Biopharmaceutical Studies on 4-(Aminoethanesulfonylamino)antipyrine and Related Compounds I

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Abstract □ Blood levels of 4-(aminoethanesulfonylamino)antipyrine in rabbits were determined, and binding of the chemical with rabbit serum *in vivo* and *in vitro* was examined. Metabolites of the chemical in rabbit urine were separated into five compounds: rubazonic acid (trace), 4-aminoantipyrine, 4-acetylaminoantipyrine, 4-hydroxyantipyrine (trace), and unchanged chemical. Glucuronide in urine of rabbits administered with 4-(aminoethanesulfonylamino)antipyrine is the conjugated form with 4-hydroxyantipyrine. Some pharmacological activities were investigated; it was observed that the chemical shows analgesic, anti-inflammatory, antihistaminic, and antipyretic actions.

Keyphrases □ 4-(Aminoethanesulfonylamino)antipyrine—biopharmaceutical studies □ Taurinopyrine—rabbit serum protein binding □ Metabolites, taurinopyrine, aminopyrine in urine—separation, analysis □ Pharmacological screening—taurinopyrine

Aminopyrine has been used for many of the same symptoms and conditions as the salicylates, acetophenetidin, and acetaminophen. The relatively infrequent but possibly severe toxicity, however, has led to a gradual decline in its use.

Taurinopyrine [4-(aminoethanesulfonylamino)antipyrine] was synthesized in the hope of obtaining a potent analgesic/antipyretic agent with a metabolic pathway sufficiently different from aminopyrine so as to overcome the toxicities associated with this drug. Aminoethanesulfonic acid, which is expected to separate from taurinopyrine when ingested, is one of the physiological amino acids and is also surface active. Taurinopyrine occurs as colorless needle crystals, m.p. 185° dec., which are fairly soluble in water; sparingly soluble in ethanol, acetone, and benzene; and practically insoluble in ether, petroleum ether, and petroleum benzene.

In a previous paper (1), stabilities of taurinopyrine stored at different temperatures and pH's were reported; it was shown that the chemical is stable, in either solution or powder form, to temperature, pH, or moisture.

The present series of experiments was undertaken to determine the blood level of taurinopyrine in animals, to determine the metabolites of taurinopyrine present in rabbit urine, and to evaluate some of its pharmacological properties.

Table I—TLC of Lyophilized Rabbit Urine[a]

Drug	R_f (Assumed Substance)	Color of Spot
Taurinopyrine (400 mg./kg.)	0.06 (unchanged taurinopyrine)	Black
	0.33 (4-hydroxyantipyrine)	Pale brown
	0.43 (4-acetylaminoantipyrine)	Grey
	0.56 (4-aminoantipyrine)	Pale brown
	0.95 (rubazonic acid)	Brownish black
Aminopyrine (300 mg./kg.)	0.43 (4-acetylaminoantipyrine)	Grey
	0.56 (4-aminoantipyrine)	Pale brown
	0.65 (unchanged aminopyrine)	Black

[a] Adsorbent: diatomite (Kieselgel G), 0.25 mm. in thickness; solvent: acetone–benzene–chloroform (10:10:1); and color developer: 5% silver nitrate solution.

Table II—Glucuronides in Rabbit Urine

Given Drug	Dose per Rabbit, 2 kg. Body Weight	Number of Rabbits Used	Yield of Crude Glucuronide[a]	Component of Glucuronide
Taurinopyrine	800 mg.	2	60 mg.	H[b]
Antipyrine	1000 mg.	6	280 mg.	H
4-Aminoantipyrine	1000 mg.	6	600 mg.	H, A[c]
Methampyrone	800 mg.	6	560 mg.	H, A
Aminopyrine	800 mg.	4	270 mg.	H, A

[a] After hydrolysis of the glucuronides with hydrochloric acid, 4-hydroxyantipyrine and 4-aminoantipyrine were identified through TLC shown in Table I. [b] H: 4-hydroxyantipyrine. [c] A: 4-aminoantipyrine.

