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Ventricular fibrillation in experimental hypothermic cardiac surgery, the evaluation of antifibrillary agents

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VENTRICULAR FIBRILLATION IN EXPERIMENTAL HYPOTHERMIC CARDIAC SURGERY, THE EVALUATION OF ANTIFIBRILLARY AGENTS

by

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TOPICAL OUTLINE

I. INTRODUCTION

If drugs are to be applied toward the solution of a problem, there must be some way of testing such drugs. The sounder the test, the more reliable are the results obtained, and the more fruitful the search.

In a sense, research follows a dialectic pattern: no sooner is a thesis proposed, than it encounters an antithesis; when the smoke clears, we are enabled to arrive at a synthesis, which in turn is destined to re-enter the cycle as a new thesis.

Since Bigelow (6) first suggested the application of hypothermia as an adjunct to cardiac surgery, many of the factors influencing the incidence of ventricular fibrillation (VF) such as pH changes (9, 13, 21), choice of anesthetic agent (4, 24), mechanical irritants (17), etc., have been investigated, and clarified. The elucidation of these factors has provided a sounder basis for the interpretation of effects observed (10, 11, 3) with antifibrillary (AF) drugs.

The task here is to analyze and integrate the information derived from previous methods; to combine their desirable features, and to minimize their shortcomings, in an effort to develop a more reliable way of evaluating AF agents.

II. REVIEW OF PERTINENT BACKGROUND

1. Ventricular Fibrillation

One of the foremost problems encountered in hypothermia is the increased susceptibility of the myocardium to VF., both in the human (5) and in its experimental proxy, the dog (6).

VF is a state in which the orderly, coordinated and useful contractions of the ventricles are replaced by the supervention of irregular, disorderly, uncoordinated and useless contractions. Since cardiac output is virtually nil when the ventricles are fibrillating, the persistence of such a state is not compatible with life. Interest in this phenomenon is not new.

Based on the 1908 experiments of Mayer on the jellyfish Garrey and Mines in 1914 advanced the circus movement theory as a possible underlying factor of VF (14, 19). Their work was furthered by Lewis in 1925 (18), who postulated that circus movements tend to be perpetuated by slowed conduction, shortened refractory period, and lengthened path of conduction; whereas the converse of these factors would tend to eliminate the circus movement.

Brooks (7) states that certain conditions predispose to VF:

1. "An acceleration of the heart rate beyond

the ability of all the elements to follow, can disorganize the heart and cause fibrillation."

2. "A localized excitation" can lead to a disorganized sequence of events ending in VF.

3. Both shortening and lengthening of the refractory period can result in VF.

Prinzmetal (22) on the basis of high speed cinematographic studies has refuted the evidence for circus movements in VF; he proposed that "fibrillation originates from and is perpetuated by a single, rapidly discharging ectopic focus" (23).

Wiggers, Wegria, Moe, Harris, et al, have studied ventricular fibrillation extensively (16, 20, 26, 27, 28, 29): by means of single induction shocks (29), direct current (28) and alternating current (27), they obtained electrical fibrillation thresholds as an index of the susceptibility of the myocardium to VF.

Despite over 50 years of investigation, the exact nature of VF remains to be defined.

2. Earlier Methods of Testing Antifibrillary Agents

Most of the literature concerned with the evaluation of AF agents pertains to normothermia; in hypothermia, few approaches have found common acceptance.

Before an antiarrhythmic drug can be tested, there must be an arrhythmia on which to test it. Since arrhythmias are not usually encountered in the normothermic dog,

experimental arrhythmias have been produced by a variety of methods which may involve damage to the myocardium of greater or lesser degree, and include procedures such as ligation of the coronary vasculature as described by Harris (15); various types of stimulation such as electrical or mechanical; and pharmacologic induction with substances such as Aconitine, as standardized by Prinzmetal (23), or involving the administration of a combination of cyclopropane and epinephrine.

In hypothermia, the production of arrhythmias is easier; a majority of the dogs succumb spontaneously to VF during the process of cooling, usually below 25^{0}C.

One method for evaluating AF agents (11) takes advantage of this fact. Dogs are "cooled to terminus", i.e. either VF occurs somewhere below 25^{0}C, or the heart rate slows progressively until asystole supervenes. It is then determined whether a given drug, administered at some empirically selected temperature significantly lowers the mean temperature at which VF or asystole occur. While information of this nature is extremely important, Angelakos has pointed out (1) that protection against spontaneous VF during cooling need not be synonymous with protection against VF during surgery in hypothermia. Our experiences with Antergan (vide infra) tend to corroborate this view.

