Functional Characterization of the Betaine/\(\gamma\)-Aminobutyric Acid Transporter BGT-1 Expressed in \textit{Xenopus} Oocytes*

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Betaine is an osmolyte accumulated in cells during osmotic cell shrinkage. The canine transporter mediating cellular accumulation of the osmolyte betaine and the neurotransmitter \(\gamma\)-aminobutyric acid (BGT-1) was expressed in \textit{Xenopus} oocytes and analyzed by two-electrode voltage clamp and tracer flux studies. Exposure of oocytes expressing BGT-1 to betaine or \(\gamma\)-aminobutyric acid (GABA) depolarized the cell membrane in the current clamp mode and induced an inward current under voltage clamp conditions. At 1 mM substrate the induced currents decreased in the following order: betaine = GABA > diaminobutyric acid = \(\beta\)-alanine > proline = quinidine > dimethylglycine > glycine > sarcosine. Both the \(V_{\text{max}}\) and \(K_m\) of GABA- and betaine-induced currents were voltage-dependent, and GABA- and betaine-induced currents and radioactive tracer uptake were strictly Na\(^+\)-dependent but only partially dependent on the presence of Cl\(^-\). The apparent affinity of GABA decreased with decreasing Na\(^+\) concentrations. The \(K_m\) of Na\(^+\) also depended on the GABA and Cl\(^-\) concentration. A decrease of the Cl\(^-\) concentration reduced the apparent affinity for Na\(^+\) and GABA, and a decrease of the Na\(^+\) concentration reduced the apparent affinity for Cl\(^-\) and GABA. A comparison of \(^{22}\)Na\(^{+}\)-, \(^{36}\)Cl\(^-\), and \(^{14}\)C-labeled GABA and \(^{14}\)C-labeled betaine fluxes and GABA- and betaine-induced currents yielded a coupling ratio of Na\(^+\)/Cl\(^-\)/organic solute of 3:1:1 or 3:2:1. Based on the data, a transport model of ordered binding is proposed in which GABA binds first, Na\(^+\) second, and Cl\(^-\) third. In conclusion, BGT-1 displays significant functional differences from the other members of the GABA transporter family.

Because osmotic equilibrium across most cell membranes occurs rapidly via water flux down its gradient, cells regulate their volume by adjusting their solute content. To this end they employ a variety of mechanisms including ion transport, formation or degradation of glycogen and proteins, as well as accumulation of organic osmolytes (1, 2). The osmolytes most frequently utilized by mammalian cells include the polyols sorbitol and myo-inositol, amino acids and amino acid derivatives such as taurine, as well as methylamines such as glycerophosphorylcholine and betaine (3, 4). In contrast to inorganic ions, the organic osmolytes do not destabilize proteins and are thus compatible with cellular protein function even at very high concentrations (5, 6). Whereas sorbitol and glycerophosphorylcholine are accumulated by cellular formation, myo-inositol, taurine, and betaine are largely accumulated by Na\(^+\)-coupled transport processes (4–6).

In recent years considerable progress has been made toward understanding the molecular mechanisms underlying osmotic regulation. Hypertonically-regulated signal transduction pathways have been identified (for review, see Ref. 1), and several osmolyte transporters have been cloned, such as BGT-1 for betaine/GABA (7–10), SCTR/TAUT for taurine (11, 12), and SMIT for myo-inositol (13). BGT-1 was originally cloned from Madin-Darby canine kidney cells (7) but has subsequently been found to be expressed ubiquitously in most mammalian tissues including the central nervous system (8–10, 14). BGT-1 and SCTR/TAUT belong to the family of Na\(^+\)- and Cl\(^-\)-coupled transporters for neurotransmitters, amino acids, and osmolytes. Four different GABA transporters have been cloned (7, 10, 15–18) and are named GAT-1, GAT-2, GAT-3, and GAT-4 in rat and man or GAT-1, GAT-2, GAT-3, and GAT-4 in mice, respectively. Most of these transporters accomplish either neuronal or glial transport of GABA in the central nervous system, whereas GAT/GAT-2 is also widely expressed outside the central nervous system (14). The mechanism of GABA uptake via GAT-1 has been studied in detail in cRNA-injected \textit{Xenopus} oocytes and transfected mammalian cells (19–24). Considerable differences have been found with respect to ion- or transport stoichiometry, binding order, and leak- and transport-associated currents in this family (25, 26), but not much is known about the functional properties of BGT-1. Additionally, pharmacological properties of BGT-1 are less well studied than those other three known GAT transporter isoforms (14, 15), and only little is known about the functional properties of BGT-1 in general. In this study the canine BGT-1 was expressed in \textit{Xenopus} oocytes and examined using the two-electrode voltage clamp technique and radioactive tracer studies.

