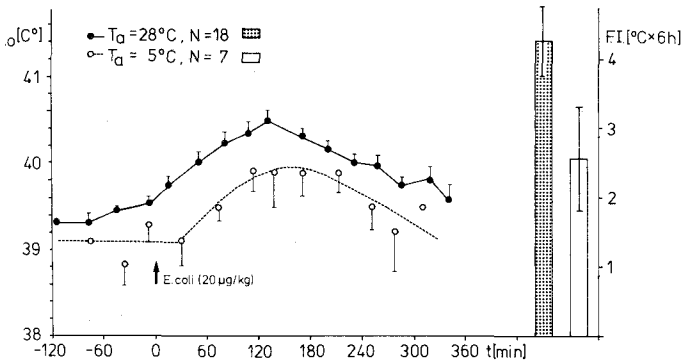

Figure 3. Comparison of fever responses to bacterial pyrogen (*E. coli*, 20 µg/kg i.m.) in groups of warm-adapted (black symbols, shaded column) and cold-adapted (white symbols and column) guinea pigs. The fever indices of cold- and warm-adapted animals differed significantly ($p < 0.05$).

that there is a causal relationship between stimulation of ascending vasopressinergic projections to the septum and fever reduction after pyrogen application during cold-adaptation of guinea pigs. The biological significance of enhanced AVP release in different stressful situations and the related antipyretic reaction has not been fully elucidated yet. The interactions between centrally released neuropeptides and autonomic functions merit further endocrinological, physiological and pharmacological studies.

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Gamma-aminobutyric acid uptake by rat kidney brush-border membrane vesicles

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Summary. Brush-border membrane vesicles (BBMV) from rat kidney cortex possessed two uptake systems for γ-aminobutyric acid (GABA), a high affinity system (Km = 10.9 µM) and a low affinity system (Km = 1203 µM). Both uptake systems were inhibited by p-hydroxymercuribenzoic acid and ouabain, and by the action of neuraminidase, whereas the GABA analogs nipecotic acid, β-alanine, 2,4-diaminobutyric acid and 4,5,6,7-tetrahydroisoxazolo-[4,5c]-pyridin-3-ol had no effect on the GABA uptake activity. The BBMV uptake systems were clearly different from the GABA transport systems present in brain tissue.
Key words. GABA; transport; kidney; brush-border membrane vesicles.

The existence of two γ-aminobutyric acid (GABA) uptake systems in brain tissue had been recognized for many years[1], but more recently, using a wide range of substrate concentrations and computer-assisted analysis, the authors demonstrated the presence of three GABA uptake systems in synaptosomes and brain slices[2,3]. Since the presence of a high affinity GABA transport system in brush-border membrane vesicles (BBMV) from rat kidney cortex had been reported[4], the current study was initiated, again with a wide range of substrate concentrations and computer-assisted analysis, to determine whether multiple uptake systems for GABA existed in kidney tissue. The identification of two uptake systems and a comparison of their properties with those of the brain tissue transport systems are described below.

Methods

Renal cortex was obtained from male Wistar rats (200–250 g b. wt), and brush-border membrane vesicles prepared from the tissue by the method of Goodyer et al.[4]. GABA uptake was measured as described previously[3]. In essence, the vesicle preparation was preincubated at 25 °C for 15 min, ^{3}H-GABA was added, and a further 10-min incubation was carried out. The vesicles were then recovered by Millipore filtration and counted by liquid scintillation spectrometry. Non-specific binding to

the filter, determined by carrying out the standard uptake procedure in the absence of vesicles, was subtracted from the observed uptake values. Non-specific binding to the vesicles was negligible compared to the amount of GABA transported into the vesicles, as can be seen from the almost complete abolishment of GABA uptake by some of the experimental treatments (table 2). Uptake of GABA by the vesicles was linear with time during the 10-min incubation for the entire range of substrate concentrations. Compounds tested as inhibitors of GABA uptake were added to the medium prior to the preincubation. The effect of low sodium ion concentration on GABA uptake was evaluated by the substitution of choline chloride for sodium chloride in the standard Krebs Ringer buffer used in the study, thereby lowering the sodium level in the buffer from 135 mM to 16 mM. Treatment of BBMV with neuraminidase (acylneuraminyl hydrolase; EC 3.2.1.18; from Sigma Chemical Co.) was carried out as described by Zaleska and Ericinska [5] whereby BBMV (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 appropriate buffer for the uptake assay. Control BBMV were subjected to the same procedures, but in the absence of neuraminidase. The protein content of BBMV was measured by the method of Lowry et al. [6] as modified by Hartree [7].

Data obtained from the uptake experiments were subjected to Eadie-Hofstee plots. Computer-assisted analysis of the plots 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 uptake versus one-system or three-system uptakes. Statistical analysis of the data from the inhibition experiments was performed using the Student t-test.

