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The γ-aminobutyric acid transporter and its interaction with taurine in the apical membrane of the bovine retinal pigment epithelium

Subramanyan SIVAKAMI, Vadivel GANAPATHY, Frederick H. LEIBACH and Yusei MIYAMOTO*
Department of Biochemistry and Molecular Biology, Medical College of Georgia, Augusta, GA 30912–2100, U.S.A.

The characteristics of γ-aminobutyric acid (GABA) uptake were investigated in apical membrane vesicles prepared from the bovine retinal pigment epithelium. An inwardly directed NaCl gradient stimulated GABA uptake markedly, and the time course of uptake exhibited an overshoot phenomenon indicating the presence of an active transport mechanism for GABA in these membranes. Other monovalent cations were not capable of substituting for Na^+. In addition to this obligatory requirement for Na^+, the GABA uptake also exhibited a Cl^--dependence, evident from the observations that the uptake was negligible in the presence of NaF or sodium gluconate in place of NaCl. NO_3^- and SCN^- could substitute for Cl^- to some extent. The uptake process was electrogenic, with a Na^+/Cl^-/GABA stoichiometry of 2:1:1 or 3:1:1. Substrate-specificity studies showed that the β-amino acids such as taurine, hypotaurine and β-alanine interacted with the GABA uptake process. Uptake of GABA could be completely inhibited by an excess of taurine and, similarly, uptake of taurine could be completely inhibited by an excess of GABA, suggesting that common transport processes operate in the uptake of these two compounds. However, a number of compounds which are specific inhibitors of GABA uptake inhibited taurine uptake only to a maximum of 50%. Kinetic analysis of GABA uptake in the concentration range 0.1–10 μM revealed that the uptake occurred via a single system and that taurine was a competitive inhibitor of this system. The Michaelis–Menten constant (K_t) for GABA was 0.94 μM and the apparent inhibition constant (K_i) for taurine was 230 μM. On the contrary, even though the kinetic analysis of taurine uptake in the concentration range 25–150 μM revealed participation of a single system in the uptake process, the inhibition of taurine uptake by GABA was not competitive. The presence of GABA decreased the maximal velocity of the taurine uptake process and also decreased the K_t for taurine. Based on these data, it is proposed that: (i) there are two distinct transport systems, namely the GABA transporter and the taurine transporter, in these membranes which accept both GABA and taurine as substrates, (ii) the affinities of these systems for taurine are very similar and cannot be kinetically distinguished under the experimental conditions employed, and (iii) the difference between the affinities of these systems for GABA is much greater than for taurine.

INTRODUCTION

Recently we reported on the characteristics of taurine uptake in apical membrane vesicles prepared from the retinal pigment epithelium of bovine eyes (Miyamoto *et al.*, 1991). Taurine uptake in these membrane vesicles occurs via a NaCl-dependent transport system. Taurine transport in the retinal pigment epithelium has been studied by other investigators using either the isolated tissue or cultured cells (Edwards, 1977; Ostwald & Steinberg, 1981). Ours was the first report on taurine uptake in apical membrane vesicle preparations from this tissue. The taurine transport system in this membrane exhibits many characteristics that are common to the β-amino acid transport system known to be present in the brush-border membranes from the kidney, the small intestine and the placenta (Barnard *et al.*, 1988; Miyamoto *et al.*, 1988, 1989; Wolff & Kinne, 1988; Kulanthaivel *et al.*, 1989; Zelikovic *et al.*, 1989; Karl & Fisher, 1990). One major difference, however, is in the interaction of the system with γ-aminobutyric acid (GABA). In the brush-border membranes of the kidney and the placenta, the system responsible for taurine uptake has much less affinity for GABA than for taurine. In contrast, in the apical membrane of the retinal pigment epithelium, the system responsible for taurine uptake has much greater affinity for GABA than for taurine. Based on these data, we concluded that the uptake of taurine in the apical membrane vesicles of the retinal pigment epithelium investigated in our previous study (Miyamoto *et al.*, 1991) actually occurred via the GABA transporter rather than the taurine transporter. To our knowledge, there is no information available on the transport of GABA in this tissue. The GABA transport process has been characterized in great detail in the brain, using synaptosomal membrane vesicles (Kanner, 1978; Pastuszko *et al.*, 1982; Radian & Kanner, 1983; Kanner & Bendahan, 1990). Two transport systems, type A and type B, catalyse the uptake of GABA in the brain. Many characteristics of these two transport systems are similar to those of the β-amino acid transport system. These characteristics include the dependence on Na^+ as well as Cl^-, electrogenicity and the Na^+/Cl^-/substrate stoichiometry. These two systems can, however, be distinguished on the basis of their substrate and inhibitor specificities. The type A transporter, present predominantly in neuronal cells of the brain, has no or little affinity for β-amino acids and is specifically sensitive to inhibition by *cis*-3-aminocyclohexanecarboxylic acid. On the other hand, the type B transporter, which is present primarily in glial cells, has an appreciable affinity for β-amino acids and is specifically sensitive to inhibition by 4,5,6,7-tetrahydroisoxazolo-[5,4-*c*]pyridin-3-ol (THPO).

As our previous study (Miyamoto *et al.*, 1991) on the inhibition of taurine uptake by GABA indicated that the apical membrane of the bovine retinal pigment epithelium possesses a GABA transport mechanism, the purpose of the present investigation was to confirm this finding by using GABA as the transport

Abbreviations used: GABA, γ-aminobutyric acid; THPO, 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol; FCCP, carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone; DABA, (*S*)-(+)-2,4-diaminobutyric acid; HNA, (±)-*cis*-4-hydroxynipecotic acid; AIB, aminoisobutyric acid.

