3. I. V. Komissarov, G. I. Zholob, and R. N. Glebov, Byull. Éksp. Biol. Med., 100, No. 10, 400 (1985).
4. F. Hajos, Brain Res., 93, 485 (1975).
5. L. L. Iversen, Science, 188, 1084 (1975).
6. Z. F. Kometiani, L. G. Tsakhadze (L. G. Tsakadse), and T. L. Dzhariashvili (T. L. Jariashvili), J. Neurochem., 42, 1246 (1984).
7. O. H. Lowry and J. A. Lopez, J. Biol. Chem., 162, 421 (1946).
8. R. Y. Miller, A. S. Horn, and L. L. Iversen, Molec. Pharmacol., 10, 759 (1974).
9. P. Seeman, Pharmacol. Toxicol., 32, 229 (1980).
10. K. Starke, Ann. Rev. Pharmacol. Toxicol., 21, 7 (1981).

DETERMINATION OF ^{3}H-SEROTONIN REUPTAKE IN SYNAPTOSOMES: CORRECT APPRAISAL OF THE CONTRIBUTION OF NONSPECIFIC UPTAKE

A. B. Katasonov, V. Yu. Kolosova, and O. S. Brusov

UDC 612.822.2.018:577.175.832/.014.46:615.214.32

KEY WORDS: synaptosomes; rat brain; serotonin uptake; nonspecific uptake.

Interest in the study of the mechanisms of active serotonin (5-hydroxytryptamine, 5-HT) reuptake is due primarily to changes in the kinetic characteristics of this process in various diseases [3, 8, 9]. Inhibition of 5-HT transport into the interior of the serotoninergic terminal is regarded as the central mechanism of action of tricyclic (imipramine) and some new heterocyclic (zimelidine) antidepressants. The study of the mechanism of regulation of 5-HT reuptake is also promising in connection with the discovery of endogenous inhibitors of 5-HT reuptake in the rat brain [4] and in human blood plasma [2, 6].

Experimental studies of 5-HT reuptake in different brain regions have been undertaken most frequently on suspensions of synaptosomes. However, the methods used for this purpose have significant differences, which are concerned mainly with assessment of the contribution of nonspecific 5-HT uptake to its total uptake by synaptosomes. Nonspecific 5-HT uptake by synaptosomes is usually determined at a low temperature [10], in the presence of micromolar concentrations of inhibitors of active transport [4].

The aim of this investigation was to study the effect of these conditions on the experimentally determined nonspecific 5-HT uptake, and it showed that these methods give an estimate of the contribution of nonspecific 5-HT uptake to its total transport that is too low. The writers suggest a method based on the sensitivity of active 5-HT transport to Na^+ ions, capable of giving the most accurate assessment of nonspecific 5-HT uptake by synaptosomes, in consequence of which it enables the parameters of serotonin reuptake to be determined for accuracy.

EXPERIMENTAL METHOD

Male Wistar rats weighing 120-160 g were used. A partially purified synaptosomal fraction (P2) was obtained by the method in [11]. To determine the total uptake of ^{3}H-5-HT (14 Ci/mmole, Amersham International, England) aliquots of the synaptosomal suspension were added in a volume of 100 µl to preincubated samples (5 min, 37°C) 0.5 ml in volume, containing different concentrations of ^{3}H-5-HT (from 0.07 to 3 µM) in incubation buffer (buffer A) in the presence of 150 mM NaCl. The composition of buffer A was: 50 mM Tris-HCl, 1.3 µM KH_2PO_4, 2.8 mM $CaCl_2$, 0.6 mM $MgCl_2$, 7.7 mM glucose, and 0.2% EDTA (pH 7.5). The samples were incubated for 30 sec at 37°C with continuous shaking. Nonspecific ^{3}H-5-HT uptake was determined in samples containing different concentrations of this ligand, in incubation buffers of the

Laboratory of Clinical Biochemistry, All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Snezhnevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 10, pp. 433-435, October, 1987. Original article submitted October 1, 1986.

