DRUGS AND HEART MUSCLE

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Some inconsistencies exist in the literature pertinent to the action of drugs on heart muscle, in regard to the question of how the action on myocardial contractility should be measured in order to get the appropriate information for an adequate evaluation and classification of an inotropic drug. The reason for this derives mainly from the fact that the mechanical properties of heart muscle are more complicated than those of skeletal muscle and that concepts and methods originally developed for the study of skeletal muscle have been transferred more or less uncritically during the last two decades to the work on heart muscle, giving rise partly to misleading conclusions. The differences in mechanical properties need to be recalled here only as far as necessary for the topical discussion, since they have been described elaborately and expertly in recent reviews (1–3): (*a*) only an isometric twitch tension and no tetanus tension can be obtained with heart muscle, which prevents the measurement of its maximum force by tetanic stimulation; (*b*) high resting tensions develop in heart muscle after relatively short extensions of its length, indicating the relative importance of a parallel elastic element in heart muscle in contrast to skeletal muscle; (*c*) whereas in skeletal muscle full contractile activity exists early in contraction, there is a relatively slow onset in contractility in cardiac muscle. Measurements of the time course of the cardiac active state at constant length of the contractile element by controlled stretch have shown the active state rising somewhat ahead of isometric tension in the unstretched response and reaching a slightly earlier maximum (1); (*d*) there is a clear distinction between the influence on the isometric tension curve of skeletal and heart muscle by several inotropically acting agents, such as epinephrine (4, 5) and different anions (6, 7).

Since the intention of the pharmacological study of an inotropic intervention is to explain, if possible, its cellular mechanism, it is understandable that different experimental efforts have been undertaken in trying to analyze various mechanical factors involved in the inotropic change. It seems to be necessary to compare the different approaches to find out which of them procures the most relevant information and appears to be most feasible. It then will be evident that an analysis of the change in mechanical properties alone will not be sufficient for the evaluation of an inotropic drug because the possibilities of the heart muscle to develop an inotropic response by a distinguishable application of its mechanical means are extremely lim-

ited. An increase in force of contraction, for instance, can be achieved only by an acceleration of the force development or by a prolongation of the duration of the activity of the muscle (8).

Different Approaches for the Measurement of the Inotropic Response

Type of tissue.—In choosing between atrial and ventricular muscle one has to keep in mind that the former differs from the latter not only in the shorter duration of its contraction cycle (9) and action potential (10) but also in the biphasic dependence of its contractility upon the frequency of contractions (9) and in the response to some drugs (11). The papillary muscles of the rabbit, cat, or guinea pig have proved to be suitable specimens of ventricular muscle for quantitative mechanical measurements, provided they are carefully selected in regard to their shape and diameter (12, 13). They require an appropriate mounting that keeps stray compliance to a minimum (14, 15) and a stimulation device that prevents the liberation of stored catecholamines (2, 16). Mostly, work on papillary muscles will be reviewed in this article.

Inotropic effect and the "intensity of the active-state."—A positive inotropic effect is, by definition, one that increases force development in the isometric twitch and enlarges the amount of shortening in the isotonic contraction. The effect is to be measured in the latter case by the change in length (ΔL) and in the former by the increase in isometric force (ΔF_0). According to the terminology, as used by A. V. Hill (17), the "intensity of the active-state" in a twitch is defined as the force a muscle can bear after a quick stretch, i.e. when the contractile element (CE) is prevented from shortening. Therefore, both designations "positive inotropic effect" and "increase in active-state intensity" should have the same meaning in describing an inotropic intervention. It is irrelevant in this connection that the CE is not actually constant in its length during isometric contraction, despite constancy in muscle length. An internal shortening of CE between 4 and 12% of the initial muscle length has been reported as taking place together with a corresponding extension of the series elastic element (18–20). The isometric force of the heart muscle clearly always reflects the force of its CE at constant length, and the difference between both, corrected for internal shortening, is relatively small, as shown by Brady in experiments in which he kept CE length constant by appropriate stretch (1). Therefore, it should be evident that the term "active-state intensity," at least in its original meaning, does not add to an understanding of the mechanism of the inotropic effect, whether it is used in describing an increase in isometric twitch or a shift in the maximal force value of the force-velocity relation curve as derived either from a series of afterloaded isotonic contractions or by the quick release technique.

