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Inhibition of Glucose Transport and Direct Interactions with Type 1 Facilitative Glucose Transporter (GLUT-1) by Etomidate, Ketamine, and Propofol

A COMPARISON WITH BARBITURATES

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ABSTRACT. Ketamine, etomidate, propofol, and pentobarbital were compared for effects on and interactions with the type 1 facilitative glucose transporter (GLUT-1). Fluxes of radiolabeled hexoses were used to determine the effects of anesthetics on GLUT-1 function. Hypotonic hemolysis of human erythrocytes was used to assess perturbations of membrane integrity. Quenching of intrinsic protein fluorescence was used to assess the direct interactions of anesthetics with purified GLUT-1. Pentobarbital, ketamine, etomidate, and propofol inhibited glucose transport in murine fibroblasts with IC_{50} values of 0.8, 1.6, 0.1, and 0.4 mM, respectively. Pentobarbital, ketamine, etomidate, and propofol also inhibited sugar transport in human erythrocytes. The IC_{50} values for pentobarbital and ketamine exhibited substrate dependence for equilibrium exchange but not unidirectional effluxes. This was not observed for etomidate. Propofol did not inhibit equilibrium exchange but did inhibit unidirectional efflux with little substrate dependence. Pentobarbital protected against hemolysis, whereas etomidate and ketamine promoted hemolysis of erythrocytes. Propofol had no effect on membrane integrity. Pentobarbital, ketamine, and etomidate all interacted directly with GLUT-1, with apparent K_d values of 2.2, 0.8, and 0.5 mM, respectively. Like barbiturates, ketamine, etomidate, and propofol inhibited GLUT-1 at concentrations near to those used pharmacologically. Inhibition of GLUT-1 by these intravenous general anesthetics was complex, exhibiting differential kinetic effects on equilibrium exchange versus unidirectional fluxes and contrasting substrate dependencies. Like barbiturates, ketamine and etomidate bound to GLUT-1 with affinities that paralleled inhibition of glucose transport. As a class, intravenous general anesthetics, in contrast to inhalation anesthetics, inhibit GLUT-1-mediated glucose transport. BIOCHEM PHARMACOL **60**;5: 651–659, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. binding specificity; cerebral metabolism; membrane transporters; protein conformation

Barbiturates, which are commonly used anesthetics, have been demonstrated to alter cerebral metabolism and impact on neurophysiology [1, 2]. Barbiturates act by reducing local cerebral blood flow, neuroexcitation, and metabolism [2, 3]. Barbiturates suppress glucose transfer across the blood–brain barrier in rats [4, 5]. Since GLUT-1† is the predominant facilitative glucose transporter in the blood–brain barrier *in vivo* [6], this isoform was proposed as a potential target for barbiturates [7]. In support of this, Honkanen *et al.* [8] have shown barbiturates to inhibit glucose transport in cultured mammalian cells and human erythrocytes, as well as to interact directly with purified GLUT-1. This inhibition of glucose transport by barbiturates does not correlate with a generalized alteration of the physical state of the lipid bilayer [8]. Kinetic studies have suggested that barbiturate inhibition of GLUT-1 function is of the non-competitive type and is likely to involve preferential interaction with a form of the carrier that is unoccupied by substrate [9]. Recently, Naftalin and Arain [10] have reevaluated the kinetics of barbiturate inhibition of GLUT-1, using more sensitive methods. They demonstrated that this inhibition is of the mixed uncompetitive type and suggested that the anesthetic interacts with a non-catalytic site on the carrier. Both kinetic analyses demonstrated that equilibrium exchange sugar flux is less sensitive to barbiturate inhibition than unidirectional sugar flux. Furthermore, both interpretations are consistent with barbiturate preferentially interacting with GLUT-1 unoccupied by substrate and thereby trapping it in a catalytically

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† *Abbreviations*: FB, fluorescence buffer; GLUT-1, type 1 facilitative glucose transporter; HLB, hypotonic lysis buffer; and TB, transport buffer.

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inefficient state that impedes the glucose-induced conformational changes necessary for glucose translocation. This is supported by the seminal inhibition studies of Widdas and associates [11, 12] and the very recent inhibitor binding studies of Carruthers and coworkers [13]. Barbiturate inhibition of GLUT-1 was shown to correlate approximately with the overall lipid solubility and pharmacology of barbiturates, and to require the presence of hydrophobic side chains on the core barbiturate structure [14]. Inhibition of GLUT-1 by barbiturates is specific with respect to anesthetic class, in that halogenated hydrocarbon anesthetics do not alter GLUT-1 function significantly [8, 14]. Recent results also indicate that this process exhibits molecular specificity, in that not all isoforms of the facilitative glucose transporters are inhibited by barbiturates [14].

Like barbiturates, several other i.v. general anesthetics, such as etomidate, ketamine, and propofol, can alter cerebral metabolism and have an impact on neurophysiology [15]. In the present study, we tested the possibility of GLUT-1 as a target for etomidate, ketamine, and propofol and compared their effects on glucose transport with those of barbiturates. The effect of these i.v. general anesthetics on glucose transport in cultured murine fibroblasts, which predominantly express GLUT-1, was examined. We also studied the effects of these i.v. general anesthetics on the kinetic properties of GLUT-1 in human erythrocytes. The effects of these i.v. general anesthetics on the general integrity of erythrocyte membranes were compared. The direct interaction of these i.v. general anesthetics with purified GLUT-1 was assessed with a quenching of intrinsic fluorescence assay.

