Short communications

Experimentally induced automatism in rat isolated ventricle

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Exposure to isoprenaline plus electrical stimulation of the isolated right ventricle of the rat produces stable ventricular automatic activity of various types. The incidence of these different types of ventricular arrhythmia is tabulated and analysed. The technique allows the antiarrhythmic activity of drugs to be studied at cellular level where extracardiac factors are excluded.

Two methods have been commonly used for the experimental production of cardiac arrhythmias. One of them is based upon the creation of a long circuit in atrial muscle, allowing the excitation to progress undirectionally. This technique was used by Rosenblueth & García Ramos (1947) in the anaesthetized dog. The other method is based upon the establishment of ectopic foci which generate an automatism similar to atrial fibrillation. An example of this is the use of aconitine by Scherf, Schaffer & Blumenfeld (1953). Mixed procedures have also been used. Burn (1961) induced automatism in the heart-lung preparation by means of high frequency stimulation of the right atrium associated with venous infusion of acetylcholine. Atrial fibrillation in the dog has also been produced by combining electrical stimulation of the right vagus with venous infusion of catecholamines (García de Jalón, Lastra & Serrano, 1969).

A new technique for induction of reproducible and persistent automatism at the simpler level of ventricular muscle is now described using the isolated right ventricle of the rat. In this method high frequency electrical stimulation and inotropic challenge by isoprenaline are combined; this combination may be effective because the balance between the demand and supply of oxygen is severely compromised in deeper layers of the tissue.

Methods.—The right ventricle of the rat was isolated by a technique similar to that described for the guinea-pig by Stewart (1958). Sprague-Dawley rats of either sex were used. A 15 ml Allihn tube was used as the organ bath; the porous base plate had pore diameters between 3 and 5 μ to produce effective aeration. Tyrode solution of the following composition (mM) was used: NaCl 136·9, KCl 2·7, $MgCl_2$ 1·05, $CaCl_2$ 1·8, NaH_2PO_4 0·4, $NaCO_3H$ 11·9, dextrose 5·0. The solution was maintained at 37° C and bubbled with 95% O_2 and 5% CO_2. Contractions were recorded on a Beckman-Offner Polygraph using a Grass FT 03 force-displacement transducer. The resting tension of the muscle was set at 1 g. The muscle was stimulated by square-wave pulses of 5 ms duration and a voltage twice the threshold, using Grass stimulators models SD5 or S4. The experimental procedure was as follows:

Step 1: Electrical stimulation. The muscle was stimulated according to the following sequence, (i) stimulation at 0·4 Hz, (ii) 10 min rest, (iii) repeat stimulation at 0·4 Hz, (iv) change to 10 Hz for 5 s, (v) finally, 10 min rest.

Step 2: Electrical stimulation plus isoprenaline. If step 1 failed to induce stable automatic activity, then (i) the muscle was stimulated at 0·4 Hz, (ii) isoprenaline 10^{-6} M was added, (iii) when inotropic change reached a maximum, stimulation was changed to 10 Hz for 5 s, (iv) rest. Isoprenaline chlorhydrate was used.

Step 3: Drug antagonism. Stable automatic activity usually developed after step 2. Activity was recorded for 30 min before the drug to be tested for antiarrhythmic activity was added. Drug left in contact for 20 minutes.

Step 4: Electrical challenge. If a drug suppressed the automatic activity steps 1 and 2 were repeated, without washing out the drug, to test for persistence.

Results.—As shown in Table 1, a few preparations showed a degree of spontaneous activity during step 1. The high rate of stimulation alone induced automaticity in some preparations. When the ventricular muscle was exposed to the provoking factors of step 2, automaticity usually appeared which tended to persist unless treated. The ventricular rhythm

TABLE 1. *Experimentally induced automaticity in isolated ventricle of the rat*

Type of automatic activity elicited	Initially or after 0·4 Hz stimulation	During resting pause	After 10·0 Hz stimulation	After isoprenaline+ 10·0 Hz stimulation
Total No. of experiments	337	265	212	323
No automaticity	183 (54·3)	118 (44·5)	65 (37·7)	18 (5·6)
Automaticity	154 (45·7)	147 (55·5)	147 (69·3)	305 (94·4)
Irregular automaticity	90 (58·4)	87 (59·2)	81 (55·2)	45 (14·7)
Regular automaticity	34 (22·1)	26 (17·7)	20 (13·6)	70 (23·0)
Mean rate ± S.E.M.	109±11	119±18	123±20	204±14
Volleys of automatism	27 (17·5)	22 (15·0)	30 (20·3)	129 (42·3)
Mixed arrhythmia	0	6 (4·1)	10 (6·8)	23 (7·5)
Interconversion of one type into another	3 (1·9)	6 (4·1)	6 (4·1)	38 (12·4)

Percentages, in parentheses, refer to the total number of cases where automaticity appeared.

elicited in this preparation was not of a single type and interconversion sometimes occurred during an experiment. The following types of automatic activity were seen:

(a) *Volleys of automatic activity.* These consisted of discharges of automaticity presumably arising from the Purkinje system. The discharges had a maximal frequency between 300 and 400 per minute. The frequency and duration of these discharges changed continuously.

