Effects of Enzyme Inhibitors on the Contractility and Membrane Potentials of the Rat Atrium


Enzyme inhibitors alter the rate of ion movement across the membrane of the rat atrial cell during the repolarization phase. There is simultaneously no constant change in the resting potential while the action potential amplitude is usually depressed to a moderate degree. The relationship between these membrane changes and the altered contractility is investigated. It is concluded that metabolic depression reduces the contractility by two mechanisms: one is a membrane effect, the result of the shortening of the action potential duration, and the other is an effect more directly on the contractile processes.

DISTURBANCE of metabolism in rat atrium alters the contractile and membrane electric properties in a characteristic manner.1 The present study was undertaken to extend the types of metabolic depression, using a variety of enzyme inhibitors which exert their actions on different metabolic systems. A comparison of the different effects of these inhibitors may provide some insight into the relationships between specific metabolic pathways and the functional activity of the atrium. The effects that are common to all types of metabolic depression may indicate how the phenomena of ion transfer and contraction depend on the energy-supplying oxidative processes.

METHODS

The membrane potentials and mechanical activity of rat atria (electrically stimulated at a rate of 200/min. in Krebs-Ringer-bicarbonate medium at pH 7.4 and 30 C.) were determined simultaneously using microelectrodes and a strain gage as previously described.2 The following minor changes have been made in the original procedure and methods of recording. It has been found that an equilibration period of one hour instead of two hours is now satisfactory and provides stable preparations. The duration of the action potential was measured from the initiation of depolarization until the potential returns to within 20 mv. of the resting potential, eliminating fluctuations frequently present as the potential approaches the resting value. Since the depolarization time is very short, the average rate of repolarization can be calculated by dividing the amplitude of the action potential minus 20 mv. by the action potential duration. The area of the action potential was measured with a polar planimeter on an enlarged tracing of the record. That part of the total area was measured which was bounded by the rising and falling potential and a line at 20 mv. depolarization from the base line. It is assumed that the effect of depolarization on the contractile systems is negligible until the depolarization reaches a minimum of 20 mv, and the area here expressed would be one possible measure of the magnitude of the effective action potential. It is also possible that once a critical level of depolarization is achieved, further change in potential is without additional effect on the contractility, in which case the action potential duration would perhaps be a more reliable criterion of effectiveness. Solutions of the inhibitors tested were prepared in tissue medium, adjusted to pH 7.4, brought to 30 C. and saturated with the standard gas mixture of 95 per cent oxygen and 5 per cent carbon dioxide.

RESULTS

The effects of the enzyme inhibitors are summarized in tables 1 and 2. In addition, the following points not covered in the tables may be noted.

Inhibitor Concentrations. The results from only one concentration for each inhibitor are given in the tables; however, other concentrations were tested in most cases. The concentrations chosen for presentation gave definite effects within an hour; lower concentrations (e.g., 0.2 to 0.4 mM iodoacetate and iodoacetamide, 5 to 10 mM arsenate and 1 to 2 mM fluoroacetate) gave similar results but required a longer period to act. Such data are
not considered as accurate inasmuch as the possibility of spontaneous change in the atria over periods longer than one hour must be considered. Concentrations of malonate and dehydroacetate are higher than generally used but lower concentrations did not produce any significant changes. It is interesting that very similar, and equally slight, changes were produced by acetylazolamide (Diamox) at concentrations from 10^{-3} to 10^{-2} mM. Since carbonic anhydrase is inhibited by diamox at concentrations from 10^{-3} to 10^{-1} mM, these results indicate that any action observed was probably due solely to an effect on this enzyme.

Initial Stimulation of Atria. It was observed that those inhibitors generally considered to act by reaction with sulfhydryl groups, namely iodoacetate, iodoacetamide, p-chloromercuribenzoate and arsenite, evoked an initial and temporary stimulation of the contractility, frequently accompanied by an increase in the action potential duration. None of the other inhibitors produced this response.