* To whom correspondence should be addressed.

substrate and to delineate the characteristics of this transport process, with emphasis on the interaction of the system with taurine.

EXPERIMENTAL

Materials

γ-[2,3-^{3}H(n)]aminobutyric acid (specific radioactivity 100 Ci/mmol) and [2-^{3}H(n)]taurine (specific radioactivity 25.6 Ci/mmol) were purchased from DuPont–New England Nuclear, Boston, MA, U.S.A. Valinomycin and unlabelled amino acids were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone (FCCP) and (*S*)-(+)-2,4-diaminobutyric acid dihydrochloride (DABA) were from Aldrich Chemical Co., Milwaukee, WI, U.S.A. *RS*-(±)-Nipecotic acid, guvacine hydrochloride, THPO and (±)-*cis*-4-hydroxynipecotic acid hydrochloride (HNA) were from Research Biochemicals, Natick, MA, U.S.A. All other chemicals were of analytical grade.

Preparation of apical membrane vesicles

The procedure for the preparation of apical membrane vesicles from the bovine retinal pigment epithelium has been reported previously from our laboratory (Miyamoto *et al.*, 1991). About 40–50 bovine eyes, which were obtained from a local slaughterhouse, were used for each preparation. The eyes were kept in ice-cold Krebs–Ringer solution for 30 min in the dark to facilitate the detachment of the neutral retina from the underlying pigment epithelial layer. All subsequent steps were also carried out at 4 °C. The eyes were cut in half and the pigment epithelium was collected after the removal of the vitreous humour and the neural retina. A 5% (w/v) homogenate of the pigment epithelial tissue was made in 2.4 mM-Tris/NaOH buffer (pH 7.1) containing 60 mM-mannitol and 1 mM-EGTA using a Waring blender for 1.5 min at high speed. A stock solution of 1 M-$MgCl_2$ was added to the homogenate to give a final concentration of 30 mM-$MgCl_2$. After stirring for 1 min, the mixture was kept for 15 min. It was then centrifuged at 2500 *g* for 15 min. The supernatant, collected by filtration though 12 layers of cheesecloth, was subjected to the same centrifugation and filtration steps one more time. The resulting supernatant was centrifuged at 46000 *g* for 45 min. The pellets containing the apical membranes were suspended in a desired preloading buffer using a 1 ml syringe and 25 gauge needle. The suspension was again centrifuged at 46000 *g* for 45 min. The resulting pellets were suspended in a small volume of the preloading buffer. The exact composition of the preloading buffer varied from experiment to experiment, and is given to the respective Table or Figure legend. The protein concentration of the membrane suspension was adjusted to 5 mg/ml. The final suspension was then stored in 1 ml aliquots in liquid nitrogen until use. The purity of the apical membrane preparations was routinely determined by measuring the activity of Na^+–K^+-ATPase. This enzyme is the most reliable marker for the apical membrane of the retinal pigment epithelium (Ostwald & Steinberg, 1980; Caldwell & McLanghlin, 1984). The activity of this marker enzyme was enriched about 10–12-fold in our apical membrane preparations compared with the homogenate.

Uptake measurements

A rapid filtration technique, as previously described (Miyamoto *et al.*, 1986, 1991), was used to measure the uptake of taurine and GABA. Millipore filters (DAWP type, 0.65 μm pore size) were used for this purpose. The composition of the uptake buffer varied depending upon the individual experiment and is given in the respective Table or Figure legend. In all experiments except the one dealing with the Cl^- kinetics, the stop buffer was 5 mM-Hepes/Tris, pH 7.5, containing 155 mM-KCl. In the Cl^- kinetics experiment, the stop buffer consisted of 5 mM-Hepes/Tris, pH 7.5, and 255 mM-KCl to adjust for the osmolality of the membrane preloading buffer and of the uptake buffer employed in this particular experiment. The radioactivity associated with the filter was determined by liquid scintillation spectrometry.

Statistics

Uptake measurements were usually made in duplicate or triplicate, and each experiment was repeated with two or three different membrane preparations. The results are given as means ± S.E.M. Kinetic analyses were done using a commercially available computer package called Statgraphics (STSC, Rockville, MD, U.S.A.). Stastical significance was determined using Student's *t* test, and $P < 0.05$ was considered significant.

RESULTS

Na^+-dependence of GABA uptake

The time course of GABA uptake was studied with the apical membrane vesicles isolated from the bovine retinal pigment epithelium. The membrane vesicles were preloaded with 300 mM-mannitol and 10 mM-Hepes/Tris, pH 7.5. An inwardly directed Na^+ gradient was generated by the presence of Na^+ in the uptake buffer. The composition of the uptake buffer was 150 mM-NaCl

