tabulated in Table VII. As can be seen in Table VII, the average increase in partition coefficient due to a methylene group addition within a linear homologous series of acids has similar values if one selects either the octanol–water or *n*-heptane–0.1 *N* hydrochloric acid system. This is in disagreement with the position constants obtained from the buccal absorption analysis. It would indeed be interesting if partitioning data with the isobutanol–water system were available.

Although one may obtain some information about the transport of nonionized drug molecules across the buccal membrane by the use of the regression equation suggested by Lien *et al.* (6) as a one-point comparison at any one particular pH, the physical model Eqs. 1–4 suggested in this report can provide absorption profiles of each acidic drug for the entire experimental buffer pH range.

The data presented here provide further evidence that the diffusion model suggested earlier by Ho and Higuchi (1) for buccal absorption is consistent in the cases of *p*-alkyl phenylacetic, *p*-halogen phenylacetic, and toluic acids. The model underscores the importance of the diffusion layer and its effect on the transport of nonionized drug molecules in the buccal absorption situation.

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Biopharmaceutical Studies on Aminoethanesulfonylphenetidine and Related Compounds III: Drug in Blood

SHUN-ICHI NAITO▲ and KAZUO FUKUI

Abstract □ No effects of taurinophenetidine and nicotinoyltaurinophenetidine on erythrocytolysis and methemoglobin production were observed. It was also found that about 40% of the nicotinoyltaurinophenetidine, which is absorbed after its oral administration, is hydrolyzed in the blood of rabbits, rats, and mice.

Keyphrases □ Aminoethanesulfonylphenetidine—effect on erythrocytolysis and methemoglobin production, plasma levels in rabbits □ Taurinophenetidine—effect on erythrocytolysis and methemoglobin production, plasma levels in rabbits □ Nicotinoylaminoethanesulfonylphenetidine—effect on erythrocytolysis and methemoglobin production, blood levels in rabbits, rats, mice □ Nicotinoyltaurinophenetidine—effect on erythrocytolysis and methemoglobin production, blood levels in rabbits, rats, mice

The binding ratio of aminoethanesulfonylphenetidine (taurinophenetidine) or nicotinoylaminoethanesulfonylphenetidine (nicotinoyltaurinophenetidine) with serum protein in rabbits and the excretion of taurinophenetidine and its nicotinoyl derivative in rat feces and in rat and rabbit bile were previously investigated (1). It was also observed that taurinophenetidine has some analgesic and antipyretic activities and that nicotinoyltaurinophenetidine has some analgesic and anti-inflammatory activities but no antipyretic action (1).

In the present study, the effect of taurinophenetidine and nicotinoyltaurinophenetidine on erythrocytolysis and methemoglobin production was examined to determine the toxicity of these drugs before undertaking clinical studies. The blood levels of nicotinoyltaurinophenetidine and its hydrolysis product following its oral administration to mice, rats, and rabbits were also investigated.

EXPERIMENTAL

***In Vitro* Osmotically Induced Hemolytic Action**—The hemolytic effects of taurinophenetidine and nicotinoyltaurinophenetidine on rat blood were determined by the method reported by Okui and Uchiyama (2).

A suspension of 0.1 ml. of rat blood in 2 ml. of sodium chloride–sodium citrate solution (0.6 g. of sodium citrate in 100 ml. of 0.9% sodium chloride solution) was centrifuged for 2 min. The residue of blood corpuscles thus obtained was suspended in 2 ml. of 0.9% sodium chloride solution. After another centrifugation, the residue was again resuspended in 1 ml. of 0.9% sodium chloride solution and this suspension was used for the following procedures.

Procedure A—To 0.25 ml. of this suspension, 0.25 ml. of 0.2 *M* phosphate buffer (pH 7.4) was added and the mixture was incubated at 37 ± 2° for 15 min. After standing for 45 min. at room temperature, the mixture was centrifuged for 2 min. To 0.2 ml. of the supernate, 3.3 ml. of water was added and the absorbance at 550 nm. was determined.

