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Comparison of Antiarrhythmic Effects of Procainamide, *N*-Acetylprocainamide, and *p*-Hydroxy-*N*-(3-diethylaminopropyl)benzamide (41325)

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Abstract. The antiarrhythmic effects of *p*-hydroxy-*N*-(3-diethylaminopropyl)benzamide (PP), procainamide (PA), and *N*-acetylprocainamide (NAPA) were compared. ED_{50} values for PP, PA, and NAPA in the mouse chloroform arrhythmia model were not significantly different. Mean effective doses in the ouabain-intoxicated dog were: PP 34, PA 25, and NAPA 122 mg/kg. PP was effective in 3/5, PA in 5/6, and NAPA in 0/5 dogs at 24 hr after ligation of the left anterior descending coronary artery. Thus, PP has antiarrhythmic potency and efficacy intermediate to PA and NAPA.

Procainamide (PA) is an antiarrhythmic agent which is highly effective in the treatment of atrial and ventricular arrhythmias. However, long-term treatment with this drug results in the development of serious immunologic reactions such as a systemic lupus erythematosus-like syndrome (SLE) (1, 2). This toxicity appears to be related to the presence of an aromatic primary amino group on PA (3–5). It has been demonstrated that *N*-acetylprocainamide, the *p*-*N*-acetylated metabolite of PA, also possesses antiarrhythmic activity, but with less tendency to induce SLE (4). Additionally, it has been reported that *p*-hydroxy-*N*-(2-diethylaminoethyl)benzamide (PHOPA), the *p*-hydroxy isostere of PA, possesses antiarrhythmic activity similar to PA in certain experimental models (6). The purpose of this study was to compare the antiarrhythmic effects of PA, NAPA, and the synthetically prepared methylene-extended analog of PHOPA, *p*-hydroxy-*N*-(3-diethylaminopropyl)benzamide (PP), in various arrhythmia models. The chemical structures for these compounds are presented in Fig. 1.

Materials and Methods. *Mouse chloroform arrhythmia.* The antiarrhythmic activity of PA, PP, and NAPA was determined using the procedure of Lawson (7). Drug was injected intraperitoneally into 28- to 35-day-old Charles River ICR male mice, average weight 25 g. Ten minutes later mice were placed in a covered beaker containing a surgical sponge saturated with chloroform. After respiratory arrest, a bipolar electrocardiogram (ECG) was obtained on a cathode ray oscilloscope. Mice that did not show intermittent or continuous ventricular fibrillation were considered protected by the drug. Mice that showed definite ventricular fibrillation were considered unprotected even if the fibrillation subsequently changed to another rhythm. A dose–response relationship was ascertained using eight mice at each dosage level.

LD_{50} determinations. The acute toxicity of PA, PP, and NAPA was determined in groups of 10 ICR mice (28–35 days old, average weight 26 g). After the drug was injected intraperitoneally, the mice were observed for up to 210 min to determine mortality caused by the drug.

Ouabain arrhythmia in the dog. Adult male mongrel dogs (10–17 kg) were anesthetized with pentobarbital (30 mg/kg iv) and intubated for spontaneous respiration. Lead II of the ECG was recorded. Ouabain was administered intravenously in bolus doses (8): 40 μg/kg, followed in 30 min by 20 μg/kg and then in 15-min intervals by 10 μg/kg until a stable ventricular arrhythmia (>95% ectopic ventricular complexes) was present for 15 min. PA, PP, or NAPA was administered intravenously un-

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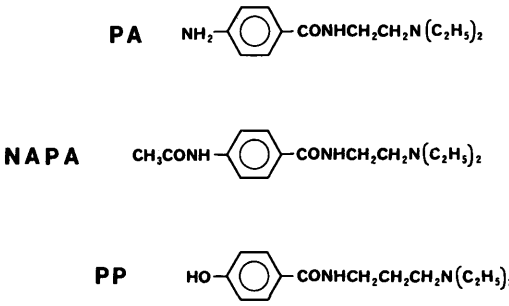

FIG. 1. Structures of procainamide (PA), *N*-acetylprocainamide (NAPA), and *p*-hydroxy-*N*-(3-diethylaminopropyl) benzamide (PP).

til arrhythmia reverted to normal sinus rhythm for at least 10 min.

Coronary artery ligation arrhythmia in the dog (Harris dog). Mongrel dogs of either sex (10–18 kg) were anesthetized with pentobarbital (30 mg/kg iv), intubated, and ventilated with room air (Harvard respirator Model 607) at a rate of 10 cycles/min and stroke volume of 30 cm^3/kg. Sterile surgical techniques were used. Catheters were implanted in the left jugular vein and left carotid artery and exteriorized at the nape of the neck. These were used for the future administration of drug and recording of mean arterial pressure, respectively. A left lateral thoracotomy was performed at the fifth intercostal space and the pericardium was incised to expose the left anterior descending coronary artery which was ligated using the two-stage technique of Harris (9). The chest was closed and once spontaneous respiration was restored the dogs were returned to their cages. Ampicillin (Polyflex, Bristol Laboratories) (6.7 mg/kg im) was administered prior to and again approximately 6 hr after surgery.

