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Production of Atrial Fibrillation in Dogs by Thyroid Administration and Acetylcholine Injection

By PHILLIP E. LEVEQUE, PH.D.

The observation of susceptibility to atrial fibrillation in the thyrotoxic patient suggests a method by which a similar condition may be produced in dogs. This has been accomplished by long-term thyroid extract feeding. Administration of acetylcholine to these dogs by intravenous injection has resulted in a high percentage (81 per cent) of atrial fibrillation.

THE experimental study of atrial arrhythmias has been hampered by lack of a satisfactory technic for producing sustained fibrillation in animals. Most of the present experimental methods do not provide ample perpetuation or consistent reproducibility of the fibrillation so that comparative evaluation of antifibrillatory drugs may be made.

A study to determine whether the production of the experimental thyrotoxic condition might mimic that of the clinical patient in a satisfactory percentage of experimental subjects was published by Surtshin and Rucknagel¹ who administered thyroid substance to dogs in the feed and also by injection over periods of several weeks. They found few normal dogs susceptible to atrial fibrillation by injected acetylcholine and only a slight increase in susceptibility to atrial fibrillation in 1 of 15 animals under the influence of heavy doses (0.25 Gm./Kg./day or more) of thyroid substance. This work was published while the investigation herein reported was in progress.

This report describes a similar experimental procedure designed to evoke consistent and prolonged atrial fibrillation in dogs using long term thyroid feeding of about the same dosage to cause thyrotoxicosis with concomitant cardiac hypersensitivity to intravenous injected acetylcholine.

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METHOD

Mongrel dogs in apparent good health, weighing about 12 Kg., were anesthetized by intraperitoneal injection of sodium pentobarbital (35 mg./Kg.) and secured to an animal board. Intravenous injections were made into a saphenous vein by the use of an indwelling 19 to 23 gage hypodermic needle secured to a supported 25 ml. syringe and three-way stopcock. Acetylcholine injections (0.1 to 2.0 ml.) were made through the side opening of the stopcock directly into the vein. Acetylcholine (chloride or bromide) injections were made at intervals of about five minutes. With the very low doses, a shorter time was allowed to elapse between injections; with the higher doses a longer time. This was done in an attempt to eliminate respiratory embarrassment due to acetylcholine-induced bronchiolar constriction and copious salivation which occurred routinely and accidentally caused the death of two of the experimental animals.

A Sanborn Viso-Cardiette electrocardiograph was used to record cardiac changes. The electrocardiograph was allowed usually to run continuously for a period of about 10 seconds before the acetylcholine was injected and continuously thereafter until changes produced by the drug reverted to normal. Measurements of the heart rate were taken from the ECG records made on the trained dog while resting before anesthesia and again during anesthesia.

Before starting thyroid feeding, each dog was subjected to anesthetization and to one or more control observations.

Production of Experimental Thyrotoxicosis: Control experiments were made first to find the sensitivity and response to intravenously injected acetylcholine. If the animal showed no natural cardiac arrhythmias or gross respiratory difficulties which would interfere with continual testing, it was placed in an individual cage and fed a regular diet consisting of kibbled dog biscuits plus a small amount of horse meat. For each daily feeding, 3 Gm. of thyroid extract powder was mixed with the food.

After starting thyroid intake, the animals were examined daily for evidence of thyrotoxicosis. At frequent intervals, they were anesthetized and tested regularly as outlined above for any change in sensitivity to acetylcholine. In this procedure each animal served as its own control for the electrocardiographic studies, for the influence of injected acetylcholine and for the effect of injected acetylcholine during thyroid extract administration.

RESULTS

Untreated Dogs: We were able to produce atrial fibrillation in 7 of 23 untreated dogs (30 per cent) with dosages of acetylcholine of 1 mg./Kg. or less. The dose of 1 mg./Kg. was felt to be the maximum safe dose for immediate intravenous administration.

A summary of table 1, showing the effects of various doses of intravenous acetylcholine on cardiac rhythm, indicates that the mean threshold dose producing atrial fibrillation was 0.12 mg./Kg. (range 0.05 to 0.20 mg./Kg.). The mean duration of fibrillation at the minimum dose was 22 seconds (range 9 to 37 seconds). Although 2:1 block and fibrillation have both been utilized as objective criteria for the experimental evaluation of antifibrillatory drugs, no correlation was found between animals manifesting low 2:1 block dose and tendency to atrial fibrillation. Nor did the heart rate of the animals, whether anesthetized or not, appear to have any correlation with fibrillation. Examination of the table indicates several animals with high heart rates both before and during anesthesia. It is felt that these results are a reflection of methodology rather than gross abnormality of the animals used. Slight modifications and improvements in technic throughout the series certainly caused some minor changes in the response of the animals. With a study of this kind, it is felt these modifications produced changes of minor significance.