EXPERIMENTAL

Binding of Taurinopyrine with Rabbit Serum Protein *In Vitro*— A mixture of 0.1 ml. of taurinopyrine solution (150 mg. %) and 0.9 ml. of normal rabbit serum was treated as reported earlier (2). The modified Folin method (2, 3) was used to quantitate the taurinopyrine.

Binding of Taurinopyrine or Aminopyrine with Rabbit Serum *In Vivo*— Taurinopyrine (300 mg./kg.) was administered orally to four rabbits, and serum samples were removed after 2 hr. One milliliter of this serum was mixed with 2 ml. of absolute ethanol and cooled in ice water for 20 min. This solution was centrifuged to separate the protein precipitate, and 0.5 ml. of the supernatant was removed for analysis. The supernatant was diluted with 0.5 ml. of water, and the modified Folin method was used to assay for free taurinopyrine. Total taurinopyrine was determined after 0.5 ml. of the plasma was mixed with 0.5 ml. of 5 *N* HCl and kept at 37 ± 2° for 2 hr. by using the modified Folin method and *in vitro* procedure with aminopyrine.

Two hours following aminopyrine (200 mg./kg.) administration to four rabbits, blood was collected and 1 ml. of serum was treated according to the *in vitro* procedure employed for taurinopyrine. Both free aminopyrine and total aminopyrine in serum were determined.

Separation and Assay of Metabolites of Taurinopyrine and Aminopyrine in Urine— About 500 ml. of urine collected from each of four rabbits (average body weight, 2 kg.), following the ingestion of 400 mg./kg. of taurinopyrine or 300 mg./kg. of aminopyrine, was lyophilized and assayed as in the case of taurinophenetidine (2) and evaluated by TLC as described previously (1).

To assay the metabolites of taurinopyrine or aminopyrine in urine, a mixture of 1 ml. of 5 *N* HCl and 200 mg. of the freeze-dried residue of urine, obtained from rabbits (average weight, 2 kg.) dosed with either taurinopyrine (400 mg./kg.) or aminopyrine (300 mg./kg.), was stored in an incubator at 37 ± 2° for 1 hr. One milliliter of 5 *N* sodium carbonate solution was added to each sample, and the resulting mixture was centrifuged. Fifty microliters of the supernatant was spotted on a chromatographic plate and submitted to TLC as described in Table I. After development with a mixed solvent of acetone, benzene, and chloroform (10:10:1), the spots corresponding to the unchanged drug, 4-acetylaminoantipyrine and 4-aminoantipyrine, were each scratched off the TLC plate and extracted 3 min. with 3 ml. of water

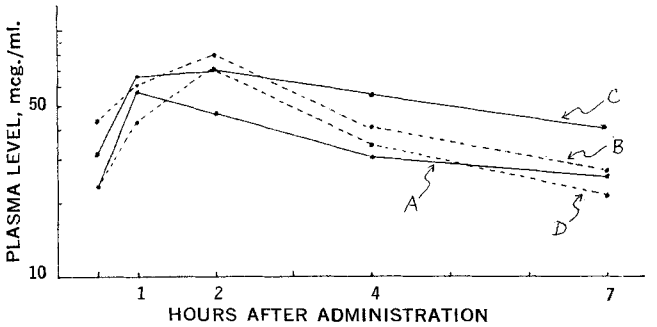

Figure 1—*Mean plasma level of taurinopyrine after oral administration to rabbits at different doses. Each group consisted of four female rabbits weighing 2.3 ± 0.3 kg. Key: A, 200 mg./kg.; B, 300 mg./kg.; C, 400 mg./kg.; and D, 500 mg./kg.*

on a boiling water bath. The mixture was centrifuged, and 1 ml. of the supernatant was treated in the same way to determine the amount of *p*-aminophenol and *p*-acetamidophenol (2), the metabolites of taurinophenetidine in rabbit urine.

A calibration curve for each of the two unchanged drugs and the two major metabolites was prepared, using known concentrations of each and the same quantitative procedure as previously described.

