Influence of Antiarrhythmic Agents on Calcium-Induced Cardiac Arrhythmias in the Rat

By Martin M. Winbury, Ph.D. and Mihaim L. Hemmer, M.S.

The effect of quinine, procaine amide and five new antiarrhythmic compounds on calcium-induced arrhythmias in the rat are described. When the compounds prevented fibrillation, the other actions of calcium on cardiac rhythm were revealed.

It has been well established that calcium can produce various alterations in cardiac rhythm such as fibrillation, arrest and/or bradycardia. Recently, Malinow presented a standardized method for producing ventricular fibrillation in the rat by calcium injection. This method was employed in the present study to evaluate a series of anti-arrhythmic compounds which were previously found effective in the treatment of several atrial and ventricular arrhythmias in the dog. During the present studies it was observed that when fibrillation was prevented other effects of calcium, particularly cardiac arrest, appeared. This report describes the effect of these compounds on calcium-induced alterations of cardiac rhythm. The compounds studied include quinidine and procaine amide in addition to SC-3920, N-(γ-isopropylaminopropyl)-α,α-diphenylacetamide hydrochloride; SC-3323, β-diisopropylaminoethyl-4-phenyl-4-tetrahydropropyran-carboxylate hydrochloride; SC-3755, O-(4-chlorobenzyl)phenoxyacetamidine hydrochloride; SC-2919, N,N,N'-triphenyl-N'-{β-diethylaminoethyl}urea hydrochloride; and SC-3412, β-diethylaminoethyl-2,6-dimethyl-5,6-dihydro-4H-pyran-3-carboxylate hydrochloride.

Methods

Male rats of the Sprague-Dawley strain weighing between 140 and 220 gm. were anesthetized with sodium pentobarbital, 45 mg. per Kg., intraperitoneally. The electrocardiogram was recorded on a Grass electroencephalograph, using needle electrodes on each side of the chest. The compounds were administered into the femoral vein in aqueous solution, usually in a volume less than 0.5 ml. Three minutes later a dose of calcium chloride dihydrate, 200 mg. per Kg., was administered intravenously in 10 per cent solution. The electrocardiogram was observed continuously during and after administration of the calcium. If death did not occur within six to ten minutes after the first dose of calcium chloride a second dose of 200 mg. per Kg. was given.

Both authors examined the electrocardiograms independently after the entire group of experiments was completed; to minimize personal bias, the records were evaluated without knowing the treatment. Changes in the electrocardiogram were classified for each animal as follows: no change, bradycardia, ventricular arrest, short periods of fibrillation and/or long periods of fibrillation. The distinction between short and long periods of fibrillation was arbitrary; short periods consisted of only a few ectopic beats in contrast to long periods which represented prolonged continuous runs.

Each anti-arrhythmic agent was studied at three or four dose levels using a two-fold increment in dosage. The various doses of the compounds were studied at random until a total of five to eight rats had been treated with each dose of each compound.

Results

Calcium chloride uniformly produced immediate death by ventricular fibrillation in a group of ten control animals. When rats were pretreated with an effective anti-arrhythmic agent there was a decrease in the incidence of fatal ventricular fibrillation and a concomitant increase in survival, or there was fatal cardiac arrest. However, cardiac arrest was frequently preceded by a short period of ventricular fibrillation, demonstrating two of the actions of calcium in the same animal. Further, the
onset of fibrillation was frequently delayed and in a few cases Bradycardia was the initial effect of calcium.

The actions of the anti-arrhythmic compounds were considered from two aspects: (a) the incidence of fibrillation following calcium chloride without regard to death or survival (fig. 1); and (b) the influence of the compounds on mortality following calcium chloride, with details as to the mode of death (fig. 2). The two aspects have been combined in reporting the results for each compound.

Procaine amide prevented fibrillation in all the animals pretreated with 160 mg. per Kg., but 67 per cent of these animals succumbed to cardiac arrest. No electrocardiographic changes occurred before the arrest. At 80 mg. per Kg., the fibrillation produced by calcium was preceded by an initial short period of Bradycardia.

Quinidine sulfate did not reduce the incidence of fibrillation at 4 and 8 mg. per Kg., although long fibrillatory periods yielded to short periods. There was a progressive decrease in death from fibrillation even though the percentage survival did not increase until the dose reached 16 mg. per Kg.

SC-3920 was the most active compound in these experiments. It produced a progressive increase in survival with the two smallest doses. However, none of the animals survived at the highest dose, but all died of cardiac arrest after the calcium without any other electrocardiographic change.

SC-3755 did not decrease the over-all incidence of fibrillation, but reduced the severity by a shift from long to short periods. The increase in the incidence of periods of short fibrillation paralleled an increase in cardiac arrest. The percentage survival remained unchanged.

SC-3412 provided little protection against calcium fibrillation until 32 mg. per Kg. were administered. At this dose 50 per cent of the rats survived and thirty-three per cent died of cardiac arrest.

SC-2919 was one of the more active com-
pounds. Neither the incidence of long fibrillation nor the percentage of death by fibrillation exceeded 33 per cent. The administration of calcium did not alter the electrocardiogram of the survivors.

SC-3323 was most effective in preventing fibrillation at a dose of 4 mg. per Kg. At this dose it also produced the greatest percentage of survival. Higher doses reduced the percentage survival and increased the percentage of animals which developed fibrillation.

