ORTHO SUBSTITUTED BENZOIC ACID ESTER OF DIALKYL AMINOALKANOL IN EXPERIMENTAL CARDIAC ARRHYTHMIAS

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Procaine hydrochloride and tridiurecaine, which resemble each other in their chemical configuration, have been reported to exhibit antiarrhythmic activity.[1,2] Since the ortho substituted benzoic acid ester of dialkyl aminoalkanol (McN-A-29-11*) a recently synthesised local anæsthetic, is another chemically similar compound, it seemed worthwhile to determine if it also shares with procaine and tridiurecaine the ability to combat arrhythmias. Hence the investigations now reported.

As there are ostensibly endless variables when recourse is taken to only one biological test, it was, therefore, deemed fit to employ a variety of experimental techniques in order to increase confidence in the results. Preliminary studies were made on the refractory period of isolated rabbit auricles; this was followed by tests on acetylcholine-induced auricular fibrillation, auricular flutter produced by injury-stimulation procedure, aconitine-evoked auricular fibrillation and hydrocarbon-adrenaline induced ventricular arrhythmias in dogs. In addition, a comparison was made of the effects of McN-A-29-11 and quinidine on the electrocardiogram of cats.

The ortho substituted benzoic acid ester of dialkylamino alkanol is a white, crystalline, odourless and stable substance with a bitter taste. It is freely soluble in water. The aqueous solution was used throughout. The action of this drug was compared with that of quinidine sulphate.

METHODS

I. *Isolated rabbit auricles.* This method, developed by Dawes[3], is based on the observation that quinidine-like drugs prolong the refractory period of isolated rabbit auricles. After washing the chambers of a rabbit heart free of blood, the auricles were isolated, care being taken not to injure the tissues near the sinus node. The preparation was then quickly transferred to an organ bath containing oxygenated Locke's solution at 29°C. The electrode arrangement used was the same as that described by Dawes. After the auricles had been immersed in the bath for 30 minutes, they were stimulated each time for 15 seconds by break shocks of increasing frequency until a point reached where they would no longer respond to every stimulus applied. This was recorded as 'maximal response rate' which is considered to be reciprocal of the refractory period. The drug being tested was then added to the bath. After 10 minutes the maximal response rate was redetermined and reduction in maximum frequency was noted.

* Code name of McNeil Laboratories, Philadelphia for the drug.

II. *Acetylcholine-induced auricular fibrillation.* This procedure for producing auricular fibrillation in dogs is based on the work of Scherf and Chick[4] and followed in all essential details the technique described by Schallek[5].

III. *Auricular flutter.* Experiments were performed on adult mongrel dogs of both sexes weighing between 12 and 16 kg. The animals were anæsthetised with morphine sulphate (10 mg/kg.) subcutaneously followed in half an hour by sodium pentobarbitone intravenously 30mg./kg. Blood pressure was recorded from carotid artery by a mercury manometer. Under artificial respiration, parts of the sternum and ribs directly over the heart were removed. To permit free access to the right side of the auricle, the animal was turned so that the heart fell towards the left side. The split pericardium was sutured over the chest walls.

Auricular flutter was initiated in all experiments by the injury-stimulation procedure described by Rosenblueth and Garcia Ramos.[6,7,8] A narrow band of auricular tissue connecting the superior and inferior vena cava was crushed by means of hæmostats and stable flutter was produced by subsequent stimulation of the auricle with square waves (duration, one millisecond, volts 15 to 20, and frequency 15 to 20 per second). The average auricular and ventricular rates during flutter were 461 and 242 respectively. The drugs were injected after the flutter had continued for at least 35 minutes. Spontaneous reversions of an arrhythmia of this duration were rare.

IV. *Aconitine-induced auricular fibrillation.* Auricular fibrillation was produced in dogs by employing Scherf's[9] method. In exactly the same manner as described above, chest and pericardium were opened under pentobarbitone anæsthesia. A cotton pledget, soaked in 0·05 per cent. solution of aconitine nitrate, was placed on the auricle. Within four minutes, persistent auricular fibrillation was produced. The intravenous administration of the drug was continued until 1 : 1 rhythm with the rate below 200 beats per minute (end-point) was reached.

