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DIFFERENCE IN HEPATIC UPTAKE KINETICS OF ASPIRIN AND SALICYLAMIDE IN RATS*

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Abstract—Immediately after intraportal administration to rats, the ratio of liver to plasma concentrations for total aspirin was close to unity, whereas that for total salicylamide ranged from about 3 to 7. The hepatic accumulation of salicylamide appeared to be capacity-limited because the ratio decreased with increases in the dose. *In vitro* experiments with isolated hepatocytes indicated that aspirin was slowly transported into the hepatocytes by an apparently linear process only, while salicylamide was taken up very rapidly by both saturable and apparently linear transport processes. The cell to medium concentration ratio estimated for the initially net transported component of the unchanged drug was significantly larger with salicylamide, which give ratios from 3.5 to 19, than with aspirin which gave an almost constant value lower than 2 despite wide variations in the initial concentration. For the capacity-limited uptake process of salicylamide, the kinetic parameters were estimated as $V_{max} = 0.325$ nmole · (mg cellular protein)$^{-1}$ · sec^{-1} and $K_m = 201$ μM. Among various metabolic inhibitors, 2,4-dinitrophenol (50 μM) inhibited the uptake of salicylamide most extensively. The present comparison of the *in vivo* and *in vitro* data for aspirin with those for salicylamide confirmed the previously reported difference in the hepatic first-pass effect of these two drugs.

Aspirin (ASA) and salicylamide (SAM) have been reported to exhibit extensive first-pass effects when they are administered orally to rats [1, 2], more than about 60 and 75% of the oral dose of ASA and SAM, respectively, being removed by intestinal and hepatic extraction and/or metabolism during first passage through these organs. However, the relative contributions of the intestine and liver to this effect are different for these two drugs. Pharmacokinetic data have suggested that the intestinal first-pass effect is greater for ASA than for SAM, while the liver plays a more important role for SAM removal. In support of this suggestion, our previous studies demonstrated that conjugation (glucuronidation and sulfation) by the gut wall during absorption is more extensive for ASA than for SAM [3], while no comparative investigation on the hepatic extraction and/or metabolism of these two drugs has been reported elsewhere.

Based on comparisons of drug metabolism in subcellular liver preparations and in isolated hepatocytes with metabolism in perfused liver, Billings *et al.* [4] have suggested that drug metabolism in isolated hepatocytes correlates better with *in vivo* drug metabolism than does metabolism in 9000 *g* supernatant fractions or microsomes. Since the first and prerequisite step for the hepatic metabolism of drugs or chemicals is the transport or passage of the compounds into liver cells, the previous suggestion would emphasize the importance of studying the transport mechanism and kinetics for various compounds in hepatocytes. In the last decade, isolated rat hepatocytes have been widely used to characterize the mode of hepatic accumulation or to determine the rate of hepatic uptake and/or metabolism of amino acids [5], antipyrine [6], bile acids [7–9], bilirubin [10], cadmium [11], copper [12], corticosterone [13], estradiol [14, 15], ethoxybenzamide [16], ethylmorphine [17], methotrexate [18], morphine [19], nalorphine [19], ouabain [20], parathion [21], phenytoin [22], sulfobromophthalein [23–26] and zinc [27]

Because translocation across the hepatocyte sinusoidal membrane may limit the rate of hepatic removal of ASA and SAM from the circulation, the present investigation was designed to characterize and compare the uptake processes (kinetics) for these two drugs into isolated rat hepatocytes. In addition, these *in vitro* uptake data were compared with *in vivo* hepatic accumulation data after intraportal administration of ASA and SAM to rats.

MATERIALS AND METHODS

Materials. Chemicals were purchased from the following sources: [*carboxyl*-^{14}C]ASA with specific activity of 33.35 mCi/mmole and radiochemical purity of more than 99.0% from the New England Nuclear Corp. (Boston, MA, U.S.A); unlabeled ASA from the Maruishi Seiyaku Co. Ltd. (Nagoya, Japan); SAM from the Tokyo Kasei Chemical Co. (Tokyo, Japan); ^3H_2O with specific activity of 1 mCi/ml from the New England Nuclear Corp.; β-glucuronidase/arylsulfatase (from *Helix pomatia*, EC 3.2.1.31/EC 3.1.6.1) and collagenase type I (from *Clostridium histolyticum*, EC 3.4.24.3) from the Boehringer Co. (Mannheim, GmbH, F.R.G.); bov-

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ine serum albumin (Fraction V) from the Sigma Chemical Co. (St. Louis, MO, U.S.A.); choline chloride, 2,4-dinitrophenol (DNP), iodoacetic acid, KCN, lithium chloride, phloridine and trypan blue from the Wako Pure Chemical Co. (Nagoya, Japan). All other chemicals used were of analytical grade.