Isotonic force-velocity curves.—In isotonic experiments with increasing

afterloads it was found that some inotropic interventions (epinephrine, higher frequency of contraction, calcium, cardioactive glycosides, nitroglycerin) cause a parallel shift of the force-velocity curve (18, 21–23) and others do not. With an extension of the muscle length, for instance, which according to the length-force relationship leads to an increase in maximal force, the inotropic effect was not accompanied by an increase in the maximal velocity value (Vmax), the intersection on the velocity axis at zero muscle force (neither afterload nor preload) of the extrapolated force-velocity curve. The extrapolated force-velocity curves derived from experiments with different initial muscle lengths merged into a common Vmax value on the ordinate. It was thought, therefore, that changes in Vmax, as determined by muscle force-velocity curves, would help to characterize an inotropic intervention (21). However, this conception has been strongly criticized on theoretical and methodical grounds by Jewell & Blinks (2) and by Pollack (24). It has been pointed out that the muscle force-velocity relation is only equivalent to the force-velocity relation of the contractile element (CE) when the two-element model (consisting only of a contractile and a series elastic element) is adopted, but not with a three-element model, including a parallel elastic element (PE), which is the minimal configuration necessary to represent most phenomena in heart muscle (24a). In considering the PE, which carries the muscle force under a preload before isotonic shortening begins, it can be deduced that the force of the CE at the onset of isotonic shortening is zero under preloaded conditions, when the afterload is zero. Therefore, the shortening of the muscle against the preload alone occurs at zero CE force. In plotting the velocity of the CE versus the force of the CE, the velocity values obtained from a muscle under different preloads (different initial muscle lengths, mainly below the optimal length, Lmax) intersect the velocity ordinate at different points, yielding different values of CE Vmax (24). A comparison with other inotropic interventions showed that the percent shifts in CE Vmax due to change in fiber length and due to positive inotropic substances are very similar (24).

Another argument against the significance of Vmax as obtained from afterloaded isotonic contractions is the influence of the time course of the activity of the CE on the shape of the force-velocity curve. It must be remembered that the force-velocity curve is characterized by data points derived from a wide spectrum of the contractile cycle since, as the afterload is increased, it is lifted progressively later after excitation (21). An alteration of the time course alone of the activity of the CE by an inotropic intervention must have its influence upon the curve and, accordingly, also on the extrapolated value of Vmax. Thus, muscle Vmax measured in afterloaded isotonic experiments appears to be a product of the particular experimental conditions rather than a general property of cardiac muscle. It should be evident that, as has been emphasized before by Jewell & Blinks (2), the study of the muscle force-velocity curve by isotonic afterloaded contractions is not a useful method for the investigation of the effects of drugs on heart muscle.

Quick-release force-velocity curves.—Many of the problems connected with the afterloaded isotonic contractions can be eliminated by using the quick release technique (25, 26) for the determination of the force-velocity curve. With this technique the muscle contracts isometrically until a predetermined time during the contraction period when the force on the muscle is suddenly released to a lower force for the remainder of the contraction. There is a sudden shortening at the time of release as a result of the recoil of the stretched series elastic element and then a slower shortening as the contractile element shortens isotonically against the new force. The force to which the muscle is released is varied in order to obtain the force-velocity curve. This method allows the determination of the force-velocity curve at a constant intensity of the active-state when the time of release is kept constant. Likewise it is to be assumed that length of contractile element is the same for all points of the force-velocity curve.

Two inotropic interventions have been studied with this method: the effects of epinephrine by Sonnenblick (27) and of an increase in muscle length by Noble et al (28). The quick-release force-velocity curves for four lengths in one muscle showed no tendency to merge at the velocity axis. Rather, muscle Vmax appeared more sensitive to changes in muscle length than peak isometric force. There was no fundamental difference between the results obtained with increasing muscle length and the addition of epinephrine. Therefore, it seems that also by quick-release force-velocity curves it is not possible to characterize different inotropic interventions.

The inverse relation between force and velocity in skeletal muscle is characterized by a hyperbolic curve described by Hill's equation (29). The force-velocity curves of cardiac muscle so far obtained by the quick-release method in four different laboratories (20, 27, 28, 30) are not uniformly hyperbolic. Sonnenblick (27) as well as Edman & Nilsson (20) found hyperbolic quick-release force-velocity curves, but those of Brady (30) are not hyperbolic. Noble et al (28) found hyperbolic curves only at muscle length near Lmax (the optimal length of muscle in regard to force development) but their force-velocity relationships were not hyperbolic at muscle length appreciably below Lmax. Since Sonnenblick (27) as well as Edman & Nilsson (20) did not indicate whether their muscle lengths were near Lmax, it is not possible to decide whether there is a discrepancy between the different results. Anyhow, the finding that in cardiac muscle, in contrast to skeletal muscle, force-velocity curves in a certain range of muscle length are not hyperbolic makes it doubtful whether Hill's equation does apply to heart muscle (28).

In summarizing the different investigations on the influence of inotropic interventions on the force-velocity relation of heart muscle it can be said that the concept of Vmax as a determinant for the differentiation of inotropic influences on heart muscle could not be proved as being well founded.

Velocity active-state curves.—One principal deduction of the foregoing discussion is the importance of the time course of the activity of the heart

muscle as influenced by inotropic interventions. This has been studied specifically by determining the velocity of isotonic shortening after releasing the muscle from an isometric contraction to the same (small) load at various times after stimulation (27, 30). The post-release velocities give a measure of the time dependence of the shortening capacity of the muscle. With this procedure it was regularly found that the shortening activity in the papillary muscle does not reach its maximum until shortly before the peak of an isometric contraction and that it then declines slightly ahead of the relaxation without an apparent plateau of maximum activity, thereby reflecting the course of the isometric contraction curve.

