PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, 146 358-363 (1974)

N-Acetylprocainamide: An Active Metabolite of Procainamide[1] (38104)

DENNIS E. DRAYER, MARCUS M. REIDENBERG, AND ROGER W. SEVY

Departments of Pharmacology and Medicine, School of Medicine, Temple University, Philadelphia, Pennsylvania 19140

Procainamide (PA), *p*-amino-*N*-(2-diethylaminoethyl)benzamide, is used clinically as an effective agent in the treatment of cardiac arrhythmias (1–3). Fifty to sixty-five percent of an administered dose of PA is excreted unchanged by man. A small amount, 2–10%, is excreted as *p*-aminobenzoic acid or its conjugates (4). Recently, *N*-acetylprocainamide (NAPA) has been found in the pooled urine of four patients receiving PA administered orally (5). The purpose of our study is to determine if this metabolite is present in human plasma and if it is pharmacologically active.

Materials and Methods. *N*-Acetylprocainamide hydrochloride was prepared from PA and acetyl chloride by the method of Dreyfuss *et al.* (6). The resulting white crystalline solid has a mp of 186–188°.

Anal. Calcd for $C_{15}H_{24}N_3O_2Cl$: C, 57.41; H, 7.71; N, 13.39; O, 10.20; Cl, 11.30. Found: C, 57.48; H, 7.62; N, 13.14; O, 10.44; Cl, 11.37.

Detection of NAPA and PA in human plasma. Human plasma, 8 ml, was alkalinized by the addition of 1.6 ml of 5 *N* NaOH and then extracted with 80 ml of chloroform. The residue of the organic extract was spotted on an activated thin-layer plate coated with aluminum oxide F-254 type-T (Brinkmann). The R_f of NAPA = 0.23 and the R_f of PA = 0.33 when developed with a solution of benzene, ammonia, and dioxane (10:1.3:80). Gas chromatographic–mass spectroscopic analysis of the organic extract and authentic NAPA was performed using the same conditions described by Strong and Atkinson (7) with the exception that the column temperature was 245°. At these conditions PA and NAPA have retention times of 3 min and 11 min, respectively. A sample of blood bank plasma to which known amounts of PA·HCl and NAPA·HCl were added was also analyzed by the above thin-layer chromatographic (tlc) procedure.

PA plasma levels were determined by the fluorimetric method of Koch-Weser and Klein (3).

Measurement of Pharmacologic Activity. The antiarrhythmic activity of NAPA and PA was determined in mice according to the procedure of Lawson and Rahdert (8). NAPA·HCl was injected ip into 42- to 49-day-old ICR male mice, av wt 30 g. Ten minutes later the mice were placed in a covered beaker containing a surgical sponge saturated with chloroform. After respiratory arrest of the mice, a bipolar electrocardiogram was obtained on a cathode ray oscilloscope. Eight mice were used at each dosage level. Mice receiving PA·HCl were handled in the same manner.

The antiarrhythmic activity of NAPA was determined in mongrel dogs weighing between 14 and 17 kg according to the following procedures: Anesthesia was induced with sodium pentobarbital, 30 mg/kg iv. Under positive-pressure artificial respiration, the chest was opened between ribs 3 and 4 and a pericardial cradle prepared to permit easy access to the right atrium. Direct atrial and ventricular electrograms were recorded via stainless-steel electrodes sewn into the atrium and ventricle. Standard limb electrocardiograms and femoral blood pressure were also recorded simultane-

[1] Supported in part by N.I.H. Grants GM 18276, 5-T0-HL05362, and Grant RR349 from the General Clinical Research Program of the Division of Research Resources, N.I.H., and a grant from the Smith, Kline, and French Foundation. Dr. Reidenberg is the recipient of U.S.P.H.S. Research Career Development Award 5K04-GM 09766 from the N.I.G.M.S.

ously on a multichannel, cathode ray oscillograph (Electronics for Medicine Recorder).

Atrial flutter was produced in dogs 1 and 2 by the procedure described by Rosenblueth and Garcia Ramos (9), but spontaneous conversion to normal sinus rhythm occurred within ½ hr. Atrial arrhythmias (fibrillation/flutter) were then induced in these dogs by intramural injection of aconitine into multiple sites in a small area of the atrium (10). In dogs 3 and 4 aconitine-induced arrhythmias were produced without prior attempts to establish a circus path. NAPA or PA injections or infusions were given and the effects observed.

