Effect of Enzyme Inhibitors on Acetylcholine-Induced Atrial Arrhythmias

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An investigation was made of the effects of enzyme inhibitors on the acetylcholine-induced atrial arrhythmias. It was found that an interference with normal metabolic processes abolishes the electrical activity of arrhythmic atria. Azide, cyanide, and iodoacetate were the most effective in causing the disappearance of all potentials. Arsenate, fluoride, fluoroacetate, and malonate were considerably less active, while pyrophosphate was least efficacious. It is felt that the inhibition of the Na^+ pump may play an important role in the consistent conversion from the induced arrhythmias to quiescence.

THE REPORT OF CHANG (1), in which iodoacetate was shown to inhibit aerobic as well as anaerobic activity of the isolated rabbit auricle led the way to numerous investigations of enzyme inhibitors on mechanical and electrical atrial parameters (2–10). In keeping with this, Webb and Hollander (11) observed that several enzyme inhibitors produced changes in the membrane electrical properties of rat atrial cells—one of the most characteristic is a shortening of the action potential duration. Since a connection between the amount of energy available, the duration of the action potential, the ionic movements, and the initiation of arrhythmia has been suggested (12, 13), the present work was an attempt to investigate the influence of an interference with normal metabolic processes on the acetylcholine-induced atrial arrhythmias.

EXPERIMENTAL

The methods used for the dissection of rabbit atria, the induction, and the recording of arrhythmias have been described previously (14). Two modifications in the technique need to be pointed out: (*a*) the use of a larger bath containing 150 ml. of Ringer's solution, (*b*) the atria were permitted to recover a regular rhythm in normal Ringer's solution for 90 minutes. The experiments to be described are of two types:

1. Control Acetylcholine (ACh)—induced atrial arrhythmias, the duration of which was noted on surface electrograms until spontaneous arrest.

2. A series of experiments consisting in inducing first the arrhythmias with ACh. On cessation of stimulation, a 5-minute period of arrhythmia was always recorded before the addition of the enzyme inhibitors to the bathing solution. At the end of that time, 1 ml. of their solutions (as sodium salts) was added to make the final concentrations (11) listed in Table I. A reliable criterion of the inhibitory activity was the disappearance of all potentials appearing as quiescence and observed for at least 5 minutes on the surface electrograms. Thereafter, the inhibitor was washed out and (except with iodoacetate and fluoroacetate) the tendency of atria to return to their normal rate and amplitude was usually rapid (10–20 minutes).

RESULTS

Duration of Control ACh-Induced Atrial Arrhythmias.—A summary of these studies is presented in Table I. Of 11 atria, eight developed stable arrhythmias lasting 80 minutes or more, while only three stopped spontaneously within 80 minutes. The period of fibrillation varied from 24 to 155 minutes with a mean duration of 82 minutes. This is not too surprising since the production and maintenance of atrial arrhythmias are dependent upon numerous factors (12, 13).

Enzyme Inhibitors and Atrial Arrhythmias.—The effect of these substances on the ACh-induced arrhythmias is interesting. The data presented in Table I illustrate clearly the capacity of these inhibitors to abolish the electrical activity of arrhythmic atria. It is quite obvious that a quiescence ensues after an interference with the normal metabolic processes of the atrial cells.

Furthermore, there seems to exist a certain relationship between the inhibitory potency and the time required for complete quiescence. Powerful inhibitors, such as azide, cyanide, and iodoacetate proved to be most effective since the disappearance of all potentials was very rapid. As for arsenate, fluoroacetate, fluoride, and malonate—they occupied an

TABLE I.—EFFECT OF ENZYME INHIBITORS ON THE ACh-INDUCED ATRIAL ARRHYTHMIAS

Trials, No.	Inhibitor	Mean Time Required for Complete Quiescence, min. ± S.E.
11	...	82[a] ± 10.9
10	Azide (1 m*M*)	9 ± 1.9
10	Cyanide (0.5 m*M*)	3 ± 0.2
10	Iodoacetate (1 m*M*)	11 ± 0.5
10	Arsenate (20 m*M*)	34 ± 4.6
10	Fluoride (0.25 m*M*)	28 ± 4.6
10	Fluoroacetate (4 m*M*)	32 ± 4.2
10	Malonate (15 m*M*)	38 ± 6.6
10	Pyrophosphate (1.5 m*M*)	69 ± 9.7

[a] Mean duration of control ACh (3.5×10^{-3} *M*)-induced atrial arrhythmias.

