of 5 h and then poured over 750 g of ice/H_2O. The crude product was filtered, stirred briefly in 200 mL of 50% aqueous EtOH, and refiltered. Two recrystallizations from DMF–H_2O yielded the mononitro acid **6f** as a hemihydrate (5.7 g, 56%), fine yellow needles of mp 295 °C dec.

4,5-Dihydro-5-methyl-6,8-dinitro-4-oxopyrano[3,2-*b*]indole-2-carboxylic Acid (6g). Acid **6a** (6.0 g, 0.025 mol) was added over a few minutes to 22 mL of concentrated H_2SO_4 cooled in ice. Concentrated HNO_3 (3.0 mL, 0.048 mol) was then added in one portion, and the mixture was stirred and heated on the steam bath for 30 min. The cooled mixture was added to 300 g of ice/H_2O, and the crude product was filtered and washed with cold H_2O. Two recrystallizations from DMF–H_2O yielded the dinitro acid **6g** as a DMF complex (5.5 g, 54%), yellow needles of mp 265 °C dec.

Acknowledgment. We are indebted to C. E. Childs and staff for microanalyses and Dr. F. A. MacKellar and staff for spectral data. Biological assays were performed by Ms. P. R. Schumann and Dr. R. Adolphson and staff.

Note added in proof: After this manuscript had been submitted, the preparation of several of the compounds described was reported by other workers.[12]

(12) K. Görlitzer and E. Engler, *Arch. Pharm.*, **313**, 405 (1980).

Antiarrhythmic Activity of Amitriptyline Analogues in Conscious Dogs after Myocardial Infarction: Cyproheptadinium Methiodide[1]

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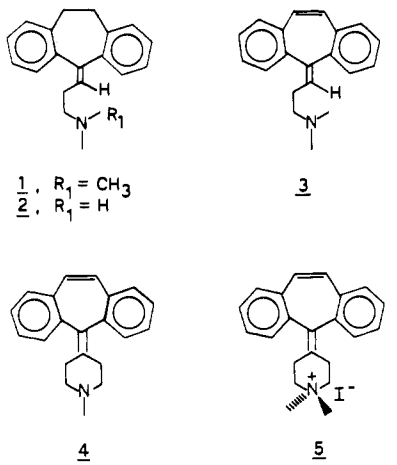
The antiarrhythmic effects of amitriptyline (1), its secondary amine metabolite nortriptyline (2), as well as cyclobenzaprine (3) and cyproheptadine (4), tertiary amine analogues of 1, were studied in conscious dogs 24 h after myocardial infarction. Since the sedative side effect of 4 presents a potential problem for its clinical use, a quarternary derivative of 4, cyproheptadine methiodide (5), was prepared and its effects also studied in this model. Complete conversion to a normal sinus rhythm occurred in all animals studied after cumulative doses of 1700 μg/kg (6.17 μmol/kg) of 3, 1300 μg/kg (4.69 μmol/kg) of 1, 300 μg/kg (1.04 μmol/kg) of 4, and 25 μg/kg (0.058 μmol/kg) of 5. While 2 significantly decreased ventricular ectopic activity, it did not convert any of the animals studied to a sinus rhythm at doses up to 3000 μg/kg. Thus, the order of potency for conversion to a normal sinus rhythm appears to be 5 ≫ 4 > 1 > 3 ≫ 2. These data suggest that 5 is very potent in converting ventricular arrhythmias associated with myocardial infarction.

Although the cardiotoxic effects of tricyclic antidepressant drugs have been thoroughly described,[2-4] recent evidence suggests that these drugs may exert a potentially beneficial effect on cardiac rhythm abnormalities.[5,6] This antiarrhythmic action also has been observed in a limited number of patients with ventricular and supraventricular arrhythmias being treated for depression with imipramine.[7]

Recently, it has been demonstrated that imipramine exerts cardiac electrophysiologic effects similar to other antiarrhythmic drugs.[8,9] Imipramine decreased the rate of phase 0 depolarization and shortened the action potential duration in Purkinje fibers.[8,9] Imipramine also decreased membrane responsiveness and conduction velocity in isolated Purkinje preparations.[9] Unlike classical antiarrhythmic drugs, however, imipramine did not decrease the rate of diastolic depolarization in concentrations sufficient to render Purkinje fibers unresponsive to external stimuli.[8]