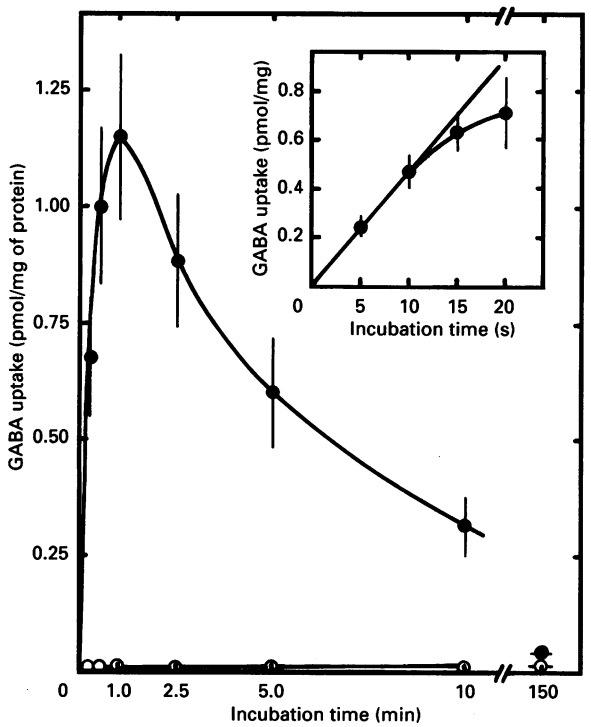

Fig. 1. Time course of GABA uptake in the presence and the absence of an inwardly directed NaCl gradient

Apical membrane vesicles prepared from the bovine retinal pigment epithelium were suspended in 300 mM-mannitol buffered with 10 mM-Hepes/Tris, pH 7.5. The composition of the uptake buffer was 10 mM-Hepes/Tris, pH 7.5, containing either 150 mM-NaCl (●) or 150 mM-choline chloride (○). The concentration of radiolabelled GABA was 4 nM. The inset shows the Na^+-dependent uptake of GABA in short-time incubations. The Na^+-dependent uptake represents the uptake in the presence of NaCl minus the uptake in the presence of choline chloride. The results are given as means, with bars representing S.E.M., for four determinations. When not shown, the error bars lie within the symbol.

Table 1. Effects of inorganic monovalent cations and anions on GABA uptake

Apical membrane vesicles were preloaded with 300 mM-mannitol and 10 mM-Hepes/Tris, pH 7.5. The uptake buffer consisted of 10 mM-Hepes/Tris, pH 7.5, containing 150 mM of the respective inorganic salt. GABA uptake (4 nM) was measured with a 8 s incubation. The results are given as means ± S.E.M. for four determinations.

Inorganic salt	GABA uptake (pmol/8 s per mg of protein)	GABA uptake (% of control)
NaCl	0.392 ± 0.021	100
LiCl	0.002 ± 0.001	1
KCl	0.002 ± 0.001	1
RbCl	0.002 ± 0.001	1
CsCl	0.001 ± 0.001	0
NaF	0.013 ± 0.001	3
Sodium gluconate	0.003 ± 0.001	1
NaSCN	0.141 ± 0.023	36
$NaNO_3$	0.227 ± 0.008	58

and 10 mM-Hepes/Tris, pH 7.5. To study the time course of GABA uptake in the absence of Na^+, NaCl in the uptake buffer was replaced by choline chloride. As shown in Fig. 1, the inwardly directed Na^+ gradient stimulated GABA uptake markedly, and the accumulation of GABA into the vesicles measured at 1 min was approx. 400-fold greater in the presence of Na^+ than in its absence. The time course of GABA uptake exhibited an overshoot (uptake at 1 min being about 30-fold greater than the uptake at 150 min). These data indicate that GABA is transported by a Na^+-dependent active process in the apical membrane of the retinal pigment epithelium. To evaluate the initial uptake rates of GABA, the Na^+-dependent uptake of GABA, representing the uptake in the presence of Na^+ minus the uptake in the absence of Na^+, was measured in short-time incubations. As shown in the inset of Fig. 1, the Na^+-dependent uptake of GABA was linear at least up to 10 s, showing that GABA uptake in the first 10 s represents the initial uptake rate of GABA in these membrane vesicles. Accordingly, a 8 s incubation was used in the following experiments.

Effects of inorganic monovalent cations and anions on GABA uptake

Since the presence of Na^+ in the uptake buffer stimulated GABA uptake to a marked extent, we investigated whether any other inorganic monovalent cation in place of Na^+ could have a similar effect. This was done by comparing the initial rates of GABA uptake measured in the presence of chloride salts of different cations in the uptake buffer (Table 1). The uptake rate was found to be the highest in the presence of NaCl. Substitution of Na^+ with Li^+, K^+, Rb^+ or Cs^+ decreased the GABA uptake rate drastically. Thus the GABA transport system in the apical membranes of the retinal pigment epithelium exhibits an obligatory requirement for Na^+. We also investigated the role of anions in the Na^+-dependent uptake of GABA. This was done by comparing the initial uptake rates measured in the presence of Na^+ salts of different anions in the uptake buffer (Table 1). The uptake rate was negligible in the presence of NaF or sodium gluconate, showing that the presence of Na^+ alone is not enough to support the catalytic activity of the GABA transport system. The co-presence of Cl^- with Na^+ is mandatory for the maximal activity of the system. NO_3^- and SCN^- were found to be able to replace Cl^- to a large extent.