MATERIALS AND METHODS

Materials

Most chemicals, including pentobarbital and ketamine, were obtained from the Sigma Chemical Co. Pentobarbital and ketamine were prepared as 200 mM stocks in water. Stocks were divided into single-use aliquots, lyophilized, and stored desiccated at 4°. Immediately before use, aliquots were dissolved in TB consisting of 140 mM NaCl, 2.5 mM $MgSO_4$, 5 mM KCl, 1 mM $CaCl_2$, and 20 mM HEPES, pH 7.4. Etomidate, a Janssen Biotech product, was obtained from Research Diagnostics, Inc. Propofol was obtained from the Aldrich Chemical Co. Concentrated solutions of etomidate and propofol were prepared fresh daily in 10% DMSO and diluted in TB. Radioisotopes were obtained from Dupont–NEN.

Glucose Transport in Cultured Murine Fibroblasts

3T3-C2 murine fibroblasts were obtained and maintained as previously described [8, 14]. [^{3}H]-2-dGlc uptake was assayed and analyzed essentially as described previously [8, 14].

Equilibrium Exchange and Zero-trans Unidirectional Sugar Efflux by Human Erythrocytes

Fresh human erythrocytes were isolated as described previously [8, 9, 14]. Cells either were used immediately or were stored overnight in a solution of TB with the addition of 5 mM Glc. Stored cells were washed three times with TB prior to use. Sugar effluxes under equilibrium exchange ([^{3}H]Glc) and unidirectional ([^{3}H]MeGlc) conditions were assayed and analyzed essentially as described previously [8, 9, 14].

Hemolytic Protection Assay

The effects of the i.v. general anesthetics on hypotonic hemolysis of human erythrocytes were determined essentially as described by Motais *et al.* [16]. Briefly, washed human erythrocytes were diluted to a hematocrit of 40% in TB. Aliquots of these cells (100 μL) were incubated with the indicated agents, added from a concentrated stock (20x), for 15 min at room temperature. TB was diluted ~53% with H_2O to obtain an HLB, which caused ~25% total lysis in the absence of any drug. Hemolysis was initiated by diluting (80-fold) small aliquots (5 μL) of pretreated cells with a large volume of HLB (0.4 mL). After a 5-min incubation at 4°, cells/membranes were sedimented by centrifugation (10,000 *g*, 1 min, 4°). Then hemoglobin content of the supernatant was determined by absorbance at 413 nm. Total (100%) hemolysis was determined in parallel by using H_2O instead of HLB. Hemolysis for each condition is expressed as a percentage of total lysis for that condition.

Fluorescence Quenching Studies for Purified GLUT-1

GLUT-1 was purified from alkali-stripped human erythrocyte membranes and reconstituted into endogenous phospholipids as previously described [8, 14]. Purified GLUT-1 was dialyzed into FB consisting of 100 mM NaCl, 1 mM EDTA, and 75 mM HEPES, pH 7.4, and stored frozen at −70° in ~1-mL aliquots. Before use, a fresh aliquot of GLUT-1 (~300 μg/mL) was thawed rapidly, probe-sonicated at 50 W for 30 sec at 20°, and stored at 4° for ≤ 24 hr. Intrinsic fluorescence of purified GLUT-1 was measured and analyzed during titrations with anesthetics essentially as described previously [8, 14]. Also as described previously [8, 14], absorbance determinations at the wavelengths used for excitation and determination of emission intensities were utilized to correct for the small inner filter effects.

RESULTS

Inhibition of GLUT-1-Mediated Glucose Transport in 3T3-C2 Murine Fibroblasts

The predominant glucose transporter of murine fibroblasts is GLUT-1 [8, 14]. The i.v. general anesthetics pentobar-