Chart I



Antiarrhythmic activity of tricyclic antidepressant drugs demonstrated against arrhythmias induced by ouabain administration in anesthetized dogs suggested that tertiary amine tricyclics were considerably more potent than secondary amine members of this group.[5] This may be related to the fact that tertiary amine tricyclics are less effective inhibitors of norepinephrine uptake than secondary amines,[10] since inhibition of catecholamine uptake and the

(1) Portions of this work were presented at the 179th ACS National Meeting; see: "Abstracts of Papers", 179th National Meeting of the American Chemical Society, Houston, TX, Mar 24–27, 1980, American Chemical Society: Washington, D.C., 1980; Abstr MEDI 35.
(2) Kantor, S. J.; Bigger, J. T.; Glassman, A. H.; Macken, D. L.; Perel, J. M. *J. Am. Med. Assoc.* **1975**, *231*, 1364.
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(5) Wilkerson, R. D. *J. Pharmacol. Exp. Ther.* **1978**, *205*, 666.
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(7) Bigger, J. T.; Giardina, E. G. V.; Perel, J. M.; Kantor, S. J.; Glassman, A. H. *N. Engl. J. Med.* **1977**, *286*, 206.
(8) Weld, F. M.; Bigger, J. T.; Swister, D.; Bordiuk, J. *Am. J. Cardiol.* **1978**, *41*, 386.
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(10) Bopp, B.; Biel, J. H. *Life Sci.* **1974**, *14*, 415.
(11) "The Merck Index", 8th ed.; Merck & Co., Inc.: Rahway, NJ, 1968; p 316.

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Table I. Antiarrhythmic Activity of Amitriptyline (1) Analogues

compd	dose, μmol/kg[a] for 50% reduction in ectopic rate	dose, μmol/kg[a] for conversion to sinus rhythm	N
1	3.97 ± 0.79	4.69 ± 0.36	5
2	b	c	6
3	3.99 ± 0.90	6.17 ± 1.09	5
4	0.69 ± 0.17	1.04 ± 0.28	6
5	0.030 ± 0.007	0.042 ± 0.007	5
lidocaine hydrochloride	5.54 ± 1.10	9.97 ± 1.85	5

[a] Data are expressed as the mean plus or minus SE. [b] Ectopic rate was not reduced by 50% in any experiment. [c] Conversion to sinus rhythm did not occur.

resulting increased availability of norepinephrine would be expected to aggravate arrhythmias. In addition, tertiary amine tricyclic compounds may exert other cardiac actions unrelated to their effect on amine uptake which may be important to the observed antiarrhythmic activity.

The present investigation had three objectives. First, the antiarrhythmic effects of amitriptyline (1, see Chart I) and nortriptyline (2) were studied in conscious dogs 24 h after myocardial infarction to ascertain whether or not the tertiary amine 1 was more effective than its secondary amine metabolite 2 in this model as had previously been demonstrated in ouabain-induced arrhythmias.[5] Second, the antiarrhythmic effects of cyclobenzaprine (3) and cyproheptadine (4) were studied to determine if the structural modifications present in these analogues of 1 would provide for enhanced activity. Finally, the effect of quaternization of a tertiary amine tricyclic on its antiarrhythmic activity was determined by preparation and testing of cyproheptadinium methiodide (5).

Results

Compounds 1–5 were administered iv to conscious dogs 24 h after coronary occlusion, during the period of maximum ventricular ectopic activity. A dose-dependent decrease in ventricular ectopic activity was observed with complete conversion to a sinus rhythm produced by 1 and 3–5, but not by 2, in cumulative doses up to 11.4 μmol/kg. These data are summarized in Table I. Lead II electrocardiogram (ECG) recordings from an experiment with 4 are shown in Figure 1. The conversion to a sinus rhythm by 3 was accompanied by a slight but statistically significant increase in heart rate. The dose-dependent increase in normal sinus beats (expressed as a percentage of total heart rate) produced by 5, the most potent of the analogues studied, is shown in Figure 2. Ten minutes after conversion by 5, four of the five animals studied still had a normal sinus rhythm.