In addition to the bipolar lead II, electrograms directly from the auricle and ventricle were recorded by a 3 channel Grass inkwriting oscillograph in procedures III and IV. Also, the dosage scheme used herein was the "titration procedure" as employed by Winbury and Hemmer[10], that is, 1 mg./kg. of the drug was intravenously injected every minute until reversion to normal sinus rhythm occurred in flutter or 'end-point' was reached in fibrillation.

V. *Hydrocarbon-adrenaline induced ventricular arrhythmias.* This method was described by Riker and Wescoe[11]. In our experiments, it consisted in anæsthetising dogs with 30 mg./kg. of sodium pentobarbitone intravenously and intratracheal administration of 0·1 ml./kg. of light petroleum, a mixture of lower aliphatic hydrocarbons, followed by 60 μg./kg. of adrenaline intravenously via the cannulated femoral vein. This would invariably result in the production of ventricular arrhythmias of types ranging from multifocal ectopic beats to ventricular fibrillation. When the protective action of the drug under trial was to be tested, it

was injected intravenously 1½ minutes before the administration of light petroleum.

VI. *Electrocardiogram.* Cats of both sexes weighing between 2·5 and 4 kg. were anæsthetised with a allobarbitone-urethane solution* 0·6 ml./kg. intraperitoneally. The electrocardiogram was recorded with a Grass inkwriting oscillograph by employing bipolar conventional lead II. The drug to be tested was injected intravenously and an electrocardiographic tracing was taken for the first two minutes and then at 5 minutes' intervals. Changes in refractory period and conduction time were measured from Q–T and P–Q intervals respectively. Only one drug was tried in one animal.

TABLE I

EFFECT OF DRUGS ON CARDIAC ARRHYTHMIAS

No. of animals	Drug	Dose	Result	
Isolated rabbit auricles				
			Average percent. reduction in max. frequency	
4	Quinidine	$2·5 \times 10^{-6}$ mg./ml.	13	± 1·22***
4	McN-A-29-11	$2·5 \times 10^{-6}$ mg./ml.	11	± 0·05
Acetylcholine-induced auricular fibrillation in dog				
			Average percent. reduction in duration of fibrillation	
6	Quinidine	2·5 mg./kg.	52	± 2·83
6	McN-A-29-11	2·5 mg./kg.	61	± 2·68
Aconitine-induced auricular fibrillation in dogs				
6	Quinidine	16 mg./kg.	End-point*	± 2·65
6	McN-A-29-11	11·8 mg./kg.	,, ,,	± 1·32
Auricular flutter in dogs				
6	Quinidine	21 mg./kg.	Reversion**	± 2·16
6	McN-A-29-11	8 mg./kg.	,,	± 1·29
Hydrocarbon-adrenaline-induced ventricular arrhythmias in dogs				
6	Quinidine	5 mg./kg.	+	± 0·78
6	McN-A-29-11	15 mg./kg.	+	± 1·41

* End-point is the establishment of 1:1 rhythm with the rate below 200 beats per minute in aconitine-induced auricular fibrillation.
** Reversion means restoration to normal sinus rhythm.
\+ Stands for complete protection against ventricular arrhythmias.
*** Standard deviation.

RESULTS

Preliminary experiments on the isolated rabbit auricles indicated that here McN–A–29–11 had an activity equivalent to that of quinidine. More extensive comparisons were then made in whole animals, results of which are summarised in Table I. In acetylcholine-induced auricular fibrillation, 2·5 mg./kg. of McN–A–29–11, like quinidine, brought about a significant reduction in the duration of fibrillation. Both of these compounds were successful in the treatment of auricular flutter (Fig. 1)

* An aqueous solution which contains, per ml., 0·1 g. allobarbitone, 0·4 g. urethane, and 0·4 g. monoethylurea, kindly supplied by Ciba Pharma Ltd., Bombay.