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The abbreviations used are: BGT-1, betaine/GABA transporter; GABA, \(\gamma\)-aminobutyric acid; GAT, GABA transporter; DABA, diaminobutyric acid.
MATERIALS AND METHODS

cRNA encoding the canine BGT-1 was synthesized in vitro as described previously (7). Dissection of *X. laevis* ovaries and collection and handling of the oocytes have been described in detail elsewhere (27). Oocytes were injected with 15 ng of cRNA and 50 nl of water/oocyte; noninjected oocytes served as controls. All experiments were performed at room temperature 3–8 days after injection.

Flux studies have been performed utilizing the radiochemicals [14C]GABA, 22NaCl, and H36Cl (NEN Life Science Products, Brussels, Belgium) and [14C]betaine (Biotrend, Cologne, Germany). BGT-1-expressing oocytes and noninjected control oocytes were washed twice with ice-cold OR21 buffer (calcium-containing oocyte Ringer OR21: 82.5 mM NaCl, 2.5 mM KCl, 1 mM MgCl2, 1 mM Na2HPO4, 1 mM CaCl2, 5 mM HEPES, pH 7.8 (28)). Uptake was initiated by addition of 100 μl of OR21 buffer supplemented with 1 mM GABA and one of the radioactive substrates [14C]GABA, [14C]betaine (final specific activity 63.0 Bq/nmol), or 22NaCl (final specific activity 21.9 Bq/nmol). Chloride uptake was studied in transport buffer in which unlabeled NaCl was replaced by H36Cl (final specific activity 6.7 Bq/nmol) titrated with NaOH to a pH of about 8; in addition, the HEPES concentration was elevated to 20 mM to increase buffering capacity. Groups of seven oocytes were incubated for each experimental condition in 5-ml polypropylene tubes containing 100 μl of supplemented OR21 buffer. Uptake was stopped after 30 min or 1 h by washing the oocytes with ice-cold OR21 buffer.

Single oocytes were lysed by adding 200 μl of 10% SDS; 3 ml of scintillation fluid was added, and radioactivity was determined by liquid scintillation counting. Each measurement was performed at least twice.

Current induced by GABA was measured by two-electrode voltage clamp on the same batch of oocytes and on the same day the transport measurements were performed.

Two-electrode voltage clamp recordings were performed at a holding potential of −50 mV if not otherwise specified. The data were filtered at 10 Hz and recorded with MacLab digital-to-analog converter and software for data acquisition and analysis (AD Instruments, Castle Hill, Australia). The external control solution (superfusate/ND96) contained 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, and 5 mM HEPES, pH 7.4. For experiments studying the dependence on external sodium or chloride, sodium was replaced by choline or N-methyl-D-glucamine and chloride by gluconate, NO3, or SO4. When necessary, the osmolarity was adjusted by adding glucose. Betaine or GABA was added to the solutions at the indicated concentrations. The final solutions were titrated to the pH indicated using HCl or KOH. The flow rate of the superfusion was 20 ml/min, and a complete exchange of the bath solution was reached within about 10 s. The currents given are the maximal values measured during a 30-s substrate superfusion. Usually the maximal current reached after 25 substrate superfusion. To rule out effects of the different solution used (OR21 versus ND96) control exper-