Intravenous administration of 5 in a dose of 25 μg/kg did not significantly alter arterial blood pressure or the PR interval or QRS duration of the ECG. Myocardial contractility, as measured by dp/dt/50, was significantly increased from 2333 ± 363 mmHg/s prior to treatment to 3250 ± 250 mmHg/s 5 min after administration of 5 (25 μg/kg). Similarly, heart rate was increased from 148 ± 6.6 beats/min prior to treatment to 174 ± 9.8 beats/min 5 min after this same dose of 5. Subsequent administration of an additional 50 μg/kg of 5 30 min after the initial dose also did not significantly alter blood pressure or the ECG parameters studied, but this dose did produce an additional increment in heart rate from 174 ± 3.5 to 196 ± 4 beats/min. No additional increment in myocardial contractility was noted with this dose of 5.

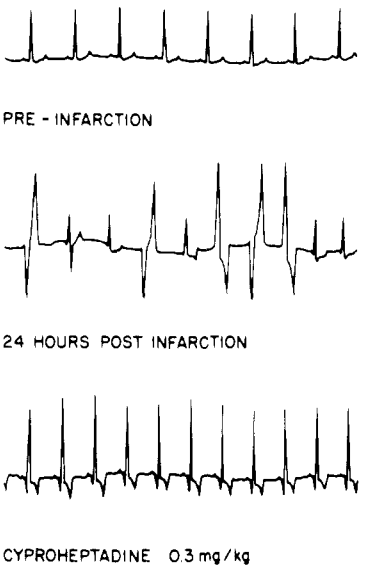

Figure 1. Lead II ECG recordings from an experiment with 4.

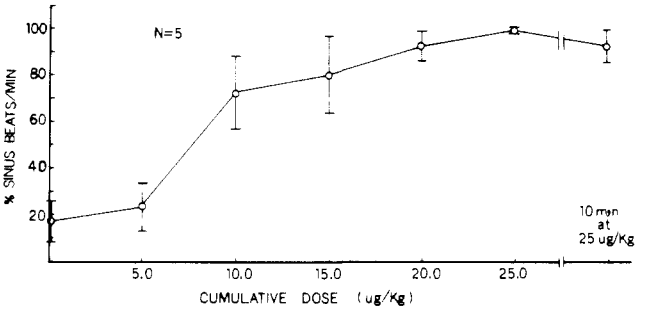

Figure 2. Effect of cyproheptadinium methiodide on arrhythmias 24 h after myocardial infarction in conscious dogs. Data points represent the mean ± SE of five values.

The increase in heart rate observed after administration of 5 was undoubtedly due to an anticholinergic action of this agent, since the blood-pressure response to 1 μg/kg of acetylcholine was also significantly reduced by the 25 μg/kg dose of 5. It should be noted that significant increases in heart rate after administration of 5 occurred only in the anesthetized animals used for measurement of hemodynamic parameters not subjected to any cardiac insult. Only transient increases in heart rate were observed in the conscious animals used for evaluation of antiarrhythmic activity of 5; in all cases the tachycardia quickly subsided, so that 10 min after conversion heart rates had returned to pretreatment values.

Discussion

Recently it was demonstrated that 1 is effective in the treatment of cardiac arrhythmias associated with cardiac glycoside toxicity and myocardial ischemia in anesthetized dogs.[5,6] Results of the present study are consistent with these findings and clearly demonstrate the effectiveness of 1 in the treatment of ventricular arrhythmias associated with myocardial ischemia in conscious dogs 24 h after coronary artery occlusion.