Animals. All animals used in the present study were male Wistar rats (300–340 g) purchased from the Shizuoka Laboratory Animal Farm (Hamamatsu, Japan) and fed *ad lib.*

Hepatic accumulation of ASA and SAM after intraportal administration. Rats were anesthetized (with 800 mg/kg urethane, i.p.), and the femoral artery and the middle ileocolic vein were cannulated with PE-50 and PE-10 tubing, respectively. The body temperature was kept at 37° with a thermostatically controlled hot plate and lamp. The drugs (5, 10, 20 or 30 μmoles/kg, in saline) were infused into the ileocolic vein (intraportally) over 30 sec. An arterial blood sample (0.25 ml) was withdrawn, and the whole liver was removed simultaneously from the rat 2 min after the start of the infusion. Total and unchanged drug concentrations in the plasma samples (100 μl) were determined as previously reported [1, 2]. The liver lobes were rinsed twice with fresh saline chilled to 4° to remove the residual hepatic blood. After weighing the whole liver, the tissue was homogenized (1 to 10 parts) in chilled saline. For the determination of total and unchanged drug, an aliquot (200 μl) of the homogenates was treated in the same way as the plasma sample.

Preparation and incubation of isolated rat hepatocytes. Isolated hepatocytes were prepared by a modification of the original procedure of Berry and Friend [28] as described earlier by Baur *et al.* [29]. Detailed procedure for this collagenase (0.05%) perfusion method has been reported previously [19, 20]. The compositions of the main buffer solutions that were oxygenated with 95% O_2–5% CO_2 were as follows: (a) perfusion buffer (pH 7.30): 121 mM NaCl, 6 mM KCl, 0.6 mM $MgSO_4$, 0.74 mM KH_2PO_4, 12 mM $NaHCO_3$ and 5 mM glucose; (b) wash buffer (pH 7.40): 131 mM NaCl, 5.2 mM KCl, 0.9 mM $MgSO_4$, 0.12 mM $CaCl_2$ and 3 mM Na_2HPO_4; and (c) incubation buffer: wash buffer added with 10 mM Tris–HCl. Stock solutions of both ASA and SAM were prepared in the incubation buffer to give final drug concentrations of 10 μM to 2 mM. All the incubation experiments were carried out at 37° unless otherwise specified. After 5 min of preincubation of cells (6.3 to 8.4 mg as protein that approximately corresponded to 6×10^5 to 8×10^5 cells) in 3.5 ml, 0.5 ml of the drug solution was added at time zero. No cofactor was added to the incubation mixture. The incubation mixture used for ASA uptake initially contained 0.1 μCi of [^{14}C]aspirin. For relative inhibition experiments on SAM uptake, 0.1 ml of various metabolic inhibitors was preincubated with 3.4 ml of the cell suspension to give a concentration of 50 μM. In Na^+-replacement experiments, all the sodium salts in the buffer, 131 mM NaCl and 3 mM Na_2HPO_4, were replaced by equimolar substitution with choline or lithium chloride and K_2HPO_4 respectively. The effect of lowering the incubation temperature was tested only at 27°.

To determine the overall velocity of uptake (V_o) of ASA or SAM, 0.1 ml of the incubated cell suspension was placed at various time intervals into polyethylene microcentrifuge tubes (Niplon Products Co., Nagoya, Japan) which had been layered previously with 50 μl of 0.7 M $HClO_4$ solution and 100 μl of silicone oil. The tubes were centrifuged for 3 sec at 4000 rpm in a tabletop microfuge, Kubota KA-1000 (Kubota Seisakusho Co., Tokyo, Japan), which is capable of extremely rapid acceleration. The amount of ASA taken into the hepatocytes was quantitated by simply cutting the centrifuged tube just above the oil–acid interface and placing the pellet layer in a scintillation vial containing 10 ml of toluene–Triton X-100 liquid scintillator [2,5-diphenyloxazole (PPO), 5 g; 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)]benzene (POPOP), 300 mg; toluene, 700 ml; Triton X-100, 300 ml] for radioactivity counting. On the other hand, the similarly separated pellets incorporating SAM were transferred directly into 2 ml of McIlvain buffer (pH 4.3) and analyzed for SAM as previously described [2]. Initial substrate concentrations in the medium were determined using the supernatant fraction obtained after centrifugation of zero-time samples. In the preliminary incubation experiments, the constituent taken into the hepatocytes even at the end of the incubation period (3 min for ASA, 24 sec for SAM) was identified to be principally unchanged ASA or SAM and not their metabolites. Since the mean volume of adherent fluid (incubation medium) that passed together with the cell pellets through the oil phase upon centrifugation has been reported to be approximately 0.8 μl/mg of cellular protein [19, 20], net content of ASA or SAM transported into the hepatocytes was evaluated by correcting for the content accompanied by the adherent fluid.