### TABLE I

| Substrate            | Chemical structure | Current (nA) |
|----------------------|--------------------|--------------|
| GABA                 | ![GABA structure](https://example.com/gaba_structure.png) | −75.3 ± 4.1 |
| Betaine              | ![Betaine structure](https://example.com/betaine_structure.png) | −73.0 ± 1.9 |
| DABA                 | ![DABA structure](https://example.com/daba_structure.png) | −33.3 ± 1.2 |
| β-alanine            | ![β-alanine structure](https://example.com/beta-alanine_structure.png) | −29.7 ± 0.6 |
| Proline              | ![Proline structure](https://example.com/proline_structure.png) | −15.1 ± 1.1 |
| Quinidine            | ![Quinidine structure](https://example.com/quinidine_structure.png) | −11.7 ± 2.6 |
| Glycine              | ![Glycine structure](https://example.com/glycine_structure.png) | −9.8 ± 1.1 |
| Putrescine           | ![Putrescine structure](https://example.com/putrescine_structure.png) | −9.7 ± 0.7 |
| Dimethylglycine      | ![Dimethylglycine structure](https://example.com/dimethylglycine_structure.png) | −7.9 ± 0.6 |
| Choline              | ![Choline structure](https://example.com/choline_structure.png) | −5.1 ± 0.2 |
| Carnitine            | ![Carnitine structure](https://example.com/carnitine_structure.png) | −4.8 ± 1.3 |
| Sarcosine            | ![Sarcosine structure](https://example.com/sarcosine_structure.png) | −4.8 ± 1.5 |
| TEA                  | ![TEA structure](https://example.com/tea_structure.png) | −7.7 ± 1.0 |
| Taurine              | ![Taurine structure](https://example.com/taurine_structure.png) | −3.1 ± 1.1 |
| Betaine aldehyde     | ![Betaine aldehyde structure](https://example.com/betaine_aldehyde_structure.png) | −0.8 ± 0.8 |
| Creatine             | ![Creatine structure](https://example.com/creatinne_structure.png) | −0.1 ± 0.1 |

![Fig. 1. Panel A, substrate-induced current in oocytes expressing BGT-1.](https://example.com/fig1_panel_a.png)

Original tracings for GABA, betaine, DABA, β-alanine, and proline (1 mM) are shown. Panel B, voltage dependence of GABA-induced currents (1 mM) expressed as a fraction of the current of GABA at −90 mV (I/I\text{max}). Currents were measured during voltage ramps from −100 to +40 mV in the absence (control) and presence of GABA, and currents under GABA were subtracted from control currents.
RESULTS

In current clamp studies superfusion of BGT-1 expressing Xenopus oocytes with 1 mM GABA or betaine caused a depolarization of 27.6 ± 2.0 mV (n = 5) and 24.2 ± 1.7 mV (n = 5), respectively. In the voltage clamp modus 1 mM GABA or betaine induced a mean inward current of −73.9 ± 3.8 nA and −75.3 ± 4.1 nA, respectively, as illustrated in Fig. 1A. In noninjected control oocytes GABA did not induce significant currents (0.9 ± 0.3 nA, n = 5), whereas betaine led to an inward current of −7.2 ± 1.5 nA (n = 5). The betaine-induced current in noninjected oocytes was independent of the presence of Na⁺ and had a low affinity for betaine (data not shown). Thus, for further kinetic analysis mostly GABA was used; and if betaine was used, betaine-induced currents in noninjected oocytes were subtracted from the currents obtained from BGT-1-injected oocytes.

To test the selectivity of BGT-1 the current elicited by a 1 mM concentration of different substrates was measured (Table I). The rank order of magnitude of induced currents was GABA > betaine > DABA > β-alanine > proline > quinidine > glycine > putrescine > choline > sarcosine = tetraethylammonium = taurine = carnitine > betaine aldehyde = creatine (Table I; all substances were tested on the same oocytes, n = 5). Among those substrates, only betaine induced currents in noninjected oocytes (see above). Serotonin, histamine, noradrenaline, dopamine, and L-DOPA did not induce any currents in BGT-1- or noninjected oocytes (data not shown, n = 5).

As shown in Fig. 1B, the GABA-induced currents in BGT-1-expressing oocytes were voltage-dependent, increasing with hyperpolarization. In ND96 buffer the GABA-induced currents were strictly inwardly rectifying in the range between −90 and +40 mV (n = 5). The holding potential affected both the maximum velocity and the apparent \( K_m \) of GABA- and betaine-induced currents. As shown in Fig. 2 the GABA concentration required for half-maximal current (apparent \( K_m^* \)) increased from 0.009 ± 0.001 mM at −100 mV holding potential to
0.031 ± 0.004 mM at a more depolarized holding potential of −30 mV. On the other hand, a depolarization from −100 to −50 and −30 mV decreased the maximal current by 31 ± 2% and 51 ± 2%, respectively (Fig. 2A, n = 4). The apparent \( K_m \) for betaine-induced currents was shifted significantly from 0.51 ± 0.04 mM at −80 mV holding potential to 2.1 ± 0.2 mM at −30 mV (n = 4). The maximal betaine-induced current was not significantly altered by depolarization (Fig. 2B).