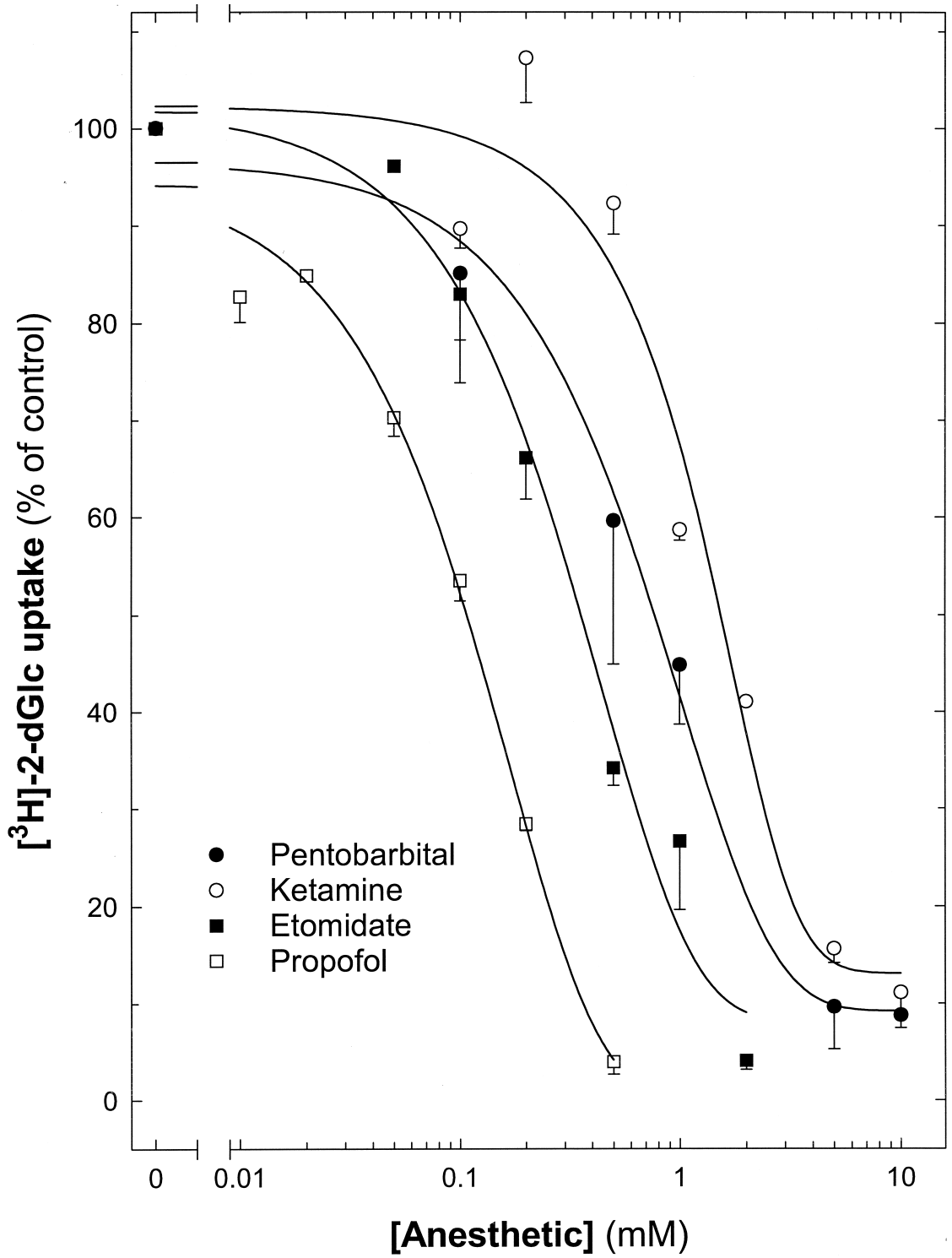


FIG. 1. Inhibition of hexose transport in 3T3-C2 murine fibroblasts by i.v. general anesthetics. 3T3-C2 cells were grown to confluence in 35 mm six-well dishes and re-fed 12–24 hr before use. Then cells were preincubated at 23° for 15 min with different concentrations of (●) pentobarbital, (○) ketamine, (■) etomidate, or (□) propofol. [^{3}H]-2-dGlc (100 μM, 1 μCi/mL) uptake then was assayed at 23°, for 15 min in the continued presence of anesthetic. Results are expressed as percent of control (2.67 ± 0.44 nmol [^{3}H]-2-dGlc/15 min·mg cellular protein from 15 representative experiments). The means of several experiments (N ≥ 3) are presented. Each data set is normalized to its respective control. The error bars indicate ± SD. The lines are meant to guide the eye of the reader.

bital, ketamine, etomidate, and propofol inhibited GLUT-1 mediated glucose transport in 3T3-C2 murine fibroblasts by ≥ 90% at 10 mM (Fig. 1). The vehicle [0.1% (v/v) DMSO] did not alter glucose transport. The relative potency of these i.v. general anesthetics was determined from the respective IC_{50} values (Table 1) and was etomidate (0.1

TABLE 1. IC_{50} **and** K_d **values for inhibition and interaction with GLUT-1 by i.v. general anesthetics**

Assay system	Substrate concentration	Assay parameter	Intravenous general anesthetic			
			Pentobarbital	Ketamine	Etomidate	Propofol
[^{3}H]-dGlc uptake by 3T3-C2 murine fibroblasts*	100 μM Glc	IC_{50} (mM)	0.8	1.6	0.1	0.4
Equilibrium exchange Glc efflux by human erythrocytes†	5 mM Glc	IC_{50} (mM)	2.5	8.0	1.0	> 10
	100 mM Glc	IC_{50} (mM)	6.0	> 10	0.9	> 10
Unidirectional Glc effflux by human erythrocytes‡	5 mM MeGlc	IC_{50} (mM)	1.0	2.1	0.3	0.4
	100 mM MeGlc	IC_{50} (mM)	0.6	1.8	0.1	0.2
Quenching of intrinsic protein fluorescence of purified GLUT-1§	Not applicable	K_d (mM)	2.2	0.8	0.5	Not done

*Values were obtained from fits of simple four-parameter logistic functions of data from Fig. 1.
†Values were obtained from fits of simple four-parameter logistic functions of data from Fig. 2.
‡Values were obtained from fits of simple four-parameter logistic functions of data from Fig. 3.
§Values were obtained from fits of simple rectangular-hyperbolic functions of data from Fig. 5.

mM) > propofol (0.4 mM) > pentobarbital (0.8 mM) > ketamine (1.6 mM).

Inhibition of Equilibrium Exchange Glucose Efflux in Human Erythrocytes

The effects of pentobarbital, etomidate, ketamine, and propofol on equilibrium exchange glucose efflux were examined at sub- and supersaturating substrate concentrations (5 and 100 mM Glc) (Fig. 2). Pentobarbital, etomidate, and ketamine inhibited equilibrium exchange efflux in a concentration-dependent manner at both 5 and 100 mM glucose. The IC_{50} values for pentobarbital, ketamine, and etomidate at 5 mM Glc were 2.5, 8.0, and 1.0 mM, respectively, and the IC_{50} values at 100 mM Glc were 6.0 mM, > 10 mM, and 0.9 mM, respectively (Table 1). For pentobarbital and ketamine, less inhibition was observed at the 100 mM Glc concentration, and inhibition therefore was dependent on substrate concentration. In contrast, etomidate did not exhibit substrate dependency under these conditions. Propofol, at concentrations up to 0.5 mM, did not inhibit equilibrium exchange efflux at either substrate concentration. Propofol could not be tested at higher concentrations due to solubility problems, as well as spontaneous hemolysis of erythrocytes under these conditions.