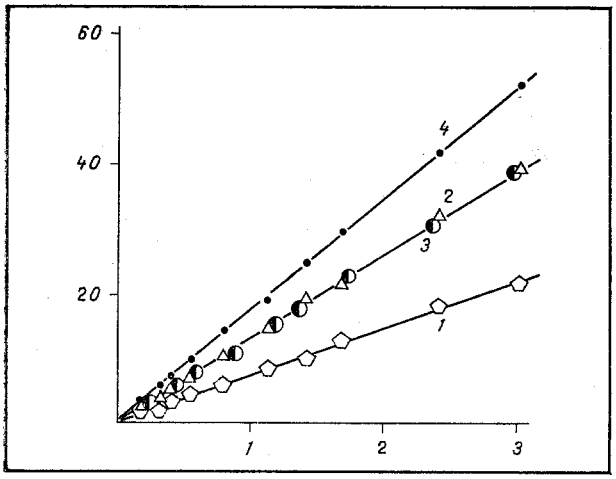

Fig. 1. Dependence of nonspecific ^{3}H-5-HT uptake on its concentration in synaptosomal suspensions when using controls I (1), IIa (2), IIb (3), and III (4). Abscissa, concentration of labeled ligand (in μM); ordinate, quantity of label taken up (in pmoles/mg protein/min). Standard relative error of the means for each point does not exceed 10% (n = 6). Reduced velocity of nonspecific uptake estimated from the angular coefficients of the regression line plotted by the method of least squares. Significance of differences estimated by Student's t test.

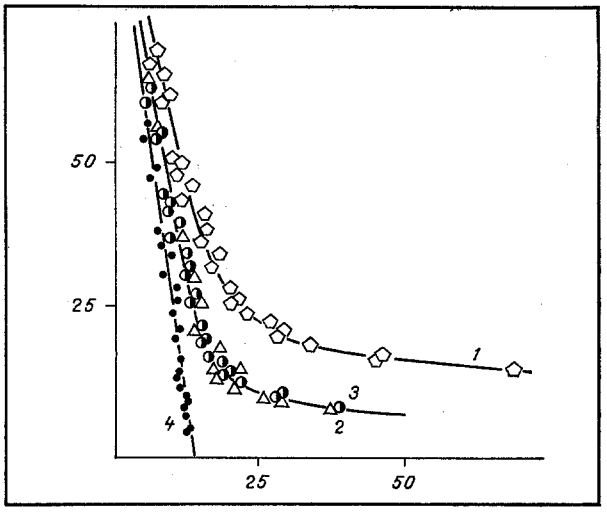

Fig. 2. Analysis of saturation curves of ^{3}H-5-HT reuptake on Scatchard plot, using controls I, IIa, IIb, and III for nonspecific uptake at 0°C I (1), in the presence of 100 μM imipramine, IIa (2) of 100 μM zimelidine, IIb (3), and in sodium-free medium, III (4). Abscissa, initial velocity (V) of ^{3}H-5-HT reuptake (in pmoles/mg protein/min). Ordinate, ratio of V to concentration of free (F) ^{3}H-5-HT (in fmoles/mg protein/min/1 nM). Value of ^{3}H-5-HT reuptake determined as the difference between total and nonspecific uptake. Values of V_{max} and K_m of ^{3}H-5-HT reuptake during the use of control III (4) are equal to 16.8 ± 0.7 pmoles/mg protein/min and 174 ± 24 nM respectively.

following compositions: a) control I — buffer A, 150 mM NaCl [10]; b) control IIa — buffer A, 150 mM NaCl, and 100 μM imipramine (IMI); c) control IIb — buffer A, 150 mM NaCl, and 100 μM zimelidine (ZIM); d) control III — buffer A, 150 mM LiCl [10]. To determine nonspecific uptake, the control samples were incubated at 0°C (control I) or at 37°C (controls IIa, IIb, III) for 30 sec with continuous shaking. After incubation the experimental and control samples were treated with 4 ml of cold (0°C) buffer A, containing 150 mM NaCl (pH 7.5), after which the ^{3}H-5-HT taken up was separated from the free by filtration through filters of the GF/F type (Whatman, England). The filters were washed with cold buffer A (4 times, 4 ml each time), spread out in the flasks, covered with 7 ml of Bray's fluid, and counted on an LS 1801 liquid scintillation counter (Beckman, Austria). Protein was determined by Lowry's method [6].