The betaine-induced currents were strictly \( Na^+ \)- and partially \( Cl^- \)-dependent. The apparent \( K_m \) for \( Na^+ \) was 93.3 ± 3.9 mM and for \( Cl^- \) 68.3 ± 0.8 mM (n = 4, Figs. 2A and B). The Hill coefficient for \( Na^+ \) was 2.5 ± 0.2 and for \( Cl^- \) 1.7 ± 0.1.

Also, the GABA-induced currents were strictly \( Na^+ \)-dependent. In the absence of \( Na^+ \), the addition of 1 mM GABA failed to induce any current. The GABA (1 mM)-induced current increased with the extracellular \( Na^+ \) concentration (at 103.6 mM \( Cl^- \) concentration) with a half-maximal current at 65.9 ± 8 mM \( Na^+ \) concentration at −90 mV and at 89 ± 3 mM \( Na^+ \) concentration at −50 mV (Fig. 4A, n = 4). Thus, hyperpolarization of the cell membrane increased the affinity of the carrier to \( Na^+ \). The Hill coefficient for \( Na^+ \) was 2.2 ± 0.4 at −50 mV and was not altered significantly by hyperpolarization to −90 mV. A decrease of ambient \( Na^+ \) concentration decreased both maximal current and apparent affinity for GABA. The apparent \( K_m \) for GABA increased slightly from 0.021 ± 0.002 mM to 0.056 ± 0.006 mM. Curves were obtained by fitting the data to the Hill equation. The Hill coefficient was 1.5 ± 0.2 for 130 mM \( Na^+ \) and 1.1 ± 0.1 for 80 mM \( Na^+ \). Inset, Lineweaver-Burk plot for the same data.

As illustrated in Fig. 6, GABA-induced currents were also partially \( Cl^- \)-dependent. Reduction of extracellular \( Cl^- \) from 150 to 0 mM reduced the GABA-induced current by about 80% at −90 mV and by 90% at −50 mV (Fig. 6A and B). Replacing \( Cl^- \) with anions other than gluconate (\( NO_3^- \) and \( SO_4^{2-} \)) showed similar results. Varying the extracellular \( Cl^- \) concentration revealed that the half-maximal current induced by 1 mM GABA was reached at 78 ± 4 mM \( Cl^- \) concentration at −90 mV and at about 114 ± 5 mM \( Cl^- \) concentration at −50 mV. For fitting the curves an offset (\( Cl^- \)-independent GABA-induced current) of 0.2 and 0.08 for −90 and −50 mV, respectively, was assumed. The apparent \( K_m \) for \( Cl^- \) at a holding potential of −50 mV could only be estimated because saturating conditions could not be reached under experimental conditions (n = 5). The apparent \( Cl^- \) affinity of the carrier was decreased by depolarization. The Hill coefficient for \( Cl^- \) was close to 1 irrespective of the holding potential. Furthermore, a reduction of the extracellular \( Cl^- \) concentration decreased both maximal current and apparent affinity for GABA.
Fig. 6. Cl⁻ dependence of GABA-induced current. Panel A, original tracing showing GABA-induced currents at −90 mV in the presence (150 mM) and the absence (0 mM) of Cl⁻. The start of a 30-s superfusion period is indicated by the arrows. Panel B, GABA-induced currents were a curvilinear function of Cl⁻ concentration in the superfusate. The apparent Km for Cl⁻ was voltage-dependent and increased from 78 ± 4 mM to 114 ± 5 mM with depolarization of the holding potential from −90 to −50 mV. The apparent Km for −50 mV is only an estimation but clearly indicates a significant shift. Curves were obtained by fitting the data to the Hill equation considering an offset of 0.1. All data were normalized against the maximal current at 150 mM Cl⁻.

Panel C, Lineweaver-Burk plot for the same data. The uptake of GABA was linear and 1 mM GABA. The Hill coefficient for GABA was 1.2 ± 0.05 for 130 mM Cl⁻ and 1.09 ± 0.07 for 50 mM Cl⁻. Inset, Lineweaver-Burk plot of the same data.

The apparent Km value for GABA from 0.033 ± 0.001 to 0.049 ± 0.003 mM, pointing to a small but significant decrease of GABA affinity (Fig. 6C, n = 5). The maximum velocity of GABA uptake decreased significantly with decreasing Cl⁻ concentration.