State of the Atria at Terminal Standstill. The resting potential was not markedly altered by most inhibitors up to the time when contractions ceased (azide and fluoroacetate being the exceptions), but the action potential was both depressed in magnitude and shortened. The resting potential after contractile failure generally proceeded to fall slowly. For example, with iodoacetate and iodoacetamide, the resting potential fell steadily to values around 5 mv. an hour after contractile failure; in the case of p-chloromercuribenzoate the fall was slower, reaching 20 mv. at the end of an hour. It is interesting to note that with fluoroacetate, although the resting potential was slightly depressed when contraction ceased, the potential remained at approximately this level for the next two hours. Such results probably reflect the degree to which the various inhibitors suppress the metabolic systems responsible for the operation of the ion pump, which normally maintains the relatively high intracellular K^+ concentration. The atria were not stopped...
by the concentrations of cyanide, azide, malonate, dehydroacetate, acetazolamide or arsenate used here.

Occurrence of Contracture. The most marked and rapidly developing contracture occurred with iodoacetate and iodoacetamide. The resting tension, however, did not begin to rise until contractions ceased, and then rose rather suddenly to reach maximal contracture around 15 to 20 min. Arsenite acted similarly but required twice as long for contracture to develop and the degree of contracture was less. No contracture was noticed with p-chloromercuribenzoate even two hours after contractile failure. In most cases, no contracture was observed after fluoroacetate, but in one case it developed from 25 min. after contractile failure and progressed over the next 85 min. However, this may not have been due to the direct action of the inhibitor but to secondary changes. No changes in resting tension were produced by the other inhibitors at any time.

Reversibility of Inhibition. Upon removing the inhibitor and washing the atria in fresh medium, rapid and complete recovery was observed after cyanide, azide and malonate; no evidence of reversibility could be obtained for the other inhibitors, and if these inhibitors were removed before the depression was complete, the inhibition continued in the same manner as if the inhibitor were present.

Effect on Depolarization Rate. The depolarization rate was not accurately measured in most of this study, but it was evident that no marked slowing occurred with any of the inhibitors. Changes in depolarization rate have been accurately measured for cyanide and dinitrophenol and only very slight slowing was found (less than 10 per cent). Thus, any changes recorded for action potential duration were due to changes in the repolarization rate.

Effect of Na+ Introduced with Certain Inhibitors. In the case of arsenate, dehydroacetate and malonate, it was necessary to increase simultaneously the Na+ concentration of the medium by 10 to 20 mM. It was observed that the addition of 15 mM NaCl did not produce significant changes in the resting or action potentials, but decreased the action potential duration and developed tension by 7 to 10 per cent. Thus the effects recorded for malonate may be attributed mainly to this factor. Such ionic effects occur rapidly and are easily separated from actions due to an inhibitory anion. Reduction of free Ca++ in the medium by these inhibitors would not

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<th>Table 2.—Effects of Enzyme Inhibitors on Rat Atrium</th>
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<td>Percentage changes</td>
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<td>1'-10'</td>
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<td>Resting potential</td>
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<td>Overshoot* (mv.)</td>
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<td>Penetrations, control</td>
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<td>Number of atria</td>
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*The change in overshoot is given in millivolts.
seem to have contributed significantly to the effects observed since low Ca\textsuperscript{++} produced a different pattern of response.

**DISCUSSION**

The inhibitors which did not produce marked or typical atrial depression were malonate and diamox. In the former case, since disturbance of the tricarboxylic acid cycle produced marked changes, as evident with fluoroacetate, it is likely that the ineffectiveness of malonate may be correlated with the probably low intracellular concentration at equilibrium and the failure to compete adequately with the levels of succinate in the tissue. Similar ineffectiveness of malonate on cardiac preparations at a pH around 7.4 has been frequently observed.\textsuperscript{3-5} The lack of a marked effect by acetazolamide over a wide concentration range would imply that carbonic anhydrase plays a minor or unessential role in atrial function, and that this enzyme certainly does not actively participate in the ionic transfers occurring during impulse conduction. The small changes recorded in the action potential and the developed tension are believed to be real, inasmuch as they occurred in each of the 4 experiments and were progressive in time.