Glucuronides in Rabbit Urine—Taurinopyrine, antipyrine, aminopyrine, 4-aminoantipyrine, or methampyrone was administered to determine the relationship between the chemical structure and metabolites in glucuronide form. The drug was given with water to rabbits through a stomach tube in the form of a solution or suspension (without any suspending agent), depending on the solubility of the drug. Taurinopyrine, 4-aminoantipyrine, and methampyrone were given as solutions, and antipyrine and aminopyrine were given as aqueous suspensions. About 500 ml. of urine was collected during 5 hr. and then treated according to the procedure reported by Williams (4).

The crude glucuronide thus obtained was hydrolyzed with a small amount of 5 *N* HCl at 37 ± 2° for 1 hr. and then neutralized with 5 *N* sodium carbonate solution. The solution was submitted to TLC evaluation as shown in Table I.

Metabolites of Taurinopyrine in Blood—A mixture of 1 ml. of rabbit plasma, taken 2 hr. (peak time of blood level) after oral ingestion of 500 mg./kg. of taurinopyrine, was treated by the same method as described in the previous report (2) and then submitted to TLC (Table I). The experiment was carried out on two rabbits of about 2 kg. in body weight, fasted for 24 hr.

Determination of Taurinopyrine in Plasma—The analysis of taurinopyrine in rabbit and guinea pig plasma was conducted as described previously (2).

Drug to be Tested—4-Hydroxyantipyrine was synthesized by the method of Halberkann and Tretwurst (5). Rubazonic acid was synthesized according to the method of Knorr (6, 7)

Acute Toxicity—Equal numbers of male and female dd strain mice, weighing 17 ± 2 g., were used. The animals were maintained on a commercial diet[1] and drinking water *ad libitum* at the room temperature of 20 ± 2°. Five mice were housed per cage, and from 30 to 50 were used to determine each LD_{50}.

The test solutions of taurinopyrine and aminopyrine were employed for the intraperitoneal and oral administrations. Various doses of the chemical were dissolved in water, and the volume was adjusted to 0.5 ml./10 g. body weight. The animals were fasted 5 hr. prior to drug administration, and they were allowed free access to the diet and drinking water 4 hr. after the administration. The behavioral changes were observed for 72 hr. after drug administration; the LD_{50} of the chemicals was calculated from the number of animals dying within 72 hr., according to the Litchfield–Wilcoxon method (8).

Effect of Taurinopyrine, Aminopyrine, and Related Compounds on Squirming and Capillary Permeability—Male and female dd strain mice, weighing 20 ± 1 g., were given the test compounds by stomach tube. After 20 min., each animal was given an intravenous injection of 0.1 ml. of a 4% solution of pontamine sky blue (Merck). At 30 min. after administration of a test compound, 1 mg. of acetic acid was injected intraperitoneally as 0.4 ml. of a 0.25% (v/v) solution. Each dose group consisted of 12 mice. Animals were primed by intramuscular injection of 0.05 mg. of stilbestrol 48 hr. before the test. Stilbestrol was administered as a solution in ethyl oleate containing 1 mg./ml. The method of Whittle (9) was followed.

Table III—Acute Toxicity of Taurinopyrine and Aminopyrine in Mice

Route	Compound	Dose, mg./kg.	Mortality Male	Mortality Female	LD_{50},[a] mg./kg. (Male and Female)
i.p.[b]	Aminopyrine	180	0/5	0/5	375
		200	0/5	0/5	(295–476)[c]
		230	0/5	1/5	
		260	0/5	3/5	
		280	0/5	2/5	
i.p.	Taurinopyrine	500	0/5	0/5	760
		600	1/5	0/5	(710–813)
		700	1/5	3/5	
		800	2/5	3/5	
		900	5/5	4/5	
p.o.[d]	Aminopyrine	500	1/5	0/5	720
		600	1/5	2/5	(621–835)
		700	2/5	2/5	
		800	3/5	2/5	
p.o.	Taurinopyrine	3000	0/5	1/5	5350
		4000	1/5	1/5	(4180–6848)
		5000	3/5	2/5	

[a] LD_{50} was calculated by the Litchfield–Wilcoxon method (8). [b] Intraperitoneally. [c] 95% confidence limits. [d] *Per os.*

Tail-Pinching Test—The reaction time of mice pinched on the tail with an artery clip was recorded every 15 min. for 1.5 hr. after administration of the compounds by the method described by Haffner (10)

Tail-Withdrawal Reflex in Mice—The same method for analgesimetry described by Ben-Bassat *et al.* (11) was used.

