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Comparison of GABA uptake by brain and kidney preparations

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Summary. The uptake of γ-aminobutyric acid (GABA) was measured in rat cerebral cortical synaptosomes and rat kidney brush-border membrane vesicles (BBMV). Three GABA uptake systems (Km = 1.3, 50 and 3246 μM respectively) were present in synaptosomes, but only two uptake systems (Km = 11 and 1203 μM respectively) were detectable in BBMV. The uptake systems in the two types of tissue preparations were similar in that every system was inhibited by *p*-hydroxymercuribenzoate and by the action of neuraminidase, thereby indicating that, irrespective of the tissue, both sulfhydryl and sialyl groups were necessary components of all the GABA uptake systems. In contrast, differences were observed between synaptosomal and BBMV uptake systems with respect to their sensitivity to inhibition by GABA structural analogs. While the synaptosomal GABA uptake systems were strongly inhibited by nipecotic acid, diaminobutyric acid, β-alanine and 4,5,6,7-tetrahydroisoxazolo[4,5-c]pyridin-3-ol (THPO), none of these compounds significantly inhibited the BBMV uptake systems. It is therefore concluded that the GABA uptake systems in brain and kidney tissues are quite different entities. While the role of GABA transport in the inactivation of the neurotransmitter function of GABA is well documented, the role of the kidney GABA uptake system is unclear, and the possibility exists that the latter systems are present in kidney tissues primarily to transport compounds other than GABA. The present study does however highlight a difference in drug susceptibility of the brain and kidney uptake systems, a phenomenon which may have some therapeutic relevance with respect to the use of GABA analogs as anticonvulsant agents.

Keywords: GABA uptake – Brain GABA – Kidney GABA

A recurring problem in drug therapy targetted towards specific neuronal pathways or regions in the brain is that the drug often affects other pathways

or regions in the brain, or indeed, other tissues and organs in the body. A major focus of research directed towards drug-induced enhancement of the γ-aminobutyric acid (GABA) neurotransmitter system has been an assessment of the possibility that inhibition of GABA uptake into cellular structures might prolong or enhance the action of GABA at its receptor sites in the brain. However, GABA uptake by cellular structures has also been reported in other tissues such as the adrenal medulla [1], oviduct [2] and kidney [3], and again the possibility exists that a beneficial effect of the inhibitors at the level of the brain might be accompanied by a detrimental effect at the level of these other organs. Accordingly, the present investigation was initiated to compare the properties of the GABA uptake systems in the brain and the kidney, and to determine whether the brain uptake systems could be inhibited specifically. The subcellular model systems chosen for the study were rat brain synaptosomes and rat kidney brush-border membrane vesicles (BBMV).

Experimental procedures

Animals and reagents

Male Wistar rats weighing 200–250g were used in the experiments. Neuraminidase (acylneuraminyl hydrolase; EC 3.2.1.18) from *Clostridium perfringens* was obtained from the Sigma Chemical Company, St. Louis, MO.

Preparations of synaptosomes and brush-border membrane vesicles

Synaptosomal-enriched fractions were obtained from rat cerebral cortex by differential and Ficoll-sucrose density gradient centrifugations as described by Cotman [4]. BBMV preparations were obtained from rat renal cortex by the method of Goodyer et al. [3]. The protein content of the preparations was measured by the Lowry method as modified by Hartree [5].

Determination of GABA uptake

The uptake of GABA into the subcellular preparations was measured as described previously [6]. In essence, the preparations were incubated at 25°C for 15 min., the ^{3}H-GABA substrate added, and the incubation carried out for a further 10 min. The synaptosomes and BBMV were then recovered on Millipore filters (0.65 μm pore size) and counted in a liquid scintillation counter. Non-specific binding to the filter, determined by performing the standard uptake procedure in the absence of the subcellular preparations, was subtracted from the observed uptake values. Non-specific binding to the synaptosomes and BBMV was negligible compared to the amount of GABA transported into these preparations, as can be seen from the almost complete abolishment of GABA uptake by some of the experimental treatments (Table 2). Uptake of GABA by both types of preparation was linear with time during the 10 min incubation period for the entire range (1μm-1μM) of substrate concentrations.

Compounds to be tested as inhibitors of GABA uptake were added to the incubation medium prior to the pre-incubation period. The effect of a low Na^+ concentration on GABA uptake was measured by replacing sodium chloride in the Krebs Ringer buffer with choline chloride, thereby lowering the Na^+ concentration from 135mM to 16mM. Treatment of synaptosomes and BBMV preparations with neuraminidase was carried out as detailed by

Zaleska and Ericinska [7], whereby the subcellular preparations (4 mg protein/ml) were incubated with neuraminidase (0.02 units/mg of suspended protein) for 20 min at 34°C. The organelles were then washed by successive centrifugations and resuspensions, and finally resuspended in the uptake buffer. Control preparations were subjected to the same procedure, but in the absence of neuraminidase.