Thyrotoxic Dogs: Thyrotoxicosis produced by this method in dogs closely mimics that seen in the human with the clinical signs of tachycardia, anorexia, vomiting, diarrhea, excitability, thirst and weight loss. With prolonged periods of feeding, additional signs such as dry, scaly or flaking skin or marked hair shedding was noted.

During the time the animals were being given

TABLE 1.—Effect of Intravenous Injections of Acetylcholine on Atrial Rhythm in Untreated Dogs Anesthetized with Pentobarbital Sodium

Normal Dog No.	Dose of Acetylcholine Causing:		Duration	Heart Rate	
	2:1 Block	Atrial Fibrillation		Anesthetized	Unanesthetized
	mg./Kg.	mg./Kg.	sec.	per min.	per min.
1	0.05	>1*	—	185	112
2	0.04	>1*	—	152	96
3†	0.07	0.95†	96†	207	120
4	0.09	>1*	—	177	96
5	0.035	>1*	—	186	80
6	0.055	>1*	—	167	96
7	0.03	>1*	—	144	108
8	0.026	>1*	—	178	75
9	0.012	0.08	34	214	—
10	0.057	>1*	—	200	88
11	0.06	>1*	—	180	—
12	0.06	0.2	37	225	—
13	0.04	>1*	—	139	—
14	0.066	0.09	12	147	97
15	0.07	>1*	—	206	—
16	0.04	>1*	—	140	—
17	0.03	>1*	—	128	—
18	0.04	0.05	9	232	—
19	0.08	>1*	—	128	—
20	0.04	0.2	17	120	—
21	0.12	>1*	—	151	—
22	0.08	>1*	—	155	—
23	0.04	0.12	24	153	—
High	0.12	0.20	37		
Ave.	0.05	0.12	22		
Low	0.012	0.05	9		

* No dose larger than 1 mg./Kg. was administered.

† Omitted from calculation because of its obvious inconsistency.

thyroid extract, they were selected at periodic intervals for experiments to ascertain any change in sensitivity to injected acetylcholine using the procedure outlined previously. Along with the signs of hyperthyroidism the dogs exhibited a marked increase in sensitivity to injected acetylcholine. It was found that this hypersensitivity permitted disturbances of cardiac rhythm such as reflex tachycardia or single 2:1 block which were the types most frequently seen. Other more severe and complicated arrhythmias consisted of longer conduction blocks of 3:1 ratio to 10:1 ratio or sometimes greater. Such blocks were characterized by complete cardiac irregularity with prolongation of the P-R intervals. Frequently the P

TABLE 2.—Effect of Intravenous Injection of Acetylcholine on Atrial Rhythm in Thyroid-Fed Dogs Anesthetized with Pentobarbital Sodium

"Thyroid" Dog Number	Time Fed Thyroid	Dose of Acetylcholine Producing Fibrillation	Duration of Fibrillation
	days	mg./Kg.	sec.
1 T	6	0.42	48
2 T	7	0.4	33
3 T	8	1.0	*
4 T	9	1.0	†
5 T	10	0.6	37
6 T	10	0.6	7
7 T	16	0.1	29
8 T	16	0.5	34
9 T	16	0.1	19
10 T	17	0.04	13
11 T	19	0.15	21
12 T	20	0.32	24
13 T	21	0.2	20
14 T	22	0.1	25
15 T	25	1.0	‡
16 T	28	1.0	‡
High		1.0	48
Ave.		0.47	26
Low		0.10	7

* Never fibrillated at any dose.

† Fibrillated on a later occasion. Failure to do so here probably due to technical error.

‡ Dogs died during or as a result of operation possibly because of too much acetylcholine.

waves either appear only now and then and are superseded by an indistinct ragged wave, or disappear altogether. Usually, these phenomena herald and proceed to variously prolonged episodes of frank atrial fibrillation. In the normal non-thyroid fed animal, the incidence of fibrillation was 30 per cent with 7 of the 23 dogs developing this condition (table 1). In the thyroid-fed series it was 81 per cent with 13 of the 16 dogs responding (table 2). The period of greatest sensitivity to injected acetylcholine was between the sixth to twenty-four days of thyroid administration and, in general, auricles fibrillating later than this had also done so before administration of thyroid extract.