Approximately 24 hr after coronary artery occlusion, dogs were brought to a quiet laboratory and placed in a restraining sling. After stabilization for 1 hr control mean arterial blood pressure and lead II ECG were recorded. Heart rate was determined by counting all ventricular electrocardiographic complexes (normal and ectopic) over a 3-min period. Arrhythmia was quantified by counting all ectopic complexes and expressing the result as percentage of the heart rate (% ectopic beats). This was done three times at 10-min intervals and the results were averaged to obtain control values. PA, NAPA, or PP was administered intravenously until arrhythmia reverted to normal sinus rhythm (approximately 80% or more normal complexes during a 3-min period) or severe toxicity occurred.

Statistics. ED_{50} values and 95% confidence limits (10) were calculated for the mouse chloroform model. LD_{50} values and 95% confidence limits were calculated after mortality was observed following intraperitoneal administration of the compounds.

In ouabain arrhythmia experiments, comparisons between groups were made using Student's *t* test (two-tailed) for unpaired data, while comparisons within groups were made using the paired test.

Results obtained in Harris dogs were analyzed using Fisher's exact test (two-tailed) to compare the frequencies of successful conversion to normal sinus rhythm with the different drugs.

Drugs. PA, PP, and NAPA were supplied as the hydrochloride salts and were administered in isotonic saline. Doses are expressed as the base.

Results. *Mouse chloroform arrhythmia.* ED_{50} values obtained in the mouse chloroform arrhythmia model were not significantly different among the three treatment groups although there was a tendency for PP and NAPA to be more potent than PA (Table I).

LD_{50} determinations. LD_{50} values in the mouse were similar for PA and PP. The value for NAPA was significantly higher than for PA and PP, however. The "therapeutic index" generated from ED_{50} and LD_{50} values in the mouse was highest for NAPA and lowest for PA (Table I).

Ouabain arrhythmia in the dog. Previous experiments established that the arrhythmia produced by ouabain culminated in ventricular fibrillation or was stable long enough for testing of antiarrhythmic compound (unpublished data). In the present study the cumulative doses of ouabain required to produce a stable arrhythmia were similar in the PA, PP, and NAPA groups (Table II). Test compound was adminis-

TABLE I. ED_{50} VALUES IN THE MOUSE CHLOROFORM ARRHYTHMIA MODEL AND LD_{50} VALUES IN THE MOUSE

Drug[a]	ED_{50}[b] (mg/kg)	LD_{50}[b] (mg/kg)	"Therapeutic Index"
PA	140 (108–182)	255 (220–307)	1.82
PP	74 (36–152)	220 (207–233)	2.97
NAPA	90 (56–143)	380 (362–399)	4.22

[a] Administered intraperitoneally.
[b] With 95% confidence limits in parentheses.

tered in doses of 4–18 mg/kg every 5 min until normal sinus rhythm was present for at least 10 min. Normal sinus rhythm was produced in all dogs. The effective doses of PA (25.1 ± 5.4 mg/kg) and PP (34.0 ± 7.3 mg/kg) were not significantly different from each other, but were significantly less than that of NAPA (122 ± 21 mg/kg) ($P < 0.005$). All three drugs dramatically reduced heart rate, thus conversion to normal sinus rhythm was not due to overdrive suppression (Table II).

Coronary artery ligation arrhythmia in the dog (Harris dog). Test compound was administered intravenously in doses of 8–18 mg/kg every 15 min until normal sinus rhythm (approximately 80% or more normal ECG complexes) or toxicity (vomiting or difficulty in breathing) occurred. PA was effective in five dogs (17, 26, 35, 43, and 60 mg/kg) and ineffective in one dog (121 mg/kg). PP was effective in three dogs (35, 52, and 79 mg/kg) and ineffective in two others (70 and 87 mg/kg). NAPA was not effective in any of five dogs; doses administered were 88, 106, 141, 141, and 159 mg/kg (Fig. 2). The incidence of conversion to normal sinus rhythm was significantly greater in PA- than NAPA-treated dogs ($P < 0.01$). In dogs in which normal sinus rhythm was produced, antiarrhythmic activity (>50% normal ECG complexes) persisted for at least 40 min after administration of the cumulative dose.

Prior to administration of drug, heart rate ranged from 148 to 189 beats/min. PA reduced heart rate (31–81 beats/min) in the six dogs. Effects of PP and NAPA were less dramatic and less consistent. PP reduced heart rate in four dogs (22–44 beats/min) but increased it in one dog (22 beats/min). NAPA reduced heart rate in three dogs (9–27 beats/min) and increased it slightly in two others (4–7 beats/min). Mean arterial pressure was little changed in any of the dogs.

Vomiting or difficulty in breathing occurred in 2/6 PA, 2/5 PP, and 5/5 NAPA dogs. One of the NAPA dogs (159 mg/kg) died shortly after dosing.