To examine the mutual interaction of Na⁺ and Cl⁻ we performed Na⁺ and Cl⁻ kinetics at different Cl⁻ and Na⁺ concentrations, respectively (Fig. 7). The apparent Km for Na⁺ was shifted from 54 ± 3 mM at 150 mM Cl⁻ to 82 ± 7 mM at 80 mM Cl⁻. The Hill coefficient was 2.5 ± 0.2 for both extracellular Cl⁻ concentrations (Fig. 7A, n = 5). On the other hand, a decrease of the ambient Na⁺ concentration from 150 to 80 mM turned the apparent Km for Cl⁻ from 72 ± 3 mM (Hill coefficient of 1.1 ± 0.2) into a nonsaturable chloride concentration dependence (Fig. 7C, n = 5). A summary of the kinetic constants derived from these experiments is shown in Table II.

To determine further the Na⁺ and Cl⁻ dependence and coupling ratio we carried out uptake studies with radioactive [14C]GABA, 22Na⁺, and 36Cl⁻. The uptake of GABA was linear.
over a time period of 30 min (Fig. 8). In agreement with GABA-induced currents, the uptake of labeled GABA was completely Na\(^+\)-dependent (n = 7) but only partially Cl\(^-\)-dependent (n = 7) (Table III). The cotransport stoichiometry in relation to GABA was 1:3.1 for Na\(^+\) and 1:1.3 for Cl\(^-\) (Table IV). The addition of 10 mM betaine to the incubation buffer resulted in a substrate-stimulated \(^{14}\)CNa uptake of 5.2 ± 1.2 nmol/h and a substrate-stimulated \(^{38}\)Cl\(^-\)uptake of 5.3 ± 0.7 nmol/h, reflecting a ratio of 3 Na\(^+\) to 1.9 Cl\(^-\). Because of the low specific activity of available tracer, the betaine uptake could not be evaluated properly in these experiments. Taken together, these data suggest a coupling ratio of 3:1:1 or 3:2:1 for Na\(^+\), Cl\(^-\), and organic substrate, respectively. This conclusion is supported further by the comparison of flux and current in the same batch of oocytes. In those oocytes currents of 29 ± 9 nA (n = 7) and 59 ± 6 nA (n = 7) have been determined for the Na\(^+\)/GABA and the Cl\(^-\)/GABA flux measurements, respectively. Electrophysiological experiments under conditions mimicking the flux experiments revealed that during current clamp, the addition of 1 mM GABA led to an initial depolarization (by 13.6 ± 1.0 mV, n = 9) followed by slow recovery of cell membrane potential (by 14.6 ± 1.1 mV, n = 9). At voltage clamp conditions, the GABA (1 mM)-induced current decreased by 12 ± 3% (n = 4). Correcting the measured current at −50 mV holding potential for altered voltage and current yielded a value of 1.0 ± 0.2 and 1.0 ± 0.1 mmol/h net charge transfer/nmol/h organic substrate transported (Table III). The uncorrected values amount to 1.8 ± 0.3 and 1.7 ± 0.2.

**DISCUSSION**

This is the first demonstration that BGT-1 conveys electrogenic transport of betaine and GABA together with Na\(^+\) and Cl\(^-\). The substrate-induced currents in BGT-1-expressing oocytes parallel several transport properties defined previously (7–10). In these studies it was observed that transport of betaine and GABA was inhibited by quinine, \(\beta\)-alanine, and DABA. In agreement with these studies we found a significantly higher \(K_m\) value for betaine compared with the value for GABA. According to the present results quinine, \(\beta\)-alanine, and DABA are not only inhibitors of GABA transport (7) but are substrates. The substrate recognition by BGT-1 mostly relies on the presence of an amino group. Methylated amines are accepted as well but with lower affinity, whereas guanidino groups are hardly recognized. A second negatively charged group is preferred but does not seem to be necessary. The substrate recognition also seems to be flexible with respect to the distance between both groups.