We shall discuss the remaining inhibitors first with respect to the responses they induce in common and then consider the differences between them. The general pattern of effect was quite similar to that produced by anoxia, substrate depletion and dinitrophenol.\textsuperscript{1} This consists of a progressive depression of contractile activity and associated with this a marked decrease in the duration of the action potential (or increment in the repolarization rate), a moderate decrease in the amplitude of the action potential leading to reduction or disappearance of the overshoot (since the resting potential is relatively less or not at all affected), a slowing of the rate of conduction and an increase in the latent period. In no case was contractility altered without definite changes occurring in the membrane electric behavior. Similar changes have been reported for certain enzyme inhibitors in other cardiac preparations. Goldenberg and Rothberger\textsuperscript{6} applied 1 to 2.5 mM arsenite to dog's Purkinje fibers and examination of their records indicates that the action potential was shortened and reduced. Azide has been shown to reduce the duration of the action potential plateau in frog heart.\textsuperscript{7} The action of iodoacetate on the frog heart seems to be similar to that on rat atrium,\textsuperscript{8} the most consistent change being a shortening of the action potential with decrease in stroke volume, the resting potential remaining relatively unaffected. It is probable that one major control of cardiac contractility is the course of depolarization and repolarization preceding contraction and hence the rates at which ion transfer across the membrane occurs. One might consider the effects on the membrane and the contractile systems to be separate and coincidental, but the results with substances such as acetylcholine and epinephrine,\textsuperscript{0} which do not directly alter metabolism or contractile elements, would indicate the close connection between the form of the action potential and the degree to which the contractile elements respond. However, it is evident that in certain cases there is an action in addition to the membrane effect, perhaps on metabolic systems upon which contraction directly depends or on the contractile elements themselves.

In order to estimate the relative magnitude of this non-membrane effect, the changes in developed tension were plotted against the changes in action potential duration as produced by agents believed to act only on the membrane and by the enzyme inhibitors (fig. 1). The curve is drawn through points representing the effects of 3 types of substance: (1) acetylcholine and carbachol, (2) adenosine, the adenine nucleotides and related compounds,\textsuperscript{10} and (3) sodium chloride. These substances are assumed to act only on the membrane and the curve would then show the depression in contractility resulting from a shortening of the action potential. The points lying within the designated area represent instances of metabolic depression induced by enzyme inhibitors, anoxia, and substrate depletion.\textsuperscript{1} It is important to note that the
points from every type of metabolic depression investigated fell within this area, with the exception of dehydroacetate. Under the assumptions made, the height of each point above the curve would indicate the contribution of the nonmembrane effect to the depression of contractility. It would appear from this that all types of metabolic depression exert an effect on contractility that involves more than a reduction in the action potential duration, and with some, such as arsenite, this is a major contribution to their mechanism.

Due to the possible importance of the action potential duration in the control of contractility, it is pertinent to inquire into the mechanisms whereby inhibition of metabolism accelerates the repolarization. Inasmuch as a more rapid rate of repolarization implies a greater rate of ion transfer across the membrane during this period, this could be attributed to an increased K+ permeability, based on the maintenance of normal membrane structure and permeability through the energy obtained from metabolism. It was suggested previously that depletion of charged molecules, such as adenosinetriphosphate, bound within the membrane could alter ionic permeability during reduction of metabolism. It is evident that energy-requiring ion pumps are not involved directly in the ion transfer during repolarization since depression of the energy supply would only slow this process. However, another possible mechanism is derivable from the plausible scheme outlined by Cranefield and Hoffman, whereby the efflux of K+ during the earlier phase of repolarization leads to accumulation of K+ in the region outside the membrane and this increasing K+ concentration is responsible for the later rapid K+ efflux and the return to the resting potential. This effect of heightened K+ concentration on repolarization was demonstrated experimentally by Weidmann. If one assumes that the outwardly-directed Na+ pump is activated following depolarization and that for each Na+ extruded a K+ is brought into the cell, this pump would slow down the efflux of K+ and so delay the accumulation of K+ outside the membrane. This would delay the repolarization process. Inhibition of this active pump by metabolic depression would favor an increased K+ efflux and a more rapid repolarization rate. It is impossible to estimate how much delay such a pump would induce in the K+ efflux because the rate at which the pump operates immediately following depolarization is unknown. At the present, a decision between these two mechanisms cannot be made.