The time course of the active-state has been determined with this method from papillary muscles under the influence of an increase in frequency of contractions (31, 32), a change in temperature (27), and the two different inotropic agents norepinephrine (27) and strophanthidin (33). The comparison of these records with the corresponding isometric tension curves again shows a striking parallelism with the time course of the isometric contraction. However, in comparing the post-release velocity curves with those of isometric contractions, an important difference becomes evident in regard to the influence of the several inotropic interventions on the absolute values of the two different parameters, the velocity of the post-release shortening and the force developed in the isometric contraction. While there is an appreciable increase in force development in response to all of the mentioned inotropic interventions, an increase in the velocity of shortening occurs only in those that increase also the rate of force development, such as administration of norepinephrine, of the cardioactive steroids, and a higher frequency of contractions. The shape of the velocity active-state curves of these interventions virtually reflects that of the isometric curves even in the increase of the peak height. But, with a lowering of the temperature, which leads to a pronounced increase in muscle force (34–37), the velocity of shortening was not enhanced and the peak of the active-state curve as determined by post-release velocities therefore, did not increase (27).

This difference points to the limitation of the importance of the results obtained by this method. Since the time dependence of the shortening capacity of the muscle is determined, it does not seem to be justified to make any statements in regard to the "intensity of the active-state" (27, 32) which, as may be recalled, was defined by Hill as the force development at constant contractile element length. The information that can be derived from the velocity active-state curves, therefore, is substantially less than that of the isometric contraction curve which, in the case of the inotropic effect of the lower temperature, indicates not only the increase in force, i.e. the increase in the "intensity of the active-state," but also the prolongation of the duration of the activity of the muscle with relatively little change in the velocity of force development (Fig. 1c).

Force-redevelopment active-state curves.—In another approach to the determination of the time course of the activity, Reichel & Bleichert (38,

39) measured the force redevelopment, instead of the shortening velocity of the muscle after a quick release, at various times during the twitch involving a length change only of the order of a few percent of the initial muscle length. The inotropic effect of an increase in contraction frequency has been investigated with this procedure (40). The results show an increase in the intensity of the active-state and a faster decline in its time course at the higher frequency as paralleled by the course of the isometric contraction curve. The results differ from those obtained with the quick release velocity curves in regard to an earlier appearance of maximum contractility during the contraction cycle. Since Brady had found that a reduction (30) as well as an increase (41) of stress in the muscle reduces contractility after the first half of the rising phase of force development, this difference might reflect a particular sensitivity of recovery tension to uncoupling by release displacement.

The uncoupling effect is, in principle, also an argument against the quick release velocity curves and makes it doubtful whether the time course of the active-state can be accurately defined either by the level of force redevelopment or by isotonic velocity measurements following quick releases.

Contractile element clamp.—A precise measurement of active-state time course and intensity should be best approximated in the sense of Hill's original formulation, by applying a controlled stretch to the muscle throughout the twitch that maintains the contractile element at constant length. Such a method, as devised by Brady (1, 42), which in effect applies a negative compliance to the muscle during contraction, utilizes measurements of total muscle elasticity and a continuous feedback arrangement of muscle length and tension. The method, which certainly is somewhat elaborate, has not been used so far for the investigation of an inotropic intervention. But the results obtained in regard to the time course of the active-state of the uninfluenced cardiac muscle are relevant to the problem of selecting from the more simple methods the most informative one for the study of inotropic interventions. It was found (1) that the active-state curve of heart muscle is surprisingly well represented by the isometric force curve corrected for internal shortening (along the ascending limb of the Frank-Starling relation). Therefore the isometric contraction itself seems to be a reliable approximation of the time course and intensity of the active-state in heart muscle.

The Isometric Contraction Curve

At present, the evaluation of the isometric contraction curve appears to be the most practicable and also the most informative method for the study of the influence on the mechanical events in heart muscle of an inotropic intervention. The isometric contraction curve reveals whether the increase in force of contraction (the inotropic effect) is the consequence of an acceleration of force development at constant, or even reduced contraction time or is the result, either partly or totally, of an increase in duration of the

time during which the muscle develops force. The increase in velocity of force development presents itself in an increase of the steepness of the isometric contraction curve. It may be recalled that this effect has long ago been termed *klinotropic*. Since the acceleration of the force development indicates an increase in the velocity of enzymatic reactions responsible for the contraction process, the positive klinotropic effect should be the result of an increase of the internal enzymatic activity of the muscle.

