Biopharmaceutical Studies on Aminoethanesulfonylpiperidine and Related Compounds I: Absorption, Excretion, Metabolism, and Some Pharmacological Activities

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Abstract □ Blood levels of taurinopiperidine in animals, distribution of the chemical in rat organs, and the excretion in rat bile were examined. The binding ratio of taurinopiperidine with rabbit serum protein is approximately 85% at peak time of blood level after oral administration. About 11.5% of taurinopiperidine is excreted in unconjugated form in rabbit urine, and most of the taurinopiperidine administered orally must be metabolized to other compounds no longer having a piperidinelike skeleton. Taurinopiperidine shows no anti-inflammatory activity on edema of rat hind paw induced by carrageenin, but it inhibited permeability and squirming in mice. In addition, taurinopiperidine has analgesic, antihistaminic, antianaphylactic, and antipyretic activities, although the effects are not as potent. It has no hemolytic action and no effect on the changes in hemoglobin with regard to the formation of methemoglobin.

Keyphrases □ Taurinopiperidine—blood levels, distribution, and excretion in animals, pharmacological activity □ Aminoethanesulfonylpiperidine—blood levels, distribution, and excretion in animals, pharmacological activity

Aminoethanesulfonylpiperidine (taurinopiperidine) was synthesized in the hope of obtaining a new therapeutic agent based upon the following concepts:

1. Aminoethanesulfonic acid, a partial component of taurinopiperidine, is one of the physiological amino acids present in man. It also has surface-active properties which may aid penetration of a compound into the digestive tract. Another component, piperidine, is one of the components of normal human urine. Therefore, taurinopiperidine, the condensation product of aminoethanesulfonic acid and piperidine, might be less toxic and may be useful if the compound has some pharmacological activity.

2. Among the many therapeutic agents which act on the CNS, some have a piperidine skeleton. It is known that an increase of activity of the original compound, or entirely new characteristic activity, often occurs by introduction of the piperidine skeleton (1).

Since it is very difficult to predict the action of taurinopiperidine from its chemical structure, some biopharmaceutical and pharmacological properties of this compound were examined in the present work. This preliminary work was performed using animals, but the pharmacological activities of taurinopiperidine in man will be investigated in the future.

EXPERIMENTAL

Determination of Taurinopiperidine in Blood—To 1 ml. of plasma of an animal which received taurinopiperidine orally, subcutaneously, or intravenously, 1 ml. of 10% perchloric acid solution was added. To 1 ml. of the supernatant obtained after centrifugation for 15 min., 1 ml. of 0.3% sodium β-naphthoquinonesulfonate solution and 1 ml. of 10% sodium carbonate solution were added. The mixture was allowed to stand for 10 min. at room temperature. While the mixture was being cooled with ice water, 5 ml. of chloroform was added to it; then the mixture was shaken for 20 min. The chloroform layer obtained after centrifugation for 10 min. was filtered through a filter paper (9-cm. diameter), and the absorbance of the filtrate was determined at 430 nm.

A mixture of 0.9 ml. of normal animal plasma and 0.1 ml. of taurinopiperidine solution of known concentration was treated as described to prepare a calibration curve. The procedure used was a modification of the method described by Kakemi *et al.* (2).

Distribution of Taurinopiperidine in Rat Organs—To male rats (Wistar strain, average weight 200 g.), 200 mg./kg. of taurinopiperidine was given orally and the animals were killed 2 hr. (peak time of blood level) and 24 hr. after the administration.

Each group consisted of three rats, and six organs (heart, lung, spleen, kidney, brain, and liver) were used for the determination of taurinopiperidine. The sample sizes were: 2 g., heart; 3 g., lung; 1 g., spleen; 4 g., kidney or brain; and 15 g., liver. These samples were homogenized with water using 6 ml. for heart and lung, 5 ml. for spleen, 7 ml. for kidney and brain, and 18 ml. for liver. One milliliter of the supernatant from the centrifuged homogenate was treated by the same method as used for the determination of taurinopiperidine in blood.

Organs of rats not receiving any drug were treated in the same way to serve as controls. Mixtures of control homogenate spiked with taurinopiperidine solutions of known concentration were treated as already described to prepare a calibration curve.