A second method (1), also widely used, appears somewhat more reliable for our purposes. Dogs are

cooled to a predetermined temperature, at which time a "standard" surgical maneuver is performed on the heart, i.e. right ventriculotomy and exploration. The incidence of VF or mean lethal temperature in "control" dogs is then compared to the results from dogs receiving drugs.

The work of Wiggers, Wegria, et al has provided another basis for AF drug testing: this approach was investigated by Shumway et al (25), Covino and Beavers (8), and by us. Our (unpublished) data revealed no correlation between electrical VF thresholds and the occurrence of VF during surgery.

III. EXPERIMENTAL SECTION

1. Introduction

A critical examination of past methods makes it clear that certain experimental variables have to be minimized or avoided if reliable results are to be obtained.

One of the most important variables to consider is the dog. Dawes, in his extensive review of the matter of experimental cardiac arrhythmias (12) states that "even in intricate experiments, performed on whole animals, analysis reveals individual influences which determine whether or not disorders of rhythm shall occur." Not all animals fibrillate without drug protection, hence it becomes essential to determine prior to the administration of an AF drug, whether the dog would fibrillate without it.

Investigation of the antifibrillary activity of various drugs in hypothermia without prior or concommitant determination of the duration of action in the dosages used, leads to inconsistencies. This point bears amplification. Using the paper by Covino, Wright, and Charleson (11) as a prototype one finds the following: the experimenters report that the dogs were cooled to terminus: i.e. onset of spontaneous VF or asystole. Dilantin, Prontesyl, and Darcorene-treated dogs were compared with control groups. However, Dilantin was given to 9 dogs at a rectal temperature of $25^{0}C$, and to 1 dog at $29^{0}C$, and Darcorene was administered

to 8 dogs in 2 doses of 5 mg/Kg each, the first dose at 25^0C and the second dose at 20^0C. In two other experiments, drugs were injected at 3 different temperatures. In our experience, the time required for a dog to cool from 29^0 to 25^0 is approximately 20 minutes, while from 25 to 19^0C it is in the vicinity of 60 to 75 minutes. It becomes clear that the drugs being compared in this study all bear a different time relationship to the end-point of spontaneous VF at a mean temperature of 19.6± 2.0 in control series; no real information is obtained either as a basis for, or as a result of the antifibrillary activity of the drugs being tested, for their activity may well have evanesced before the dogs reached critical temperatures with respect to VF.

Therefore, it is highly desirable that any approach used should incorporate some means of estimating the duration of action of the drug being tested.

Finally, the investigator's bias, conscious or unconscious must be accounted for. The value of "blind" experiments is a time-tested countermeasure for minimizing an investigator's propensity, albeit unconscious, for making one agent appear more effective than another.

These considerations largely influenced the molding of the method presented below.

2. Methods

Random mongrel dogs were anesthetized with intraperitoneal pentobarbital, 33 mg/Kg. Positive pressure respiration with a pump [1] permitting regulation of rate and volume was instituted as soon as depth of anesthesia permitted tracheal intubation. Temperatures were measured continuously in degrees Centigrade, with a Speedomax [2] Recorder. Deep esophageal temperatures were used throughout since in our experience this approximates heart temperatures within 0.5^0C. Continuous visual and audio monitoring of the heart action was obtained by connecting an oscilloscope and audio amplifier to the output of the EKG [3] recording lead II.

Dogs were cooled by immersion in a Frigidaire food freezer, containing water in specially constructed jacket, the water having been previously cooled to 2^0C.

The animals were removed from the cold water at 2^0C above the required temperature to allow for the invariable downward continuation of temperature after removal from the ice water. They were then allowed to stabilize (24 - 20^0C).

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[1] Respiration Pump, Model No. 1063, Harvard Apparatus Co., Inc., Dover, Mass.

[2] Speedomax Type G Recorder, Leeds and Northrup Co., Philadelphia, Pa. (range 50^0 - 0^0C.).

[3] Sanborn Viso Cardiette, Sanborn Co., Cambridge, Mass.