Anti-Inflammatory Activity—Inflammation was produced by injecting carrageenin (0.1 ml. of 2% suspension) into the plantar surface of the rat's hind paw. The test compound was administered as an aqueous solution, and the volume was kept constant at 0.1 ml./100 g. body weight for intraperitoneal dosage. One-half of the total dose was given approximately 6 hr. later. Intraperitoneal injections were given in either caudal quadrant of the abdominal wall. Control animals received injection of the vehicle alone. The results were obtained 24 hr. after administration of carrageenin.

Anaphylactic Shock in Guinea Pigs—Guinea pigs (Hartley strain) were used in the experiment following the procedure of Labelle and Tislow (12). Guinea pigs were challenged exactly 1 hr. after drug administration by rapid injection of 1 ml. of horse serum into the saphenous vein.

Antihistamine Activity—Male and female guinea pigs (Hartley strain), with a body weight of about 200 g., were used in the histamine tests. The compound was administered subcutaneously 0.5 and 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 made in fasted animals.

Antipyretic Activity—To rabbits weighing 2.3–2.5 kg., 15 mcg./kg. of nonanaphylactogenic polysaccharides obtained from *Pseudomonas fluorescens*[2] was administered intravenously, and taurinopyrine solution was given subcutaneously 2 hr. after the administration of pyrogen solution. Body temperature was recorded every 15 min. for 6 hr.

RESULTS AND DISCUSSION

Rabbit plasma, taken 2 hr. after oral ingestion of 500 mg. taurinopyrine/kg., contained no detectable metabolites of the chemical. Concentrations of taurinopyrine in blood following the oral administration of 200, 300, 400, and 500 mg./kg. are shown in Fig. 1. Since the peak blood level is not proportional to the dose, the absorption would apparently be active.

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

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

Table IV—Evaluation of Analgesic Potency in Mice Using the Tail-Pinching Test

Route	Compound	Dose, mg./kg.	Number Not Reacting[a] Male	Number Not Reacting[a] Female	ED_{50},[b] mg./kg. (Male and Female)
i.p.[c]	Aminopyrine	50	2/5	3/5	65
		75	3/5	3/5	(51.1–82.5)[d]
		100	4/5	4/5	
		125	5/5	4/5	
i.p.	Taurinopyrine	50	2/5	0/5	94
		80	2/5	2/5	(73–120)
		90	2/5	2/5	
		100	3/5	3/5	
p.o.[e]	Aminopyrine	50	1/5	2/5	142
		100	2/5	2/5	(90–223)
		150	3/5	2/5	
		200	3/5	3/5	
		300	5/5	3/5	
p.o.	Taurinopyrine	400	1/5	1/5	560
		500	1/5	2/5	(479–655)
		600	3/5	3/5	
		700	4/5	3/5	

[a] The results were obtained 30 min. after administration. [b] ED_{50} was calculated by the Litchfield–Wilcoxon method (8). [c] Intraperitoneally. [d] 95% confidence limts. [e] *Per os.*

Table V—Effect of Test Compounds on Squirming and Capillary Permeability

Compound	Oral Dose, mg./kg.	Reduction of Squirming, %	Inhibition of Squirming, ED_{50}, mg./kg.	Change in Permeability,[a] %
Taurinopyrine	100	8	475	+83
	250	24	(293–770)[b]	−31
	350	36		−41
	500	56		+30
Aminopyrine	50	28	213	0
	100	33	(83–545)	−11
	250	50		−45
4-Aminoantipyrine	50	26	165	−9
	100	35	(79–347)	−32
	250	58		−35
4-Acetylamino-antipyrine	50	12		−12
	100	25		+3
	250	25		−10

[a] Percentages are calculated from the mean of groups consisting of 12 mice compared with controls (12 mice). [b] 95% confidence limits.