Data analysis

Data obtained from the uptake experiments were subjected to Eadie-Hofstee plots. Computer analysis of the data was performed on a Victor 9000 computer with an adaptation of the program LIGAND [8]. This program uses weighted, non-linear, least squares curve fitting and provides a statistical analysis of goodness of fit of two-system versus one-system or three-system uptakes.

Statistical analysis of the data from the inhibition experiments was performed using the Student's *t*-test.

Results and discussion

The Eadie-Hofstee plots for GABA uptake by BBMV and synaptosomal preparations were clearly of different shapes (Fig. 1), and suggested the presence of three uptake systems in synaptosomes, but only of two systems in BBMV. Computer assisted analysis of the data confirmed this situation, in that the data from BBMV could not be fitted by three systems, and that two systems fitted

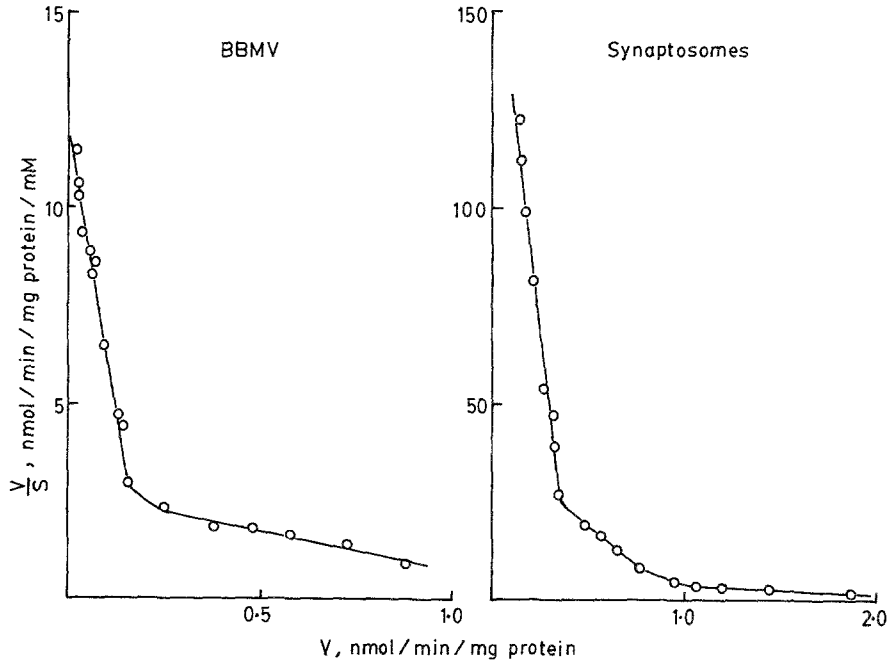

Fig. 1. Eadie-Hofstee plots of GABA uptake by rat kidney brush-border membrane vesicles and rat brain cortical synaptosomes. Each point is the mean of 5 experiments, the standard errors ranging from 3–6% of the mean values. GABA substrate concentrations ranged from 1 μm to 1 mM

Table 1. Kinetic constants for GABA uptake by rat brain cortical synaptosomes and rat kidney brush-border membrane vesicles

Kinetic Constant	Synaptosomes	BBMV
Km_1 (μM)	1.3 ± 0.1	11 ± 1
Km_2 (μM)	50 ± 7	1203 ± 105
Km_3 (μM)	3246 ± 285	
$Vmax_1$ (pmol/min/mg protein)	255 ± 19	111 ± 8
$Vmax_2$ (pmol/min/mg protein)	582 ± 33	1920 ± 142
$Vmax_3$ (pmol/min/mg protein)	4360 ± 310	

Values are the mean ± SEM for 5 experiments. Kinetic constants were obtained by LIGAND analysis. The data are taken from Wood and Sidhu [6, 12].

better than one system ($F_{2,13} = 99.1$; $p < 0.001$). In contrast, the data from synaptosomes fitted a three-system model significantly better than it did a two-system model ($F_{2,11} = 14.7$; $p < 0.001$). However, the difference in GABA uptake by the synaptosome and BBMV preparations was not due simply to the absence of one of the uptake systems in BBMV, since the two systems detected in BBMV preparations possessed Km values which were quite different from those of any of the uptake systems in synaptosomes (Table 1). It was therefore concluded that GABA uptake by BBMV was not identical to that by synaptosomes. The question "how different were the respective transport systems" was addressed in the remainder of the study.