Determination of Taurinopiperidine in Rat Bile—Rats (Wistar strain, average weight 180 g.) were anesthetized with urethan (1 g./kg. s.c.) after fasting for approximately 24 hr. A polyethylene tube was inserted into the bile duct, and the bile was collected for 7.5 hr. postadministration. At the end of the 1st (control) hr., the compound was given orally. One milliliter of rat bile was treated by the same method as for the determination of taurinopiperidine in blood.

Equilibrium Dialysis of Taurinopiperidine in Blood—*Free Taurinopiperidine*—Five milliliters of water was placed outside of a cellulose tube (Visking) in a standard tapered tube. A mixture of 1 ml. of water and 1 ml. of centrifuged serum (with citric acid), taken from two male rabbits (average weight 2.3 kg.) receiving 200 mg./kg. of taurinopiperidine orally, was placed inside the bag and then dialyzed at 5° for 120 hr. To 3 ml. of the outside solution of the tube, 1 ml. of 0.3% sodium β-naphthoquinonesulfonate solution and 1 ml. of 10% sodium carbonate solution were added; this mixture was treated by the same method as described for the determination of taurinopiperidine in blood.

Total Taurinopiperidine—Five milliliters of water was placed outside of a cellulose tube (Visking). A mixture of 1 ml. of water and 1 ml. of the supernatant from centrifugation after storage of a mixture of 1 ml. of plasma of rabbit receiving taurinopiperidine and 1 ml. of 5 *N* HCl at 37 ± 2° for 1 hr. was placed inside the bag and then dialyzed at 5° for 120 hr. Three milliliters of the outside solution of the tube was treated as described for the determination of taurinopiperidine.

Separation and Assay of Metabolites of Taurinopiperidine in Rabbit Urine—About 1400 ml. of urine, excreted from four rabbits (average weight 2 kg.) after oral administration of 250 mg./kg. of taurinopiperidine, was submitted to freeze drying. The lyophilized residue (A) of urine was about 28 g. The urine of four rabbits not receiving the chemical was collected and treated in the same way, and its lyophilized residue was designated as B. A mixture of 1 ml. of 5 *N* HCl and 100 mg. of the residue was incubated at 37 ± 2° for 1 hr. The mixture was neutralized with 1 ml. of 5 *N* sodium carbonate solution and submitted to TLC for evaluation (Table I). After alkali treatment, Residues A and B were designated, respectively, A′ and B′. No difference was observed in the number of

Table I—TLC of Lyophilized Rabbit Urine[a]

Material	Color Developer	R_f Value	Color of Spot
Sample A	Iodine tincture (JP VII)	0.73	Brown
		0.47	Brown
		0.41	Brown
		0.23[b]	Orange-yellow
Blank urine		0.73	Brown
		0.47	Brown
		0.41	Brown
Sample A′		0.73	Brown
		0.48	Brown
		0.42	Brown
		0.23[b]	Orange-yellow
Sample A	Mixture of 0.3% sodium naphthoquinonesulfonate and 10% Na_2CO_3 solution	0.23[b]	Orange-red
Sample A′		0.23[b]	Orange-red
Blank urine		Nil	

[a] Adsorbent: diatomite (Kieselgel G), 0.25 mm. in thickness; distance developed: 15 cm.; and solvent: ether–ethanol–28% ammonia (4:4:1). [b] Taurinopiperidine. A micromelting-point determination of the mixture of evaporated residue of ethanolic extract from five to 10 spots having the same R_f value and an authentic sample was used for identification.

spots in TLC between Residues A and A′ and between Residues B and B′.

To assay the metabolites of taurinopiperidine in urine, a mixture of 2 ml. of water and 100 mg. of the freeze-dried residue of urine was heated on a steam bath for 3 min. Ten microliters of the supernatant, obtained after 10 min. of centrifugation, was spotted on a glass plate and submitted to TLC. The spot corresponding to unchanged taurinopiperidine was scraped off the TLC plate after development with a mixed solvent of ether–ethanol–28% ammonia (4:4:1) (Table I).