Cell viability tests. Cell viability before and after the incubation experiments was determined only by the trypan blue exclusion method of Baur *et al.* [29]. Cell preparations in which more than 95% of the cells excluded the dye were considered to be satisfactorily viable to use for the incubation experiments. Even after 24 sec or 3 min of incubation, the exclusion percentages were still found to range from 94 to 97%. Degrees of cellular membrane permeability and coupling of oxidative phosphorylation have been previously mentioned to correspond almost directly with the results from the trypan blue exclusion test [19, 20, 29].

Determination of cellular protein and aqueous cellular volumes. Cellular protein was determined by the method of Lowry *et al.* [30], with bovine serum albumin (Fraction V) as a standard. Aqueous cellular volumes of the sedimentary pellets were determined by incubating cells with ^3H_2O (0.7 mCi/ml) and determining the volume of intracellular water in the pellet fraction, yielding an average value of 2.6 ± 0.15 μl/mg of cellular protein which is the same value as previously reported [19, 20].

Radioactivity measurement. Total radioactivity in the scintillator was determined directly in a Mark II liquid scintillation spectrometer (Nuclear-Chicago Corp., Des Plaines, IL, U.S.A.). The counting efficiencies were determined automatically by a ^{133}Ba external standardization method and cpm were converted to dpm.

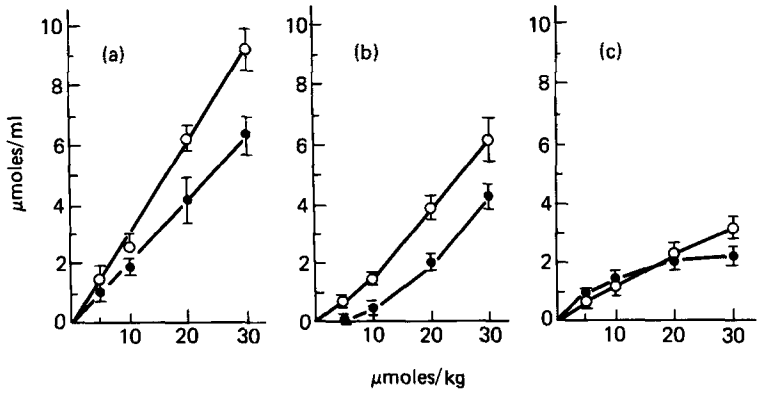

Fig. 1. Effect of an intraportal dose of ASA (○) and SAM (●) on the plasma concentration of total (a), unchanged (b) or conjugated (c) form. The plasma concentration was determined at 2 min after intraportal injection of the drug to rats anesthetized with urethane (800 mg/kg, i.p.). Each point is the mean ± S.D. of three rats. Regression line for total ASA ($r = 0.998$) and SAM ($r = 0.999$) is expressed as $Y = 0.634X$ and $Y = 0.430X$, respectively, where Y is the plasma concentration and X is the dose.

RESULTS

Comparison of hepatic accumulation of ASA and SAM after intraportal administration. Total drug concentration in the systemic circulation after intraportal administration was directly proportional to the dose for both ASA and SAM, as shown in Fig. 1. In contrast, the concentrations of unchanged and conjugated (glucuronized and/or sulfated) drugs were not proportional to the dose. Total, unchanged and conjugated concentrations of ASA or SAM accumulated in the liver at 2 min after intraportal administration, which were expressed as μmoles/g liver, are shown in Fig. 2. Although the hepatic concentration of total ASA was almost proportional to the dose, that of SAM exhibited non-linearity with dose, indicating a capacity-limited hepatic accumulation of SAM. As expected from the results in Fig. 1, both ASA and SAM accumulated in the liver immediately after the intraportal dosing were mostly as the conjugated forms, and the hepatic level of salicylic acid derived from ASA was almost insignificant. It is evident that the conjugation of SAM is capacity-limited and is much more extensive than that of ASA.