Discussion. PP is the methylene-extended homolog of PHOPA, the *p*-hydroxy isostere of PA (6). NAPA is the *p*-*N*-acetyl analog of PA and the major me-

TABLE II. EFFECTS OF PA, PP, AND NAPA ON OUABAIN-INDUCED ARRHYTHMIA IN THE DOG[a]

Drug[b]	Ouabain dose (μg/kg iv)	Effective dose of drug (mg/kg iv)	Heart rate (beats/min)	
			Before drug	After drug
PA	70 ± 3.2	25.1 ± 5.4	228 ± 8	143 ± 7[c]
PP	67 ± 3.7	34.0 ± 7.3	224 ± 13	144 ± 16[c]
NAPA	70 ± 4.5	122.0 ± 21[d]	228 ± 11	126 ± 11[c]

[a] Values are mean ± SE.
[b] $N = 5$ in each group.
[c] Heart rate was significantly reduced after test drug ($P < 0.001$).
[d] The effective dose of NAPA was significantly greater than that of PA or PP ($P < 0.005$).

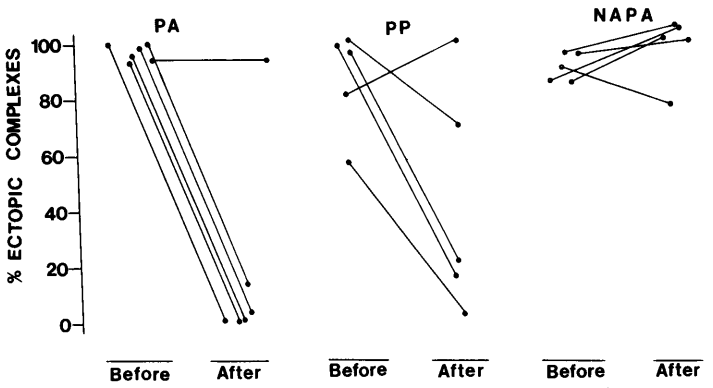

FIG. 2. Effects of PA, PP, and NAPA on arrhythmia (% ectopic complexes, ordinate) in individual conscious dogs 24 hr after coronary artery ligation. Test compound was administered in cumulative doses until production of normal sinus rhythm or toxicity. PA was effective in 5/6, PP in 3/5, and NAPA in 0/5 dogs.

tabolite of PA in the human (11). Previous investigations have demonstrated that, like PA, PHOPA and NAPA also possess antiarrhythmic activity (3, 6, 11–13). The present study demonstrates and compares the antiarrhythmic activity and toxicity of PP to NAPA and PA.

In our study PP, PA, and NAPA were all effective in the mouse chloroform arrhythmia model. There was a tendency for PP and NAPA to be more potent than PA; however, because of wide 95% confidence limits, ED_{50} values were not significantly different. ED_{50} and LD_{50} values in the mouse provided the highest therapeutic index for NAPA, and then PP, followed by PA, indicating that the two drugs may be more beneficial than PA in certain arrhythmia settings. Similar results were not obtained in the ouabain-intoxicated or coronary artery ligated dog, however (see below).

While each drug was very effective in restoring normal sinus rhythm in the ouabain-intoxicated dog, the potencies of PP (34 mg/kg) and PA (25 mg/kg) were much greater than that of NAPA (122 mg/kg). The lower potency of NAPA vs PA in the ouabain-intoxicated dog previously was reported by Bagwell *et al.* (13), although higher doses of both drugs were required than in our study.

We observed that PP was effective in the treatment of coronary artery occlusion-induced arrhythmia, albeit less so than PA; NAPA was essentially ineffective. Bagwell *et al.* (13) previously reported that NAPA (200 mg/kg) and PA (50 mg/kg) were similarly effective in the anesthetized dog approximately 8 hr after coronary ligation. In our study with 24 hr unanesthetized dogs, we were unable to approach the 200 mg/kg NAPA dose without inducing marked toxicity. In fact, one dog died after a cumulative dose of 159 mg/kg NAPA. The reason for this disparity of results is unclear but could be related to the presence or absence of anesthesia, the method of drug administration, or the time after coronary occlusion that studies were performed.

While no experimental animal model can be entirely predictive for man, each of the models used in this study can be said to relate to some clinical counterpart. Certainly cardiac glycoside and myocardial infarction arrhythmias are prevalent in man (15–18). The mouse chloroform model may simulate clinical arrhythmias which involve hypoxia and the adrenergic nervous system (catecholamine release) (19). In general, this study shows that PP has antiarrhythmic activity in various experimental models and that its efficacy and potency lie between those of PA and NAPA. Since PP, like NAPA, lacks the *p*-amino group of PA, which has been implicated in the development of antinuclear antibodies and SLE, results of this study suggest that PP may

possess a therapeutic advantage over PA in man. Clinical studies to evaluate the antiarrhythmic activity and to determine whether PP produces SLE are needed in order to fully explore this possibility.

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