Taurinopyrine (300 mg./kg.) produced a peak level of 81.3 ± 15.4 mcg./ml. 2 hr. after oral administration. Eighty-two percent of this amount was bound to serum proteins. Aminopyrine (200 mg./kg.) produced a peak blood level of 117.8 ± 8.7 mcg./ml. 2 hr. after oral administration, and 58% was bound to plasma proteins. On the other hand, the binding ratio of taurinopyrine with rabbit serum *in vitro* was about 52%. These experiments were performed because of the possibility that the binding of a drug with serum protein could be prohibited by the action of hydrochloric acid.

Urine samples collected from rabbits within 48 hr. of the administration of both taurinopyrine (400 mg./kg.) and aminopyrine (300 mg./kg.) were lyophilized, and the residue was examined by TLC for the presence of metabolites (Table I). Five spots representing rubazonic acid, 4-aminoantipyrine, 4-acetylaminoantipyrine, 4-hydroxyantipyrine, and unchanged drug (taurinopyrine or aminopyrine) were separated and identified. A micromelting-point determination of the mixture of evaporated residue of ethanolic extract from 5–10 spots having the same R_f value and an authentic sample was used for identification. Two of the metabolites, rubazonic acid and 4-hydroxyantipyrine, were present only in trace amount, whereas 48.3% was unchanged taurinopyrine, 43.7% was 4-acetylaminoantipyrine, and 8.0% was 4-aminoantipyrine. In the case of aminopyrine, 52.0% was excreted as 4-aminoantipyrine, 32.5% as the unchanged aminopyrine, and 15.5% as 4-acetylaminoantipyrine. The physiological availability of taurinopyrine or aminopyrine in rabbits could not be calculated because of incomplete urine collection.

4-Hydroxyantipyrine was detected from the glucuronides in urine when taurinopyrine was administered orally to rabbits (Table II).

According to Brodie and Axelrod (13), aminopyrine is metabolized in man at the rate of 10–30%/hr., with only about 3% being excreted unchanged. About one-half of the 0.5-g. dose is excreted in 3 days as 4-aminoantipyrine and its acetyl derivative, the latter compound being the major metabolite. Metabolism of taurinopyrine in man has not been studied, but taurinopyrine is metabolized in rabbits in a way similar to aminopyrine; it is dealkylated to 4-aminoantipyrine, which is excreted partly as such and partly acetylated.

Thus, it was clarified that the main substance excreted after ingestion of taurinopyrine is the unchanged taurinopyrine, in contrast to the main metabolite of aminopyrine, that is, 4-aminoantipyrine. Therefore, taurinopyrine may have a characteristic pharmacological action different from aminopyrine.

The details of acute toxicity of various doses of taurinopyrine and aminopyrine are shown in Table III. Although the animals receiving intraperitoneal doses below 500 mg./kg. of taurinopyrine exhibited no behavioral abnormality, the intraperitoneal dose of 700–800 mg./kg. produced a transient ataxic walk with extended hind limbs for 5–10 min. after the administration. Thereafter, the animals showed a marked reduction in spontaneous movements. Within 1 hr. after the administration of 1000 mg./kg. of taurinopyrine, all of the animals died in respiratory failure. No animal exhibited lacrimation, piloerection, or convulsions before death. The righting reflex was usually maintained until death. Absence of convulsions before death in the group receiving taurinopyrine is very different from the manifestation of convulsions before death in the group receiving aminopyrine. No significant difference in the behavioral effect or LD_{50} was observed between the male and female mice.

The results of the tail-pinching test are shown in Table IV. Taurinopyrine shows analgesic action to the tail-pinching test, but its potency is less than that of aminopyrine.