For the assay of taurinopiperidine, the two spots having the same R_f value were scraped off the TLC plate and extracted with 3 ml. of ethanol for 1.5 min. on a steam bath. After centrifugation, 2 ml. of a supernatant was obtained. Six milliliters of the supernatant from six spots having the same R_f value was evaporated to dryness, and the residue was dissolved in 2 ml. of water. To this solution, 1 ml. of 0.3% sodium β-naphthoquinonesulfonate solution and 1 ml. of 10% sodium carbonate solution were added; then the mixture was treated by the same method as for the determination of taurinopiperidine in blood.

Taurinopiperidine solution of known concentration was submitted to TLC and treated as described to prepare a calibration curve.

Acute Toxicity—Each group consisted of five male and five female mice (dd strain, average weight 15 g.). The animals were maintained on a commercial diet[1] in a room of 20 ± 2°, and drinking water was given freely. Five mice were housed in one cage.

Taurinopiperidine solution was given by both intraperitoneal and oral routes. Various doses of the chemical were dissolved in water, and the volume was limited to 0.5 ml./10 g. body weight. The animals were fasted for 5 hr. prior to drug administration, but they were allowed free access to the diet and drinking water beyond 4 hr. after drug administration. The behavioral changes were observed for 72 hr. after drug administration, and the LD_{50} of the chemical was calculated from the number of animals that died during the next 72 hr., according to the method of Litchfield and Wilcoxon (3).

Tail-Withdrawal Reflex in Mice—The analgesic method used was described in the paper of Ben-Bassat *et al.* (4).

Anaphylactic Shock in Guinea Pigs—Each group consisted of 10 guinea pigs (Hartley strain, average weight 375 g.). Guinea pigs were sensitized with two 0.5-ml. i.p. injections of horse serum 48 hr. apart. Sixteen days after the initial injection, the animals were fed the test compound in an aqueous solution.

Separate groups of guinea pigs were challenged exactly 1 hr. after drug administration by rapid injection of 1 ml. of horse serum into the saphenous vein. Guinea pigs surviving the first 10 min. after the horse serum injection were protected since sensitized control animals challenged in this manner in each experimental series invariably died of anaphylactic shock within 10 min.

Table II—Mean Plasma Levels[a] of Taurinopiperidine (Micrograms per Milliliter) following Oral Administration to Mice and Rats

Animal	Dose, mg./kg.	Hours after Dosing 0.5	1.0	2.0	4.0	7.0
Mice	150	40	24	38	0	0
	200	42	36	38	0	0
Rats	100	14	4	6	1	0
	150	43	42	27	42	13
	200	45	36	47	28	22

[a] Each group consisted of 10 female mice (dd strain) weighing about 17 g. or five female rats (Wistar strain) weighing about 170 g. Animals in each group were killed according to the sampling schedule. At the time of sacrifice, each animal was bled completely, and the equivoluminal mixture of the blood from each group was reserved for analysis.

Antihistaminic Activity—Each group consisted of five male and five female guinea pigs (Hartley strain, average weight 375 g.). The compound was administered subcutaneously 1 hr. before histamine injection. Histamine dihydrochloride was injected rapidly into the saphenous vein in a dosage of 1.1 mg./kg. The test was performed on fasting animals.

Effect on Hypercholesteremia Induced by Poly(oxyethylene Ethers)—The experiment was carried out under the conditions described in a previous paper (5). Male rats (Wistar strain, average weight 200 g.) were used, and serum cholesterol was determined by the method reported by Zurkowski (6).

Anti-Inflammatory Activity—The experiment was performed under the conditions reported previously (7). Five female rats (Wistar strain, average weight 200 g.) were used for each group.

Effect on Squirming and Capillary Permeability—Ten mice (dd strain, average weight 20 g.) were used for each group. The experiment was performed under the conditions reported previously (7).

Antipyretic Activity—To three male rabbits (average weight 2.0 kg.), 15 mcg./kg. of nonanaphylactogenic polysaccharides obtained from *Pseudomonas fluorescens*[2] was administered intravenously and taurinopiperidine solution was given subcutaneously 1.75 hr. later. Body temperature was recorded every 15 min. for 6 hr.

Methemoglobin Production—Heparinized venous blood from male rabbits (average weight 2.8 kg.) was used as the source of erythrocytes. Oxyhemoglobin and methemoglobin concentrations in incubation mixtures of hemoglobin and taurinopiperidine were determined by the method described by Harley and Mauer (8).