Groups of eight Sprague-Dawley male rats (av wt = 140 g) received either 86 mg/kg (0.044 mmole) of PA·HCl or 107 mg/kg (0.048 mmole) of NAPA·HCl. Two hours after injection the rats were anesthetized with ether and blood was obtained by aortic puncture for ferrihemoglobin determination and examination for PA and NAPA. Ferrihemoglobin was measured by the method of Evelyn and Malloy (11), except that 30 mg of granular sodium cyanide per ml of blood was used.

Results. NAPA has been detected in the individual plasma samples from each of four patients receiving procainamide orally. The metabolite was identified by comparison of its tlc and gas chromatographic retention times and mass spectrum (Fig. 1) with that of an authentic sample. Plasma PA levels for two of these patients were 4.4 and 6.9 μg/ml as determined fluorimetrically. NAPA does not

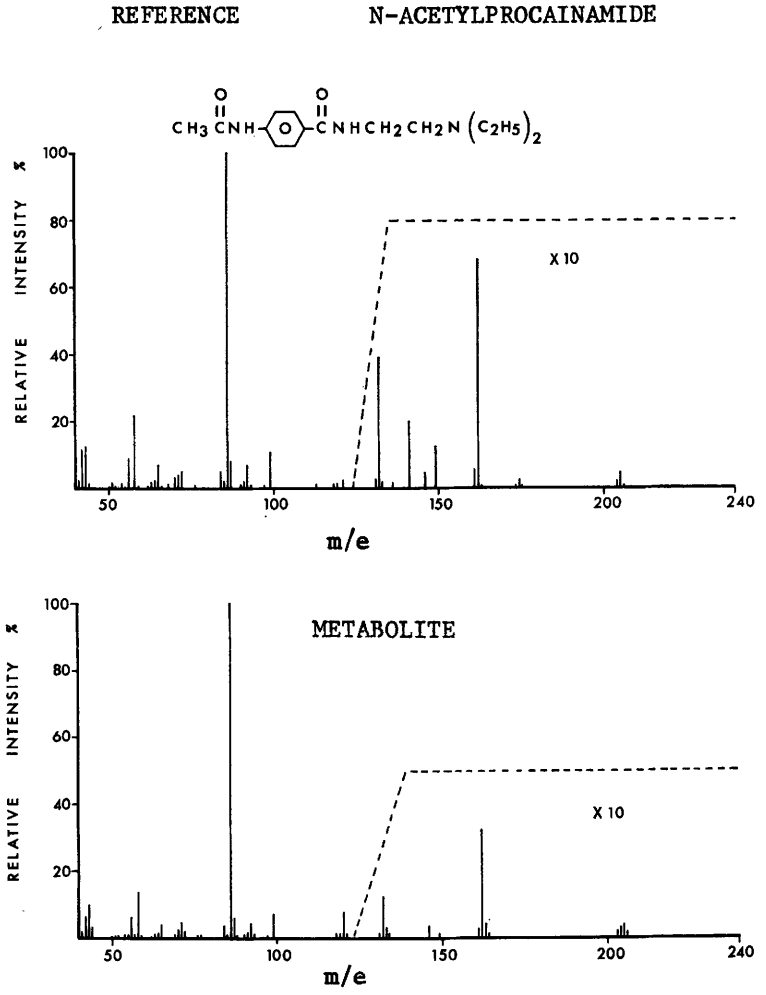

FIG. 1. Mass spectra of reference NAPA and PA metabolite.