Previous studies of the antiarrhythmic activity of tricyclic antidepressants in cardiac glycoside toxicity demonstrated that the tertiary amine members of this group of drugs were more potent and in most cases more effective than the corresponding secondary amines. It was suggested that these differences in activity may be related to

(12) Wilkerson, R. D.; Downey, J. M. J. Pharmacol. Methods 1978, 1, 39.

the fact that secondary amine tricyclic antidepressants are more effective inhibitors of the neuronal uptake of norepinephrine than tertiary amines.[10]

Since increased availability of norepinephrine at cardiac adrenergic receptors would clearly be expected to aggravate cardiac arrhythmias, it would be assumed that inhibition of norepinephrine uptake would not be favorable for antiarrhythmic activity. Thus, any antiarrhythmic activity possessed by secondary amine tricyclics would be opposed to some degree by increased adrenergic tone. The data presented here demonstrate that the same differential in antiarrhythmic activity exists in arrhythmias associated with myocardial ischemia, since 1 was much more effective than 2, its secondary amine metabolite.

In order to test our hypothesis that tertiary amine tricyclic antidepressants should be more effective antiarrhythmic drugs than secondary amine members of this group, we also studied the antiarrhythmic effects of 3, a centrally acting skeletal-muscle relaxant, and 4, a serotonin and histamine receptor antagonist. Both of these compounds are structural analogues of 1 (Figure 1), and the tertiary amine group of 4 is incorporated into a piperidine ring which should make it somewhat resistant to demethylation. On the other hand, 3 differs from 1 only in having an endocyclic double bond in the central seven-membered ring.

As might be expected from its close chemical similarity to 1, 3 resembled 1 with respect to antiarrhythmic activity. When given in 0.3 mg/kg dose increments iv, the dose–response relationships for suppression of ventricular ectopic activity for the two drugs were virtually identical, indicating that the endocyclic double bond does not alter antiarrhythmic activity.

The antiarrhythmic potency of 4, however, was much greater than either 1 or 3 when all drugs were given in the minimum cumulative dose necessary to effect a normal sinus rhythm. When administered in 0.1 mg/kg increments at 2-min intervals, the maximum cumulative dose of 4 required to convert any animal was 2.44 μmol/kg, as compared to 8.72 and 7.57 μmol/kg for 3 and 1, respectively. This minimal dose of 4 required to effect a change to normal sinus rhythm in this study did not significantly depress atrioventricular conduction or intraventricular conduction in anesthetized dogs, nor did it alter norepinephrine uptake as evidenced by lack of potentiation of the pressor response to norepinephrine (unpublished observation).

The most potent of the analogues studied was 5, as evidenced from Table I. Since 1, 3, and 4 were all capable of producing complete conversion to a sinus rhythm, the enhanced activity of 5 cannot be explained on the basis of intrinsic activity. Rather, the increased potency of 5 could be due to a change in some pharmacokinetic parameter. It is possible that 5 in its ionized quaternary form has a significantly decreased volume of distribution (V_d) compared to 4. Highest tissue concentrations of 5 should be found in the most rapidly perfused organs, including the heart, while entry into the central nervous system, muscle, fat, or other deep compartments should be insignificant because of quaternization. Alternatively, the enhanced potency of 5 might be explained on the basis of quaternization enhancing the effects of the drug on cardiac electrophysiologic events. Gillis and co-workers have previously shown that quaternization of lidocaine increased its antiarrhythmic potency approximately threefold.[13]

One of the serious drawbacks to the potential use of 4 as an antiarrhythmic agent is its sedative side effect. The ionized, quaternary derivative 5 should be virtually excluded from entry into the brain; this assumption is based on similar observations regarding central vs. peripheral actions of various autonomic drugs and their quaternized derivatives.[14]

In conclusion, 1 and two of its tertiary amine analogues are effective in treating arrhythmias in dogs initiated by coronary occlusion. The secondary amine 2 was incapable of converting any animal to a normal sinus rhythm. Compound 5 was clearly the most potent of the agents studied, being about 25 times as potent as 4. The enhanced potency (but not intrinsic activity) suggests that a pharmacokinetic alteration has occurred, possibly a decreased V_d of 5 compared to 4. Since arrhythmias associated with myocardial infarction are probably the most common type of arrhythmia encountered clinically, 5 could prove to be very beneficial in reversing ventricular arrhythmias due to coronary artery occlusion. In addition, the CNS depressant side effect of 4 should be relatively insignificant for 5 due to its expected impermeability to the blood–brain barrier.