To compare the hepatic accumulation patterns of these two drugs, the liver to plasma concentration ratio for the total drug was plotted against the dose, as shown in Fig. 3. Although the ratio for SAM was extremely high (ranging from about 9 to 18) and appeared to be inversely dependent on the dose, that for ASA was only 2 to 3 and exhibited no dose dependency. If one arbitrarily converts the left ordinate in Fig. 3 into the dimensionless ratio (right ordinate) by dividing by 2.6 ml/g, which was estimated for aqueous cellular volume per gram of hepatocyte protein and thus could be approximated to be the tissue volume per dry weight (g) of the liver, the ratio for SAM would range from 3 to 7 with the decrease of the dose while that for ASA would be close to unity.

Time course of ASA and SAM uptake by isolated

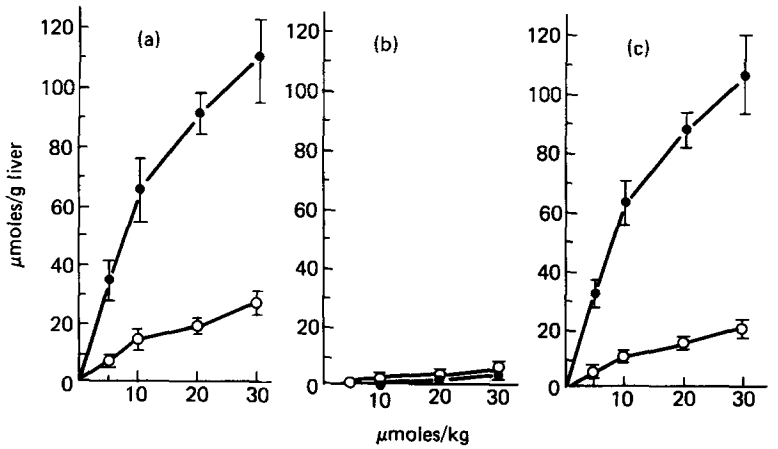

Fig. 2. Effect of an intraportal dose of ASA (○) and SAM (●) on the hepatic concentration of total (a), unchanged (b) or conjugated (c) form. The hepatic concentration was determined at 2 min after intraportal injection of the drug to the same rats as those in Fig. 1. Each point is the mean ± S.D. of three rats.

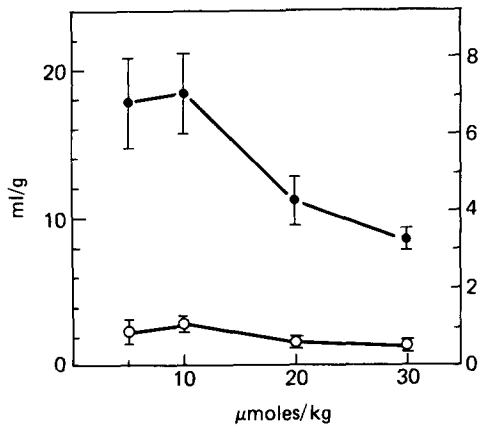

Fig. 3. Liver/plasma concentration ratio for total ASA (○) and SAM (●) at various intraportal doses. Both plasma and hepatic drug concentrations were determined at 2 min after the intraportal injection. Each point is the mean ± S.D. of three rats. Left ordinate is expressed as ml plasma/g liver, while the right ordinate is made dimensionless by dividing the left ordinate by aqueous cellular volume per gram of the hepatocytes (2.6 ml/g).

hepatocytes. Figure 4 represents the uptake of ASA and SAM per mg of hepatocellular protein with time at ten different substrate concentrations of 10 μM to 2 mM. The positive y-intercept was considered to represent instantaneous non-specific binding of the substrate to the cell surface. It increased proportionally with the initial concentration of both substrates [Y = 0.016X for SAM (r = 0.996) and Y = 0.010X for ASA (r = 0.994), where Y represents moles bound nonspecifically per mg of hepatocytes and X is the initial substrate concentration]. Uptake appeared to be linear at all concentrations tested within the incubation period of 16 sec and 3 min for SAM and ASA respectively. However, the uptake of SAM which was incubated in relatively high concentration tended to decrease after 16 sec. Initial uptake rate (V_O) for each substrate concentration was estimated from the slope of each initial linear portion.