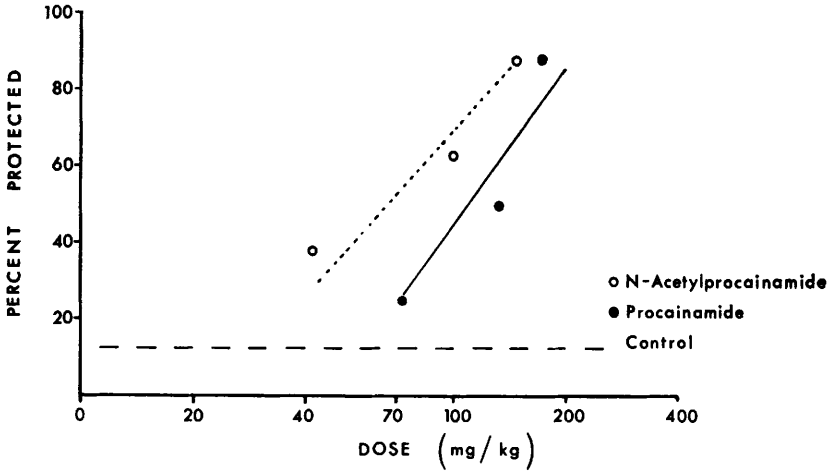

FIG. 2. Dose-response curve for mice protected against ventricular arrhythmia produced by chloroform and hypoxia.

interfere with this method since it has a fluorescent intensity 2–3% of PA. The tlc analysis of the organic extract of blank plasma to which was added equal amounts (6 μg/ml) of PA·HCl and NAPA·HCl revealed spots of approximately equal intensity. The tlc spot for NAPA from each of the above two patients' plasma extract, however, was more intense than the spot for PA. NAPA plasma levels, therefore, were higher than the simultaneously measured PA levels in these patients.

The results of the dose-response study on mice can be seen in Fig. 2. Analysis by tlc of the organic extract of whole blood withdrawn from two mice 10 min after injection of NAPA·HCl revealed the absence of PA. Similarly, tlc analysis of the organic extract of plasma, withdrawn from dog 2 after the effect was observed revealed the absence of PA.

The effects of NAPA on atrial arrhythmias produced in dogs are shown in Table I. The atrial fibrillation induced in dog 1 was accompanied by occasional ventricular extrasystoles prior to treatment. After injection of NAPA these extrasystoles progressed to multiple ventricular extrasystoles and then ventricular fibrillation. We think that this was unrelated to the small amount of drug given since death of the dog occurred only 8 min after drug administration. Injection of NAPA into dogs 2, 3, and 4 caused progressive slowing of the atrial fibrillation to flutter or tachycardia with varying degrees of A–V block. Electrogram changes produced in dog 3 after NAPA administration can be seen in Fig. 3.

The effect of PA and NAPA on ferrihemoglobin formation is summarized in Table II. Analysis by tlc of whole blood extract from two rats that received PA showed that both metabolized it extensively to NAPA after 2 hr. A similar study of two rats that received NAPA showed no deacetylation after 2 hr.

Discussion. NAPA·HCl has been shown to have antiarrhythmic activity in mice and dogs. A dose-response (inhibition of chloroform-hypoxia-induced ventricular fibrillation) study in mice indicated that NAPA has an antiarrhythmic activity similar to PA since the ED_{50} for NAPA was 90 mg/kg and the ED_{50} for PA was 140 mg/kg. Confidence limits, 95%, were 56–143 mg/kg for NAPA and 108–182 mg/kg for PA [determined by the method of Litchfield and Wilcoxon (12)]. Previously reported values were the following: NAPA, ED_{20} = 100 mg/kg (13); PA, ED_{50} = 150 mg/kg (8). Since the mice and dogs did not deacetylate NAPA during the time of pharmacologic testing, its antiarrhythmic activity cannot be attributed to the *in vivo* formation of PA.

If NAPA is pharmacologically active in man, and if it has an antiarrhythmic effect similar to PA, then NAPA would be a better therapeutic agent than PA because we anticipate it to be less toxic. The reasons why are the following:

TABLE I. Antiarrhythmic Activity of *N*-Acetylprocainamide in Dogs.

Dog	Control (NSR) beats/min	Arrhythmia before therapy		Total dose of NAPA-HCl injected iv	Effect
		A-rate	V-rate		
1	180	Aconitine fibrillation	340 with occasional V-extrasystoles	5 mg/kg in a single dose	Multiple V-extrasystoles progressing to V-fibrillation
2	160	Aconitine fibrillation	300	38 mg/kg; infusion rate, 1 mg/kg/min	A-fibrillation reduced to A-flutter (A-rate = 340, V-rate = 220). A-fibrillation recurred 20 min after cessation of NAPA[a]
3	200	Aconitine fibrillation alternating with flutter (flutter rate = 330)	180	72 mg/kg; infusion rate, 4 mg/kg/min	A-fibrillation reduced to A-flutter or tachycardia (A-rate = 240, V-rate = 120) which persisted for 1 hr[b]
4	160	Aconitine fibrillation	220	37 mg/kg; infusion rate, 3.7 mg/kg/min	A-fibrillation reduced to A-flutter (A-rate = 420, V-rate = 300). A-fibrillation recurred 20 min after cessation of NAPA[c]

Abbreviations used are as follows: A, atrial; V, ventricular; NSR, normal sinus rhythm.