The chest was opened via a right thoracotomy, the azygous vein was tied off, and the pericarium widely incised. Ligatures were passed under the vena cavae to permit occlusion during the ventriculotomy. A right ventriculotomy (3 cm. incision) was performed, and the right ventricle thoroughly explored with the index finger. The myocardium was then closed with four to five interrupted sutures. Emphasis was not on performing the operation with an eye to being as gentle as possible to avoid VF or to minimize the chances of its occurring, but rather to being as strenuous as possible without damaging the myocardium. This procedure required 5 minutes' occlusion time, and all ventriculotomies in these experiments were done with a timed occlusion of 5 minutes. If surgery resulted in VF, the heart was defibrillated with countershock [4], the right ventricle was sutured, and the ligatures released. The heart was allowed five minutes to recover, and then the procedure was repeated. If the heart fibrillated a second time, the dog was considered a "fibrillator", and as such, adequate for drug testing. Further, it had been shown that VF was brought about by introducing the finger into and exploring the right ventricle (second ventriculotomy), and not merely by the act of incising the ventricle. This is important, since it was necessary to establish in every

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[4]Heart Defibrillator, The Mark Co., Randolph, Mass. (capacities: 280 v for 1/20 sec., 220 v for 1/10 sec., 120 v for 1/4 sec., all being alternating current).

case that exploration was an adequate stimulus for VF in the heart "unprotected" by a drug.

The sine qua non of these prelminary steps was to obtain two successive VF's. Dogs surviving either ventriculotomy were cooled further and re-explored. Any animal not fibrillating by 18^0C., or requiring more than four initial ventriculotomies to establish it as a fibrillator, was discarded as being unsuitable for drug testing.

The fibrillator, having fibrillated twice, was given ten minutes' respite, and then the drug to be tested was injected into the right ventricular chamber over a 60 second period. The drugs were prepared and injected by someone other than the surgeon. The surgeon did not know at the time of the experiments which drug was being tested, if any, since many placebos (puncture of the right ventricle and withdrawal of a few cc. of blood) were used. Ten minutes after "injection," the great veins were again occluded, and the right ventricle re-explored as previously. This was repeated at 20, 30, 45, and 60 minutes respectively after the injection, in order to determine

1. Antifibrillary activity.
2. Duration of adequate protection, with VF re-occurring when drug effects wore off.

For the sake of economy, if a given dog had received a placebo instead of a drug, and did fibrillate on the first exploration after "injection," the surgeon was informed of this fact, and left the room while another "injection" was

given without any assurance, however, that the second injection was not again a placebo.

Initially, four drugs were screened on 3 dogs each by this method, these were: quinidine (10 mg/Kg), procaine amide 50 mg/Kg, Ambonestyl 50 mg/Kg, and Antergan 10 mg/Kg. The quinidine tests were then amplified to include a total of 10 dogs, intermingled with 7 others receiving placebos.

Subsequently, a confirmatory test was performed on twenty dogs, ten receiving quinidine (10 mg/Kg), and ten controls, on a blind basis. These dogs were merely cooled to $23.5^0 \pm 1^0$C. and a right ventriculotomy performed, at ten minutes after "injection" during the standard five-minute occlusion, and the outcome of surgery noted.

3. Results

The results are given in Tables 1 and 2 (pages ii and iii). The first screening series, involving three dogs per drug, were made in order to determine which if any of the four drugs being tested would be effective were it to be tested on a large number of dogs. The statistical method involved and its advantages, are discussed elsewhere (2). Briefly, judgment is based on the calculation of the exact probability (using the Bernoulli theorem) that the observed incidence of VF after treatment bears a relation to the effectiveness of the drug. For this purpose an effective drug is defined as one which would produce a high degree of protection (95%) when tested on a large number of animals. In the case of

Antergan and Pronestyl the probability that they might be effective during the first half-hour after injection was much too small ($P<0.001$) and therefore these drugs can be rejected with a high degree of confidence. For Ambonestyl the probability was larger but still rejectable ($P<0.05$) in this rather severe test. By contrast, quinidine appeared to have a great probability of effectiveness ($P>0.5$) in screening tests, and this was supported by the second series of experiments. In the latter the difference in the cumulative incidence of VF between quinidine-treated and control animals up to 30 minutes after injection is statistically highly significant ($P<0.01$).