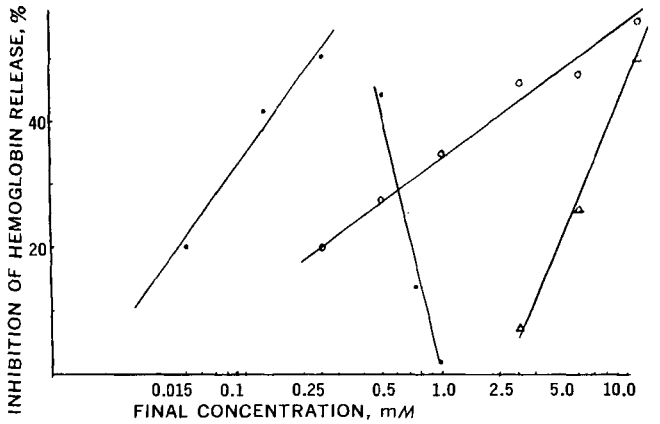

Figure 1—*Inhibition of heat-induced hemolysis. Key: ●, nicotinoyltaurinophenetidine hydrochloride; ○, sodium salicylate; and △, taurinophenetidine hydrochloride.*

Procedure B—To 0.25 ml. of the suspension, 0.25 ml. of 0.2 *M* phosphate buffer containing a test compound was added and the mixture was treated as described above. Taurinophenetidine, nicotinoyltaurinophenetidine, or saponin was used as the test compound.

Procedure C—To 0.25 ml. of the suspension, 8.5 ml. of water was added to cause complete hemolysis of the red blood cells. After centrifugation, the supernate was collected and its absorbance at 550 nm. was determined.

The absorbance of supernate B or C was determined at 550 nm. and compared with the absorbance of supernate A as a control; the percent hemolysis was determined as follows:

$$\text{hemolysis (\%)} = \frac{\text{absorbance (B} - \text{A)}}{\text{absorbance (C} - \text{A)}} \times 100 \quad \text{(Eq. 1)}$$

Effect of Sodium Salicylate, Taurinophenetidine, and Nicotinoyltaurinophenetidine on Erythrocytolysis with Heat—The procedure was the same as the method reported by Glenn *et al.* (3) and Nakanishi *et al.* (4). A blood sample was obtained from a male rat (Wistar strain, average weight 200 g.) by decapitation. The test compound was dissolved in phosphate buffer (pH 7.4, water was added to a mixture of 19 ml. of 0.2 *M* NaH_2PO_4 and 81 ml. of 0.2 *M* Na_2HPO_4 to make 200 ml.).

Methemoglobin Production—Heparinized venous blood from male rabbits (average weight 3.0 kg.) was used as a source of erythrocytes. Oxyhemoglobin and methemoglobin concentrations in the incubation mixture of hemoglobin and taurinophenetidine, nicotinoyltaurinophenetidine, or β-naphthol were determined by the method described by Harley and Mauer (5).

Blood Level of Taurinophenetidine after Intravenous Administration to Rabbits—To three male rabbits (average weight 2.0 kg.), 80 mg./kg. of taurinophenetidine (30 ml. of 0.83% NaCl solution was added to 160 mg. of taurinophenetidine to make an isotonic solution) was administered intravenously. Taurinophenetidine in rabbit plasma was determined by the modified Folin method (6) at 30-min. intervals for 2 hr. after its administration.

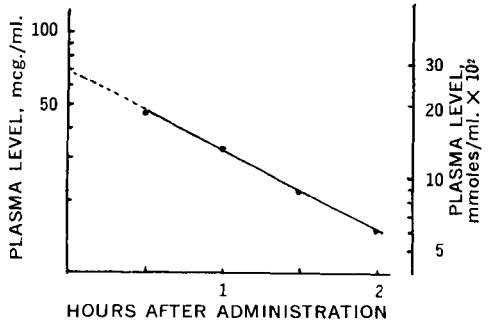

Figure 2—*Mean blood level of taurinophenetidine after intravenous administration of 80 mg./kg. of taurinophenetidine to rabbits.*