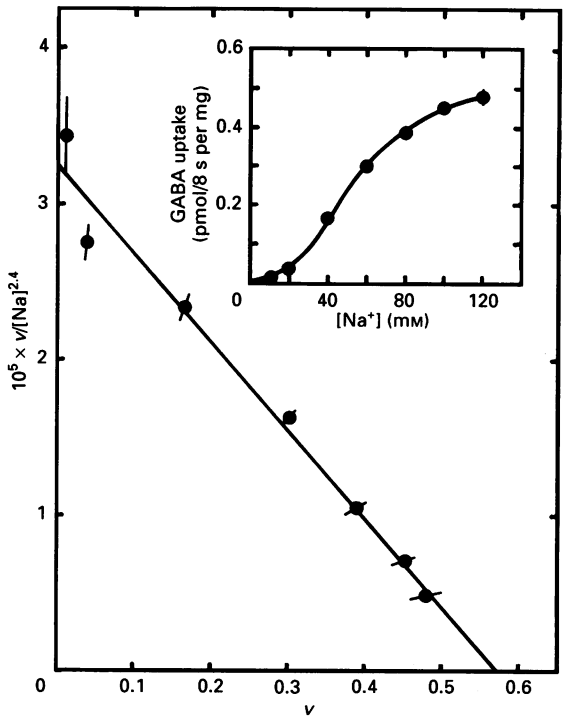

Fig. 2. Na^+ kinetics of GABA uptake

Apical membrane vesicles were preloaded with 300 mM-mannitol buffered with 10 mM-Hepes/Tris, pH 7.5. The uptake buffer consisted of 10 mM-Hepes/Tris, pH 7.5, containing various concentrations at Na^+ (0–150 mM). The concentration of Na^+ was varied by substituting LiCl for NaCl, and the concentration of Cl^- was kept constant at 150 mM. GABA uptake was determined for 8 s and the concentration of GABA was 5 nM. The inset shows the dependence of GABA uptake on the Na^+ concentration, and the data represent Na^+-dependent uptake of GABA. The data in the inset were analysed by a Hill-type equation and a straight line was obtained with a correlation coefficient of −0.99 for the plot of v versus $v/[Na^+]^n$, when n (the Hill coefficient) was assigned a value of 2.4. v is the uptake rate in pmol/8 s per mg of protein. The results are given as means, with bars representing S.E.M., for four determinations. When not shown, the error bars lie within the symbol.

Electrogenicity of the GABA uptake process

In order to determine the electrogenic nature of the GABA transport process, the effects of an inside-negative membrane potential on the uptake were studied. An inside-negative membrane potential was generated by enhancing the in-to-out diffusion of K^+ or H^+ with the appropriate ionophore under suitable experimental conditions. In the K^+-diffusion potential experiment, the membrane vesicles were preloaded with 300 mM-mannitol and 100 mM-potassium gluconate buffered with 10 mM-Hepes/Tris, pH 7.5. The composition of the uptake buffer was 10 mM-Hepes/Tris, pH 7.5, containing 150 mM-NaCl, 5 μM-valinomycin and either 100 mM-potassium gluconate (no membrane potential) or 200 mM-mannitol (inside-negative membrane potential). The inside-negative membrane potential significantly increased GABA uptake from 0.017 ± 0.001 to 0.038 ± 0.003 pmol/8 s per mg of protein ($P < 0.001$). However, the activity of GABA uptake was approx. 20 times less under the no-membrane-potential conditions of this experiment than under the regular experimental conditions, indicating that the presence of valinomycin and/or K^+ may have deleterious effects on GABA uptake. Therefore an inside-negative membrane potential was generated by H^+ diffusion and its effects on GABA uptake were studied. The membrane vesicles were preloaded with 300 mM-mannitol

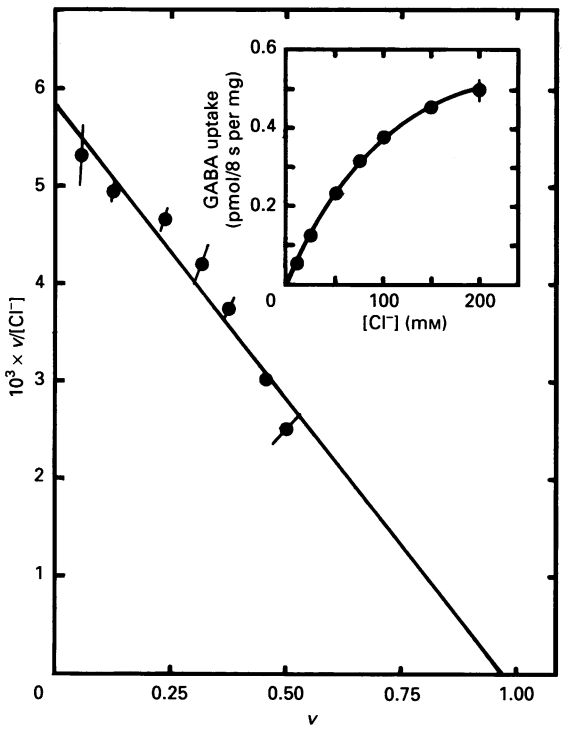

Fig. 3. Cl^- kinetics of GABA uptake

Apical membrane vesicles were preloaded with 500 mM-mannitol buffered with 10 mM-Hepes/Tris, pH 7.5. The uptake buffer was 10 mM-Hepes/Tris, pH 7.5, containing various concentrations of Cl^- (0–250 mM). The concentration of Cl^- was varied by substituting sodium gluconate for NaCl, and the concentration of Na^+ was maintained constant at 250 mM. Uptake of GABA (5 nM) was determined for 8 s. The dependence of GABA uptake on the concentration of Cl^- is shown in the inset. The Cl^--dependent uptake of GABA (uptake in the presence of Cl^- minus uptake in the absence of Cl^-) was analysed by a Hill-type equation. The plot of v versus $v/[Cl^-]^n$ was a straight line ($r = -0.98$) when n was assigned a value of 1. v is the uptake rate in pmol/8 s per mg of protein. The results are given as means, with bars representing S.E.M., for two experiments with duplicate determinations. When not shown, the error bars lie within the symbol.