Experimental Section

Melting points were determined on an electrical micro hot stage and are uncorrected. NMR spectra were determined with a Perkin-Elmer R-32 instrument in $CDCl_3$ containing Me_4Si as internal standard. Elemental analysis was performed by Galbraith Laboratories, Inc., Knoxville, TN. Where analysis was indicated by symbols of elements, the analytical results were within ±0.04% of theoretical values. Compounds 1, 3, and 4 were donated by Merck Sharp & Dohme, Inc.; 2 was donated by Eli Lilly and Co. Lidocaine hydrochloride (Xylocaine) was donated by Astra Pharmaceuticals.

Cyproheptadinium Methiodide (5). A suspension of 4 as its HCl salt (2.0 g, 6.18 mmol) in H_2O (50 mL) was warmed to dissolution, and 1 N KOH (15 mL) was added. Absolute EtOH was then added to the resulting suspension and heating continued until the turbidity disappeared. During removal of the EtOH on a Büchi rotary evaporator, a solid separated and was collected to give 4 as the free base, 1.58 g (after vacuum drying at 60 °C). A 500-mg portion of this material was recrystallized (EtOH–H_2O) to give a pure sample: white needles (380 mg); mp 116.5–118 °C (lit. 112.3–113.3[11]); NMR δ 7.25 (m, 8 H, aromatic), 6.9 (s, 2 H, olefinic), 2.7–1.9 (complex, aliphatic), 2.2 (s, $-CH_3$), total 11 H.

Methyl iodide (0.30 mL, 4.82 mmol) was added to a solution of 4 (free base; 1.0 g, 3.48 mmol) in absolute EtOH (30 mL). After refluxing for 4 h, the solution was stirred overnight at room temperature. The resulting suspension was filtered to give 5 as a white powder: 1.10 g; mp >260 °C. Dilution of the filtrate with ether gave a second crop as an off-white powder, 260 mg: total yield 1.44 g (97%); NMR δ 7.3 (m, aromatic), 6.9 (s, olefinic), 4.0–2.0 (complex, aliphatic), 3.45 (s, $-CH_3$, equatorial), 3.2 (s, $-CH_3$, axial). Anal. ($C_{22}H_{24}IN$) C, H, I, N.

Induction of Myocardial Infarction and Evaluation of Antiarrhythmic Activity. Mongrel dogs (11–14 kg) were anesthetized with sodium pentobarbital (30 mg/kg, iv). Adhesive electrocardiographic electrodes were placed on shaved areas of limbs for monitoring lead II ECG. A midline incision was made in the neck under aseptic conditions, and the left common carotid artery was identified and carefully isolated from surrounding connective tissue and the vagus nerve in order to minimize traction and trauma to the nerve. Myocardial infarction was produced by injection of a 1.5-mm plastic bead into the left anterior descending coronary artery via a modified coronary cannula introduced through the left common carotid artery and positioned in the left coronary ostium for bead injection by monitoring pressure via a sidearm of the cannula.[12] After bead injection, the

(13) Gillis, R. A.; Levine, F. H.; Thibodeaux, H.; Raines, A.; Standaert, F. *Circulation* 1973, *47*, 697.

(14) Goldstein, A.; Aronow, L.; Kalman, S. M. "Principles of Drug Action", 2nd ed.; Wiley: New York, 1974; p 195.

carotid artery was ligated, the incision was sutured, and the animal was allowed to recover from anesthesia. The ECG was continuously monitored for a period of approximately 4 h. During this observation period, only occasional, isolated premature ventricular beats occurred.