An evaluation of the isometric contraction curve comprises the parameters force, contraction time, and velocity of contraction, as well as velocity of relaxation, for which the following symbols will be used:

F_M = muscle force
F_C = force of contraction
F_R = resting force
L_M = length of muscle
t_1 = time to peak force
t_2 = relaxation time
$t_1 + t_2$ = total contraction time

$\left(\frac{dF_C}{dt_1}\right)_{max}$ = maximal velocity of force development

$\left(\frac{dF_C}{dt_2}\right)_{max}$ = maximal velocity of relaxation

$S_1 = \frac{F_C}{t_1}$ = mean velocity of force development (mean steepness of the ascending limb of the contraction curve)

$S_2 = \frac{F_C}{t_2}$ = mean velocity of relaxation (mean steepness of the descending limb of the contraction curve)

ΔF_C = inotropic effect
ΔS_1 = klinotropic effect

F_C is obtained by subtraction of F_R from total muscle force ($F_C = F_M - F_R$), and the mean steepness of the contraction curve (S_1) by dividing F_C through t_1. It is questionable whether by the determination of the maximal velocity of force development $(d\,F_C/d\,t_1)_{max}$ much is gained in comparison to S_1 since under most circumstances S_1 is directly related to $(d\,F_C/d\,t_1)_{max}$. The situation would be different if by a particular intervention the isometric contraction curve would reach a plateau. In Figure 1 examples are shown for different influences on the isometric contraction curve as caused by an increase in frequency of contraction (*a*), an increase in length of muscle by appropriate stretch (*b*), and a decrease in temperature (*c*). From the superimposed contraction curves it is evident that only in the case of the increased contraction frequency the inotropic effect is solely brought about by a positive klinotropic effect, i.e., by an increase in S_1 whereas the increase of F_C by stretch is at

least partly and that by a decrease in temperature almost exclusively caused by a prolongation of t_1.

Length-force relationship and plasticity.—Both force of contraction (F_C) and resting force (F_R) of the heart muscle depend on muscle length (L_M) in a characteristic manner (18, 21, 41) known as the Frank-Starling relationship (see legend to Fig. 1b). F_C increases with increasing L_M up to a maximal value and declines with a further stretch of the muscle. Likewise, the absolute amount of an inotropic effect (ΔF_C) varies (21) with a change in L_M. For the quantitative measurement of the effect of an inotropic drug and for the purpose of comparison, therefore, the role of L_M for cardiac contractility has to be considered. The situation is complicated by the fact that an account of the plasticity of the heart muscle (3, 18, 43) the value of F_R, as obtained by a certain amount of stretch, slowly declines if the muscle is kept under isometric conditions over a longer period of time. Together with F_R also F_C declines since the whole length-force relationship is shifted to the right towards higher values of L_M. This makes it evident that, in order to secure the same control value of contractility over the length of an experiment, i.e. the same point on the length-force relationship, one has to keep F_R constant (3, 15), and not L_M (44), by compensating for the plasticity through an adequate increase of muscle stretch.

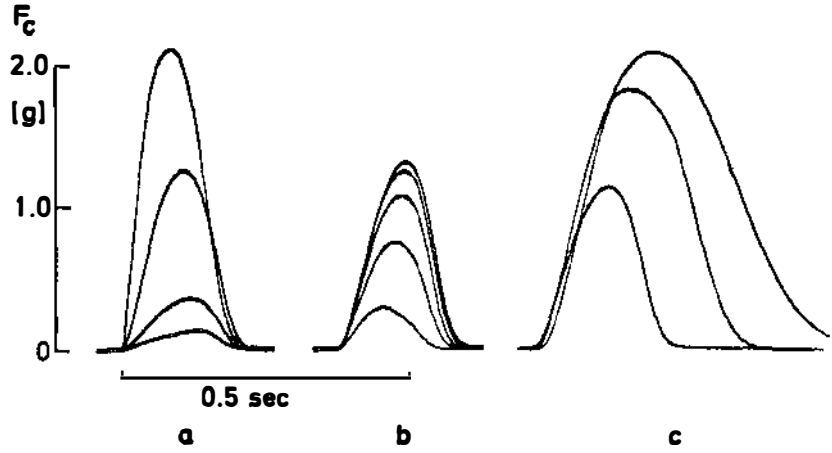

FIGURE 1. Isometric contraction curves of a guinea-pig papillary muscle. (*a*) Dependence on frequency of contractions. Increasing force of contraction with increasing frequency, from below: 0.25/sec, 0.5/sec, 1.0/sec, 2.0/sec. (*b*) Effect of increase in muscle length. Increase in muscle length from the lowest to the second curve 0.22 mm, to the third 0.16 mm, to the fourth 0.16 mm and to the fifth 0.07 mm. The corresponding values of resting force (F_R) are: 0.05, 0.1, 0.2, 0.4, 0.8 g. (*c*) Influence of temperature. From below: 35°, 30°, 25°C. Net-weight of papillary muscle 1.88 mg, length at 0.4 g F_R 4.5 mm, diameter 0.73 mm, temperature 35°C unless otherwise stated. F_R 0.4 g in *a* and *c*, contraction frequency 1.0/sec in *b* and *c*. Krebs-Henseleit-solution containing 140 mM Na^+ and 3.2 mM Ca^{2+}.