Results.

A plot of GABA uptake by BBMV against the concentration of GABA in the incubation medium revealed a discontinuity in the curve (around 50 µM GABA) which suggested the presence of more than one uptake system (fig. 1). The existence of multiple uptake systems in BBMV preparations was confirmed when the data were subjected to an Eadie-Hofstee plot (fig. 2) and computer analysis. Although the data could not be fitted to three uptake systems, the fit of two uptake systems was significantly better than that of only one system ($F_{2,13} = 99.1$; $p < 0.001$). Calculation of the kinetic constants for the uptake systems (table 1) revealed that one system was of high affinity and low capacity, whereas the second system was of low affinity and high capacity.

The presence of 1 mM RS-nipecotic acid in the incubation medium had little effect on the GABA uptake activity (fig. 2), the kinetic constants not being significantly different from those of control preparations (table 1).

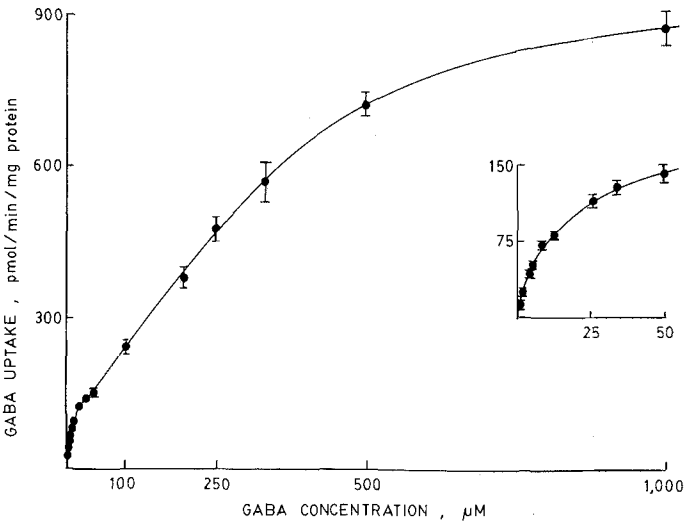

Figure 1. GABA uptake by rat kidney BBMV as a function of the GABA concentration in the incubation medium. Each point and bars represent the mean ± SEM for five experiments. The inset represents an enlargement of the plot in the 0-50 µM GABA range.

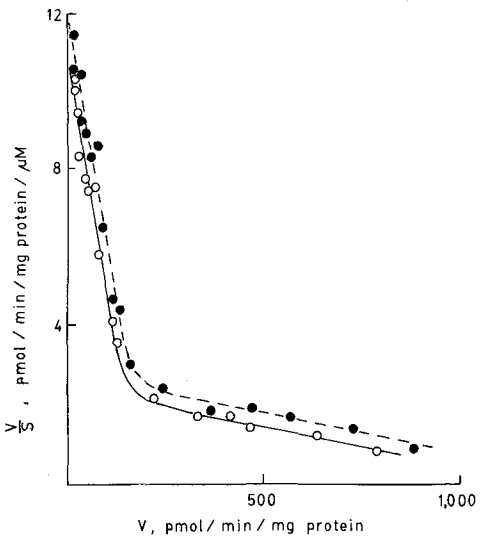

Figure 2. Eadie-Hofstee plots of GABA uptake by rat kidney BBMV. ●---●, Control; ○—○, in the presence of 1 mM nipecotic acid. Each point is the mean of 5 experiments. The standard error for each of the points ranged from 3 to 6% of the mean values. GABA substrate concentrations ranged from 1 µM to 1 mM.

Table 1. Kinetic constants for GABA uptake by rat kidney BBMV

Constant	Control	Nipecotic acid
Km_1 (µM)	10.9 ± 1.0	10.2 ± 0.6
Km_2 (µM)	1203 ± 105	1287 ± 286
$Vmax_1$ (pmol/min/mg protein)	111 ± 8	95 ± 7
$Vmax_2$ (pmol/min/mg protein)	1920 ± 142	1744 ± 213

Values are the mean ± SEM for 5 experiments. Data were obtained by LIGAND analysis of Eadie-Hofstee plots. Nipecotic acid was present in the incubation medium at a concentration of 1 mM.