Twenty-four hours after myocardial infarction, during the period of maximal ventricular ectopic activity,[12] the animals were returned to the laboratory and allowed to lie quietly on a table while an adhesive ECG electrode was again attached to each limb and a 23-gauge Butterfly infusion set was introduced into a foreleg vein and secured for drug injection. Prior to the initiation of any experimental procedure, animals were allowed a period of approximately 30–45 min to adjust to the laboratory surroundings. Throughout this period a lead II ECG was continuously recorded on a Beckman R-611 dynograph and simultaneously monitored at a fast trace speed on a storage oscilloscope. In all experiments it was noted that during this period approximately 80–85% of all ECG complexes arose from a ventricular pacemaker site and that the proportion of ectopic beats remained relatively constant. At the conclusion of the observation period, drugs being evaluated for antiarrhythmic activity were injected iv in incremental doses until conversion to a normal sinus rhythm occurred or until a total dose of 3.0 mg/kg was reached. Compounds 1–3 were administered in 0.3 mg/kg increments at 2-min intervals; 4 was administered in 0.1 mg/kg increments at 2-min intervals. Solutions of each drug were prepared fresh daily in 5% dextrose in water. Compound 5 was administered in 5 μg/kg increments at 2-min intervals in isotonic dextrose containing 5% propylene glycol (v/v). Several animals received only the 5% propylene glycol–isotonic dextrose solution to serve as controls; no change in cardiac rhythm was observed in any of the control animals. Lidocaine hydrochloride was administered in saline in 0.5 mg/kg increments at 1-min intervals. The concentration of each solution was adjusted so that each dosage increment of drugs employed was contained in 0.5 mL. Each animal received only one drug.

At the conclusion of each experiment, the animals were sacrificed and the hearts removed. At this time the location of the bead in the left coronary circulation and the location of the infarct were noted. In addition, the area of the aorta surrounding the left coronary ostium and the ostium itself were examined for damage incurred during catheterization. No evidence of trauma was noted in the area of the left coronary ostium in any animal. In all instances it was noted that the occlusion occurred distal to the first diagonal branch of the left anterior descending coronary artery, and the infarct involved only the anterior wall of the left ventricle.

Hemodynamic Effects of 5. Three dogs anesthetized with pentobarbital sodium (30 mg/kg) had cannulae positioned in a femoral artery and vein for blood-pressure monitoring and drug injection, respectively. A cardiac cannula was positioned in the left ventricle via the left carotid artery for recording left ventricular pressure, which was electronically differentiated to obtain left ventricular dp/dt. Needle electrodes were placed in each limb for recording lead II ECG and heart rate was electronically derived from the ECG signal. All parameters were recorded on a Beckman R611 dynograph at a paper speed of 100 mm/s in order to determine the left ventricular dp/dt at a developed left ventricular pressure of 50 mmHg (dp/dt/50) for evaluation of changes in myocardial contractility and to allow accurate determination of the ECG PR interval and QRS duration. All animals were allowed to stabilize approximately 30 min after surgical preparation before iv administration of 5.

Statistics. All statistical comparisons were made using Student's t test for paired or unpaired data utilizing a Monroe 1930 calculator. In each instance, statistical significance was defined as $p < 0.05$.

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Synthesis and Some Pharmacological Properties of [8-L-Tryptophan]oxytocin

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Condensation of (*tert*-butyloxycarbonyl)tocinoic acid with L-prolyl-L-tryptophylglycinamide produced the Boc derivative of a nonapeptide (disulfide) which on deprotection afforded [8-L-tryptophan]oxytocin. In assays on the rat uterus in vitro and in vivo the new analogue acts as both an agonist and an antagonist. The duration of both actions is prolonged.

The conformation of oxytocin has been extensively studied, mostly by NMR spectra.[1] It seemed to us desirable to seek confirmation of the proposed[2] architecture of the molecule of the hormone, particularly to find new, independent evidence for the folding of the C-terminal tripeptide segment over the cyclic hexapeptide. In order to measure the distance between two parts of the structure through a fluorescence energy transfer experiment,[3] a donor–acceptor pair is necessary. The presence of a tyrosine (donor) residue in the ring prompted us to synthesize an oxytocin analogue with tryptophan (acceptor) replacing

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(3) Förster, T., *Ann. Phys.* (Leipzig) 1948, *2*, 55. Cf. also Schiller, P. W. In "Biochemical Fluorescence: Concepts"; Chen, R. F.; Edelhoch, H., Eds.; Marcel Dekker: New York, 1975; Vol. I, p 285.

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