The animals that survived the initial injection of calcium (fig. 2) were challenged a second time in the same manner. This resulted in death by arrest in the rats treated with SC-3920, SC-3755 and SC-2919; death by fibrillation in the rats treated with quinidine; and either by fibrillation or by arrest in the rats treated with SC-3323, SC-3412 or procaine amide.

**DISCUSSION**

The compounds most active in preventing calcium-induced ventricular fibrillation in the rat on a dosage basis were SC-3920, quinidine and SC-2919. Procaine amide was the least potent substance, requiring from ten to twenty times the dose of SC-3920 for a comparable effect. The effective dosages for procaine amide and quinidine in the above studies compare closely with those reported by Malinow and coworkers\(^8\) (50-100 mg. per Kg. for procaine amide and 20 mg. per Kg. for quinidine).

The present work indicates that all seven compounds have some activity against ventricular arrhythmias in the rat. This contrasts with the observations previously reported\(^4\) for the dog in which only quinidine, procaine amide, SC-3920 and SC-3755 had a significant effect on the ventricular tachycardia resulting from coronary ligation, and all the compounds except SC-3755 produced a reversion of atrial flutter and fibrillation to sinus rhythm. It is evident, therefore, that several experimental procedures should be used for the evaluation of anti-arrhythmic activity.

Calcium is capable of producing several alterations in cardiac rhythm, but the only effect observed in the control animals was fibrillation; the other actions were revealed only when fibrillation was prevented or delayed by the anti-arrhythmic agents. These actions of calcium on cardiac rhythm in the rat are consistent with the findings of Hoff and coworkers\(^1\) in morphinized dogs, that calcium in doses of 1.29 to 3.9 mM per Kg. produces bradycardia leading to fibrillation or cardiac arrest. Their doses of calcium are comparable to the dose of calcium used in the present studies in the rat, namely 1.36 mM per Kg.

The decrease in percentage survival produced by the 8 mg. per Kg. dose of SC-3920 (fig. 2) is probably associated with a combined action of calcium and SC-3920 to produce immediate cardiac arrest; SC-3920 was previously shown to be a severe cardiac depressant in dogs at 10 mg. per Kg.

**SUMMARY**

The actions of quinidine, procaine amide and five new compounds were studied on calcium-induced fibrillation in rats. The compounds are hydrochlorides of the following bases: SC-3920, N-(\(\gamma\)-isopropylaminopropyl)-\(\alpha,\alpha\)-diphenylacetamide; SC-3323, \(\beta\)-diisopropylaminoethyl-4-phenyl-4-tetrahydropyranecarboxylate; SC-3755, O-(4-chlorobenzyl)-phenoxyacetamidine; SC-2919, N,N',N'-triphenyl-N'-Q(\(\beta\)-diethylaminomethyl)urea; and SC-3412, \(\beta\)-diethylaminomethyl-2,6-dimethyl-5,6-dihydro-4H-pyran-3-carboxylate. All the compounds were active in preventing calcium fibrillation. The most active on a dosage basis were SC-3920, quinidine and SC-2919. Other actions of calcium, such as cardiac arrest and bradycardia, were revealed when fibrillation was prevented by these anti-arrhythmic agents.

**REFERENCES**


Alleged Synergism of the Sympathetic Nervous and Adrenal Medullary Systems

It has become a common theory that the adrenal medullary hormones and sympathetic neuro-effectors act synergistically and indiscriminately on regulation of blood flow. The adrenal medulla has even been designated as the "loud pedal" of the sympathetic nervous system.

Many of the inferences linking humoral and nervous actions have resulted from use of pharmacologic rather than physiologic doses of humoral agents or from nerve excitation at frequencies above the established normal rates of sympathetic impulse discharges.

Using these modern methods, Swedish physiologists have recently demonstrated that the motor control of blood vessels is much more powerful than the adrenal hormonal control under physiologic conditions. For instance, in cutaneous areas low frequency stimulation of nerves reduced blood flow 10 to 20 times more than similar excitation of adrenal nerves. Furthermore, simultaneous stimulation of the two caused no significant reinforcement of effect. No evidence of an "overflow of sympathin" was discovered.

In a careful review of previous work and that in which he participated, Celander concludes that neither medullary catechols nor circulating sympathins are of quantitative importance in regulating blood flow directly. Contrary to the "key cell" theory of Cannon & Rosenblueth (Autonomic Neuro-Effector Systems, The MacMillan Co. N. Y. 1937) the evidence indicates that "every cell subordinate to the sympathetic nervous system makes a close contact with sympathetic neuro fibers, and, as a matter of fact, an overlap appears to exist, so that axon ramifications from several neurons converge upon each effector cell."

The action of the adrenomedullary hormone is apparently limited to cells that lack a sympathetic innervation. This explains not only their important metabolic effects within physiologic ranges, but also secondary circulatory adjustments. For example, it appears demonstrated that norepinephrine in a wide dosage range only constricts vessels of skeletal muscle whereas epinephrine constricts them in large doses, but dilates them in small ones. The hypothesis is favored that the latter effects are secondary to its glycogenetic action, i.e., are really induced by the ultimate release of lactate.

Influence of Antiarrhythmic Agents on Calcium-Induced Cardiac Arrhythmias in the Rat
MARTIN M. WINBURY and MIRIAM L. HEMMER

Circ Res. 1955;3:474-477
doi: 10.1161/01.RES.3.5.474

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1955 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/3/5/474

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/