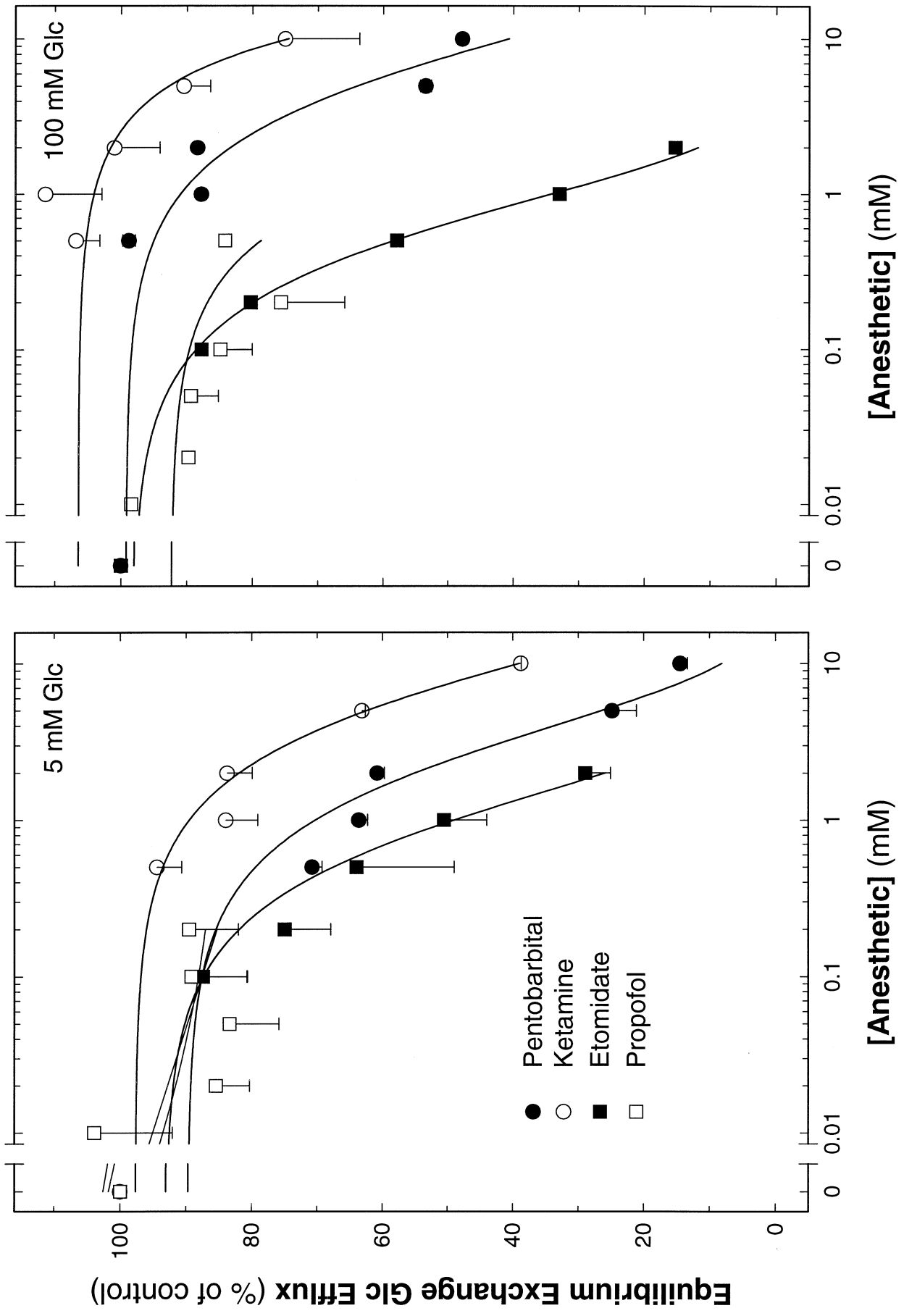
Inhibition of Unidirectional Zero-trans Glucose Efflux in Human Erythrocytes