buffered with 25 mM-Mes/Tris, pH 5.5, and the uptake buffer consisted of 25 mM-Hepes/Tris, pH 7.5, containing 150 mM-NaCl. Uptake was measured in the absence (no membrane potential) or presence of 10 μM-FCCP (inside-negative membrane potential). GABA uptake in the presence of the membrane potential was found to be significantly higher than in its absence (0.58 ± 0.03 versus 0.46 ± 0.03 pmol/8 s per mg of protein; $P < 0.01$). These data show that the transport process catalysing GABA uptake is electrogenic, resulting in the transfer of net positive charge across the membrane.

GABA has two ionizable groups, the carboxylic group and the γ-amino group, with pK values of 4.03 and 10.56 respectively. Therefore this compound exists predominantly as a zwitterion at pH 7.5. If the co-transport of Na^+, Cl^- and GABA results in the transfer of positive charge across the membrane, as suggested by the aforementioned experiments, this means that the ratio of the number of Na^+ ions to the number of Cl^- ions involved in the transport of one GABA molecule is greater than one. We therefore investigated the dependence of GABA uptake on the concentrations of Na^+ and Cl^- and, from these experimental data, determined the number of each ion that was associated with the transport of one GABA molecule. Fig. 2 describes the Na^+ kinetics. In this experiment, the initial uptake rate of GABA was determined at various concentrations of Na^+ in the uptake medium (range 0–120 mM). The concentration of Cl^- was kept constant at 120 mM. The Na^+-dependent uptake rates, calculated by subtracting the uptake rate in the absence of Na^+ from the uptake rate in the presence of Na^+, were plotted against the Na^+ concentration (Fig. 2, inset). The plot was sigmoidal, suggesting that more than one Na^+ ion was co-transported with one GABA molecule. The exact number of Na^+ ions involved in the process was calculated by the method described by Turner (1983). In this method, the experimental data were fitted to a Hill-type equation:

$$v = \frac{V_{max.} \cdot [Na^+]^n}{K^n_{t(Na^+)} + [Na^+]^n}$$

where v is the uptake rate, $K_{t(Na^+)}$ is the Michaelis–Menten constant for Na^+ and n is the number of Na^+ ions involved in the process per transport of one GABA molecule. The value of n was determined from the best fit of the experimental data to the linear plot v versus $v/[Na^+]^n$. The plot was linear ($r = -0.99$) when $n = 2.4$. These results demonstrate that 2 or 3 Na^+ ions are associated with the transport of one GABA molecule. The value for $K_{t(Na^+)}$ was found to be approx. 58 ± 1 mM.

A similar experiment was performed to analyse the Cl^- kinetics (Fig. 3). The initial uptake rate of GABA was determined with various concentrations of Cl^- in the uptake medium (range 0–200 mM). The concentration of Na^+ was maintained constant at 200 mM. The plot of the uptake rate versus Cl^- concentration was hyperbolic (Fig. 3, inset), indicating that one Cl^- ion was associated with the transport of one GABA molecule. The Hill-type plot, v versus $v/[Cl^-]^n$, gave a straight line for a value of $n = 1$ ($r = -0.98$). The $K_{t(Cl^-)}$ was found to be 150 ± 20 mM.

Substrate specificity

The effects of various amino acids on the uptake of GABA were studied to determine the substrate specificity of the GABA transporter (Table 2). The initial uptake rate of radiolabelled GABA was measured in the presence and absence of 100 μM of unlabelled amino acids. The amino acids we used were taurine, hypotaurine and β-alanine (substrates for the β system), α-aminoisobutyric acid (AIB, a representative substrate for the A system), α-alanine (a representative substrate for the ASC system) and leucine (a representative substrate for the L system). Hypotaurine and β-alanine, even though both of them are β-amino acids, were the most potent inhibitors. Taurine, another β-amino acid, inhibited GABA uptake significantly, but to a much lesser extent than hypotaurine and β-alanine. Apart from these β-amino acids, none of the other amino acids tested had any effect on the GABA uptake.

Interaction between GABA and taurine during uptake

Our previous study has shown that the uptake of taurine in apical membrane vesicles from the retinal pigment epithelium is inhibited by GABA, and that the taurine uptake process has higher affinity for GABA than for taurine (Miyamoto *et al.*, 1991). The present study has demonstrated that the uptake of GABA is inhibited by taurine. These data suggested to us that common transport systems may be involved in the uptake of taurine and GABA in these membrane vesicles. This idea is supported by subsequent experiments in the present study on the mutual interaction between taurine and GABA during their uptake. Uptake of GABA was found to be completely inhibitable by an excess of taurine and, similarly, uptake of taurine was also completely inhibitable by an excess of GABA (results not shown).

The interaction between taurine and GABA during uptake in these membrane vesicles was then analysed kinetically. First, we determined the nature of the inhibition of GABA uptake by

Table 2. *Cis*-inhibition of GABA uptake by amino acids

Apical membrane vesicles were preloaded with 300 mM-mannitol and 10 mM-Hepes/Tris, pH 7.5. Uptake of radiolabelled GABA (4 nM) was measured with a 8 s incubation. The uptake buffer consisted of 10 mM-Hepes/Tris, pH 7.5, containing 150 mM-NaCl. The concentration of unlabelled amino acids was 100 μM. The results are given as means ± S.E.M. for four determinations.