As shown previously (7–10), the substrate-induced current requires the presence of extracellular Na\(^+\) and Cl\(^-\). The Hill parameters of GABA uptake in BGT-1-expressing oocytes (Table II) and the comparison of flux and current in the same batch of oocytes. Taken together, these data suggest a coupling ratio of 3:1:1 or 3:2:1 for Na\(^+\), Cl\(^-\), and organic substrate, respectively. This conclusion is supported further by the comparison of flux and current in the same batch of oocytes. In those oocytes currents of 29 ± 9 nA (n = 7) and 59 ± 6 nA (n = 7) have been determined for the Na\(^+\)/GABA and the Cl\(^-\)/GABA flux measurements, respectively. Electrophysiological experiments under conditions mimicking the flux experiments revealed that during current clamp, the addition of 1 mM GABA led to an initial depolarization (by 13.6 ± 1.0 mV, n = 9) followed by slow recovery of cell membrane potential (by 14.6 ± 1.1 mV, n = 9). At voltage clamp conditions, the GABA (1 mM)-induced current decreased by 12 ± 3% (n = 4). Correcting the measured current at −50 mV holding potential for altered voltage and current yielded a value of 1.0 ± 0.2 and 1.0 ± 0.1 mmol/h net charge transfer/nmol/h organic substrate transported (Table III). The uncorrected values amount to 1.8 ± 0.3 and 1.7 ± 0.2.

**TABLE II**

Summary of kinetic constants

| Varied substrate | Apparent \(K_m\) | Voltage | GABA concentration | Na\(^+\) concentration | Cl\(^-\) concentration |
|-----------------|----------------|---------|---------------------|------------------------|-----------------------|
| GABA            | 9 ± 1 μM       | −100    | 96                  | 103.6                  |                       |
|                 | 19 ± 1 μM      | −50     | 96                  | 103.6                  |                       |
|                 | 31 ± 4 μM      | −30     | 96                  | 103.6                  |                       |
|                 | 56 ± 6 μM      | −50     | 80                  | 103.6                  |                       |
|                 | 49 ± 3 μM      | −50     | 96                  | 130                    |                       |
|                 | 33 ± 1 μM      | −50     | 96                  | 50                     |                       |
| Na\(^+\)         | 66 ± 8 mM      | −80     | 1                   | 100                    |                       |
|                 | 89 ± 3 mM      | −50     | 1                   | 100                    |                       |
|                 | 55 ± 2 mM      | −80     | 1                   | 103.6                  |                       |
|                 | Approx. 120 mM | −80     | 0.03                | 103.6                  |                       |
| Cl\(^-\)         | 54 ± 3 mM      | −50     | 1                   | 150                    |                       |
|                 | 82 ± 7 mM      | −50     | 1                   | 80                     |                       |
|                 | Nonsaturable   | −50     | 1                   |                        |                       |
|                 | Approx. 114 mM | −50     | 1                   | 96                     |                       |
|                 | 72 ± 3 mM      | −50     | 1                   | 150                    |                       |

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**TABLE III**

Ion dependence of GABA transport in BGT-1-expressing oocytes

Uptake of \(^{14}\)C]GABA (1 mM) was determined under different ionic conditions. Groups of seven oocytes were washed, and subsequently uptake activity was determined in ND96, Na\(^+\)-free, or Cl\(^-\)-free ND96 buffer. Uptake was terminated by washing the oocytes four times in ice-cold ND96 buffer. The uptake activity of noninjected oocytes was determined under identical conditions.

| ND96 buffer with the indicated modification | BGT-1-expressing oocytes | Noninjected oocytes |
|-------------------------------------------|--------------------------|---------------------|
| pmol GABA/30 min/oocyte                   | pmol GABA/30 min/oocyte  |
| None                                      | 1,127 ± 71               | 37 ± 3              |
| Na\(^+\)-free                              | 53 ± 4                   | 24 ± 1              |
| Cl\(^-\)-free                              | 380 ± 18                 | 25 ± 1              |
Functional Properties of Betaine/GABA Transporter BGT-1

**TABLE IV**

Uptake of $[^14]{C}$GABA, $^{32}$Na $^+$, and $^{36}$Cl $^-$ by BGT-1-expressing Xenopus oocytes

All data are given in nmol/h/oocytes calculated from the respective tracer fluxes. The charge transfer was calculated from GABA-induced currents considering that 1 nA corresponds to 36 pmol/h/oocyte. The corresponding data are indicated by an asterisk (*). For a second set of experiments similar data were obtained (not shown); n = seven oocytes for each experiment.