[a] NAPA was then reinfused (18 mg/kg) over a period of 9 min with reduction of A-fibrillation to A-tachycardia (A-rate = 240, V-rate = 240) with A–V dissociation.

[b] PA was then infused (36 mg/kg) over a period of 10 min with conversion to normal sinus rhythm (A-rate = 120).

[c] NAPA dose was limited because drug supply was exhausted; PA was then infused (48 mg/kg) over a period of 13 min with conversion of the fibrillation to normal sinus rhythm (A-rate = 180).

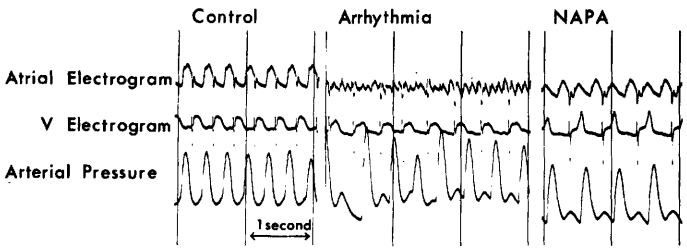

FIG. 3. Electrogram changes produced by NAPA on aconitine-induced arrhythmia in dog 3. The control reading was taken just prior to introduction of aconitine; the NAPA reading was taken after 72 mg/kg was infused. V = ventricular.

1. The toxic side effects of many drugs can be attributed to an aromatic amino or hydrazino group. The most potent drugs capable of inducing systemic lupus erythematosis (SLE) contain either of these groups. Procainamide is the most potent, followed by hydralazine and isoniazid (14). Genetic fast acetylators of hydralazine (15) and isoniazid (16) have been observed to be less likely to develop SLE-like toxic symptoms than slow acetylators. Acetylation in this instance is a detoxification mechanism. Therefore, we expect that NAPA, the acetylated metabolite of PA, would be less likely to induce SLE than PA.
2. Metabolites of aromatic amines formed by *N*-oxidation are viewed as ferrihemoglobin-forming agents (17–19). *N*-Arylacetamides, although capable of being *N*-oxidized, produce ferrihemoglobin very slowly (18). Acetylation in this case also seems to be a detoxifying mechanism. Although acetophenetidin causes ferrihemoglobin, deacetylation to aromatic amine metabolites is necessary prior to ferrihemoglobin formation (20). NAPA, an *N*-arylacetamide derivative, in agreement with the above, causes less ferrihemoglobin in rats than does PA (see Table II). Since rats that received PA extensively metabolized it to NAPA after 2 hr, less PA than that which was given caused the observed ferrihemoglobin.

Additional examples of acetylation being a detoxification mechanism are the following: Severe adverse effects of phenelzine were more common among slow acetylators than among rapid acetylators on the same treatment schedule (21). Side effects of salicylazosulfapyridine therapy were much more common in slow than in fast acetylators because slow acetylators had much greater accumulation of sulfapyridine in their bodies (22). The incidence of polyneuropathy for patients receiving isoniazid was higher in slow acetylators than in rapid acetylators (23).

Summary. *N*-Acetylprocainamide has been detected in the plasma samples of each of four patients receiving procainamide. The metabolite was identified from tlc data and its identity confirmed by gas chomatographic–mass spectroscopic analysis. The metabolite was also detected in the whole blood of Sprague–Dawley rats after administration of PA. NAPA·HCl when injected ip into 42- to 49-day-old ICR male mice prevented coarse ventricular fibrillation caused by deep chloroform anesthesia and resultant hypoxia. NAPA·HCl reduced aconitine-induced arrhythmia to atrial

TABLE II. Effect of PA and NAPA on Ferrihemoglobin Formation in Rats.