Methodological factors influencing the inotropic effect (ΔF_σ).—In order to get a useful concentration-response curve of an inotropic drug the muscle should contract under conditions that allow a sufficient increase of F_σ over the control value. This is not the case if F_σ is already at or near its maximal value as at a high frequency of contraction, a high extracellular calcium, or a low sodium or potassium concentration. The same applies to temperatures considerably below the physiological range where either negligible or no inotropic effects of cardioactive steroids have been found (45, 46) unless extracellular calcium (47), or frequency of contractions (45) were reduced in order to lower F_σ. The analog holds for the influence of an inotropic intervention on the time to peak force (t_1). At low temperatures, at which t_1 is already markedly prolonged, the influence of increasing L_M on the duration of t_1 is obviously concealed. This explains the different statements in the literature regarding the dependence of t_1 on L_M. Whereas in an experiment at 23°C of Sonnenblick (21), t_1 was not prolonged with an increase in muscle length, other authors (8, 48–51), working at temperatures near the physiological range, regularly found such a prolongation (see Fig. 1b).

Shortening of the time to peak force (t_1).—It is not possible yet to give a complete survey on the influence of the different inotropically acting agents on the time course of the active-state, since the pharmacological reports on a considerable number of inotropic drugs do not present isometric contraction curves. Even with such therapeutically important substances as the catecholamines and the cardioactive steroids a systematic comparison of their influence on the time course of the isometric contraction over a greater range of concentrations is still missing. Although agents of both groups act inotropically in a similar way and like an increase in calcium concentration, i.e. by an acceleration of force development at an almost unchanged or shortened time to peak force (5, 15, 52, 53) minor differences in regard to t_1, t_2 and S_2 can be noticed. On the papillary muscle at physiological temperature the shortening effect upon t_1 by cardioactive steroids is prominent (15, 54) whereas catecholamines hasten relaxation (S_2) which leads to a shortening of t_2 (55). In order to investigate this point more precisely, experiments should be made under strictly identical conditions to avoid erroneous results. In a recent publication (33) it was stated that norepinephrine shortens t_1 considerably more than does strophanthidin, referring to isometric contraction curves obtained from cat papillary muscles at 27°. Not only was the statement based on one single concentration of each substance, but also the contraction frequency in the experiment with norepinephrine was almost twice as high as that with strophanthidin. In papillary muscles, an increase in contraction frequency has—over a wide range—the same mechanical effect as a proportional increase in calcium concentration (56). Detailed studies of the influence of five different concentrations of a cardioactive steroid on the isometric contraction curve at different extracellular calcium concentrations on the guinea pig papillary muscle at 25°C had shown (57)

that the shortening effect on t_1 of the glycoside decreases with increasing $[Ca^{2+}]_e$. A comparison of the amount of shortening of t_1 by dihydro-ouabain (57) and adrenalin (58) at nearly equal, high calcium (7, 2 and 9.6 mM, resp.) yields quantitatively similar results for this low temperature.

Aftercontractions.—It is the shortening of t_1 under the influence of cardioactive steroids (54, 57, 59) and catecholamines (58) which, if excessive, is intimately connected with the appearance of aftercontractions: damped mechanical oscillations after the complete electrical repolarization of the muscle. The shortening for itself of t_1 cannot be the cause of the aftercontractions, since a strong reduction of t_1 alone, which can be obtained by an abbreviation of the action potential through electrical current, does not produce such an effect (60). The development of aftercontractions predominantly depends on the degree of the inotropic intervention which has to be beyond, or at least near, the maximum of the concentration (or intervention)-response relationship under the prevailing experimental condition. This is the case not only for cardioactive glycosides (57) and catecholamines (58) but also for calcium (36) and the increase in contraction frequency (56). It is consistent with this view that not only the frequency but also the number of preceding contractions influence the height (57, 61) and the wave length (62, 63) of the aftercontractions. It has been said that aftercontractions are a pharmacological curiosity that may provide a key to a better understanding of the physiology of cardiac muscle (64). Since it has been reported from active-state measurement by controlled stretch that the response during a normal contraction period is not smooth but tends to become oscillatory, both in the late rising phase and early falling phase of the active-state (1), it seems indeed possible that the "pharmacological curiosity" could serve as a useful tool for the study of the mechanical events in heart muscle.

Prolongation of the time to peak force (t_1).—Relatively few inotropic interventions lead to a prolongation of the time to peak force in heart muscle. Besides a change in temperature or in L_M, an increase in osmolarity (8, 65) and the addition of 5 mM fluoride (7) can have such an effect. Substitution of strontium in the extracellular fluid for calcium leads to a marked prolongation of t_1 (8, 66–68). Sr^{2+} apparently can replace Ca^{2+} as a coupling agent somewhat incompletely since, on a molar basis, the influence on the velocity of force development is considerably less. However, with an increase of $[Sr^{2+}]_e$ the force of contraction is augmented by an increase in S_1 and the time to peak force shortens (8, 68). A substitution of chloride in the suspension medium by bromide or iodide which, in skeletal muscle, leads to a strong prolongation of t_1 (6), in heart muscle produces a marked increase of F_0 exclusively by a klinotropic effect (7). The substitution by nitrate has, in heart muscle, only a very transient inotropic effect which, in contrast to skeletal muscle, is also caused by an increase in S_1 (7). There are two recent reports on a prolongation of t_1 in addition to an increase in the maximum rate

of force development by methylxanthines, one referring to caffeine (69), in accordance with earlier observations on guinea-pig atria (69a), and the other to theophylline (70). It was found that theophylline partly acts indirectly by releasing stored catecholamines, the prolongation of t_1 being caused by the direct effect of the substance on the muscle.