EXPERIMENTAL RESULTS

The rate of ^{3}H-5-HT uptake in all the controls was a linear function of its concentration up to 3 μM (Fig. 1), evidence of the nonspecific character of uptake of this neurotransmitter under the conditions used. Values of reduced velocities of nonspecific ^{3}H-5-HT uptake, determined in controls I, IIa, IIb, and III, differed significantly ($p < 0.01$) and were 7.5 ± 0.9, 13.0 ± 1.5, 12.9 ± 1.6, and 17.5 ± 2.0 fmoles/mg/min, respectively. The difference between the values obtained for ^{3}H-5-HT uptake under control conditions (controls IIa, IIb, III at 37°C) and the corresponding values observed during incubation of the samples at 0°C (control I) can be attributed to the existence of a number of temperature-dependent processes taking place at 37°C, and therefore not taken into consideration during the use of the "cold" control I: extraneuronal 5-HT uptake, its transport into other subcellular organelles, and passive diffusion of 5-HT into the synaptosomes [4]. Differences in the values of nonspecific ^{3}H-5-HT uptake in controls IIa and IIb, on the one hand, and control III, on the other hand, can probably be explained on the grounds that IMI, ZIM, and the other antidepressants in concentrations as low as 10 μM modify the surface charge on the membranes of the liposomes and their membrane potential significantly and approximately equally [1]. Since IMI, ZIM, and 5-HT at pH 7.5 carry a charge of the same sign, in the presence of micromolar concentrations of the antidepressants used, a decrease in nonspecific 5-HT uptake by membranes ought to be observed due to the effect of electrostatic repulsion [4]. Thus in the control tests I, IIa, and IIb, the values of passive diffusion obtained may be too low compared with values in the experimental sample.

The most correct control for nonspecific 5-HT uptake will evidently be that in which only active transport of this ligand is inhibited. Since specific 5-HT uptake can proceed only in the presence of Na^+ [11], it can be tentatively suggested that replacement of Na^+ in the incubation medium by an equimolar amount of the closely related Li ion will enable the true values of nonspecific uptake to be determined. In fact, the parameters of the saturation curve of active reuptake with the use of control III (Fig. 2) are evidence that ^{3}H-5-HT reuptake is effected mainly on an Na^+-sensitive carrier of the same type (V_{max} = 13.8 ± 0.7 pmoles/mg protein/min) with high affinity for 5-HT (K_m = 174 ± 24 nM). In the various experimental studies of the processes of specific 5-HT transport it can thus be confidently shown that a change in ^{3}H-5-HT reuptake reflects the state of the specialized system for transport of this neurotransmitter. Meanwhile, when the methods of control I and, to a lesser degree, of controls IIa and IIb are used, nonspecific uptake makes a significant contribution to the recorded ^{3}H-5-HT reuptake, the magnitude of which is determined by the difference in velocities of reuptake between control III and controls I, IIa, and IIb (Fig. 1). Saturation curves of this type, on Scatchard plots (Fig. 2, controls I, Ia, and IIb) are nonlinear in character, which makes their interpretation more difficult. In particular, the heterogeneity of the ^{3}H-5-HT reuptake sites, discovered in [10], may in the light of the facts described above, be explained by the use of an incorrect control for nonspecific uptake (control I).

It can thus be concluded from the results of this investigation that correct determination of the velocity of specific 5-HT reuptake by synaptosomes can be undertaken by the use of a control for nonspecific uptake, in medium not containing Na^+ (control III).

LITERATURE CITED

1. N. A. Avdulov, Neuropharmacology of Antidepressants [in Russian], Moscow (1984), p. 159.
2. L. Ahtee, M. Briley, R. Raisman, et al., Life Sci., 29, 2323 (1981).
3. M. L. Barbaccia, O. Gandolfi, D.-M. Chuang, et al., Proc. Nat. Acad. Sci. USA, 80, 5134 (1983).
4. J. M. Boeynaems and J. E. Dumont, Outlines of Receptor Theory, Amsterdam (1980).
5. O. S. Brusov, A. M. Fomenko, and A. B. Katasonov, Biol. Psychiatry, 20, 235 (1985).
6. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
7. J. Modai, L. Zemishlany, and Z. Jerushalmy, Neuropsychobiology, 12, 93 (1984).
8. R. Raisman, M. S. Briley, F. Boushami, et al., Psychopharmacology, 77, 332 (1982).
9. E. G. Shaskan and S. H. Snyder, J. Pharmacol. Exp. Ther., 175, 404 (1970).
10. J. Talvencheimo, H. Fishkes, P. J. Nelson, et al., J. Biol. Chem., 258, 6115 (1983).
11. V. P. Whittaker, I. A. Michaelson, and R. J. A. Kirkland, Biochem. J., 90, 293 (1964).