(b) *Irregular automatism.* In these arrhythmias groups of faster and slower frequencies alternated at irregular intervals.

(c) *Regular automatism.* This consisted of automatic discharges of constant frequency at rates from 95 to 263 per minute.

(d) *Mixed arrhythmias.* The automatic activity did not present a single type of automatism during a given period of time.

The distribution of the automaticity observed, along with the procedure used, is given in Table 1. In some instances, preparations very resistant to the induction of automatism were met, where no arrhythmias could be induced even when step 2 of the procedure was repeated several times.

From Table 1, it is apparent that the pattern of automatic activity, either spontaneous or induced by step 1 of the procedure, was independent of the provoking factor. In any instance, irregular automaticity appeared more frequently than any other type of arrhythmia. The pattern was reversed if isoprenaline was used when volleys of automatism and regular automaticity were the predominant forms of arrhythmia. The rate of regular automaticity was significantly higher when induced by isoprenaline plus 10·0 Hz stimulation, then when it appeared spontaneously or after 0·4 Hz stimulation ($P<0·05$ in group comparison).

Discussion.—The technique described in this paper constitutes a direct approach for studying abnormal automaticity in ventricular muscle and may help in clarifying its mechanism. Some of the disadvantages common to most techniques used to induce cardiac arrhythmias are excluded in this preparation. In the isolated right ventricle all kinds of arrhythmias produced by conduction disturbances and those that require a long perimeter to appear (Moe, Rheinholdt & Abildskov, 1964) are excluded.

The arrhythmias are probably produced by changes in the potential automaticity of the Purkinje system of the heart. To produce those changes volleys of high frequency stimuli associated with the addition of high doses of isoprenaline to the bath have been used. Both these facors act in a well-known manner. Catecholamines produce automatism by increasing the slope of phase 4 in the action potential and by diminishing the threshold (moving threshold potential closer to resting potential) according to Otsuka (1958). Similar effects may be obtained during hypoxia and when the extracellular calcium concentration is decreased. It must be remembered that high doses of adrenaline may provoke ventricular multifocal activity (Hoffman & Cranefield, 1960). When the ventricular muscle is contracting maximally under the positive inotropic effect of isoprenaline, the high frequency stimulation breaks the myo-

cardial adaptive capacity for maintaining equilibrium between oxygen supply and demand; this favours the establishment of absolute or relative hypoxic foci. Such imbalance is furthermore favoured in ventricular muscle, as compared to atrial muscle, because of the greater thickness of the muscle wall.

In summary, two possible mechanisms may be involved in the production of these experimental arrhythmias: (a) the existence of hypoxic foci in the ventricular muscle wall and (b) the influence of afterpotentials which Matsuda, Takeshi & Shigenori (1959) offered as an explanation for aconitine-induced arrhythmias.

REFERENCES

BURN, J. H. (1961). The cause of fibrillation. *Can. med. Ass. J.*, **84**, 625–632.

GARCÍA DE JALÓN, P. D., LASTRA, L. A. & SERRANO, J. S. (1969). Differential effects of isoproterenol and noradrenaline in the induction of cardiac arrhythmias during digitalization. *Abstracts Fourth Intl. Congress Pharmacol., Basel*, p. 262.

HOFFMAN, B. F. & CRANEFIELD, P. H. (1960). The electrophysiology of the heart. New York: McGraw Hill.

MATSUDA, K., TAKESHI, H. & SHIGENORI, K. (1959). Effects of aconitine on the cardiac membrane potential of the dog. *Jap. J. Physiol.*, **9**, 419–425.

MOE, G. K., RHEINHOLDT, W. C. & ABILDSKOV, J. A. (1964). A computer model of atrial fibrillation. *Am. Heart J.*, **67**, 200–220.

OTSUKA, M. (1958). Die Wirkung von Adrenalin auf Purkinje-Fasern von Säugetierherzen. *Pflügers Arch. ges. Physiol.*, **226**, 512–517, and **267**, 312.

ROSENBLUETH, A. & GARCÍA RAMOS, J. (1947). On flutter and fibrillation. *Am. Heart J.*, **33**, 677–688.

SCHERF, D., SCHAFFER, A. I. & BLUMENFELD, S. (1953). Mechanism of flutter and fibrillation. *Archs intern. Med.*, **91**, 333–352.

STEWART, G. A. (1958). The actions of digitalis leaf preparations and of cardiac glycosides on the isolated right ventricle of the guinea-pig. *J. Pharm. Pharmac.*, **10**, 741–754.

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