Slowing of relaxation.—Tyramine which in concentrations between 10^{-6} M and 10^{-4}M increases F_c as an indirectly acting sympathomimetic amine, in concentrations higher than 10^{-4}M produces a positive inotropic effect in guinea-pig papillary muscles which is neither influenced by pretreatment with reserpine nor by the presence of a β-sympatholytic agent (71). This direct inotropic effect of tyramine is caused by an acceleration of force development and is connected with a reduction in the duration of t_1. However, the speed of relaxation is diminished and the relaxation time t_2 increases in dependence upon the tyramine concentration. Finally, at 10^{-2}M tyramine, which is beyond the inotropic concentration-response curve, t_1 is prolonged also (71). A prolongation of t_2 can also be seen from an isometric contraction curve under the influence of the peptide angiotensin (10^{-6}M), as presented by Koch-Weser (72). The inotropically acting veratrum esteralkaloids, veratridine and cevadine, produce a retardation of relaxation, cevadine in the early phase and veratridine in the second half of the descending limb of the isometric contraction curve, the latter effect giving rise to mechanical oscillations (73). The influence upon the relaxation process by high concentrations of tyramine and by cevadine and veratridine are paralleled by characteristic prolongations of the transmembrane action-potentials.

Possible Differentiation of Klinotropic Effects

The classification of inotropic drugs according to their influence on the time course of the isometric contraction curve can only be the first step in the effort to clarify the cellular mode of action. In the case of the prolongation of t_1 and t_2 by lowering the temperature, certain reflections can be made regarding the temperature dependence of enzymatic reactions responsible for the contraction process as well as for the binding and elimination of intracellularly released calcium ions (36, 43). The velocity of force development (S_1) decreases with lowering of the temperature if it has been high before in accordance with a high extracellular calcium concentration (8, 36); but there is an increase of the low value of S_1 at low calcium (8, 36) which, at least partly, might be caused by an increase in stiffness of the series elastic element (SE) which is associated with hypothermia (37). An increase in SE stiffness might also play a role in the influence of hyperosmotic solutions on the time course of activity (74).

From the therapeutical point of view those drugs seem to be most important that have a predominant klinotropic effect, i.e. act by an acceleration of force development. It seems reasonable that these effects are brought about by a greater disposition of calcium ions at places from which they are

released during stimulation (8). The question then arises whether there exists a uniform mechanism to produce such an increase of calcium at certain sites of the cardiac muscle cell. Still another problem would be whether an inotropic agent could act simply by increasing the susceptibility of the contractile proteins to calcium ions.

Sodium dependence.—It has been found that the inotropic effects of cardioactive steroids (54) differ from those of catecholamines (55) in the way they are influenced by the extracellular sodium concentration: while the inotropic effect of the steroids is greatly diminished when the sodium concentration is reduced by one half, the inotropic effect of catecholamines is not. Other experiments reveal that the sodium dependence of the cardioactive steroids is shared by the inotropic effect of a reduction in extracellular potassium concentration (75) and that of an increase in frequency of contractions (56). These results have been extended by the observation of the sodium dependence of the rate of onset of the ouabain-induced positive inotropic effect on the frog heart (76) and of the independence on the sodium concentration of the inotropic effects of histamine (77) and theophylline (78).

The dependence of some inotropic effects on the sodium concentration makes it reasonable to assume that sodium has a key position in those processes which can lead to an increase in calcium at some cellular sites. An experimental basis for such a conception is provided by the observation that the calcium influx increases in squid axon (79) as well as in guinea pig atria (80) when the intracellular sodium concentration ($[Na^+]_i$) is raised. For the above mentioned sodium dependent inotropic interventions it is very likely that they lead to an increase of $[Na^+]_i$: the enhanced frequency of contractions through an increase in sodium influx per unit of time, the reduction of $[K^+]_e$ as well as the cardioactive steroids through an inhibition of the sodium transport to the outside. The diminution of these inotropic effects by lowering the extracellular sodium concentration is likely to be the consequence of a reduced sodium influx (81) which leads to a decrease in $[Na^+]_i$ (82).

It would be interesting to know whether the inotropic effects of p-chloromercuribenzene sulfonic acid (83) and of some dipolar aprotic solvents (84) which have recently been reported on are sodium dependent since in both cases an inhibition of Na^+-K^+-dependent ATPase has been found in vitro. Likewise, the inclusion in the pharmacological investigation of the inotropically acting peptide glucagon (85–88) of its sodium dependence would be of value. An independence on $[Na^+]_e$ should be expected if the inotropic effect of glucagon is mediated by cyclic 3′,5′-AMP as has been postulated (85, 86). However, in investigating the effect of sodium upon an inotropic intervention, one carefully has to consider that sodium ions extracellularly inhibit contractility by an antagonism with calcium in heart muscle of the warm-blooded animal (8, 54) as well as of the frog (89, 90). A diminution of $[Na^+]_e$ alone leads to an increase in force of contraction

which, if sufficiently great, conceals the degree of an inotropic drug effect. It is not possible, for instance, to make a clear statement about the dependence or independence of the inotropic action of angiotensin on the sodium concentration from experiments in which solely $[Na^+]_e$ has been changed (72). Instead, the calcium concentration must be reduced simultaneously to keep the ratio $[Ca^{2+}]/[Na^+]^2$ constant, and thereby the control value of the contraction force.