Hemolytic Action *In Vitro*—Hemolytic action of taurinopiperidine on rat blood was determined by the method reported by Okui and Uchiyama (9).

Effect on Erythrocyte Lysis—The procedure followed was the same as that described by Glenn *et al.* (10). The blood sample was obtained from male rats (Wistar strain, average weight 220 g.).

Stability Test—Taurinopiperidine was assayed every 10 days during 10 weeks after 3% of a taurinopiperidine solution was stored at 40, 50, 60, and 70°. Two spots, having the same R_f value as the authentic sample of pure taurinopiperidine after development of 5 μl. of the test solution by TLC, were combined and extracted with 2 ml. of water on a boiling water bath for 5 min. After centrifugation, 1 ml. of the supernatant was used for determination of taurinopiperidine. The assay procedure was similar to that used with blood. The TLC solvent was ethanol–ether–28% ammonia (4:4:1); the color developer consisted of 0.3% of β-naphthoquinone sulfate solution and 10% of sodium carbonate solution.

RESULTS

The peak blood levels of taurinopiperidine in rabbits, mice, and rats were almost the same for the same dose. The difference in time courses lies in the fact that the curves of blood levels of taurinopiperidine show two peaks with an intervening valley in mice and rats, as shown in Table II, but not in rabbits (Fig. 1). To clarify this phenomenon, the distribution of taurinopiperidine in rat organs and the excretion of the drug in rat bile were investigated.

[1] CA-1, CLEA Japan, Inc.

[2] T.T.G No. 1, Fujisawa Yakuhin Kogyo Co. Ltd., Osaka, Japan.

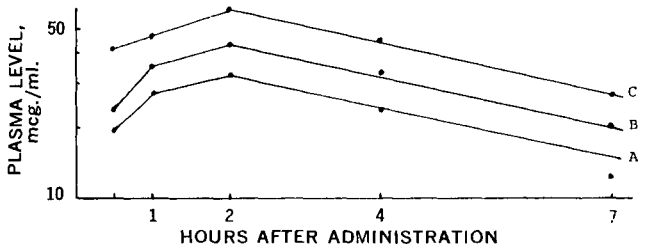

Figure 1—*Mean plasma level of taurinopiperidine after oral administration of different doses to rabbits. Key: A, 100 mg./kg.; B, 150 mg./kg.; and C, 200 mg./kg. Four male rabbits weighing about 2.2 kg. each were used.*

The distribution of taurinopiperidine (micrograms per gram) in rat organs 2 hr. after its oral administration to three groups was: 29.9 ± 3.2 in the heart, 44.8 ± 2.6 in the lung, 29.6 ± 3.0 in the liver, 83.7 ± 9.4 in the spleen, 180.7 ± 23.4 in the kidneys, and 84.5 ± 5.2 in the brain (mean value ± standard error). Taurinopiperidine in all rat organs had disappeared entirely 24 hr. after the administration.

The amount of taurinopiperidine excreted in rat bile during 7.5 hr. after oral administration in a 200-mg./kg. dose to each of five rats was 334 ± 38 (mcg./kg.) (mean value ± standard error). At the same time, no effect of taurinopiperidine on bile volume was observed.

The distribution of taurinopiperidine in the organs and its excretion in bile could explain the double peaks in the blood level curves in rats. It may be assumed that taurinopiperidine is absorbed and distributed to some organs and that the drug reappears in blood, the drug from organs combining with the drug from enterohepatic circulation.

The binding of taurinopiperidine with rabbit serum protein was investigated by equilibrium dialysis; the results revealed that about 85 and 83% of taurinopiperidine were bound 1 and 2 hr., respectively, after the administration. The experiment was made with the consideration that binding of the chemical with serum protein can be inhibited by the action of hydrochloric acid. It is also assumed that the blood level of taurinopiperidine shown in Fig. 1 represents the total chemical, including that bound with protein and the unbound fraction, because perchloric acid was used for the determination. The binding of taurinopiperidine with serum protein was examined to understand the pharmacological activities of taurinopiperidine.