| Na$^+$ dependence | BGT-1-expressing oocytes | Noninjected oocytes | Δ |
|-------------------|--------------------------|---------------------|---|
| $^{32}$Na (+GABA)  | 0.266 ± 0.103            | 0.210 ± 0.032       | 0.156 ± 0.108 |
| $^{32}$Na (ΔGABA)  | 2.000 ± 0.180            | 0.229 ± 0.025       | 1.768 ± 0.181 |
| $[^14]{C}$GABA     | 1.630 ± 0.207            | 0.018 ± 0.041       | 1.612 ± 0.211 |
| Charge             | 0.643 ± 0.107            | 0.067 ± 0.007       | 0.577 ± 0.107 |
| Ratio $[^14]{C}$GABA/Na$^+$ | 1.062 ± 0.316* | 0.032 ± 0.011* | 1.030 ± 0.316* |

| Cl$^-$ dependence  |                          |                     | |
|-------------------|--------------------------|---------------------|---|
| $[^36]{Cl}$ (ΔGABA) | 1.378 ± 0.022            | 1.287 ± 0.050       | 0.090 ± 0.055 |
| $[^36]{Cl}$ (+GABA) | 2.639 ± 0.209            | 0.890 ± 0.036       | 1.749 ± 0.212 |
| $[^36]{Cl}$ (ΔGABA) | 1.261 ± 0.210            | -0.398 ± 0.061      | 1.659 ± 0.219 |
| GABA              | 1.333 ± 0.057            | 0.071 ± 0.004       | 1.263 ± 0.057 |
| Charge            | 2.166 ± 0.229*           | 0.032 ± 0.011*      | 2.134 ± 0.229* |
| Ratio $[^36]{Cl}$/[^14]{C}GABA |                    | 1.3                |    |

**FIG. 9. Proposed model for ordered binding of GABA/betaine, Na$^+$, and Cl$^-$ from the exterior side of the membrane to the BGT-1 transporter.** GABA/betaine binds first to the transporter, and Na$^+$ binding occurs before Cl$^-$ binding. However, for the translocation of the transporter to the interior side of the membrane (dotted line), Cl$^-$ is not required. About 20% of the GABA/betaine-induced current and uptake is Cl$^-$-independent. Full transport is only evoked after Cl$^-$ binding. The coupling ratio of GABA/betaine to Na$^+$ and Cl$^-$ may be either 1:3:2 or 1:3:1. The order of substrate release at the interior side is completely hypothetical and not known so far.

cotransported with 3 Na$^+$ instead of 2 (14, 18–20). The maximal amplitude and apparent $K_m$ of GABA- and betaine-induced currents were voltage-dependent but not the Hill coefficients for GABA, Na$^+$, and Cl$^-$. The currents were strictly inwardly rectifying as described for the GAT family (14, 19, 20, 29). Depolarization of the cell membrane or a decrease of the GABA concentration strongly decreased the apparent Na$^+$ affinity. Presumably the Na$^+$ binding site is within the cell membrane and senses part of the electrical field. Hyperpolarization of the cell membrane favors entry of Na$^+$ and thus increases the Na$^+$ concentration at the binding site. We have tried to deduce the binding order from the effects of substrate and ion concentration on the maximum velocity (30). Because binding usually is not rate-limiting for transport, the uptake velocity should be dictated by the number of fully occupied transporters. In an ordered binding mechanism maximum velocity can therefore be reached with saturating concentrations of the last binding substrate, even in the presence of subsaturating concentrations of earlier binding substrates (30). The maximum velocity of GABA uptake decreases with decreasing Na$^+$ and Cl$^-$ concentrations. On the other hand, the maximum transport current can still be reached at subsaturating GABA concentrations (see Fig. 5B). This is indicated by the intersection of the straight lines on the y axis in Fig. 5B. We therefore tentatively suggest that GABA binds prior to Na$^+$. The significant Cl$^-$-independent GABA transport that was visible in flux and electrophysiological studies can be explained best by assuming a limited translocation of the GABA-3Na$^+$ transporter complex. Alternatively, cotransport of the replacement anions gluconate, NO$_3^-$ or SO$_4^{2-}$ has to be assumed. However, in the face of the similar current in the presence of these completely different anions, this possibility is considered unlikely. It is very likely that binding of Cl$^-$ should occur after binding of Na$^+$. Based on the presented data we propose a transport model of ordered binding (Fig. 9) in which GABA or betaine binds first to the extracellular side of the transporter. Na$^+$ binding occurs after GABA/betaine but before Cl$^-$ binding. Cl$^-$ facilitates the translocation of the transporter, but there is a substantial transport rate even in the absence of extracellular Cl$^-$.  

