Influence of Anesthetic Regimens on Intestinal Absorption in Rats

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We compared the influence of anesthetic regimens using urethane (U), pentobarbital (P), ether (E), and ketamine/midazolam (K) on the intestinal absorption of several probes using a single-pass perfusion technique in rats. The selected probes were D-glucose (1 mM) for the resistance of the unstirred water layer (UWL), D-glucose (100 mM) for the capacity of carrier-mediated D-glucose transport, L-glucose, and urea for membrane-limited passive transport, and tritiated water (H2O) for blood flow at the absorption site. The absorbed fraction of D-glucose (1 mM) was the smallest for U and the largest for P, suggesting that the resistance of UWL is the largest for U and the smallest for P. The absorbed fraction of D-glucose (100 mM) was the largest for P (U = E = K < P), suggesting a higher capacity of carrier-mediated D-glucose transport for P. The absorbed fraction of urea was similar for all anesthetics, while that of L-glucose was the smallest for K (U = P = E > K). Although the results for these two markers of membrane-limited passive transport were inconsistent, the passive permeability of the intestinal membrane may be lower when treating with K. The intestinal absorptions of D-glucose (1 and 100 mM), L-glucose, and urea were, in general, lower with any of the anesthetics than under nonanesthesia (N), suggesting increased resistance of UWL and decreased intestinal membrane permeability by carrier-mediated and passive transport under anesthesia. The only exception was the absorption of D-glucose (100 mM) under P, which was comparable to that under N. The results were similar when considering the membrane permeability clearance estimated by correcting for the resistance of UWL. The blood flow at the absorption site, estimated from the absorption of H2O, was decreased under U, compared with N, and increased under K (U < P = E = N < K). The information obtained in this study is useful for the comprehensive interpretation of intestinal absorption data obtained under different anesthetic regimens and the prediction of intestinal absorption in vivo.

KEY WORDS: intestinal absorption; rat; anesthetic; urethane; pentobarbital; ether, ketamine; midazolam.

INTRODUCTION

Intestinal perfusion techniques have been used to characterize the intestinal absorption of drugs. Perfusion experiments using laboratory animals such as rats have been performed mostly under anesthetic regimens, e.g., using urethane or pentobarbital (1-5), to facilitate the handling of animals and perfusion procedures. However, anesthesia could affect intestinal absorption and drug disposition. In a comparative study on cardiac output and regional blood flow under various anesthetic regimens, Gumbleton et al. found the most severe effects on the hemodynamic profile to occur in rats anesthetized with urethane (6). Another study by

Materials

[14C]glucose (9.6 GBq/mmol), [14C]urea (2.1 GBq/mmol), tritiated water (37.0 MBq/g), [3H]inulin (15.8 GBq/g), [14C]inulin (74.0 MBq/g), and Biofluor scintillation cocktail were purchased from New England Nuclear Co. (Boston, MA). Urethane (Tokyo Kasei Kogyo Co., Tokyo), pentobarbital (Nembutal, Dainippon Pharmaceuticals Co., Osaka), ketamine (Ketalar, Sankyo Co., Tokyo), and midazolam (Dormicum, Yamanouchi Pharmaceuticals Co., Tokyo) were commercially obtained. All other reagents were of analytical grade.

Anesthetic Regimens

Male Wistar rats (250-300 g) were used without fasting prior to experiments. The rats were given one of the following anesthetic regimens: U, urethane (1.13 g/4.5 mL/kg, i.p.); P, pentobarbital sodium (50 mg/1 mL/kg, i.p.); E, ether by inhalation through natural spontaneous respiration (animal dose); and K, ketamine (80 mg/1.6 mL/kg) with midazolam (5 mg/1 mL/kg) as an initial i.p. dose followed by ketamine (20 mg/0.4 mL/kg, i.p.) every 30 min for maintenance (6). The anesthetic regimens of urethane and pentobarbital followed those generally reported in the literature.

Perfusion Experiments

Perfusion solutions consisted of 20.1 mM Na2HPO4 · 12H2O, 47.0 mM KH2PO4, 101.0 mM NaCl (pH 6.4) and contained a 14C-labeled probes with a tracer amount of [3H]inulin, nonabsorbable marker, or tritiated water as a probe with [14C]inulin. The concentration of each probe was adjusted by adding the unlabeled probe.

In situ intestinal single-pass perfusion was carried out using male Wistar rats anesthetized by one of the anesthetic regimens described above. The abdomen of each rat was opened by a midline incision, and a 10-cm intestinal segment, starting 20 cm below the duodenojejunal flexure or
about 30 cm below the pylorus, was selected. The segment was internally flushed with saline to remove intestinal contents, attached with inflow and outflow cannulas made of polyethylene tubing (internal diameter, 0.3 cm), placed on a flat plate on the abdomen, and perfused at 0.15 mL/min with a peristaltic pump (Minipulse III, Gilson Co., France). The outflow solution was collected for 20 min at 5-min intervals, starting 25 min after the initiation of perfusion. The temperature was monitored at the perfused segment and maintained at 37°C by using a thermostat and a heating lamp.