Concurrent changes in the peritoneal capillary permeability during squirming was measured according to the method of Whittle (9). Table V records the values for the ED_{50} for inhibition of squirming; since reduction of the permeability response did not approach 50% over the range of doses that inhibited squirming, there is no

Table VI—Average Pain Reaction Times in Control Mice and Mice Given Intraperitoneal Injections of Test Compounds[a]

Compound	Dose, mg./kg.	Average Reaction as Recorded in Seconds after Injection 15′	30′	60′	90′	120′	150′	PRT[b]
Control		1.3	1.4	1.2	1.2	1.1	1.2	1.2
Aminopyrine	50	1.7	2.0	2.6	2.8	2.9	3.8	2.6
		1/10[c]	4/10	6/10	7/10	6/10	7/10	
	75	1.5	2.6	2.6	2.5	3.0	2.7	2.5
		5/10	8/10	8/10	6/10	7/10	7/10	
	100	2.7	3.9	4.3	4.7	4.7	4.3	4.1
		7/10	9/10	9/10	8/10	8/10	8/10	
Taurinopyrine	50	1.4	2.0	1.8	2.7	2.6	2.9	2.2
		2/10	2/10	3/10	4/10	4/10	4/10	
	100	1.3	1.7	2.4	2.4	2.5	2.2	2.1
		2/10	3/10	4/10	4/10	4/10	6/10	
	150	2.1	2.5	3.0	3.0	3.4	3.7	2.9
		4/10	4/9	5/9	6/9	6/9	6/9	

[a] Each group consisted of 10 mice. [b] PRT: average pain reaction time. [c] Number of effective mice/number of mice used.

Table VII—Effect of the Edema Induced by Subplantar Injection of Carrageenin[a]

Compound	Dose, mg./kg.	Normal Foot Volume ± SE[b]	Mean Foot Volume ± SE	Inhibition, %
Control		0.302 ± 0.056	0.988 ± 0.039	
Aminopyrine	100	0.260 ± 0.071	0.960 ± 0.048	
	200	0.230 ± 0.093	0.910 ± 0.055	
	300	0.305 ± 0.039	0.982 ± 0.036	2
Taurinopyrine	100	0.286 ± 0.042	0.900 ± 0.037	13
	200	0.322 ± 0.056	0.878 ± 0.046	19
	300	0.274 ± 0.075	0.736 ± 0.050[c]	33

[a] Each group consisted of 10 rats, each weighing 150 ± 15 g. [b] Standard error of the mean. [c] Statistically significant effect ($p < 0.05$).

Table VIII—Blood Level of Taurinopyrine (mcg./ml.) following its Subcutaneous Administration to Guinea Pigs[a]

Dose, mg./kg.	Hours after Dosing 0.25	0.5	1.0	2.0	4.0	7.0
100	84 ± 33[b]	35 ± 3	21 ± 8	4 ± 5	0	0
200	95 ± 23	87 ± 11	87 ± 23	52 ± 13	19 ± 12	3 ± 2
300	122 ± 17	101 ± 8	90 ± 7	54 ± 6	16 ± 7	3 ± 4

[a] Each group consisted of three male guinea pigs; the animals of each group were killed for collection of blood every sampling time. At the time of sacrifice, each animal was bled completely, and the blood was reserved for analysis. Average body weight of the guinea pigs was 200 g. [b] Mean value ± standard error.

corresponding ED_{50} for the permeability effect. Although the dose of taurinopyrine at ED_{50} is higher than that of aminopyrine, taurinopyrine does inhibit squirming but not permeability.

Study results of the tail-withdrawal reflex in mice (11) are shown in Table VI. Computation of ED_{50} and its confidence limits was made by probit analysis. This was made by dichotomizing the reactions after 60 and 90 min. into two groups: (*a*) "refractory," *i.e.*, pain reaction time of 1.8 sec. or more; and (*b*) "nonrefractory," for aminopyrine and taurinopyrine. The ED_{50} and 95% confidence limits were 42 (30–59) mg./kg. for aminopyrine and 89 (59–135) mg./kg. for taurinopyrine. Analgesic effect of taurinopyrine seems to be less potent than that of aminopyrine, according to the ED_{50} of the tail-withdrawal reflex in mice.