No alteration in the 2:1 atrioventricular block dose was noted between the untreated and the thyroid fed group. The average dose of acetylcholine to produce auricular fibrillation varied considerably between the untreated "fibrillators" and those which were fed thyroid.

The normals "fibrillating" at an average of 0.12 mg./Kg. (range 0.05 to 0.20) and the thyroid-fed 0.47 mg./Kg. (range 0.10 to 1.0). It must be remembered that 16 of 23 normals did not fibrillate at any dose up to 1.0 mg./Kg. whereas only 3 of 16 of the experimental dogs did not fibrillate at doses of 1.0 mg./Kg. or considerably below. Further explanation of this apparent difference must await more exhaustive study.

The duration of fibrillation was quite comparable between the two groups. It is felt that future refinement of method and equipment may possibly alter the results to provide more consistent results.

Further observation showed that long continued administration of thyroid extract powder finally resulted in a condition which might be considered a refractory state to the intravenously administered acetylcholine. Strong refractoriness appeared usually about the third or fourth week of administration. Usually, after this time, the only sign of thyrotoxicosis was an increased heart rate and the atria would fibrillate only with much greater doses of acetylcholine or not at all at doses considered to be consistent with preventing death of the dogs.

DISCUSSION

It has been possible by this method to produce, in a high percentage of cases, a preparation in which atrial fibrillation can be produced at will for periods of time which would allow comparative evaluations of antifibrillatory drugs. The dogs so prepared may be reused repeatedly with little apparent discomfort or change.

It is felt that the experimental employment and duplication in the dog of the long-known clinical observation, that an intimate correlation exists between thyrotoxicosis and heart disease, provides the closest approach to clinical atrial fibrillation yet devised.

An explanation of the failure of the other group¹ to produce satisfactory experimental atrial fibrillation under similar conditions is offered. While these workers continued thyroid administration for about 28 days, they tested the animals for fibrillation on the tenth and

seventeenth days and sometimes later. It was observed that this is the period during which the animals are no longer sensitive or are developing decreased sensitivity to injected acetylcholine. Secondly, the doses of acetylcholine they gave were in most cases far less than those which were used in this series. Had larger or increasing doses of acetylcholine been used, it is felt that a higher incidence of fibrillation might have resulted, regardless of the fact that the animals were tested during the later period of decreasing sensitivity to acetylcholine.

Although Surtshin and Rucknagel¹ found no increased sensitivity to acetylcholine in experimental canine thyrotoxicosis during the second and third weeks, our earlier testing with larger doses provides experimental circumstances in which such an increase can be found and may be used for the evaluation of procedures used to control arrhythmia. Investigations are being planned to further elaborate the value of this new tool in a comparative evaluation of certain "antifibrillatory" drugs.

SUMMARY

Atrial fibrillation in dogs was produced by the administration of thyroid extract and intravenous acetylcholine. It was found that all types of disturbed atrial rhythms, from simple tachycardia to sustained atrial fibrillation, may be induced in thyrotoxic dogs by intravenous injection of provocative doses of acetylcholine. The incidence of induced atrial fibrillation, caused by acetylcholine injection, has been increased from that of 30 per cent in normal dogs to that of 81 per cent in those made thyrotoxic.

This technic provides a suitable low cost, experimental method, easily duplicated in the same animal repeatedly, for the evaluation of "antifibrillatory" drugs.

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SUMMARIO IN INTERLINGUA

Fibrillation atrial in canes esseva producite per le administration de extracto thyroide e acetylcholina intravenose. Il esseva constatate que omne generes de arrhythmias atrial—ab simple tachycardia a continue fibrillation atrial—pote esser inducite in canes thyrotoxic per le injection intravenose de doses provocative de acetylcholina. Le frequentia de inducite fibrillation atrial que resulta del injection de acetylcholina esseva 30 pro cento in canes normal e accresceva a 81 pro cento in le canes que nos habeva rendite thyrotoxic.

Iste technica provide un commode e incostose methodo experimental, que es facile a duplicar e a repeter in le mesme animal, pro le evaluation de drogas antifibrillatori.

REFERENCE

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