Amino acid	GABA uptake (pmol/8 s per mg of protein)	(% of control)
Control	0.457 ± 0.025	100
Taurine	0.317 ± 0.013	69
Hypotaurine	0.015 ± 0.003	3
β-Alanine	0.021 ± 0.001	5
AIB	0.525 ± 0.017	115
α-Alanine	0.516 ± 0.004	113
Leucine	0.506 ± 0.036	111

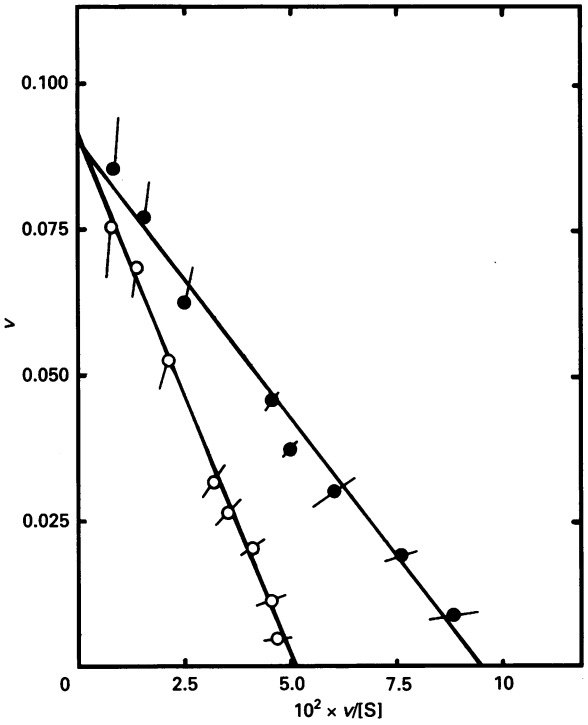

Fig. 4. Kinetics of GABA uptake in the presence and absence of taurine

Uptake of GABA was measured in the apical membrane vesicles with a 8 s incubation in the presence of an inwardly directed NaCl gradient. The concentration range for GABA was 0.1–10 μM. Uptake measurements were made in the absence (●) and in the presence (○) of 200 μM-taurine. The results are given as Eadie–Hofstee plots (uptake rate/GABA concentration versus uptake rate). The values represent means ± S.E.M. for four determinations. *v*, uptake rate in nmol/8 s per mg of protein; [S], GABA concentration has units of μM.

taurine (Fig. 4). The uptake rate of GABA was measured over a concentration range of 0.1–10 μM in the presence and in the absence of 200 μM-taurine. When the results were plotted as the uptake rate/substrate concentration versus the uptake rate (Eadie–Hofstee plot), the plots were linear ($r^2 > 0.98$) in the presence as well as in the absence of taurine, apparently indicating the participation of a single transport system in the uptake

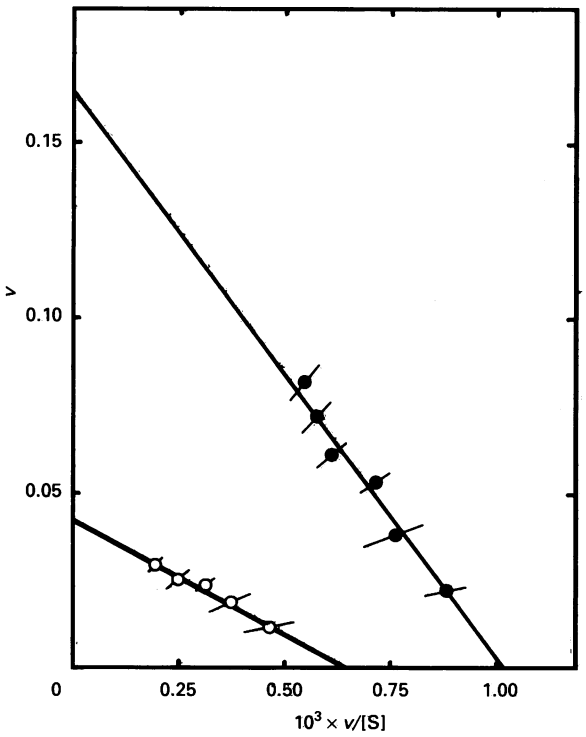

Fig. 5. Kinetic analysis of taurine uptake in the presence and absence of GABA

Uptake of taurine was measured in the apical membrane vesicles with a 8 s incubation in the presence of an inwardly directed NaCl gradient. The concentration range for taurine was 25–150 μM. Uptake measurements were made in the absence (●) and in the presence (○) of 2.5 μM-GABA. The results are given as Eadie–Hofstee plots (uptake rate/taurine concentration versus uptake rate). The values represent means ± S.E.M. for four determinations. *v*, uptake rate in nmol/8 s per mg of protein; [S], taurine concn. in μM.

process. Kinetic analysis revealed that the inhibition by taurine was competitive in nature, resulting in a decrease in the apparent affinity of the transport system for GABA (the K_t values for GABA in the absence and in the presence of taurine were 0.94 ± 0.04 μM and 1.76 ± 0.08 μM respectively), with no change in the maximal velocity (0.09 ± 0.01 nmol/8 s per mg of protein). The apparent inhibition constant (K_i) for taurine, calculated from these data, was 230 μM. We then studied the nature of the inhibition of taurine uptake by GABA (Fig. 5). In this experiment the uptake rate of taurine was measured over a concentration range of 25–150 μM in the presence and in the absence of 2.5 μM-GABA. The Eadie–Hofstee plots were linear ($r^2 > 0.96$) in the presence as well as in the absence of GABA, apparently indicating the involvement of a single transport system in the uptake process. Kinetic analysis revealed that the inhibition of taurine uptake by GABA was not competitive. The maximal velocity decreased markedly in the presence of GABA (control, 0.16 ± 0.01 nmol/8 s per mg of protein; GABA, 0.04 ± 0.01 nmol/8 s per mg of protein). An interesting aspect of the inhibition was that the affinity of the transport process for taurine increased significantly in the presence of GABA (the K_t values for taurine in the absence and in the presence of GABA were 161 ± 5 μM and 66 ± 10 μM respectively).