Effect of substrate concentration on V_O. When the overall initial uptake rate (V_O) was plotted against the initial substrate concentration (Fig. 5), a straight line was obtained with ASA while the uptake of SAM was curvilinear. The overall uptake rate of SAM was much greater than that of ASA. The overall uptake process for SAM was considered to be a combination of a saturable process and an apparently linear process which was completely independent of the wide initial substrate concentrations ranging from 10 μM to 2 mM, because the relationship between the uptake rate and the initial concentration was almost linear above 400 μM. In contrast, the process for ASA seemed to be completely linear up to 2 mM and to be lacking in any saturable process. The linear transport rate constant for SAM was approximately 2.7 times greater than that for ASA as indicated by the slope of the two linear portions of the curves (Fig. 5). In the case of SAM, this linear portion was then subtracted from the overall uptake rate at each substrate concentration to yield the initial uptake rate for the saturable component and to be plotted as a hyperbolic curve as in Fig. 6a. A single reciprocal linear transformation of these mean data yields a straight line (Fig. 6b). Kinetic parameters were determined from the reciprocal of the slope (V_{max} = 0.325 nmole·mg^{-1}·sec^{-1}) and from the negative intercept on the abscissa (K_m = 201 μM).

Initial intracellular accumulation of ASA and SAM. The degree of initial intracellular accumulation of total substrate, expressed as cell to medium concentration ratio (C/M ratio) at different initial concentrations of the substrate in the medium, is shown in Fig. 7. Since relatively prolonged incubation of longer than 5 min to obtain the equilibrated C/M ratios was considered to involve the problem that viability of the hepatocytes would decrease while formation of the metabolites would increase with time, temporary comparison was made in the present study as to the net uptake which was calculated by

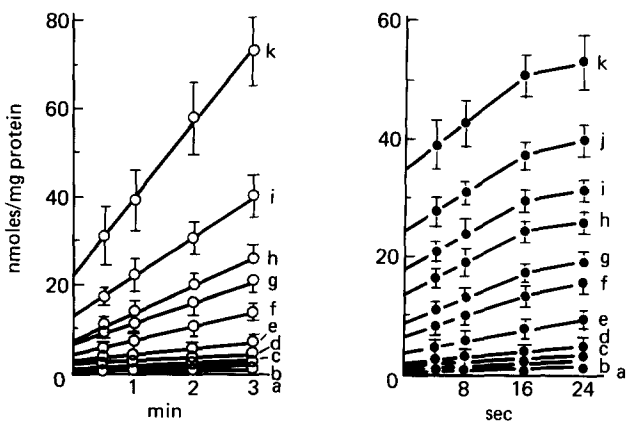

Fig. 4. Time course of ASA (○) and SAM (●) uptake by isolated rat hepatocytes. Uptake is expressed as nmoles of total drug/mg of cellular protein. Incubation was performed at pH 7.4 and 37° using 6.3 to 8.4 mg cellular protein in 4.0 ml buffer. Initial drug concentration was 10 (a), 25 (b), 50 (c), 100 (d), 200 (e), 400 (f), 600 (g), 800 (h), 1000 (i), 1500 (j) or 2000 (k) μM. Incubation mixture for ASA contained initially 0.1 μCi of [^{14}C]aspirin. Each point is the mean ± S.D. of five experiments (rats).

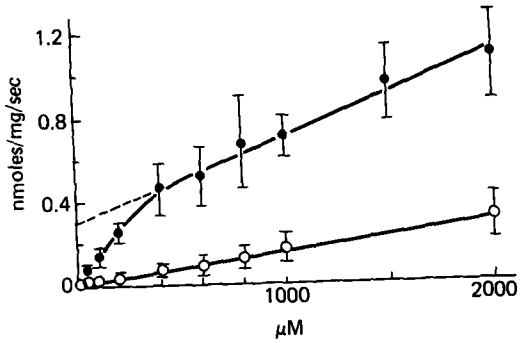

Fig. 5. Effect of initial substrate concentration on the overall initial uptake rate for ASA (○) and SAM (●) by isolated rat hepatocytes. The overall uptake rate was estimated from the slope of the linear portion in Fig. 4 and expressed as nmoles · (mg cellular protein)$^{-1}$ · sec^{-1}. Each point is the mean ± S.D. of five experiments. The linear regression line shown for ASA uptake ($r = 0.998$) is Y = 0.000151X, while that shown for 400 to 2000 μM SAM ($r = 0.984$) is Y = 0.000404X + 0.310 as extrapolated to zero concentration with the broken line, where Y and X are overall uptake rate and initial concentration respectively.