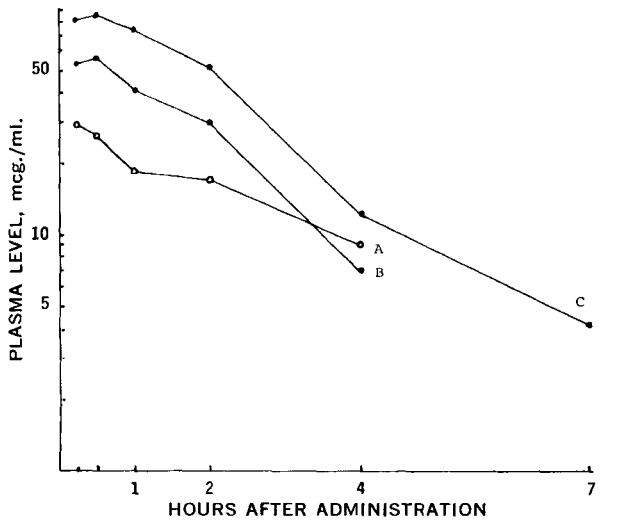

Figure 2—*Mean plasma level of taurinopiperidine after subcutaneous administration of different doses to guinea pigs. Key: A, 45 mg./kg.; B, 90 mg./kg.; and C, 135 mg./kg. Groups consisted of three male guinea pigs (Hartley strain) weighing about 210 g. each; animals were killed according to the sampling schedule. At the time of sacrifice, each animal was bled completely and the blood was reserved for analysis.*

Urine collected from rabbits receiving taurinopiperidine was lyophilized, and the residue was examined by TLC for the presence of metabolites (Table I). Urine was collected for only 72 hr. after oral administration of the chemical, since urine collected from 72 to 120 hr. after administration contained no chemical or its metabolites, as evidenced by TLC. Only unchanged taurinopiperidine (no metabolites) was detected in rabbit urine by TLC. On the other hand, in the case of other derivatives of 2-aminoethanesulfonic acid [*e.g.*, taurinophenetidine (11) and taurinopyrine (7)], many metabolites were detected.

About 11.5% of taurinopiperidine was excreted in the unchanged form, and it is assumed that most of taurinopiperidine administered must be metabolized to other compounds having no piperidine nucleus. Among the color developers used in TLC, iodine tincture is sensitive to many organic compounds, and a mixture of naphthoquinonesulfonate and sodium carbonate solution can develop some color for piperidine and its derivatives. Therefore, the color developer shown in Table I must be positive if piperidine and its derivatives exist in urine samples. It can also be stated positively that no metabolites are present in the conjugated form, because urine samples A and A′, described in the *Experimental* section, gave the same spots on TLC (Table I).

The LD_{50}'s of taurinopiperidine after intraperitoneal and oral administration to mice are 350 mg./kg. (334–368 mg./kg.) and 950 mg./kg. (876–1031 mg./kg.), respectively. Numbers in parentheses are the 95% confidence limits—both above and below. To compare the experimental conditions, the LD_{50} of aminopyrine after intraperitoneal administration was determined; this value was 320 mg./kg. (300–350 mg./kg.). Although the animals receiving intraperitoneal doses below 270 mg./kg. or oral doses below 700 mg./kg. of taurinopiperidine exhibited no behavioral abnormality, the intraperitoneal dose of 320 mg./kg. or the oral dose of 800 mg./kg. produced transient ataxic walk with extended hind limbs 5–10 min. after the administration. Within 15 min. after intraperitoneal or oral administration of 450 or 1200 mg./kg. of taurinopiperidine, all of the animals died after exhibiting shock symptoms (such as progressive emaciation and respiratory failure), jumping, and convulsions. No significant difference was observed between male and female mice in the behavioral effect and the LD_{50}.

The ED_{50} of taurinopiperidine (90 min. after intraperitoneal administration) for the tail-withdrawal reflex in mice was 35 mg./kg. (26–47 mg./kg.). For the sake of comparing experimental conditions, the ED_{50} of aminopyrine 60 min. after intraperitoneal administration was determined and found to be 42 mg./kg. (30–59 mg./kg.). In these experiments, each group consisted of 10 male mice (dd strain, average weight 15 g.). The computation of the ED_{50} was made by dichotomizing the reactions after 60 or 90 min. into two groups: "refractory," *i.e.*, pain reaction time of 1.8 sec. or more, and "nonrefractory" for aminopyrine and taurinopiperidine, respectively. The analgesic effect of taurinopiperidine seems to be about the same as that of aminopyrine according to the ED_{50} of the tail-withdrawal reflex in mice.