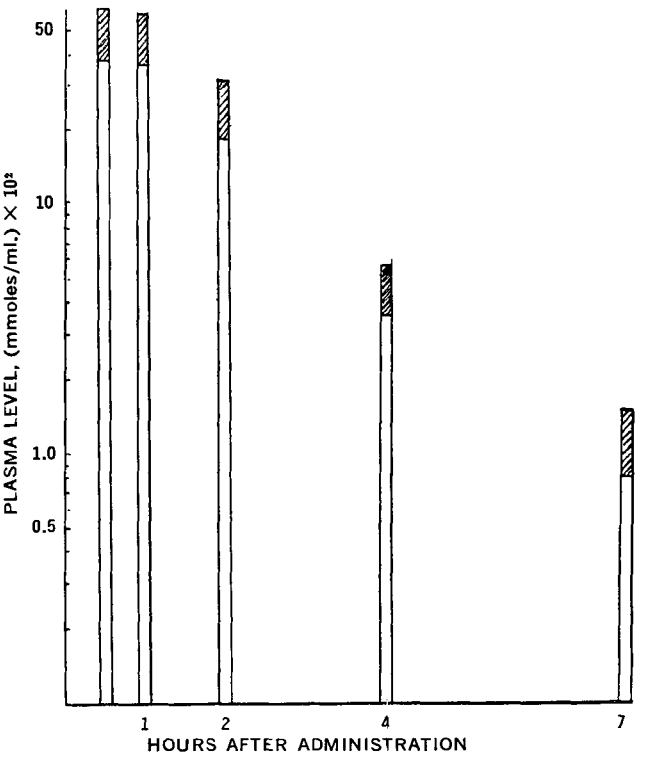

Figure 3—*Mean plasma levels of nicotinoyltaurinophenetidine and nicotinic acid in three rabbits (body weight 3.2 kg.) after oral administration of nicotinoyltaurinophenetidine in 220-mg./kg. dose each. Key: □, unchanged nicotinoyltaurinophenetidine; and ▨, nicotinic acid from hydrolysis of nicotinoyltaurinophenetidine in blood.*

Determination of Nicotinoyltaurinophenetidine and Its Hydrolyzed Product in Animal Blood—Nicotinoyltaurinophenetidine, screened through 100 mesh, was suspended in 0.2% tragacanth solution and this suspension was administered to animals orally. Unchanged nicotinoyltaurinophenetidine and its hydrolysis product, free nicotinic acid, were assayed in animal blood by a method reported previously, using barbital buffer (7) and ammonia buffer (1, 7), respectively.

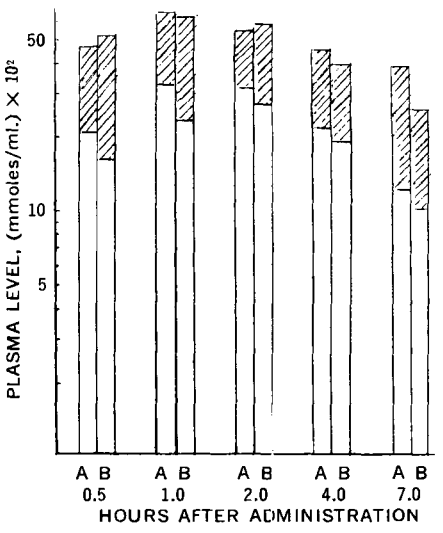

Figure 4—*Mean plasma level of nicotinoyltaurinophenetidine and nicotinic acid in rats after oral administration of nicotinoyltaurinophenetidine. Key: A, 350-mg./kg. dose; B, 225-mg./kg. dose; □, unchanged nicotinoyltaurinophenetidine; and ▨, nicotinic acid. Blood sample was collected from three rats (Wistar strain, body weight 210 ± 10 g.) after decapitation at every sampling time.*

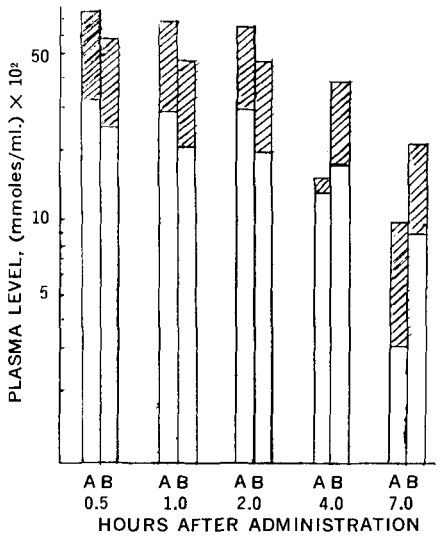

Figure 5—*Mean plasma level of nicotinoyltaurinophenetidine and nicotinic acid in mice after oral administration of nicotinoyltaurinophenetidine. Key: A, 350-mg./kg. dose; B, 225-mg./kg. dose;* □, *unchanged nicotinoyltaurinophenetidine; and* ▨, *nicotinic acid. Blood sample was collected from 10 mice (dd strain, body weight 21 ± 1 g.) after decapitation at every sampling time.*

RESULTS AND DISCUSSION

The hemolytic action of taurinophenetidine, nicotinoyltaurinophenetidine, and saponin[1] on rat blood was determined at three concentration levels, and their hemolysis rates (percent) were calculated. The hemolytic actions of taurinophenetidine on rat blood were 0.5 ± 0.3, 0.6 ± 0.2, and 0.3 ± 0.2% (mean ± *SE*) at 50, 100, and 200 mcg./ml. in final concentration, respectively; those of nicotinoyltaurinophenetidine were 0.3 ± 0.2, 0.4 ± 0.3, and 2.1 ± 0.2% at 50, 100, and 200 mcg./ml. in final concentration, respectively. The hemolytic action of saponin was determined for the sake of comparison, and the values were 15.3 ± 5.2, 78.7 ± 6.8, and 90.0 ± 8.5% at 40, 80, and 120 mcg./ml. in final concentration, respectively. These results indicate that the percentage of hemolysis due to taurinophenetidine is not significantly different from zero at any concentrations employed ($t_{0.025} = 3.2$, $df = 3$), while that of the nicotinoyl derivative is significant only at 200 mcg./ml.