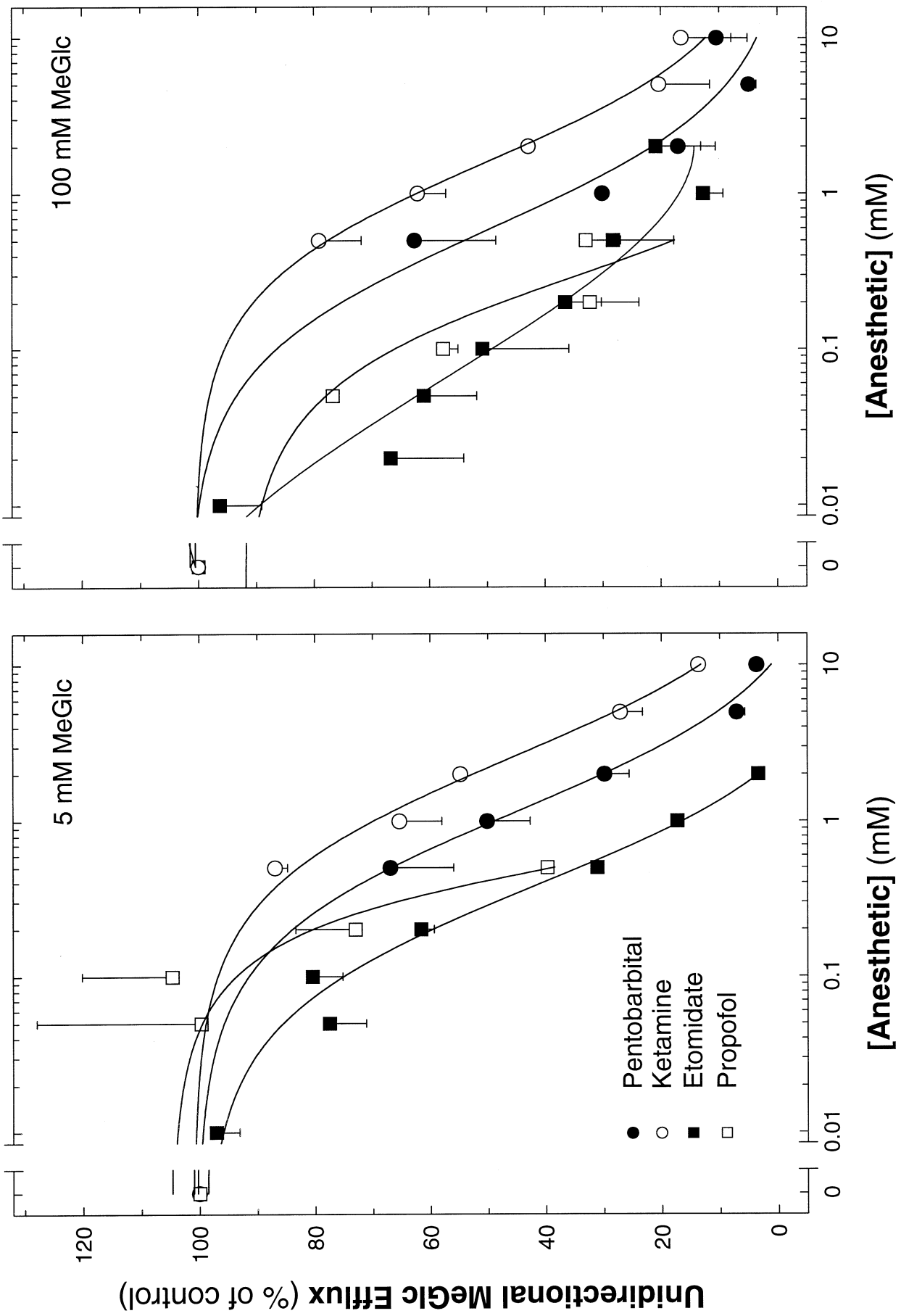
The effects of pentobarbital, ketamine, etomidate, and propofol on unidirectional zero-trans glucose efflux also were examined at sub- and supersaturating substrate concentrations (5 and 100 mM MeGlc) (Fig. 3). All of the i.v. general anesthetics tested inhibited unidirectional zero-trans glucose efflux in a concentration-dependent manner. The IC_{50} values for pentobarbital, ketamine, etomidate, and propofol at 5 mM MeGlc were 1.0, 2.1, 0.3, and 0.4 mM, respectively, and the IC_{50} values at 100 mM MeGlc were 0.6, 1.8, 0.1, and 0.2 mM, respectively (Table 1). Inhibition of unidirectional efflux by pentobarbital, ketamine, etomidate, and propofol was essentially independent of substrate concentration. For all of the i.v. general anesthetics tested, inhibition was much greater for unidirectional than for equilibrium exchange efflux.

Membrane Integrity Determined by Hypotonic Hemolysis

It has been shown previously that some anesthetics protect erythrocytes from hypotonic hemolysis [16]. The membrane expansion hypothesis suggests that a change in membrane area allows for greater volume changes before hypotonic hemolysis will occur. The effects of pentobarbital, etomidate, ketamine, and propofol on hypotonic hemolysis of human erythrocytes were examined (Fig. 4). Pentobarbital protected the membrane from hypotonic hemolysis, but this effect was small at the K_d for inhibition of glucose transport. Etomidate and ketamine induced hypotonic hemolysis at concentrations greater than 0.5 mM. These effects were also small at the corresponding K_d values for inhibition of glucose transport. Propofol had no effect on hypotonic hemolysis at the concentrations tested.

FIG. 2. Inhibition of equilibrium exchange efflux in human erythrocytes by i.v. general anesthetics. Isolated human erythrocytes were loaded with 5 mM (left panel) or 100 mM Glc (right panel), radio-loaded with [^{3}H]Glc, and preincubated (15 min) with different concentrations of (●) pentobarbital, (○) ketamine, (■) etomidate, or (□) propofol. Equilibrium exchange sugar efflux was assayed in the continued presence of the indicated agents with external Glc concentrations equal to the loaded Glc concentrations. K_{app} values were calculated from the first-order exponential fits of the data. Percent of control values (K_{app} = 0.149 ± 0.027 sec^{-1} from 5 representative experiments at 5 mM Glc and 0.071 ± 0.020 sec^{-1} from 5 representative experiments at 100 mM Glc) was calculated from the K_{app} values at each concentration of anesthetic as follows: 100 × [$^{anesthetic}K_{app}/^{control}K_{app}$]. The error bars indicate ± SD. The data shown are the average of several experiments (N ≥ 3). The lines are meant to guide the eye of the reader.


Equilibrium Exchange Glc Efflux (% of control)
100
80
60
40
20
0
5 mM Glc
100 mM Glc
Pentobarbital
Ketamine
Etomidate
Propofol
0
0.01
0.1
1
10
[Anesthetic] (mM)


Unidirectional MeGlc Efflux (% of control)
120
100
80
60
40
20
0
5 mM MeGlc
100 mM MeGlc
Pentobarbital
Ketamine
Etomidate
Propofol
0
0.01
0.1
1
10
[Anesthetic] (mM)

←

FIG. 3. Inhibition of unidirectional zero-trans efflux in human erythrocytes by i.v. general anesthetics. Isolated human erythrocytes were loaded with 5 mM (left panel) or 100 mM (right panel) MeGlc, radio-loaded with [^{3}H]MeGlc, and preincubated (15 min) with different concentrations of (●) pentobarbital, (○) ketamine, (■) etomidate, or (□) propofol. Unidirectional zero-trans sugar efflux was assayed in the continued presence of the indicated agents, with osmotically equivalent external concentrations of mannitol replacing the loaded MeGlc. K_{app} values were calculated from the first-order exponential fits of the data. Percent of control values (K_{app} = 0.251 ± 0.066 sec^{-1} from 7 representative experiments at 5 mM MeGlc and 0.062 ± 0.014 sec^{-1} from 5 representative experiments at 100 mM MeGlc) was calculated from the K_{app} values at each concentration of anesthetic as follows: 100 × [$^{anesthetic}K_{app}$/$^{control}K_{app}$]. The error bars indicate ± SD. The data shown are the average of several experiments (N ≥ 3). The lines are meant to guide the eye of the reader.