Moreover, no uptake of ^{3}H-nipecotic acid by BBMV was detected at substrate concentrations of 1 µM, 100 µM and 1000 µM (results not shown). With a knowledge of the values for the control kinetic constants, it was possi-

Table 2. Effect of various compounds on GABA uptake by rat kidney BBMV

Treatment	GABA uptake (pmol/min/mg protein) 1 μM GABA	1 mM GABA
Control	11.7 ± 0.7	917 ± 92
PHMB (0.5 mM)	1.2 ± 0.1*	147 ± 50*
DABA (1.0 mM)	11.0 ± 0.8	864 ± 119
β-Alanine (1.0 mM)	11.4 ± 0.7	839 ± 73
THPO (1.0 mM)	12.0 ± 0.9	844 ± 104
Ouabain (1.0 mM)	0.5 ± 0.1*	23 ± 16*
Low Na^+	0.3 ± 0.1*	87 ± 25*
Control	8.4 ± 1.0	643 ± 127
Neuraminidase	0.5 ± 0.1*	105 ± 31*

Values are the mean ± SEM for 4 experiments (ouabain and low Na^+) and for 5 experiments (other groups). The vesicles were preincubated with the chemical agents for 15 min prior to the measurement of uptake activity. Treatment with neuraminidase was carried out prior to the uptake assay as described in the methods section. The control group for the neuraminidase study was subjected to the same pretreatment step as done with neuraminidase, but without the enzyme. * indicates difference from controls was significant $p < 0.01$.

ble to calculate the contribution of each uptake system to the observed overall uptake at any given substrate concentration. For example, at 1 μM GABA the high affinity system was responsible for 85% of the total activity, while at 1 mM GABA the low affinity system accounted for 89% of the total uptake. Thus, a study of the effect of inhibitors on uptake activity at these two GABA concentrations would provide information on the effects of the compounds on the high and low affinity uptake systems respectively. Such a protocol was used for the 'inhibitory' experiments described hereafter.

The data in table 2 clearly indicate that the sulfhydryl agent p-hydroxymercuribenzoic acid (PHMB) strongly inhibited both the high affinity and low affinity GABA uptake systems, as did ouabain, an inhibitor of ($Na^+ + K^+$)-ATPase activity. In contrast, the GABA analogs 2,4-diaminobutyric acid (DABA), β-alanine and 4,5,6,7-tetrahydroisoxazolo[4,5-c]-pyridin-3-ol (THPO) did not significantly affect GABA uptake by either system. Pretreatment of BBMV with neuraminidase caused a significant inhibition of both uptake systems, while a reduction in the sodium ion concentration in the incubation medium caused a drastic loss of activity in both high and low affinity systems (table 2).

Discussion. GABA transport systems in rat kidney brush-border membrane vesicles were clearly different from those of brain tissue. Whereas three uptake systems were present in synaptosomes from most brain regions (3) only two systems were detected in BBMV (table 1). Moreover, although the Km value of the BBMV low affinity system fell within the range of values obtained for synaptosomes from different brain regions (Km = 1100–4400 μM), the Km value of the BBMV high affinity uptake system corresponded neither to values for the high affinity system (Km = 0.9–1.7 μM) nor to values for the medium affinity system (Km = 31–58 μM) in synaptosomes. The effects of GABA analogs on GABA uptake in preparations from brain and kidney were also totally different. Whereas nipecotic acid, β-alanine, DABA and THPO were potent inhibitors of GABA uptake by all three systems in synaptosomes (manuscript submitted), none of the compounds significantly inhibited either of the BBMV uptake systems (table 2). On the other hand, the various GABA transporters in both synaptosomes and BBMV all required sulfhydryl and sialyl groupings for activity, as witnessed by the inhibitory effects of PHMP and neuraminidase. As was the case for all three GABA uptake systems in synaptosomes, the driving force for both BBMV GABA uptake systems appeared to be the sodium ion gradient, since reduction in the gradient or the inhibition of ($Na^+ + K^+$)-ATPase activity led to a large decrease in the uptake activities.

Although the Km value for BBMV GABA uptake (30–36 μM) reported previously by Goodyer et al.[4] appeared at first sight to be at variance with the present results (table 1), the difference was more apparent than real. If analysis of the data in figure 1 was limited to those points representing a substrate concentration range similar to that employed by the previous workers (10–100 μM GABA), only one uptake system was detected and its Km value was 36 μM, a value identical to that found by Goodyer et al. The lack of effect of DABA and nipecotic acid on GABA uptake observed here (table 2) corroborates the previous finding of Goodyer et al.[4], but whereas the latter researchers reported a significant inhibition by β-alanine, no such phenomenon was observed in the present study. The reason for this discrepancy was not known.

In conclusion, it can be stated categorically that the GABA uptake systems in brain tissue are different from those in kidney tissue. While the function of GABA uptake in brain is to terminate the neurotransmitter action of GABA in the synaptic cleft, the function of the uptake systems in kidney is less apparent. Indeed, the question arises as to whether the prime purpose of the kidney system is to transport GABA per se, or whether the purpose is to transport some other substrate, with the observed GABA uptake being a creation of the experimental protocol.

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