Received December 6, 1962, from the Department of Pharmacology, Faculty of Medicine, University of Montreal, Montreal, Quebec, Canada.
Accepted for publication January 29, 1963.
This investigation was supported by a grant from the Fondation Rheaume, University of Montreal.
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intermediary position, being less active than the former, but more active than pyrophosphate (the least efficacious). Apparently, the lack of activity of pyrophosphate may be associated with the fact that pyrophosphate combines with Ca^{++} and that the walls of the tissue bath slowly became covered with a white layer following its addition. It is not unlikely that the decrease of ionized Ca^{++} neutralized the inhibitory effect of pyrophosphate. The finding that a lack of Ca^{++} in the medium increases the incidence of arrhythmias which tend to be of longer duration supported this suggestion (15).

DISCUSSION

Previous investigations (16) suggested that a mechanism is present in cardiac tissue which actively delays repolarization under normal conditions; this mechanism is maintained by metabolic processes and seemingly is inactivated by ACh. Then, Burn (17) postulated that the onset of atrial fibrillation appears to be linked to the diminution in membrane resistance, to the increased permeability to K^+, and to the increased rate of loss of K^+ which ACh causes. This was extended and confirmed by subsequent works of Klein and Holland (18) and Holland and Briggs (19). They showed that the onset of atrial fibrillation is accompanied by a marked increase in the inward movement of Na^+ and K^+ leaving the tissue in exchange. More recent studies (20–22) established a net increased Cl^- influx during fibrillation that may play an important role in inactivating the marked increase in the inward movement of Na^+. The thesis was developed that maintenance of fibrillation depends upon the rate of entry of Cl^- into a thin barrier, probably the cell membrane, which is the seat of chemical processes driving Na^+ out of the cell (23). According to Woodbury (24), this process whereby the cell continuously uses metabolic energy to maintain an efflux of Na^+ is called active Na^+ transport; the detailed mechanism for utilization of metabolic energy to carry Na^+ out of the cell is not yet known. Nonetheless, Hodgkin and Keynes (25) reported very interesting findings relative to the effect of enzyme inhibitors on the active Na^+ transport. It is beyond the scope of this paper to summarize them all and we shall mention only three: (*a*) Na^+ efflux is decreased to values near zero by the addition, at appropriate concentrations, of a metabolic inhibitor to the bathing fluid. (*b*) K^+ influx is greatly reduced by metabolic inhibitors. (*c*) Na^+ influx and K^+ efflux are not greatly affected by metabolic inhibitors. These observations are well in agreement with those of Leaf (26) who concluded that inhibition of metabolism slows or stops the ion pump so that the Na^+ entering the cell by diffusion cannot be extruded and the K^+ lost from the cell cannot be replaced. The cell membrane potential diminished and Cl^- enters the cell. This requires further entry of Na^+ into the cell to preserve electric neutrality.

Since experimental evidence is increasing that the maintenance of auricular arrhythmias is associated with active ionic movements which are metabolic energy dependent and that the utilization pathways of this energy essential to the Na^+–K^+ exchange seem very sensitive to enzyme inhibitors, it is logical to assume that the inhibition of the Na^+ pump might probably be responsible for the conversion from the induced arrhythmias to quiescence. Indeed, it will appear that if an arrhythmia is to continue, the active Na^+ transport, which is an energy requiring reaction, need not be impaired. When the metabolism is inhibited, the energy supply is cut off through a reduction in the amount of energy available, resulting in a deficient Na^+ pump. The pump being deprived of its source of energy, the Na^+ efflux and the K^+ influx are greatly reduced. The Na^+ will accumulate inside and the K^+ outside the cell. The ionic desequilibrium will tend to lengthen the refractory period usually indicated by a lengthening of the action potential and to delay the return of excitability. Consequently, an atrial fiber which has contracted remains inexcitable for a long time. When excitation spreads to it from a neighboring fiber which is out of phase, the excitation has no result. Thus the arrhythmia stops and quiescence ensues.

In conclusion, it is emphasized that because of the lack of specificity of most enzyme inhibitors, the data presented here do not exclude the possibility that the inactivation of particular enzymes is involved in stopping the electrical activity of arrhythmic atria; they do suggest, however, that the inhibition of the Na^+ pump may be the main contributing factor.

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