Table VII shows the anti-inflammatory effects of aminopyrine and taurinopyrine in the carrageenin test. Taurinopyrine was more active than aminopyrine in these tests. However, anti-inflammatory tests using other irritants, such as formaldehyde solution or croton oil, should be performed before definite conclusions are drawn.

Antihistamine and antianaphylactic shock activities of taurinopyrine were evaluated by the method of Labelle and Tislow (12). No difference in antihistamine activity was observed between 300- and 500-mg./kg. doses of taurinopyrine when the compound was administered subcutaneously 1 hr. before histamine injection. As a result, 60% of 10 guinea pigs survived in each group. Therefore, blood levels of taurinopyrine were measured following different doses to determine if some relationship exists between antihistamine activity and blood level of the drug (Table VIII). No definite relationship was observed, although the potency of antihistamine activity seems to be stronger when the chemical is given 30 min. rather than 1 hr. before histamine injection (Table IX). Table IX also presents antianaphylactic results. From these results, it can be concluded that taurinopyrine shows antihistamine activity but its antianaphylactic action is uncertain. The results of antipyretic activity are presented in Fig. 2. Taurinopyrine, at doses of 150 and 250 mg./kg., decreased the elevated body temperature of all rabbits used, not rapidly but gradually.

In conclusion, it was shown that taurinopyrine has some analgesic, anti-inflammatory, antihistamine, and antipyretic activities. More

Table IX—Antianaphylactic and Antihistamine Activities of Taurinopyrine in Guinea Pigs[a]

Antianaphylactic Activity	
Dose, mg./kg.	Survived for 10 min.
Control	0/10
100	2/10
200	1/10
300	1/10
500	4/10

Antihistamine Activity[b]			
	Survived for 24 hr., Administration of Taurinopyrine		
Dose, mg./kg.	30 min. before Histamine Injection	1 hr. before Histamine Injection	ED_{50}, mg./kg.
150	2/10		195
175	4/10		(165–230)[c]
200	5/10		
175		2/10	250
200		4/10	(197–318)
250		4/10	

[a] Guinea pigs weighing 250–300 g. were used. [b] No control animals receiving histamine injection survived for 24 hr. [c] 95% confidence limits.

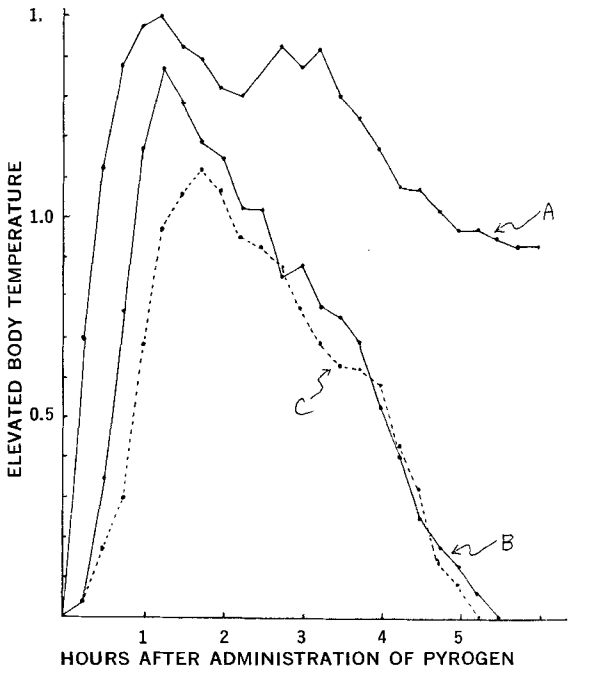

Figure 2—*Mean fall of elevated body temperature of male rabbits receiving taurinopyrine 2 hr. after administration of pyrogen. Each group consisted of three male rabbits weighing 2.4 ± 0.2 kg. and having 39.3 ± 0.3° of basal body temperatures. Key: A, control group; B, 150 mg./kg.; and C, 250 mg./kg.*

extensive investigations are required to determine its practical therapeutic application.