Drug injected	Mean ferrihemoglobin concentration [g/100 ml blood (± SD)]	*P* value (experimental vs control)
Control	0.061 ± 0.031	—
PA • HCl	0.182 ± 0.159	< 0.05
NAPA • HCl	0.095 ± 0.069	< 0.2

flutter or atrial tachycardia with varying degrees of A–V block in three dogs. Analysis by tlc indicated that the mice, dogs, and rats did not deacetylate NAPA during the period of pharmacologic testing. NAPA was found to cause less ferrihemoglobin in Sprague–Dawley rats than did PA.

The authors thank the following people: Dr. Stanley Glauser for performing the ferrihemoglobin study with the assistance of Miss Mary McCafferty; Miss Janet Bendrick, Mr. Robert Faust, and Mr. Remus Berretta for their assistance in the pharmacologic activity study (dog); Dr. Arthur Atkinson, Jr., and Dr. John Strong for providing the mass spectral data; and Mrs. Marilyn Drayer for her help in preparing the manuscript.

1. Bigger, J.T., and Heissenbuttel, R.H., Progr. Cardiovasc. Dis. **11**, 515 (1969).

2. Giardina, E.G., Heissenbuttel, R.H., and Bigger, J.T., Ann. Intern. Med. **78**, 183 (1973).

3. Koch-Weser, J., and Klein, S.W., J. Amer. Med. Ass. **215**, 1454 (1971).

4. Mark, L.C., Kayden, H.J., Steele, J.M., Cooper, J.R., Berlin, I., Rovenstine, E.A., and Brodie, B.B., J. Pharmacol. Exp. Ther. **102**, 5 (1951).

5. Dreyfuss, J., Bigger, J.T., Cohen, A.I., and Schreiber, E.C., Clin. Pharmacol. Ther. **13**, 366 (1972).

6. Dreyfuss, J., Ross, J.J., and Schreiber, E.C., Arzneim. Forsch. **21**, 948 (1971).

7. Strong, J.M., and Atkinson, A.J., Anal. Chem. **44**, 2287 (1972).

8. Lawson, J.W., and Rahdert, E.R., Pharmacologist **1**, 75 (1959).

9. Rosenblueth, A., and Garcia Ramos, J., Amer. Heart J. **33**, 677 (1947).

10. Mendez, R., Aceves, J., and Kabela, E., Acta Cardiol. **20**, 1 (1965).

11. Evelyn, K.A., and Malloy, H.T., J. Biol. Chem. **126**, 655 (1938).

12. Litchfield, J.T., and Wilcoxon, F., J. Pharmacol. Exp. Ther. **96**, 99 (1949).

13. Schreiber, E.C., Ger. Offen. 2,062,978 (Cl.CO7c, A61k) July 15, 1971. U.S. Patent applied for Jan. 9, 1970.

14. Alarcón-Segovia, D., Mayo Clin. Proc. **44**, 664 (1969).

15. Perry, H.M., Tan, E.M., Carmody, S., and Sakamoto, A., J. Lab. Clin. Med. **76**, 114 (1970).

16. Alarcón-Segovia, D., Fishbein, E., and Alcalá, H., Arthritis Rheum. **14**, 748 (1971).

17. Kiese, M., Pharmacol. Rev. **18**, 1091 (1966).

18. Kiese, M., Xenobiotica **1**, 553 (1971).

19. Cucinell, S.A., Israili, Z.H., and Dayton, P.G., Amer. J. Trop. Med. Hyg. **21**, 322 (1972).

20. Shahidi, N.T., and Hemaidan, A., J. Lab. Clin. Med. **74**, 581 (1969).

21. Evans, D.A.P., Davison, K., and Pratt, R.T.C., Clin. Pharmacol. Ther. **6**, 430 (1965).

22. Das, K.M., Eastwood, M.A., McManus, J.P.A., and Sircus, W., New Eng. J. Med. **289**, 491 (1973).

23. Devadatta, S., Gangardharam, P.R.J., Andrews, R.H., Fox, W., Ramakrishnam, C.V., Selkon, J.B., and Velu, S., Bull. World Health Organ. **23**, 587 (1960).

Received Jan. 12, 1974. P.S.E.B.M. 1974, Vol. 146.