With a knowledge of the kinetic constants in Table 1, it was possible to calculate the contribution of each of the uptake systems to the observed GABA uptake at any particular concentration of GABA. For example, at 1 μM GABA the high affinity uptake system was responsible for 85% and 90% of the total uptake by BBMV and synaptosomes respectively. Similarly, at 1 mM GABA the lower affinity uptake system in BBMV accounted for 89% of the total uptake, while the medium affinity and low affinity uptake systems in synaptosomes accounted for 30% and 56% of the total uptake by these organelles. Accordingly, if a treatment strongly inhibited (by more than 70%) the observed GABA uptake at either of these substrate concentrations, it would indicate that the major contributing system(s) at that particular substrate concentration was affected by the treatment. Based on such reasoning, it would appear that all three synaptosomal and both BBMV uptake system were sodium-dependent, since negligible GABA uptake activity occurred at either 1 μM or 1mM GABA concentrations when the Na^+ concentration was low, or when the sodium pump (Na^+K^+-ATPase) was inhibited by ouabain (Table 2). Likewise, all five GABA uptake systems were inhibited by *p*-hydroxymercuribenzoate (PHMB) and neuraminidase, thereby demonstrating the essential role of sulfhydryl and sialyl groups for activity of the uptake systems. In contrast to these similarities between the synaptosomal and BBMV uptake systems, the active sites of these trans-

Table 2. Effects of various *in vitro* treatments on GABA uptake by rat brain cortical synaptosomes and rat kidney brush-border membrane vesicles

	GABA Uptake (pmol/min/mg protein)				% Inhibition			
	Synaptosomes		BBMV		Synaptosomes		BBMV	
Treatment	1 μM GABA	1 mM GABA	1 μM GABA	1 mM GABA	1 μM GABA	1 mM GABA	1 μM GABA	1 mM GABA
Control	137 ± 5 (15)	1958 ± 73 (20)	12.1 ± 0.6 (15)	938 ± 44 (15)				
RS-Nipecotic Acid	9 ± 3 (6)	286 ± 87 (6)*	10.4 ± 0.6 (5)	788 ± 30 (5)	93 ± 2	85 ± 4	12 ± 4	10 ± 4
DABA	15 ± 3 (5)*	72 ± 5 (3)*	11.0 ± 0.8 (5)	864 ± 119 (5)	89 ± 3	97 ± 2	4 ± 7	6 ± 10
β-Alanine	11 ± 3 (5)*	490 ± 67 (5)*	11.4 ± 0.7 (5)	839 ± 73 (5)	92 ± 2	71 ± 5	3 ± 4	6 ± 10
THPO	4 ± 1 (5)*	40 ± 20 (5)*	12.0 ± 0.9 (5)	844 ± 104 (5)	97 ± 1	98 ± 1	2 ± 6	7 ± 11
PHMB	11 ± 2 (5)*	309 ± 63 (5)*	1.2 ± 0.1 (5)*	147 ± 50 (5)*	91 ± 2	85 ± 3	90 ± 2	86 ± 5
Ouabain	3 ± 1 (5)*	30 ± 7 (5)*	0.5 ± 0.1 (4)*	23 ± 16 (5)*	98 ± 1	97 ± 1	95 ± 2	98 ± 2
Low Na^+	1 ± 0.1 (5)*	295 ± 42 (5)*	0.3 ± 0.1 (4)*	87 ± 25 (4)*	99 ± 1	87 ± 4	97 ± 1	92 ± 3
Control	96 ± 12 (5)	1288 ± 167 (5)	8.4 ± 0.1 (5)	643 ± 127 (5)				
Neuraminidase	14 ± 4 (5)*	134 ± 50 (5)*	0.5 ± 0.1 (5)*	105 ± 31 (5)*	86 ± 2	93 ± 3	92 ± 2	81 ± 6

Values are mean ± SEM for the number of samples in parenthesis. Concentration of the compounds in the incubation medium was 1 mM except for PHMB which was 0.5 mM. Composition of the low Na^+ medium and the treatment with neuraminidase were as described under Experimental procedures. The values for the % inhibition were based on comparisons with the appropriate paired control samples. Data for BBMV are taken from Wood and Sidhu (12).

* indicates significantly different from control values ($p < 0.01$).

port systems differed greatly in their specificities. The GABA analogs 2,4-diaminobutyric acid (DABA), β-alanine, nipecotic acid and 4,5,6,7-tetrahydroisoxazolo[4,5-c] pyridine-3-ol (THPO) are known competitive inhibitors of brain GABA uptake [9–11], and the present study indicated that the four compounds were potent inhibitors of all three GABA uptake systems in synaptosomes. On the other hand, none of these GABA analogs had any significant effect on the GABA uptake activity in BBMV (Table 2).

In conclusion, although the GABA uptake systems in synaptosomes share some properties with those in BBMV preparations, the transport systems in brain tissue and kidney tissue clearly differ, particularly with respect to the active sites. Thus, it should be possible to develop inhibitors of GABA uptake which could enhance the functioning of the GABA neurotransmitter system in brain without causing concomitant, possibly deleterious effects in the kidney.

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