Quenching of Intrinsic Fluorescence of Purified GLUT-1

Pentobarbital has been shown previously to interact directly with GLUT-1, a result demonstrated by assaying quenching of the intrinsic fluorescence of the carrier [8, 14]. We observed similar concentration-dependent quenching of the intrinsic fluorescence of GLUT-1 upon titration with pentobarbital, ketamine, and etomidate (Fig. 5). The K_d values for pentobarbital, ketamine, and etomidate were 2.2, 0.8, and 0.5 mM, respectively (Table 1). The values calculated for maximal quenching were ~60, ~20, and ~50%, respectively. The direct interactions of propofol with GLUT-1 could not be studied under these conditions due to a very large inner filter effect that was not observed for pentobarbital, ketamine, or etomidate.

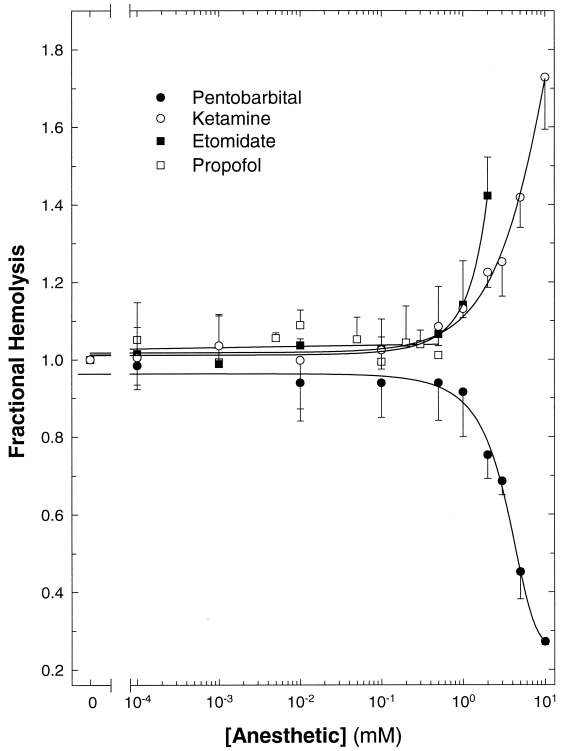


FIG. 4. Effects of i.v. general anesthetics on hypotonic hemolysis of human erythrocytes. Washed human erythrocytes were diluted to 40% hematocrit in TB and preincubated with different concentrations of (●) pentobarbital, (○) ketamine, (■) etomidate, or (□) propofol for 15 min at room temperature. They were subjected to hypotonic hemolysis by adding 5 μL of cell suspension to 400 μL HLB. After incubation at 4° for 5 min, cells/membranes were centrifuged, and hemoglobin content of the supernatant was determined. Hemolysis for each condition is expressed as a percentage of total (H_2O-mediated) hemolysis for that condition. The error bars indicate ± SD. Data shown are the average of several experiments (N ≥ 3). The lines are meant to guide the eye of the reader.

DISCUSSION

Inhibition of Glucose Transport by i.v. General Anesthetics

Etomidate, ketamine, and propofol, like barbiturates [8, 9, 14], inhibited glucose transport in murine fibroblasts (Fig. 1) at concentrations near to those used pharmacologically. In fact, the IC_{50} of 100 μM for etomidate (Table 1) is similar to the concentration employed by Krasowski *et al.* [17] to elicit a maximum activation (~50%) of the type A γ-aminobutyric acid receptor. Intravenous general anesthetics, unlike halogenated hydrocarbon inhalation anesthetics [14], can therefore specifically inhibit GLUT-1. These findings for GLUT-1-mediated glucose transport are consistent with those of Krasowski *et al.* [17], who have demonstrated for the type A γ-aminobutyric acid receptor that the structural requirements for modulation by volatile and i.v. general anesthetics may be quite distinct. Barbiturates are known to suppress glucose transfer across the blood–brain barrier *in vivo* [4, 5]. It is not known if other i.v. general anesthetics, such as etomidate, ketamine, and propofol, or, for that matter, volatile anesthetics, have similar *in vivo* effects.