It is possible that these effects, common to all metabolic depression, are the result of interference with the supply of energy in the form of high energy phosphate compounds, that is, that every depression of oxidative reactions or an uncoupling of phosphorylation would result in the pattern of response discussed above. In the case of inhibitors, this might be called the nonspecific part of their

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**Fig. 1.** Relationship between shortening of action potential duration and depression of contractility. The curve is drawn from points (X) representing the action of substances assumed to act only on the membrane (see text). The points (•) enclosed within the designated oval area represent various types of metabolic depression as follows: 1, 2, 3, increasing degrees of anoxia; 4, 5, increasing degrees of substrate depletions; 6, dinitrophenol; 7, iodoacetate; 8, iodoacetamide; 9, 10, increasing effects of p-chloromercuribenzoate; 11, 12, increasing effects of arsenite; 13, cyanide; 14, azide; 15, 16, increasing effects of arsenate; 17, 18, 19, increasing effects of fluoracetate. DT = developed tension and AP-D = action potential duration.
action. Since there are differences in action between the various enzyme inhibitors, one is led to postulate that, in addition to the above, there are what one might call specific effects. Within the group of sulfhydryl inhibitors there were definite and reproducible differences in the progression of the effects or in the relative degrees to which the different atrial characteristics were altered. Interesting differences were observed between iodoacetate and iodoacetamide: the latter gave a more pronounced and longer initial period of stimulation and produced a more sudden failure, while the former led to the characteristic depression much earlier. This is surprising inasmuch as iodoacetamide is generally considered to penetrate into cells more readily than iodoacetate. A possible origin of this difference might lie in the fact that iodoacetate imposes a negatively charged group on the protein with which it reacts, whereas iodoacetamide is neutral. Reactions in the membrane could alter ionic movements differently. There was the tendency of all sulfhydryl inhibitors to raise the resting potential at some time during their action, although this effect was erratic. This action on the resting potential could have been due to a direct action on membrane components to alter ionic permeability. In fact, it is impossible to conclude that any specific effects of these sulfhydryl agents were the result of enzyme inhibition, since reaction with proteins involved in cell function is possible. On the contrary, fluoroacetate induced no stimulation at any time, and since this inhibitor probably acts quite specifically to block the tricarboxylic acid cycle, the results may be interpreted in terms of this action only. It is not implied that the action here is only on the mitochondria, for it is possible that the cycle reactions also occur in the membrane or adjacent to it. Dehydroacetate was the only inhibitor that slowed the repolarization while it depressed the contractility. This might be taken as evidence that it exerted an action other than interference with oxidative reactions in the tricarboxylic acid cycle.13

The resistance of the resting potential to reduction by metabolic inhibition is noteworthy and indicates that most enzyme inhibitors depressed the atria and stopped them before sufficient lowering of the resting potential had occurred to alter function. The membrane activity and contractility failed before the normal ionic distribution between medium and cell interior was significantly altered. The contractile failure was brought about mainly, if not entirely, by a combination of two effects: a disturbance in the ionic movements across the membrane during activity, especially at the repolarization phase, and a depression of contractile ability, probably by reduction in the supply of energy necessary for the cyclical operation of the elements involved in contraction. The question arises as to whether the contracture that occurred with certain inhibitors was related to the gradual fall in the resting potential subsequent to failure of contractile mechanism. This would appear not to be the case, since in some instances the resting potential fell without contracture occurring, and in others contracture developed fairly rapidly while the resting potential was not changing markedly. The lack of relationship between membrane depolarization and contracture is substantiated by the fact that depolarization by high K+ does not lead to contracture.

**Summary**

Several enzyme inhibitors produced changes in the membrane electrical properties of rat atrial cells. The most marked action was an acceleration of repolarization, leading to a shortening of the action potential duration. It is believed that this was responsible for part of the observed alteration in contractility. There was also a more direct effect on contraction apparently unassociated with action on the membrane. A provisional method for estimating the relative contributions of these two effects is given. A theory was formulated for the mechanism by which metabolic depression induces a shortening of the action potential duration.
SUMMARIO IN INTERLINGUA

Plure inhibitores de enzyma produceva alterationes in le proprietates electric membranal de cellulas atrial in rattos. Le plus marcate effecto esseva un accelerate repolarisation con le consequentia de un reducete duration del potential de action. Es opinate que isto esseva responsabile in parte pro le observate alteration del contractilitate. Esseva etiam constatate un plus directe effecto super le contraction, apparentemente non associate con un action super le membrana. Es presentate un metodo provisori pro le estimation del contributiones relative de iste duo effectos. Esseva formulate un theoria concernente le mechanismo per le qual un depression metabolic induce un reduction del duration del potential de action.

REFERENCES

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