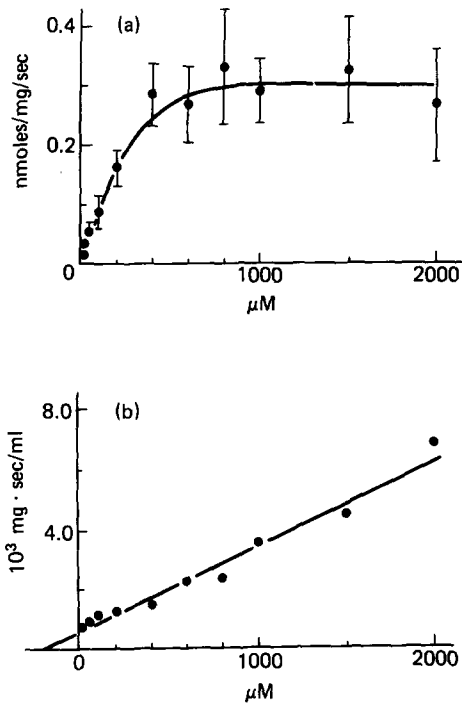

Fig. 6. (a) Saturable uptake rate by the isolated hepatocytes at various SAM concentrations. This rate was estimated by subtracting each simple diffusion rate from each overall uptake rate. Each point is the mean ± S.D. of five experiments. (b) Single reciprocal plots for the mean saturable uptake rate against initial SAM concentration. Linear regression analysis yields the relationship ($r = 0.984$) expressed as Y = 3.08X + 619, where Y and X are initial SAM concentration divided by the saturable uptake rate and the initial concentration respectively. Hence, the uptake kinetic parameters are obtained as follows: V_{max} = 0.325 nmole · mg^{-1} · sec^{-1}, K_m = 201 μM. Each point is the mean of five experiments.

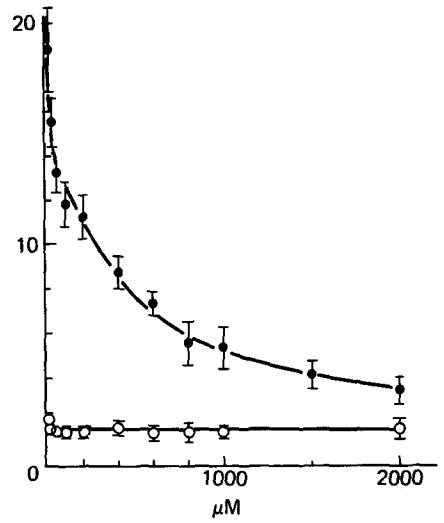

Fig. 7. Cell/medium concentration ratio of ASA (○) and SAM (●) at various initial concentrations. Cellular drug concentration for the net uptake was obtained by subtracting the non-specifically bound amount at t = 0, which was the intercept in Fig. 4, from the overall uptake amount at 30 sec for ASA or from that at 24 sec for SAM and then by dividing this net amount by aqueous cellular volume per mg protein (2.6 μl/mg cellular protein). Each point is the mean ± S.D. of five experiments.

subtracting the non-specifically bound amount at t = 0 from the overall uptake amounts at 30 and 24 sec for ASA and SAM respectively. The net uptake amount was then converted to the concentration using an intracellular volume of 2.6 μl/mg of cell

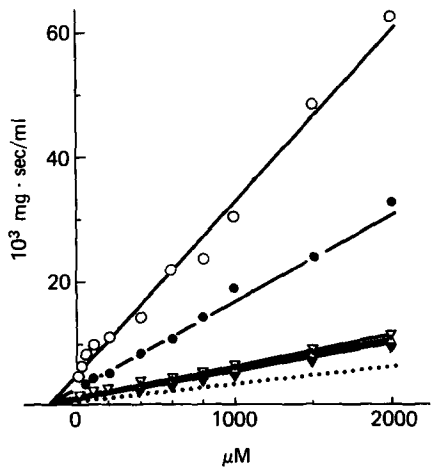

Fig. 8. Single reciprocal plots for the mean saturable uptake rate of SAM in the presence of various metabolic inhibitors (50 μM) against the initial SAM concentration. Dotted line refers to the result of the control experiment as shown in Fig. 6b. Incubation conditions were the same as those described in Fig. 4. Key and parameter value: 2,4-DNP (○, K_m = 190 μM, K_i = 0.159 μM^{-1}); KCN (●, K_m = 188 μM, K_i = 0.054 μM^{-1}); phloridine (▽, K_m = 221 μM, K_i = 0.018 μM^{-1}), ouabain (△, K_m = 288 μM, K_i = 0.015 μM^{-1}); and iodoacetic acid (▼, K_m = 194 μM, K_i = 0.0082 μM^{-1}). Each point is the mean of four experiments.