Perfusion experiments in unanesthetized rats were performed as described in our previous report (1). Briefly, the surgical operation to attach cannulas was carried out under light ether anesthesia and perfusion was initiated right after the rat regained consciousness in a Bollman cage.

Scintillation cocktail (1.5 mL; Biofluor) was added to the 100-μL aliquots of inflow and outflow solutions, and the radioactivity was determined by liquid scintillation counting.

Data Analysis

The fraction absorbed \((F_a)\) of each probe was estimated as the fraction disappeared from the intestinal lumen, correcting for a minor volume change by using inulin as a non-absorbable marker:

\[
F_a = 1 - \frac{C_{in,1}}{C_{out,1}} \cdot \frac{C_{out}}{C_{in}} \tag{1}
\]

where \(C_{in,1}\) and \(C_{out,1}\) are the concentrations of inulin in inflow and outflow solutions, respectively, and \(C_{in}\) and \(C_{out}\) are the concentrations in inflow and outflow solutions, respectively. The \(F_a\) was determined as the average of those for four sampling periods.

A tube model incorporated with the unstirred water layer to consider the preepithelial diffusional resistance (film model) was used to estimate the intestinal membrane permeability (1). The apparent membrane permeability clearance, or the product of the apparent membrane permeability coefficient and the surface area, for the unit length of intestinal segment was estimated as follows:

\[
CL_{a,app} = -\frac{Q}{L} \cdot \ln(1 - F_a) \tag{2}
\]

where \(CL_{a,app}\) is the apparent membrane permeability clearance; \(Q\) is the perfusion rate, 0.15 mL/min in this study; and \(L\) is the length of the perfused segment, 10 cm in this study. The \(CL_{a,app}\) is related to the membrane permeability clearance \((CL_{a,m})\) and the permeability clearance of the unstirred water layer \((CL_{a,aq})\) as follows:

\[
\frac{1}{CL_{a,app}} = \frac{1}{CL_{a,m}} + \frac{1}{CL_{a,aq}} \tag{3}
\]

Since the intestinal absorption of d-glucose was reported to be unstirred water layer limited at a low concentration such as 1 mM and at a low perfusion rate such as 0.15 mL/min (1), we assumed that \(CL_{a,app}\) is equal to \(CL_{a,aq}\) for the absorption of d-glucose at 1 mM. Considering that \(CL_{a,aq}\) is proportional to the diffusion coefficient, which is inversely proportional to the square root of the molecular weight \((M)\), the \(CL_{a,aq}\) of each probe was estimated as follows:

\[
CL_{a,aq} = CL_{a,aq}(G, 1\text{ mM}) \cdot \frac{\sqrt{M_g}}{\sqrt{M}} \tag{4}
\]

where \(CL_{a,aq}(G, 1\text{ mM})\) is the \(CL_{a,aq}\) of d-glucose at 1 mM and \(M_g\) is the molecular weight of d-glucose. Finally, \(CL_{a,m}\) was estimated from the estimates of \(CL_{a,app}\) and \(CL_{a,aq}\) using Eq. (3).

Statistical significance was examined by Student’s \(t\) test, taking the data in unanesthetized rats as controls and also taking those in urethane-anesthetized rats as controls. The anesthetic regimen using urethane is widely used and is the standard procedure in our laboratory.

RESULTS

The intestinal absorption of d-glucose is known to be mostly carrier mediated, with a minor passive transport component (1). At a concentration of 1 mM, carrier-mediated d-glucose transport was reported to be in the linear phase of the Michaelis–Menten kinetics, with an apparent \(K_m\) of 30 to 50 mM, and the overall absorption process is limited by the preepithelial diffusional process in the lumen, or the permeation of the unstirred water layer (UWL) in the UWL model. The resistance of the UWL was reported to represent 93 and 85%, respectively, of the total resistance in urethane-anesthetized and unanesthetized rats at a perfusion rate of 0.16 mL/min. Thus, the absorption of d-glucose at 1 mM was assumed to serve as the marker of the resistance of UWL or preepithelial diffusion. Figure 1 shows the absorbed fraction of d-glucose at 1 mM under various anesthetic regimens. The smallest fraction absorbed in rats anesthetized with urethane suggests the largest resistance of UWL. The fraction absorbed in pentobarbital-anesthetized rats was the largest but still smaller than that in unanesthetized rats in our preceding report (1). Thus, the resistance of the UWL was found to be increased by all anesthetic regimens tested in this study, compared to that in unanesthetized rats.

For all anesthetic regimens tested, the absorbed fraction