**REFERENCES**

1. Lang, F., Buseh, G. L., Ritter, M., Volki, H., Waldegger, S., Gubins, E. J., and Haussinger, D. (1998) *Cell. Physiol. Biochem.* **8**, 1–45
2. Lang, F., Buseh, G. L., and Volki, H. (1998) *Physiol. Rev.* **78**, 247–306
3. Burg, M. B. (1995) *Am. J. Physiol.* **268**, F983–F996
4. Handler, J. S., and Kwon, H. M. (1993) *Am. J. Physiol.* **265**, C1449–C1455
5. Burg, M. B. (1994) *J. Exp. Zool.* **268**, 171–175
6. Burg, M. B., Kwon, E. D., and Kultz, D. (1996) *FASEB J.* **10**, 1598–1606
7. Yamauchi, A., Uchida, S., Kwon, H. M., Preston, A. S., Robey, R. B., Garcia-Perez, A., Burg, M., and Handler, J. S. (1992) *J. Biol. Chem.* **267**, 649–652
8. Rasola, A., Galietta, L. J. V., Barone, V., Romeo, G., and Bagnasco, S. (1995) FEBS Lett. 373, 229–233
9. Borden, L. A., Smith, K. E., Gustafson, E. L., Branchek, T. A., and Weinshank, R. L. (1995) J. Neurochem. 64, 977–984
10. Lopez-Corcuera, B., Liu, Q.-R., Mandiyan, S., Nelson, H., and Nelson, N. (1992) J. Biol. Chem. 267, 17491–17493
11. Uchida, S., Kwon, H. M., Yamauchi, A., Preston, A. S., Marumo, F., and Handler, J. S. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 8230–8234
12. Smith, K. E., Borden, L. A., Wang, C.-H. D., Hartig, P. R., Branchek, T. A., and Weinshank, R. L. (1992) Mol. Pharmacol. 42, 563–569
13. Kwon, H. M., Yamauchi, A., Uchida, S., Preston, A. S., Garcia-Perez, A. Burg, M. B., and Handler, J. S. (1992) J. Biol. Chem. 267, 6297–6301
14. Borden, L. A. (1996) Neurochem. Int. 29, 335–356
15. Liu, Q.-R., Lopez-Corcuera, B., Mandiyan, S., Nelson, H., and Nelson, N. (1993) J. Biol. Chem. 268, 2106–2112
16. Borden, L. A., Smith, K. E., Hartig, P. R., Branchek, T. A., and Weinshank, R. L. (1992) J. Biol. Chem. 267, 21098–21104
17. Liu Q. R., Mandiyan S., Nelson H., and Nelson N. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 6639–6643
18. Guastella, J., Nelson, N., Nelson, H., Czyzyk, L., Keynan, S., Miedel, M. C., Davidson, N., Lester, H. A., and Kanner, B. I. (1996) Science 249, 1303–1306
19. Kavanaugh, M. P., Arriza, J. L., North, R. A., and Amara, S. G. (1992) J. Biol. Chem. 267, 22007–22009
20. Mager, S., Naeve, J., Quick, M., Labarca, C., Davidson, N., and Lester, H. A. (1993) Neuron 10, 177–188
21. Cammack, J. N. (1994) Neuron 13, 949–960
22. Cammack, J. N., and Schwartz, E. A. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 723–727
23. Risso, S., DeFelcie, L. J., and Blakely, R. D. (1996) J. Physiol. (Lond.) 490, 691–702
24. Su, A., Mager, S., Mayo, S. L., and Lester, H. A. (1996) Biophys. J. 70, 762–777
25. Lester, H. A., Mager, S., Quick, M. W., and Corey, J. L. (1994) Annu. Rev. Pharmacol. Toxicol. 34, 219–249
26. Sonders, M. S., and Amara S. G. (1996) Curr. Opin. Neurobiol. 6, 294–302
27. Busch, A. E., Kopp, H.-G., Waldegger, S., Samarzija, I., Suessbrich, H., Raber, G., Kunzelmann, K., Ruppersberg, J. P., and Lang, F. (1996) J. Physiol. (Lond.) 491, 735–741
28. Peng, H. B. (1991) Methods Cell Biol. 36, 657–662
29. Quick, M. W., Corey, J. L., Davidson, N., and Lester, H. A. (1997) J. Neurosci. 17, 2967–2979
30. Stein, W. D. (1986) Academic Press, Orlando, FL