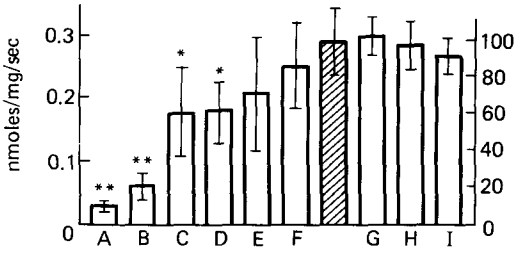

Fig. 9. Comparison of effects of various metabolic inhibitors, incubation temperature and Na^+-replacement on the saturable uptake rate of SAM tested at 400 μM. The added concentration of each metabolic inhibitor was 50 μM. The effect of incubation temperature on the uptake rate was tested only at 27°. Replacement of medium Na^+ was performed with equimolar Li^+, choline$^+$ or K^+. The shaded column refers to the mean value of five control experiments. The left ordinate represents the saturable uptake rate while the right ordinate represents percent of the control. Key: 2,4-DNP (A); KCN (B); phloridine (C); ouabain (D); iodoacetic acid (E); at 27° (F); Na^+-replacement with Li^+ (G); choline$^+$ (H); or K^+ (I). Each column is the mean ± S.D. of four experiments. A or B and C or D were significantly different from the control at $P < 0.001$ (**) and $P < 0.05$ (*) respectively.

protein. The C/M ratio for SAM was inversely dependent on the substrate concentration and extremely large in the relatively low concentrations (more than 10 below 200 μM), while that for ASA did not show any dose dependency and remained at the relatively small values of 1.5 to 2.0.

Energy dependence of SAM uptake process. Effects of five metabolic inhibitors added at 50 μM on the initial uptake rate for the saturable process of SAM are shown as single reciprocal plots in Fig. 8. The inhibitory kinetic parameters for these agents are also summarized in the figure. Since it was evident that each K_m value was rather close to the control value, the inhibitory effect seemed to be noncompetitive. Thus from the comparison of the estimated inhibition constant (K_i), it is suggested that 2,4-DNP exhibits the highest inhibitory effect. Significant reduction in the initial C/M ratio was also observed when these metabolic inhibitors were employed. Comparison of other inhibitory effects, such as reducing the incubation temperature (27°) and Na^+-replacement in the medium, with those obtained by metabolic inhibitors is demonstrated in Fig. 9 which shows saturable uptake rate measured at 400 μM SAM. 2,4-DNP or KCN ($P < 0.001$) and phloridine or ouabain ($P < 0.05$) demonstrated significant inhibitory effects on the uptake rate of SAM. However, reduction of the incubation temperature and Na^+-replacement tended to yield a considerably smaller and almost insignificant effect on the uptake of SAM respectively.

DISCUSSION

Although a different role of intestines and liver in the first-pass metabolism of ASA (10 mg/kg) and SAM (30 mg/kg) after oral dosing has been proposed in rats [1, 2], the only supporting data for this proposal are our previous observations that the conjugation (glucuronidation and/or sulfation) by the jejunal wall is much more extensive for ASA than for SAM and that the conjugation processes of both drugs are capacity-limited [3]. Levy and Matsuzawa [31] first suggested that there was a saturable mechanism for SAM conjugation in man. Schibazaki *et al.* [32] have also examined a similar phenomenon in *in situ* experiments with SAM given to rabbits and rats. Furthermore, Lin *et al.* [16] have recently compared the deethylation kinetics of ethoxybenzamine and the conjugation of the SAM which was formed from the parent compound by isolated rat hepatocytes and by hepatic microsomal preparations. However, there have been no reports investigating and/or comparing the dose dependency of ASA and SAM uptake by either the liver or isolated hepatocytes.