LITERATURE CITED

1. Brady, A. J. 1968. *Physiol. Rev.* 48: 570–600
2. Jewell, B. R., Blinks, J. R. 1968. *Ann. Rev. Pharmacol.* 8:113–30
3. Blinks, J. R., Koch-Weser, J. 1963. *Pharmacol. Rev.* 15:531–99
4. Goffart, M., Ritchie, J. M. 1952. *J. Physiol. London* 116:357–71
5. Krop, St. 1944. *J. Pharmacol. Exp. Ther.* 82:48–62
6. Hill, A. V., Macpherson, L. 1954. *Proc. Roy. Soc. London* 143:81–102
7. Reiter, M. 1965. *Experientia* 21:87–89
8. Reiter, M. 1964. *Proc. Int. Pharmacol. Meet. 2nd, Prague 1963,* ed. O. Krayer, Vol. 5:25–42. Pergamon Press, Oxford
9. Blinks, J. R., Koch-Weser, J. 1964. *Proc. Int. Pharmacol. Meet. 2nd, Prague 1963,* ed. O. Krayer, Vol. 5:53–62
10. Brooks, Ch. McC., Hoffman, B. F., Suckling, E. E., Orias, O. 1955. *Excitability of the Heart.* Grune & Stratton, New York, 1955
11. Buccino, R. A., Sonnenblick, E. H., Cooper, Th., Braunwald, E. 1966. *Circ. Res.* 19:1097–1108
12. Cranefield, P. F., Greenspan, K. 1960. *J. Gen. Physiol.* 44:235–49
13. Koch-Weser, J. 1963. *Am. J. Physiol.* 204:451–57
14. Blinks, J. R. 1965. *J. Appl. Physiol.* 20:755–57
15. Reiter, M. 1967. *Arzneimittel-Forsch.* 17:1249–53
16. Furchgott, R. F., de Gubareff, T., Grossmann, A. 1959. *Science* 129:328–29
17. Hill, A. V. 1949. *Proc. Roy. Soc. London* 136:399–420
18. Abbott, B. C., Mommaerts, W. F. H. M. 1959. *J. Gen. Physiol.* 42:533–51
19. Parmley, W. W., Sonnenblick, E. H. 1967. *Circ. Res.* 20:112–23
20. Edman, K. A. P., Nilsson, E. 1968. *Acta Physiol. Scand.* 72:205–19
21. Sonnenblick, E. H. 1962. *Am. J. Physiol.* 202:931–39
22. Edman, K. A. P., Nilsson, E. 1965. *Acta Physiol. Scand.* 63:507–08
23. Strauer, B. E., Westberg, C., Tauchert, M. 1971. *Pfluegers Arch.* 324:124–33
24. Pollack, G. H. 1970. *Circ. Res.* 26: 111–27
24a. Brady, A. J. 1967. *Physiologist* 10: 75–86
25. Jewell, B. R., Wilkie, D. R. 1958. *J. Physiol. London* 143:515–40
26. Jewell, B. R., Wilkie, D. R. 1960. *J. Physiol. London* 152:30–47
27. Sonnenblick, E. H. 1965. *Fed. Proc.* 24:1396–1409
28. Noble, M. I. M., Bowen, T. E., Hefner, L. L. 1969. *Circ. Res.* 24: 821–33
29. Hill, A. V. 1938. *Proc. Roy. Soc. London* 126:136–95
30. Brady, A. J. 1965. *Fed. Proc.* 24: 1410–20
31. Edman, K. A. P., Grieve, D. W., Nilsson, E. 1966. *Pfluegers Arch.* 290:320–34
32. Edman, K. A. P., Nilsson, E. 1969. *Acta Physiol. Scand.* 76:236–47
33. Sonnenblick, E. H. 1967. *J. Gen. Physiol.* 50:661–76
34. Trautwein, W., Dudel, J. 1954. *Pfluegers Arch.* 260:104–15
35. Katzung, B., Farah, A. 1956. *Am. J. Physiol.* 184:557–62
36. Reiter, M. 1963. *Arch. Pathol. Exp. Pharmakol.* 245:551–61
37. Yeatman, L. A. Jr., Parmley, W. W., Sonnenblick, E. H. 1969. *Am. J. Physiol.* 217:1030–34
38. Reichel, H., Bleichert, A. 1962. *Pfluegers Arch.* 275:526–33
39. Bleichert, A., Reichel, H. 1962. *Pfluegers Arch.* 276:242–49
40. Rumberger, E. 1970. *Pfluegers Arch.* 318:353–65
41. Brady, A. J. 1964. *Proc. Int. Pharmacol. Meet. 2nd, Prague 1963,* ed.