Kinetic Complexity of GLUT-1-Mediated Glucose Transport Inhibition by i.v. General Anesthetics

Etomidate, ketamine, and propofol, like barbiturates [8, 9, 14], inhibited glucose transport in human erythrocytes (Figs. 2 and 3, and Table 1) in a kinetically restrictive fashion. As observed for barbiturates [9, 10], inhibition of GLUT-1 by these i.v. general anesthetics was much greater for unidirectional flux than for equilibrium exchange flux. Substrate dependencies for this process are, however, kinetically complex. No clear dependence on substrate concentration was observed for inhibition of unidirectional flux by any of the i.v. general anesthetics tested. Inhibition by pentobarbital and ketamine of equilibrium exchange flux exhibited a clear dependence on substrate concentration that was not observed for etomidate. Inhibition of equilibrium exchange flux by propofol was not observed at the

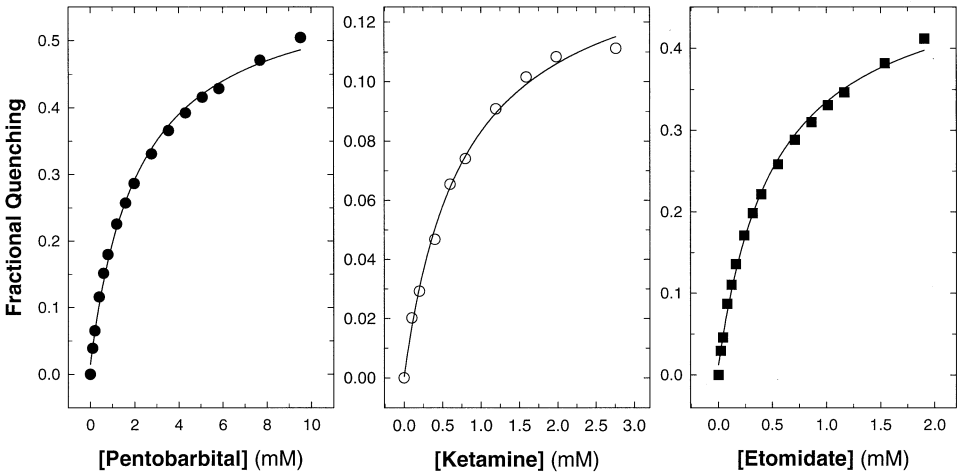


FIG. 5. Quenching of the intrinsic fluorescence of GLUT-1 by i.v. general anesthetics. GLUT-1-containing proteoliposomes (~25 μg/mL of GLUT-1 protein, 400 nM GLUT-1) in FB were incubated with different concentrations of (●) pentobarbital, (○) ketamine, or (■) etomidate. Intrinsic fluorescence intensities and absorbance were determined for each condition. Fluorescence intensities were corrected for dilution, and absorbance determinations were used to correct for the inner filter effect. Fractional quenching (FQ) was determined from the corrected fluorescence intensity values as follows: $FQ = 1 - F/F_o$, where F_o and F are the initial and experimental intensities. The curves were computer-fit to simple rectangular-hyperbolic functions of saturable binding. The error bars indicating SD are not observed because they are smaller than the plotted symbols on the graphs.

concentrations studied, so the substrate dependence of the process could not be evaluated directly. Generally, this kinetic analysis suggests that inhibition of GLUT-1 by the i.v. general anesthetics studied occurs by virtue of a preferential interaction with GLUT-1 unoccupied by substrate that traps it in a catalytically inefficient state [9, 10]. This impedes the glucose-induced conformational changes necessary for glucose translocation. This thinking is also very consistent with the classic inhibition studies of Widdas and associates [11, 12], in which the asymmetry of the carrier was defined by alternating inward and outward conformations. It is also in agreement with the recent interpretations of Carruthers and coworkers [13], in which the carrier exists as an oligomeric structure, with each GLUT-1 monomer representing a potential sugar translocation pathway with putative sugar import and export sites. There are, however, complex subtleties to the kinetics of this process that may be explained by the detailed interpretations of Naftalin and Arain [10]. Namely, carrier in the equilibrium exchange mode might have a lower affinity for i.v. anesthetics than carrier in the unidirectional mode. This may be explained by a kind of hindrance in which double occupancy of the carrier by substrate blocks access to the hydrophobic sites interacting with i.v. anesthetics. The lack of effect of substrate concentration on etomidate inhibition of equilibrium exchange is but one example in this regard. Taken in concert, this suggests that the sites and the physical chemical nature of the interactions of i.v. general anesthetics with GLUT-1 may, as observed for the type A γ-aminobutyric acid receptor [17], exhibit structural specificity and selectivity that requires a more detailed biophysical and biochemical analysis.

Effects of i.v. General Anesthetics on Membrane Integrity

We have shown previously [8] that inhibition of GLUT-1 by barbiturates does not correlate with a generalized alteration of the physical state, i.e. fluidity, of the lipid bilayer. We have also demonstrated previously [8] that band 3-mediated anion exchange (i.e. $^{35}SO_4^{2-}$ uptake) by human erythrocytes is much less sensitive to inhibition by pentobarbital than is glucose transport. We have expanded on this question, herein, by employing a hypotonic hemolysis assay as a more generalized measure of membrane integrity. Using this assay, we observed that pentobarbital protected erythrocytes from hemolysis, whereas ketamine and etomidate promoted hemolysis (Fig. 4). Propofol, at the concentrations tested, did not alter hypotonic lysis. Clearly all of the i.v. general anesthetics studied inhibited GLUT-1, yet they had diversified effects on generalized membrane integrity. Additionally, the effects of the i.v. general anesthetics on hypotonic hemolysis were small at the corresponding K_d values for GLUT-1 inhibition. The major conclusion that can be drawn from the present, as well as the past [8, 9, 14], observations is that inhibition of GLUT-1 by i.v. general anesthetics does not correlate even qualitatively with a generalized alteration in membrane integrity, and it is relatively specific.

Direct Interaction of i.v. General Anesthetics with Purified GLUT-1

Employing quenching of the intrinsic fluorescence of GLUT-1 as an assay, we have demonstrated previously [8, 14] that barbiturates, but not halogenated hydrocarbon

inhalation anesthetics, interact directly with, i.e. bind to, GLUT-1 protein and that this interaction correlates with inhibition of glucose transport. Both etomidate and ketamine, like pentobarbital, quenched the intrinsic fluorescence of purified GLUT-1 in a concentration-dependent manner (Fig. 5). This direct interaction of i.v. general anesthetics with GLUT-1 also paralleled the ability of these agents to inhibit glucose transport. Molecular biology and physical biochemistry technologies may allow us to begin to dissect the binding sites and conformational states of GLUT-1 with which these i.v. general anesthetics interact.

