PEA on nerve-cell metabolism. On the other hand, β-PEA clearly inhibited serotonin-^{14}C uptake by synaptosomes of the caudate nucleus and cerebral cortex. The effect was evidently dependent on the concentration of β-PEA, With a concentration of 50 μM, for instance, virtually total inhibition of serotonin uptake by synaptosomes of the caudate nucleus took place. In the presence of β-PEA, binding of serotonin-^{14}C by synaptosomes and glial cells of the rabbit cerebral cortex was inhibited about equally.

The data on the inhibitory effect of β-PEA on glial uptake of mediators and, in particular, of serotonin are new and have not previously been stated in the literature. It is interesting to compare the results of the present experiments to study the effect of β-PEA on synaptosomal serotonin uptake with results obtained by Baker et al. [4], who showed that β-PEA is a powerful inhibitor (75% inhibition by β-PEA in a concentration of 10^{-5}M) of uptake of noradrenalin-^{3}H by synaptosomes of the rat hypothalamus and a moderate inhibitor of uptake of dopamine-^{3}H by synaptosomes of the corpus striatum (39% by a concentration of 10^{-5}M respectively). This indicates that the inhibitory action of β-PEA on synaptosomal uptake of various biogenic monoamines (noradrenalin, dopamine, serotonin,) shares a common mechanism. Inhibition of re-uptake and potentiation of liberation of biogenic amines from nerve endings lead to an increase in the concentration of these substances in the synaptic space [4] and to a decrease in the content of biogenic monoamines in the terminals of the corresponding neurons [6]. Both these processes are affected if the deposition of biogenic monoamines in granules is disturbed [1]. Penetrating into neurons by passive diffusion [9], β-PEA possibly disturbs the deposition of biogenic monoamines. The mechanism of this effect is not clear. However, we know that D-amphetamine, a methyl derivative of β-PEA with similar stimulating effects to it on the CNS, binds with the vesicular membrane to create a "cationic barrier," preventing transport of the neurotransmitter inside the vesicles [1]. β-PEA possibly acts in the same way. This hypothesis is in agreement with the property of amphetamine, discovered previously, of inhibiting synaptosomal uptake not only of catecholamines, but also of serotonin [11]. Like β-PEA, amphetamine likewise does not affect the uptake of mediator amino acids. The results of the present investigation thus indicate that β-PEA can actively interfere in the regulation of synaptic transmission in the caudate nucleus and cerebral cortex of animals that takes place with the aid of biogenic monoamines. Disturbances of β-PEA metabolism can therefore lead to profound shifts of intermediator balance in the caudate nucleus and may be the cause of development of certain mental diseases.

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