There are several compounds which have been described as specific inhibitors of GABA uptake in the brain. In order to obtain a better understanding of the interaction between GABA and taurine during uptake in the apical membrane vesicles of the retinal pigment epithelium, we studied the effects of these GABA

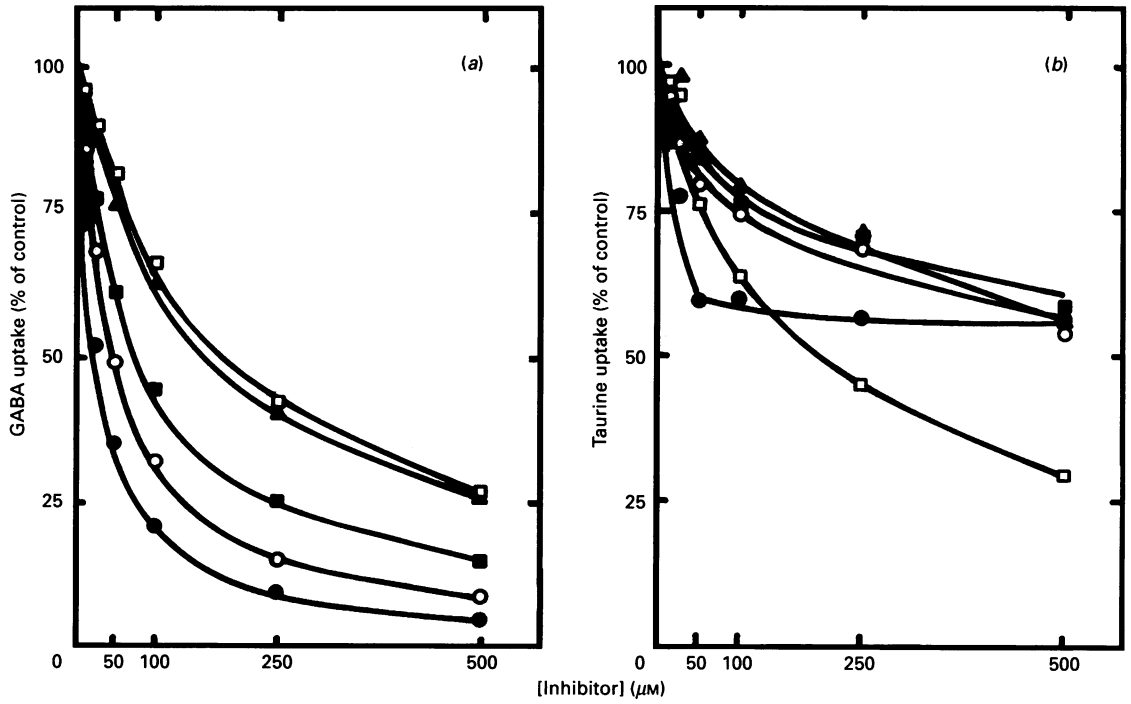

Fig. 6. Inhibition of the uptake of GABA and taurine by compounds known to be specific GABA uptake inhibitors

Uptake of 4 nM-GABA (*a*) or 0.4 μM-taurine (*b*) was measured in the apical membrane vesicles with a 8 s incubation in the presence of an inwardly directed NaCl gradient. Uptake measurements were made in the absence and in the presence of various inhibitors. The concentration of the inhibitors was varied over the range 10–500 μM. The results are given as percentages of the control uptake measured in the absence of the inhibitors. The control uptake for GABA was 0.31±0.01 pmol/8 s per mg of protein and that for taurine was 0.53±0.03 pmol/8 s per mg of protein. Inhibitors: nipecotic acid (●), guvacine (○), DABA (■), THPO (□) and HNA (▲).

inhibitors on the uptakes of GABA and taurine in these vesicles (Fig. 6). The inhibitors employed in this study were nipecotic acid, guvacine, DABA, HNA and THPO. All of these compounds inhibited the uptake of GABA and taurine, but to a variable extent. The ability of these compounds to inhibit GABA uptake was in the following order: nipecotic acid > guvacine > DABA > HNA > THPO. Uptake of taurine was also found to be inhibited by these compounds, but the inhibition was significantly less when compared with the inhibition of GABA uptake. Nipecotic acid, which inhibited GABA uptake almost completely, caused only about 50% inhibition of taurine uptake under similar conditions. This was true in the case of other inhibitors also, with the exception of THPO.