Antihistaminic and antianaphylactic activities of taurinopiperidine in guinea pigs were examined by the method of Labelle and Tislow (12). The blood level of taurinopiperidine after its subcutaneous administration to guinea pigs (Fig. 2) was determined to see the relationship between blood level and antihistaminic or antianaphylactic action. Dosages of taurinopiperidine required to protect 50% of the guinea pigs were 90 mg./kg. (62–128 mg./kg.) in antihistaminic activity and 153 mg./kg. (113–206 mg./kg.) in antianaphylactic activity, with 95% confidence limits. It is certain that taurinopiperidine has antihistaminic and antianaphylactic actions, but the dosages of the chemical to protect 50% of guinea pigs from histaminic and anaphylactic shock are very high compared with other commercially available antihistaminic agents.

The effect of taurinopiperidine on hypercholesteremia induced by poly(oxyethylene ethers)[3] was examined, but no activity was observed after intraperitoneal administration of taurinopiperidine in 45-, 90-, and 135-mg./kg. doses. Under the same conditions, taurinopiperidine had no effect on the serum cholesterol level in normal rats.

No anti-inflammatory activity of taurinopiperidine was observed on an edema of rat hind paw induced by carrageenin. The effect of taurinopiperidine on squirming and capillary permeability in mice was investigated; the ED_{50} of inhibition of squirming was 214 mg./

[3] Triton WR-1339.

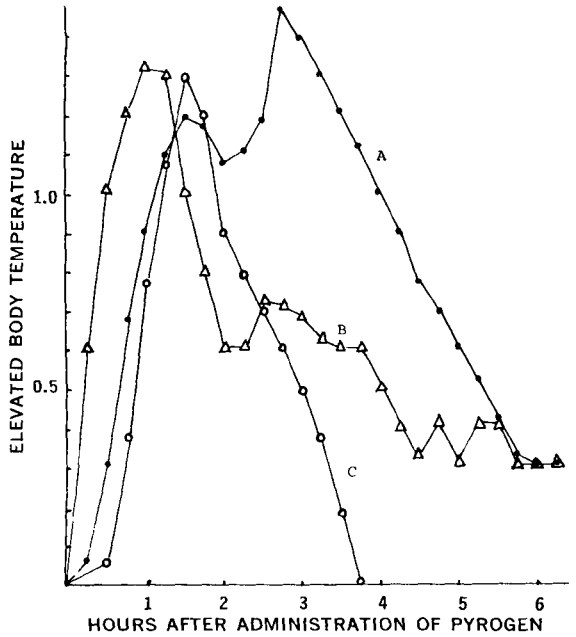

Figure 3—*Mean curve for the fall of elevated body temperature of male rabbits receiving pyrogen. Each group consisted of three rabbits. Key: A, control group, mean basal body temperature (MBBT) 39.3°; B, 45 mg./kg. of taurinopiperidine, MBBT 39.2°; and C, 90 mg./kg. of taurinopiperidine, MBBT 39.4°.*

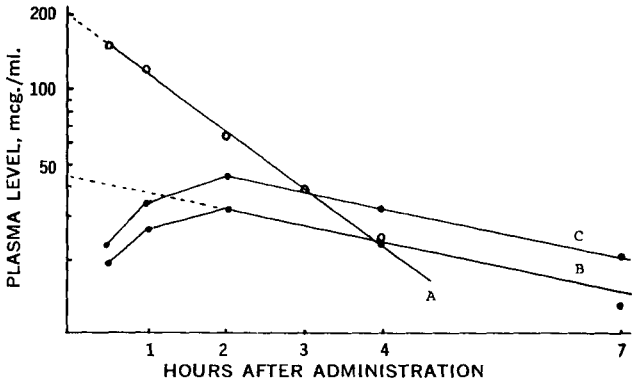

Figure 4—*Comparison of mean plasma level of taurinopiperidine after intravenous and oral administration to rabbits. Key: A, intravenous administration of 100-mg./kg. dose; B, oral administration of 100-mg./kg. dose; and C, oral administration of 150-mg./kg. dose. The data on oral administration were taken from Fig. 1. The volume of aqueous solution for intravenous injection to each rabbit was adjusted to 10 ml.; three male rabbits weighing about 2.2 kg. each were used.*

kg. (197–270 mg./kg.), and the ED_{50} of reduction of the permeability response was 142 mg./kg. (95–213 mg./kg.). Taurinopiperidine shows inhibition of permeability different from that of taurinopyrine (7) or taurinophenetidine (11).