The plasma levels of unchanged as well as conjugated SAM determined at 2 min after intraportal dosing suggest that a typical capacity-limited mechanism may be involved in the uptake and/or the conjugative metabolism of the compound by rat liver (Fig. 1). This was confirmed by a dose-dependent hepatic accumulation and conjugation at 2 min after the intraportal administration of SAM (Fig. 2). Furthermore, the dose-dependent liver to plasma concentration ratio of total SAM suggests an involvement of a capacity-limited mechanism in the uptake and/or accumulation process of this drug in rat liver. These observations with SAM are in good agreement with the other reports in man [31] and in *in situ* rabbits and rats [32]. Dose-dependent hepatic accumulation and conjugation have also been demonstrated previously with morphine [33] and nalorphine [34].

Studies utilizing intact animal, perfused liver and liver slice models have been compared to describe hepatic uptake and biliary excretion of many compounds by Klaassen [35]. Unfortunately, kinetic characterization of the sinusoidal uptake system apart from the biliary excretory system was not possible with these experimental models. It has been reported recently that one cannot regard even the isolated hepatocyte data as completely free of biliary excretory canalicular effects if the hepatocytes were paired to permit canalicular function [36]. Nevertheless, isolated hepatocytes have an advantage over other models for the study of hepatic sinusoidal uptake processes, because the amount of compound taken into the hepatocytes can be determined at relatively early and short time intervals (within a few minutes) before the biliary excretory or the metabolic process significantly interferes with the uptake amount. Schwartz [26] has reported that conjugation does not influence the initial uptake rate of sulfobromophthalein into isolated rat hepatocytes. In the present study, it was evident that metabolism (conjugation) in the incubated hepatocytes could be almost insignificant during such short intervals as 24 sec and 3 min for SAM and ASA, respectively, though both ASA and SAM accumulated in the liver, immediately after intraportal administration, existed largely as the conjugated forms. This is probably because the incubation mixture was lacking in cofactors which would be necessary for the conjugative

metabolic processes. Short sampling intervals moreover allow approximation of the initial velocities of uptake at different substrate concentrations and thus kinetic characterization of the uptake mechanism. Although it has been reported that the extents of biliary excretion of ASA and SAM are almost negligibly small in rats [1, 2], this approach utilizing the isolated hepatocytes was employed for the evaluation of the initial hepatic uptake rate of these compounds.

The overall uptake rate of SAM was much larger than that of ASA at all substrate concentrations tested (Fig. 5). Uptake of ASA by the hepatocytes was caused solely by an apparently linear transport process, while that of SAM was driven by both capacity-limited (saturable) and apparently linear processes. Of the kinetic parameters estimated for the capacity-limited uptake process of SAM, V_{max} [0.325 nmole · (mg protein)$^{-1}$ · sec^{-1}] appeared to be extraordinarily large as compared with those obtained for ouabain [20], morphine and nalorphine [19], indicating that the saturable uptake process of SAM in rat hepatocytes may have relatively high capacity (Fig. 6, A and B). *In vivo* hepatic accumulation patterns with a change in intraportal dose of ASA and SAM (Fig. 2) were exactly supported by the *in vitro* hepatic uptake profiles (Fig. 5). Furthermore, the dose-dependency profile of liver to plasma concentration ratio for total SAM after intraportal dosing (Fig. 3) was also confirmed by the *in vitro* cell to medium concentration ratio of SAM (Fig. 7). Dependency of these ratios (liver/plasma and cell/medium) on the dose and the initial concentration of SAM directly indicated an involvement of a capacity-limited process in the hepatic transport (uptake) of this drug. All the metabolic inhibitors tested in the present work appeared to act noncompetitively on the uptake process of SAM. An uncoupler of oxidative phosphorylation, 2,4-DNP, was the strongest inhibitor at the concentration (50 μM) tested (Figs. 8 and 9). Metabolic inhibitors acting at other sites of energy production within the cells also significantly reduced or tended to decrease the uptake rate of SAM (Fig. 9). However, sensitivity to the incubation temperature and effect of replacement of sodium ion were not significant. Hence, these inhibition data indicate that the uptake of SAM by rat hepatocytes is mediated by a carrier mechanism. The data also indicate that there is not a major sodium dependence for this transport system.

The present study has clarified the effect of dose on the *in vivo* hepatic accumulation and/or metabolism and, moreover, the effect of the substrate concentrations on *in vitro* hepatic uptake rates of these two compounds, ASA and SAM. Furthermore, the data presented here demonstrate that the greater hepatic first-pass effect of SAM which was reported earlier [1, 2] is directly related to more extensive and rapid transport (uptake) of the drug into the liver and the hepatocytes as compared with that of ASA.

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