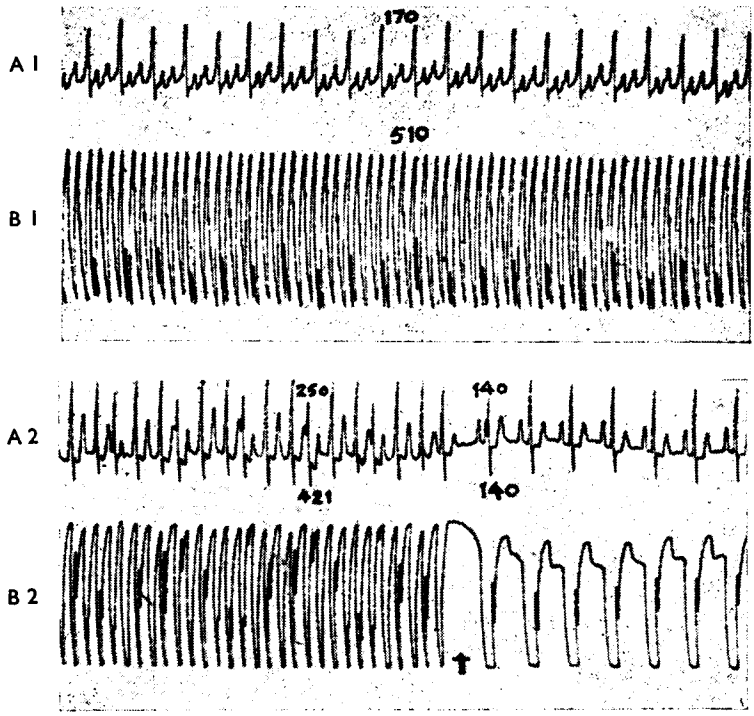

FIG. 1. McN-A-29-11 in auricular flutter.

A 1, B 1 show the auricular flutter with 3 : 1 auricularventricular block.
A 2, B 2 show an excerpt from continuous electrocardiographic record illustrating the abrupt reversion of auricular flutter to normal sinus rhythm at the arrow after McN-A-29-11 for 8 minutes.

A. Electrocardiogram lead II.
B. Electrogram directly from the auricle.

and aconitine-induced auricular fibrillation in all the cases but comparatively lower doses were required with McN-A-29-11 than with quinidine for bringing about restoration to normal sinus rhythm or 'end-point' (Fig. 2). In its ability to avert ventricular arrhythmias produced by light petroleum and adrenaline, McN-A-29-11 was, however, less effective than quinidine.

The effects on P-Q and Q-T intervals of cats (Table II) indicated that McN-A-29-11 shares with quinidine the property of prolonging conduction time and refractory period, but to a lesser degree.

TABLE II

EFFECTS OF DRUGS ON ELECTROCARDIOGRAM OF CAT.

AVERAGE PERCENTAGE CHANGE

No. of expts.	Drug	Dose mg./kg.	Average percent increase in P-Q interval.	Average percent increase in Q-T interval.
6	Quinidine	10	46	22
6	McN-A-29-11	10	26·7	14·4