The antipyretic activity of taurinopiperidine is presented in Fig. 3. Taurinopiperidine decreased the elevated body temperature of rabbits at the 90-mg./kg. dose. From this result, it is clear that taurinopiperidine has an antipyretic action although it is not very potent.

The changes in hemoglobin that occur when erythrocytes are incubated for 2 hr. with a test substance (in a molar ratio of 4:1 of hemoglobin) were examined in regard to the formation of methemoglobin. An incubation mixture of phenylhydrazine and hemoglobin at zero time showed 57% of oxyhemoglobin and 28% of methemoglobin, while that of taurinopiperidine and hemoglobin at zero time showed 100% of oxyhemoglobin. Two hours later, the former mixture showed 50% of oxyhemoglobin and 36% of methemoglobin and the latter mixture showed 99% of oxyhemoglobin and 1% of methemoglobin. It was thereby found that taurinopiperidine has no effect on "intact" hemoglobin (8), differing from phenylhydrazine which was used for the sake of comparison.

The hemolytic action of taurinopiperidine on rat blood was examined, but no effect was observed with 10, 50, and 100 mcg./ml. of the chemical in a final concentration.

No protective effect of taurinopiperidine on heat-induced erythrocyte lysis (10) *in vitro* was observed with 1, 5, and 10 mmoles of the chemical in a final concentration.

Figure 4 shows that the extrapolated blood level of taurinopiperidine at zero time is about 200 mcg./ml. after intravenous administration and about 43 mcg./ml. after oral administration of a 100-mg./kg. dose. Therefore, intravenous or subcutaneous administration of taurinopiperidine must be a better route than oral administration for the pharmacological activities of the compound if taurinopiperidine does not affect normal blood constituents.

Taurinopiperidine is very stable in aqueous solution, as shown by the fact that no decomposition was observed, within experimental error, after storage at 40, 50, 60, and 70° for 10 weeks.

DISCUSSION

Although assay procedures for taurinopiperidine are essential as an evaluation basis of absorption, excretion, and metabolism of drugs, no characteristic and quantitative color reactions of piperidine derivatives have been reported except the one described by Kakemi *et al.* (2). Kakemi *et al.* (2) showed that azacyclonol [α-(4-piperidyl)benzhydrol hydrochloride], a tranquilizer, can be assayed in urine colorimetrically by using β-naphthoquinone-4-sulfonate solution. They studied in detail the stability of the color developed by changing experimental conditions. Taurinopiperidine also shows a color reaction with β-naphthoquinone-4-sulfonate since it has a piperidine nucleus. It was established that the color developed from taurinopiperidine is very stable for at least 2 hr. in the chloroform layer under the experimental conditions used in the present work. The assay procedure was also more simplified than the method reported by Kakemi *et al.* (2).

SUMMARY

1. Taurinopiperidine in aqueous solution is very stable at room temperature; this fact indicates the possibility of making an injectable solution of this compound.

2. Taurinopiperidine is absorbed from the animal intestine, is distributed to rat organs, and is excreted in rat bile.

3. Taurinopiperidine is excreted in unconjugated form in rabbit urine, and most of this compound administered orally must be metabolized to other compounds which no longer have a piperidinelike nucleus.

4. Taurinopiperidine inhibits permeability and squirming in mice. This compound also has analgesic, antihistaminic, antianaphylactic, and antipyretic activities, although its potency is not high in any of these activities.

5. Taurinopiperidine shows low toxicity, no hemolytic action, and no formation of methemoglobin.

6. The results obtained show that taurinopiperidine has no harmful effects in animals. The possibility of clinical trials to determine pharmacological activities of this compound in man was discerned in the present work.