The protective effect of taurinophenetidine and nicotinoyltaurinophenetidine on heat-induced erythrocytolysis (3) *in vitro* was determined to see whether the chemical inhibits inflammatory reactions. Taurinophenetidine and its nicotinoyl derivative have a stabilizing action (Fig. 1), but anti-inflammatory action was not observed in taurinophenetidine on rat edema induced by carrageenin (1).

Changes in hemoglobin were investigated with respect to the formation of methemoglobin that occurs when erythrocytes are incubated with these test compounds. Erythrocytes were incubated for 2 hr. with each test substance in a molar ratio of 4:1 to hemoglobin. Oxyhemoglobin and methemoglobin concentrations of the incubation mixture of the chemical and hemoglobin at zero time were 95.0 and 2.2% in β-naphthol, 99.2 and 0.8% in taurinophenetidine, and 101.7 and 0% in nicotinoyltaurinophenetidine, respectively. Oxyhemoglobin and methemoglobin concentrations 2 hr. after the incubation were 68.8 and 7.8% in β-naphthol, 98.3 and 0.9% in taurinophenetidine, and 97.4 and 1.7% in nicotinoyltaurinophenetidine, respectively. The sum of the concentrations of oxyhemoglobin and methemoglobin decreased in the incubation mixture of β-naphthol and hemoglobin but not in that of taurinophenetidine or nicotinoyltaurinophenetidine and hemoglobin. These results indicate that neither taurinophenetidine nor nicotinoyltaurinophenetidine has any effect on "intact" hemoglobin (5), differing from the results with β-naphthol which was used as a comparison.

[1] Merck.

The mean plasma levels of taurinophenetidine after intravenous administration to rabbits are shown in Fig. 2. The extrapolated plasma level of taurinophenetidine obtained graphically at zero time is about 68 mcg./ml. On the other hand, the extrapolated plasma level of taurinophenetidine at zero time after its oral administration (250 mg./kg.) is about 90 mcg./ml. (6). The average body weight of the rabbits used for intravenous and oral administrations was the same (2.0 kg.). If the extrapolated blood level of taurinophenetidine is proportional to dose after intravenous administration, the calculated extrapolated blood level of taurinophenetidine at zero time after intravenous administration of 250 mg./kg. of taurinophenetidine would be expected to be:

$$68 \times \frac{250 \times 2}{80 \times 2} \fallingdotseq 211 \text{ (mcg./ml.)} \qquad \text{(Eq. 2)}$$

Therefore, bioavailability of orally administered taurinophenetidine is estimated by this method to be:

$$\frac{90}{211} \times 100 \fallingdotseq 42.6\,(\%) \qquad \text{(Eq. 3)}$$

However, the method of estimation used above would always give an underestimate of the true bioavailability after oral administration.

In a previous work (1), blood levels of nicotinoyltaurinophenetidine in rabbits, rats, and mice were determined using ammonia buffer. Previously, quantitative determination of nicotinoyltaurinophenetidine and its metabolites in blood was examined by TLC, but no metabolites were found. However, nicotinoyltaurinophenetidine was extracted with chloroform before the TLC, and the extraction procedure usually resulted in some quantitative loss. Therefore, in the present work, nicotinoyltaurinophenetidine and nicotinic acid from hydrolysis of nicotinoyltaurinophenetidine in the blood of rabbits, rats, and mice were determined after the oral administration to ascertain whether hydrolysis of nicotinoyltaurinophenetidine occurred in blood or not. However, the blood level of nicotinoyltaurinophenetidine after oral administration was previously reported (1) only in rabbits and, therefore, two doses of this compound were given to mice and rats. In the case of rabbits, only one dose, 220 mg./kg. of nicotinoyltaurinophenetidine was administered. This dose is almost equal to the average of the 300-, 225-, and 150-mg./kg. doses used in the previous study (1). Neither nicotinoyltaurinophenetidine nor nicotinic acid was observed in blood (rabbits, rats, and mice) 10 hr. after the administration. About 40% of nicotinoyltaurinophenetidine absorbed was hydrolyzed in the blood of rabbits, rats, and mice, as shown in Figs. 3–5.

In conclusion, taurinophenetidine and its nicotinoyl derivative were not found to cause either erythrocytolysis or the production of methemoglobin in *in vitro* and laboratory animal studies. Previous studies showed that these compounds were useful as analgesics and anti-inflammatory agents. Therefore, clinical trials appear to be warranted and should be apparently safe with respect to toxic blood effects.

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