We have demonstrated the following: (i) GLUT-1 is functionally sensitive to inhibition by the i.v. general anesthetics etomidate, ketamine, and propofol; (ii) unlike what is seen with inhalation anesthetics, inhibition of GLUT-1-mediated glucose transport by these i.v. general anesthetics is similar to that observed for barbiturates; (iii) like barbiturates, inhibition by these i.v. general anesthetics probably involves trapping the carrier in a catalytically inefficient state that impedes the glucose-induced conformational changes necessary for glucose translocation; (iv) the kinetics of inhibition by the different i.v. general anesthetics are, however, complex, exhibiting differential kinetic effects on equilibrium exchange versus unidirectional fluxes and contrasting substrate dependencies; (v) inhibition of GLUT-1 by i.v. general anesthetics does not correlate with a generalized alteration in membrane integrity; (vi) like barbiturates, ketamine and etomidate interact directly with GLUT-1, and the characteristics of this interaction parallel those of glucose transport inhibition; and (vii) the *in vivo* consequences of glucose transporter inhibition by i.v. general anesthetics to cerebral metabolism should be further evaluated and may have unforeseen clinical implications.

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References

1. Shapiro HM, Barbiturates in brain ischaemia. *Br J Anaesth* **57:** 82–95, 1987.
2. Nakashima K, Todd MM and Warner DS, The relation between cerebral metabolic rate and ischemic depolarization. A comparison of the effects of hypothermia, pentobarbital, and isoflurane. *Anesthesiology* **82:** 1199–1208, 1995.
3. Todd MM and Warner DS, A comfortable hypothesis reevaluated: Cerebral metabolic depression and brain protection during ischemia. *Anesthesiology* **76:** 161–164, 1992.
4. Gjedde A and Rasmussen M, Pentobarbital anesthesia reduced blood-brain glucose transfer in the rat. *J Neurochem* **35:** 1382–1387, 1980.
5. Otsuka T, Wei L, Bereczki D, Acuff V, Patlak C and Fenstermacher J, Pentobarbital produces dissimilar changes in glucose influx and utilization in brain. *Am J Physiol* **261:** R265–R275, 1991.
6. Vannucci SJ, Maher F and Simpson IA, Glucose transporter proteins in brain: Delivery of glucose to neurons and glia. *Glia* **21:** 2–21, 1997.
7. Fenstermacher JD, Wei L, Acuff V, Lin S-Z, Chen J-L, Bereczki D, Otsuka T, Nakata H, Tajima A, Hans F-J, Ghersi-Egea J-F, Finnegan W, Richardson G, Haspel H and Patlak C, The dependency of influx across the blood-brain barrier on blood flow and the apparent flow-independence of glucose influx during stress. In: *New Concepts of a Blood-Brain Barrier* (Ed. Greenwood J), pp. 89–101. Plenum Press, New York, 1995.
8. Honkanen RA, McBath H, Kushmerick C, Callender GE, Scarlata SF, Fenstermacher JD and Haspel HC, Barbiturates inhibit hexose transport in cultured mammalian cells and human erythrocytes and interact directly with purified GLUT-1. *Biochemistry* **34:** 535–544, 1995.
9. El-Barbary A, Fenstermacher JD and Haspel HC, Barbiturate inhibition of GLUT-1 mediated hexose transport in human erythrocytes exhibits substrate dependence for equilibrium exchange but not unidirectional sugar flux. *Biochemistry* **35:** 15222–15227, 1996.
10. Naftalin RJ and Arain M, Interactions of sodium pentobarbital with d-glucose and l-sorbose transport in human red cells. *Biochim Biophys Acta* **1419:** 78–88, 1999.
11. Basketter DA and Widdas WF, Asymmetry of the hexose transfer system in human erythrocytes. Comparison of the effects of cytochalasin B, phloretin, and maltose as competitive inhibitors. *J Physiol (Lond)* **278:** 389–401, 1978.
12. Baker GF, Basketter DA and Widdas WF, Asymmetry of the hexose transfer system in human erythrocytes. Experiments with non-transportable inhibitors. *J Physiol (Lond)* **278:** 377–388, 1978.
13. Hamill S, Cloherty EK and Carruthers A, The human erythrocyte sugar transporter presents two sugar import sites. *Biochemistry* **38:** 16974–16983, 1999.
14. Haspel HC, Stephenson KN, Davies-Hill T, El-Barbary A, Lobo JF, Croxen RL, Mougrabi W, Koehler-Stec EM, Fenstermacher JD and Simpson IA, Effects of barbiturates on facilitative glucose transporters are pharmacologically specific and isoform selective. *J Membr Biol* **169:** 45–53, 1999.
15. Cheng MA, Theard MA and Tempelhoff R, Intravenous agents and intraoperative neuroprotection: Beyond barbiturates. *Crit Care Clin* **13:** 185–199, 1997.
16. Motais R, Baroin A, Motais A and Baldy S, Inhibition of anion and glucose permeabilities by anesthetics in erythrocytes: The mechanisms of action of positively and negatively charged drugs. *Biochim Biophys Acta* **599:** 673–688, 1988.
17. Krasowski MD, Koltchine VV, Rick CE, Ye Q, Finn SE and Harrison NL, Propofol and other intravenous anesthetics have sites of action on the γ-aminobutyric acid type A receptor distinct from that for isoflurane. *Mol Pharmacol* **53:** 530–538, 1998.