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Biopharmaceutical Studies on Aminoethanesulfonylphenetidine and Related Compounds II

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Abstract □ The binding ratio of taurinophenetidine or nicotinoyltaurinophenetidine with serum protein in rabbits varies according to experimental conditions in dialysis, but the averaged binding ratios of taurinophenetidine and its nicotinate were roughly 40–50% at high doses. Taurinophenetidine and its nicotinate are scarcely excreted in rat feces and in rat and rabbit bile. The amount of both compounds distributed in organs of mice and rats is very small. It was observed that taurinophenetidine has some analgesic and antipyretic activities, and nicotinoyltaurinophenetidine reveals some analgesic and anti-inflammatory activities but no antipyretic action.

Keyphrases □ Aminoethanesulfonylphenetidine, related compounds—biopharmaceutical studies □ Metabolites, taurinophenetidine and derivatives—determination □ Taurinophenetidine and derivatives—distribution, animal organs □ Serum protein binding—taurinophenetidine and derivatives

In a previous work (1), blood levels of aminoethanesulfonylphenetidine (taurinophenetidine) in rabbits were determined and binding of taurinophenetidine with rabbit serum was found to be about 67% of the total amount administered *in vitro* and nil *in vivo*. Metabolites of taurinophenetidine in rabbit urine are separated into four compounds: *p*-aminophenol, *p*-acetamidophenol, *p*-phenetidine, and unchanged taurinophenetidine. It was also recognized that glucuronide in urine of rabbits ingesting taurinophenetidine is the conjugated form of *p*-aminophenol.

The present paper deals with the results obtained from biopharmaceutical studies and with some of the pharmacology on two compounds of *p*-phenetidine derivatives, taurinophenetidine and nicotinoylaminoethanesulfonylaminophenetidine (hereafter abbreviated as nicotinoyltaurinophenetidine).

EXPERIMENTAL

Metabolites of Taurinophenetidine in Blood—Plasma from 10 male mice (dd strain, average weight 20 g., taken at the time of peak blood levels 1.5 hr. after oral administration of 2500 mg./kg. of taurinophenetidine) or plasma from three female rats (Wistar strain, average weight 200 g., taken at the time of peak blood levels 1.5 hr. after oral ingestion of 2000 mg./kg. of taurinophenetidine) was treated by the method described in the previous paper (1).

Metabolites of Nicotinoyltaurinophenetidine in Blood—Plasma from two female rabbits (average weight 2.3 kg., taken at the time of peak blood levels 1.5 hr. after oral ingestion of 1000 mg./kg. of nicotinoyltaurinophenetidine) was treated by the same method (1). Methylene dichloride was used instead of chloroform for extraction.

TLC for separation of the metabolites was carried out under the following conditions: solvent, benzene–acetone–ethyl acetate (1:1:2); adsorbent, diatomite (Kieselgel G); color developer, 0.4% potassium ferricyanide solution followed by spraying with 1% ferric nitrate in 0.7 *N* nitric acid; R_f values in plasma of rabbits ingested the chemical, 0.33; R_f values in control plasma, nil; and R_f value of nicotinoyltaurinophenetidine, 0.33.

Determination of Acetophenetidin in Blood—Four female rabbits (average weight 3.0 kg.) were used. After oral administration of 250 mg./kg. of acetophenetidin, blood samples were taken according to the sampling schedule. To assay the free form of acetophenetidin, a mixture of 1 ml. of plasma and 3 ml. of water was shaken with 7 ml. of chloroform for 15 min. After centrifugation and filtration, a 3-ml. aliquot of the chloroform layer was evaporated to dryness on a water bath, and the residue was treated by the same method as previously reported for the determination of isopropylantipyrine (2).

A mixture of 1 ml. of plasma and 1 ml. of 5 *N* HCl was incubated at 37 ± 2° for 1.5 hr., and 2 ml. of water was added to this mixture. The plasma was treated with the acid due to the consideration that the binding of a chemical with plasma protein can possibly be prohibited by the action of hydrochloric acid. This mixture was shaken with 7 ml. of chloroform and treated by the same procedure as described for determination of total acetophenetidin.

Determination of Nicotinoyltaurinophenetidine in Blood—Each group consisted of four female rabbits (average weight 2.3 kg.).