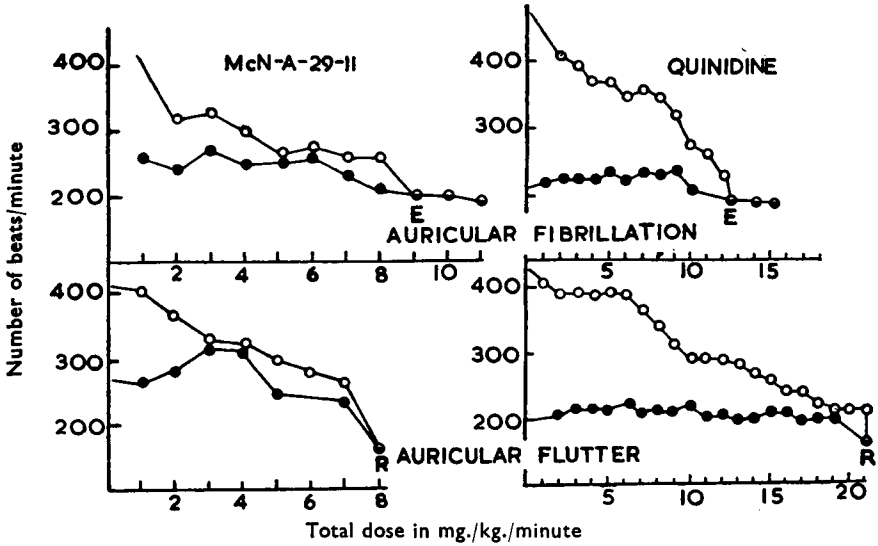

FIG. 2. The effect of McN-A-29-11 and quinidine on aconitine-induced auricular fibrillation and auricular flutter induced by injury-stimulation procedure. Each graph represents results from a different animal. —○— auricle, —●— ventricle. E. End-point. R. Reversion.

DISCUSSION

In tests on the isolated rabbit auricles, McN–A–29–11 exhibited an activity which was equivalent to that of quinidine, but as measured from the Q–T interval of the electrocardiogram of cats, quinidine caused a greater increase in refractory period. As a matter of fact there is no longer any need to cavil over the terms absolute, relative or effective refractory period because there is no real proof that a prolongation of refractory period is responsible for the antiarrhythmic activity of a drug[12]. It, therefore, seemed expedient to carry out further experimental studies to determine whether McN–A–29–11 was more effective than quinidine. Also, our present knowledge regarding the underlying mechanism causing these arrhythmias is limited despite the work of many investigators[13]. The three theories, most compatible with known facts, that have been postulated to explain the patho-physiologic disturbances responsible for these arrhythmias, are: (1) the classical circus movement theory[14], (2) the multiple ectopic focus theory[15] and (3) the single ectopic focus theory[16].

Accordingly, in the present study, in order to ensure a more correct interpretation of the results, use was made of selected experimental procedures which, as cited by Dick and McCawley[17], are representative of each theory: auricular flutter produced by injury-stimulation procedure (Circus wave); acetylcholine-induced auricular fibrillation (multiple focus) and aconitine-induced auricular fibrillation (single focus). The results obtained in these experiments indicate that McN–A–29–11 is stronger in its activity than quinidine in auricular arrhythmias. It is, however, weaker than quinidine in averting ventricular arrhythmias.

Electrocardiographic changes caused by the two agents include a greater prolongation of conduction time by quinidine. Slowing of conduction rate is the most deleterious property of quinidine because in the presence of conduction defects, it may precipitate ventricular tachycardia and ominous ventricular fibrillation.[18] It is significant to note here that McN-A-29-11 shares with quinidine the propensity to slow conduction, but to a lesser extent.

The intraperitoneal LD50 in mice of McN-A-29-11 was 180 mg./kg.[19] while that of quinidine was found to be 135 mg./kg.[3] Keeping in mind this reduced toxicity and the greater efficacy of McN-A-29-11 than quinidine in aconitine-induced auricular fibrillation and auricular flutter, it seems that McN-A-29-11 shows sufficient promise to warrant clinical trials.

SUMMARY

1) McN-A-29-11 was shown to exhibit an activity stronger than quinidine in experimental auricular flutter and aconitine-evoked auricular fibrillation, but was equivalent to it in its effects on the refractory period of isolated rabbit auricles and acetylcholine-induced auricular fibrillation in dogs.

2) It was, however, found less effective than quinidine in averting ventricular arrhythmias produced by hydrocarbon-adrenaline in dogs.

3) Electrocardiographic changes in cats produced by McN-A-29-11 and quinidine included greater lengthening of both the refractory period and conduction time by the latter drug.

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