DISCUSSION

GABA is a major inhibitory neurotransmitter in the vertebrate retina (Yazulla, 1986). Active re-uptake of GABA plays an important role in the termination of the action of GABA in GABAnergic neurons. The existence of GABA transporters in the neural retina has been demonstrated by autoradiographic techniques (Marshall & Voaden, 1975). The retinal pigment epithelium is a single layer of cells interposed between the neural retina and the choroid. This is a polarized cell, with its apical membrane facing the subretinal space between the neural retina and the retinal pigment epithelium and its basolateral membrane facing the choroid. There is no information available on the handling of GABA by the retinal pigment epithelium. The present paper provides evidence for the first time that the apical membrane of this cell possesses an active mechanism for the transport of GABA. This raises the possibility that the retinal pigment epithelium may play a role in the regulation of the GABA concentration in the subretinal space.

We have described in this paper the characteristics of GABA uptake in apical membrane vesicles prepared from the bovine retinal pigment epithelium. Uptake of GABA in these vesicles is an active process, energized by an inwardly directed NaCl gradient. The process is electrogenic, resulting from the co-transport of two or three Na^+ and one Cl^- ions with each molecule of GABA. Thus, in intact retinal pigment epithelium, three different driving forces are expected to participate in the uphill transport of GABA: the transmembrane Na^+ gradient, the transmembrane Cl^- gradient and the membrane potential. We have shown earlier that the apical membrane also possesses a transport mechanism for taurine with characteristics similar to those of the GABA transporter (Miyamoto *et al.*, 1991). There was evidence from our previous study that the taurine transport system interacted with GABA. Therefore it was necessary to carry out detailed studies on the interaction between GABA and taurine during uptake in these membrane vesicles. In the present study we have done a systematic kinetic analysis of the uptake of both GABA and taurine, and have also analysed the interaction between these two compounds during uptake.

Uptake of GABA in these vesicles occurred via a single transport system when the uptake was measured over a concentration range of 0.1–10 μM; the K_t for GABA was 0.94 μM. In addition to GABA, this transport system interacts with β-amino acids such as taurine, hypotaurine and β-alanine. Interestingly, the affinity of the system for β-alanine and hypotaurine is much higher than for taurine. The K_i for taurine to inhibit GABA uptake was 230 μM, which is at least two orders of magnitude greater than the K_t for GABA. The competitive nature of the inhibition of GABA uptake by taurine indicates that both of these compounds interact with the transporter at a common substrate-binding site. The interaction of the GABA transporter with the β-amino acids in the apical membrane of the retinal

pigment epithelium is interesting. Of the two types of GABA transporters which have been described in the brain, only the type B transporter interacts with β-amino acids. It therefore appears that the GABA transporter which we have described in the present paper belongs to type B rather than type A. Distribution studies in the brain have shown that the type A GABA transporter is localized primarily in neuronal cells, whereas the type B GABA transporter is localized primarily in glial cells (Kanner & Bendahan, 1990). The retinal pigment epithelium is not a neuronal cell, and hence it is not surprising that the GABA transporter present in this cell is of type B.

Kinetic analysis of taurine uptake in the apical membrane vesicles prepared from the retinal pigment epithelium revealed that the uptake occurs via a single transport system over a concentration range of 25–150 μM. The K_t for taurine is 160 μM. This transport system interacts with GABA, as taurine uptake in these vesicles is inhibited by GABA. Even though taurine is a substrate for the GABA transporter, uptake of taurine in the apical membrane vesicles does not occur solely via this transporter, as the GABA transport inhibitors such as nipecotic acid are unable to inhibit taurine uptake completely. This suggests that there is another transport system, in addition to the GABA transporter, which is responsible for taurine uptake in these vesicles. However, the presence of multiple systems is not readily apparent in the kinetic analysis. This would be expected if the systems have similar affinities for taurine. Since taurine uptake can be completely inhibited by excess amounts of GABA in apical membrane vesicles from the retinal pigment epithelium, this shows that both transport systems which function in the uptake of taurine interact with GABA. However, kinetic analysis reveals that the inhibition of taurine uptake by GABA is not competitive in nature. Furthermore, there is the surprising finding that the apparent affinity of the transport system for taurine is higher in the presence of GABA than in its absence. These results can be explained if taurine uptake occurs via the GABA transporter as well as via a second transport system and the presence of GABA blocks the uptake of taurine via the GABA transporter, leaving the second uptake component largely intact. Tissues such as the kidney and the placenta possess a taurine transporter whose substrate specificity is quite distinct from that of the GABA transporter. The taurine transporter interacts with taurine as well as with GABA, but its affinity for taurine is several-fold greater than for GABA. Therefore it is very likely that the second transport system represents a taurine transporter similar to the one described in these other tissues.

The following conclusions can therefore be drawn from the results presented in this paper. The apical membrane of the retinal pigment epithelium possesses a GABA transporter as well as a taurine transporter. The GABA transporter, most likely representing the type B, exhibits an affinity for GABA which is at least 2–3 orders of magnitude greater than for taurine. The taurine transporter also interacts with both taurine and GABA, but its affinity for the former is significantly greater than for the latter. In addition, the GABA transporter has an affinity for GABA which is 2–3 orders of magnitude greater than the affinity of the taurine transporter for GABA. In contrast, the affinities of these two transporters for taurine are only slightly different. These characteristics of the transporters suggest that, under the experimental conditions employed, the uptake of GABA in these membrane vesicles occurs predominantly via the GABA transporter. The taurine transporter does not contribute to GABA uptake to any significant extent. On the contrary, the uptake of taurine occurs via the GABA transporter as well as the taurine transporter, both systems contributing to the uptake to an appreciable extent.

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