4. Discussion

The results indicate that quinidine is 100% effective in protecting the hypothermic heart from induced VF for 15 minutes after the injection of 10 mg/Kg. The observation that this protection decreases to 90% in 30 minutes, 70% after 45 minutes and 50% within an hour, indicates that the result is due to quinidine, the effectiveness of which diminishes and disappears, leaving the heart still able to respond with VF to the exploratory procedure. In retrospect, the evidence in favor of this point would have been more reliable had all the dogs subjected to multiple exploration been re-explored up to 60 minutes after injection regardless of the occurrence of VF during

any post-injection exploration, to establish the response of the myocardium to subsequent exploration after the drug protection had receded, according to our criteria. Also, it is conceivable that the allotted 10 minutes was not sufficient to allow maximum drug protection in every case. Post-fibrillary explorations would again have provided data on this point.

The additional 20 dogs served to clarify another point. According to the method of "multiple exploration," the incision into the myocardium was made in every case, prior to drug administration. It was important to determine whether a drug shown to protect against subsequent explorations would also deter VF during actual incision of the myocardium. In the case of quinidine, this was shown to be so.

The most salient aspect of the method of "multiple exploration" is that in effect, each dog serves as its own control. While the incidence of VF under similar conditions (artificial ventilation throughout) in controls has been previously reported as 40 to 60% (4), a recent review of such experiments performed in this laboratory reveals that in any small series, the incidence of VF in controls may be anywhere in the 30 to 70% range, hence unless confirmed fibrillators are used, one is faced with the possibility of attaining 70% control over induced VF, with placebos.

This method also provides a semi-quantitative assessment of the duration of action of the agent tested, in the dosage used.

Of the four drugs selected for initial screening by this approach, quinidine and Pronestyl were included because they were classical drugs in this field; Ambonestyl was selected because of the report by Covino and Hegnauer (10) concerning its AF activity; Antergan was included because it had been shown to be effective in reducing the incidence of spontaneous VF in dogs during cooling (3). Our experience with Antergan confirms the view of Angelakos (1) that protection against spontaneous VF is not necessarily synonymous with protection against induced (surgical) VF, in hypothermia.

It is hoped that the anti-fibrillary capacity of quinidine may find a more effective application in human hypothermia, if it is employed with the awareness of its relatively short duration of action.

IV. SUMMARY

A new method for evaluating anti-fibrillary drugs for use in hypothermia is presented. This method involves serial surgical exploration of the hypothermic myocardium. Initially, two explorations were performed to determine if that particular myocardium was subject to ventricular fibrillation. Subsequently, after a drug had been given, serial explorations were repeated in order to evaluate any protection conferred on that myocardium by the drug being tested, and the duration of the protection conferred.

The method when used to screen four well-known drugs in this field (quinidine, Pronestyl, Ambonestyl, and Antergan) clearly indicated the superiority of quinidine.

When the original screening of quinidine was amplified, then checked by another method, the screening method was found to be reliable, as was the antifibrillary action of quinidine.

As evidenced by this method, quinidine is 100% effective in protecting against induced (surgical) ventricular fibrillation at temperatures in the range of $22^{0}C \pm 2^{0}C$. This protection declines to 90% in 30 minutes, 70% in 45 minutes, and 50% in 60 minutes after injection of 10 mg/Kg.

TABLE 1. Cumulative Incidence of Ventricular Fibrillatia From Surgical Manipulations of The Hypothermic Heart.*

First Series:	Before Injec. 1.	Before Injec. 2.	Minutes after Injec. 10-15	Minutes after Injec. 30-35	Minutes after Injec. 40-45	Minutes after Injec. 60-65
Ambonesty1	3/3	3/3	1/3	1/3	3/3	
Antergan	3/3	3/3	1/3	2/3	3/3	
Pronesty1	3/3	3/3	2/3	2/3	2/3	2/3
Quinidine	3/3	3/3	0/3	0/3	1/3	2/3
Second Series:						
Controls	7/7	7/7	6/7	7/7		
Quinidine	7/7	7/7	0/7	1/7	1/7	3/7

* Expressed as the ratio - number fibrillated/total.

Table 2. Incidence of VF During Surgery at 23.5 ± 1°C.

Drug	No. of Dogs	No. Succumbing to VF	%VF
Quinidine (10 mg/Kg)	10	0	0
Placebo	10	6	60

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