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Effect of Ionic Strength on Chemical Stability of Potassium Penicillin G

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Abstract □ Ionic strength up to 0.5 does not affect the chemical stability of potassium penicillin G unless one of the ions contributing to the ionic strength is also catalytic. Thus, ionic strength adjusted with NaCl had no effect on the chemical stability of 0.01 *M* potassium penicillin G solutions in 0.06 *M* citrate buffer at pH 7.0 where the major buffer species was the noncatalytic citrate ion. However, in penicillin solutions buffered with 0.06 *M* citrate buffer, the ionic strength had a direct effect at pH 6.5 or lower. The magnitude of the ionic strength effect was inversely related to pH. The monohydrogen citrate and dihydrogen citrate ions were found to be catalytic. At 45°, the catalytic rate constants were 0.272 and 1.92 l. mole^{-1} hr.$^{-1}$, respectively. At pH's where either of these catalytic species was present, an increase in the ionic strength by the addition of NaCl caused an increased rate of degradation.

Keyphrases □ Penicillin G, potassium—effect of ionic strength on chemical stability □ Ionic strength—effect on chemical stability of potassium penicillin G □ Degradation, penicillin—effect of ionic strength on chemical stability □ Potassium penicillin G—effect of ionic strength on chemical stability

The chemical instability of penicillin has led to the development of reconstitutable liquid dosage forms which demonstrate limited shelflife after reconstitution but possess the advantages of oral or parenteral liquid dosage forms. These dosage forms normally have a relatively high ionic strength due to the buffers, preservatives, and chelating agents which are required components of the formula. Most reports involving penicillin stability adjust the ionic strength to a constant value and study the effect of variables such as pH and temperature. The authors are aware of no studies which have examined the effect of ionic strength on the chemical stability of penicillins, although both Finholt *et al.* (1) and Hou and Poole (2) suggested that it may affect the chemical stability.

Carstensen (3) recently reviewed the applications of the primary salt effect to pharmaceuticals. He pointed out 14 drugs including penicillin that are affected by ionic strength.

This variable may have an important effect because liquid penicillin dosage forms have various ionic strengths depending on the individual formulation. In most cases, pharmaceutical products have a relatively high ionic strength. In addition, the ionic strength of gastric juice is reported to be 0.09–0.24 (4). The effect of ionic strength may be pH dependent, and a different effect may occur at the low pH of the stomach compared to the more neutral pH of liquid dosage forms.

The objective of this study was to investigate the effect of ionic strength on the chemical stability of potassium penicillin G with the aim of increasing the understanding and ability to control the degradation of penicillin.

EXPERIMENTAL

Materials—Potassium penicillin G[1] was used. All other chemicals were reagent grade.

Methods—The chemical stability of 0.01 *M* potassium penicillin G solutions at 45° in 0.06 *M* citrate buffer was studied at pH values from 4.0 to 7.0 and at ionic strengths of 0.30 to 0.50. The pKa values for citric acid at 60° and ionic strength 0.50 are 2.72, 4.30, and 5.47 (1). These values were used to calculate the concentration of each citrate species present in the solutions studied (Table I) because they were determined at temperature and ionic strength conditions very similar to the experimental conditions of this study.

The ionic strength of the 0.01 *M* potassium penicillin G solutions with 0.06 *M* citrate buffer was calculated at each pH from the data in Table I and from the reported pKa for potassium penicillin G of 2.78 at 60° and ionic strength 0.5 (1). The concentration of NaCl needed to obtain ionic strengths of 0.30, 0.35, 0.40, 0.45, and 0.50 was determined at each pH. Solutions were prepared using 0.01 *M* potassium penicillin G, 0.06 *M* citric acid, and the calculated concentration of NaCl. The volume of 1 *N* NaOH needed to adjust each solution to the desired pH was determined, and a final small correction in ionic strength was made to include the concentration of sodium ion used in adjusting pH. The final composition of each solution is given in Table II.

Stability samples were prepared by adding the calculated amounts of citric acid, NaCl, and 1 *N* NaOH to doubly distilled water. One hundred milliliters of these solutions was placed in a 45° water bath, and 0.001 mole of potassium penicillin G was added when the solution reached 45°. An initial sample was taken immediately after the addition of the potassium penicillin G to the 45° solution.

[1] Eli Lilly and Co.