O. Krayer, Vol. 5:15–23. Pergamon Oxford Press
42. Brady, A. J. 1967. *Physiologist* 10: 130
43. Reichel, H. 1960. *Muskelphysiologie,* Springer-Verlag, Berlin
44. Katzung, B. 1968. *Selected Pharmacological Testing Methods,* Vol. 3:193–234. Marcel Dekker, Inc., New York
45. Saunders, P. R., Sanyal, P. N. 1958. *J. Pharmacol. Exp. Ther.* 123: 161–63
46. Meyer, H. F., Kukovetz, W. R. 1962. *Arch. Pathol. Exp. Pharmakol.* 242:409–13
47. Reiter, M., Stickel, F. J. 1970. *Klin. Wochenschr.* 48:935–38
48. Penefsky, Z. J., Hoffman, B. F. 1963. *Am. J. Physiol.* 204:433–38
49. Reiter, M. 1968. *Verh. dtsch. Ges. Kreisl.-Forsch., Herzdilatation und Insuffizienz, 34. Tagung,* 87–99 Dr. Dietrich Steinkopff Verlag, Darmstadt
50. Blinks, J. R. 1970. *Fed. Proc.* 29:611 Abstr.
51. Naidu, S., Fisher, V. J. 1971. *Fed. Proc.* 30:268 Abstr.
52. Reiter, M. 1958. *Pfluegers Arch.* 267: 158–71
53. Engstfeld, G., Antoni, H., Fleckenstein, A., Nast, A. v. Hattingberg, M. 1961. *Pfluegers Arch.* 273:145–63
54. Reiter, M. 1963. *Arch. Pathol. Exp. Pharmakol.* 245:487–99
55. Reiter, M., Schöber, H. G. 1965. *Arch. Pathol. Exp. Pharmakol.* 250:9–20
56. Reiter, M. 1966. *Arch. Pathol. Exp. Pharmakol.* 254:261–86
57. Reiter, M. 1962. *Arch. Pathol. Exp. Pharmakol.* 242:497–507
58. Reiter, M., Schöber, H. G. 1965. *Arch. Pathol. Exp. Pharmakol.* 250:21–34
59. Reiter, M. 1961. *Biochem. Pharmacol.* 8:37–38
60. Morad, M., Trautwein, W. 1968. *Pfluegers Arch.* 299:66–82
61. Katzung, B. 1964. *J. Cell. Comp. Physiol.* 64:103–14
62. Braveny, P., Sumbera, J., Kruta, V. 1966. *Arch. Int. Physiol. Biochim.* 74:169–78
63. Feigl, E. O. 1967. *Circ. Res.* 20:447–58
64. Feigl, E. O. 1968. *Paired Pulse Stimulation of the Heart,* ed. P. F. Cranefield, B. F. Hoffman, 155–63
65. Koch-Weser, J. 1963. *Am. J. Physiol.* 204:957–62
66. Thomas, L. J., Jr. 1957. *J. Cell. Comp. Physiol.* 50:249–64
67. Weyne, J. 1966. *Arch. Int. Physiol. Biochim.* 74:449–60
68. de Hemptinne, A., Weyne, J., Leusen, I. 1967. *Arch. Int. Physiol. Biochim.* 75:96–108
69. Blinks, J. R. 1971. *Fed. Proc.* 30:267 Abstr.
69a. de Gubareff, T., Sleator, W., Jr. 1965. *J. Pharmacol. Exp. Ther.* 148:202–14
70. Marcus, M. L., Skelton, C. L., Grauer, L. E., Epstein, S. E. 1971. *Fed. Proc.* 30:632 Abstr.
71. Brandt, W., Reiter, M. 1971. *Arch. Pharmakol. Exp. Pathol.* 269: 439–40
72. Koch-Weser, J. 1965. *Circ. Res.* 16: 230–37
73. Reiter, M. 1963. *Arch. Pathol. Exp. Pharmakol.* 246:45
74. Wildenthal, K., Skelton, C. L., Coleman, H. N. III, 1969. *Am. J. Physiol.* 217:302–06
75. Reiter, M., Seibel, K., Stickel, F. J. 1971. *Arch. Pharmakol. Exp. Pathol.* 268:361–78
76. Talbot, M. S. 1968. *Nature* 219:1053–54
77. Bernauer, W., Dörfler, G., Gross-Hardt, M. 1971. *Arch. Int. Pharmacodyn.* 189:72–89
78. Scholz, H., de Yazikof, E. 1971. *Arch. Pharmakol. Exp. Pathol.* 270:R 128
79. Baker, P. F., Blaustein, M. P., Hodgkin, A. L., Steinhardt, R. A. 1969. *J. Physiol. London* 200:431–58
80. Glitsch, H. G., Reuter, H., Scholz, H. 1970. *J. Physiol. London* 209:25–43
81. Glynn, I. M. 1956. *J. Physiol. London* 134:278–310
82. Reiter, M. 1956. *Arch. Pathol. Exp. Pharmakol.* 227:300–15
83. From, A. H. L., Probstfield, J. L. 1971. *Fed. Proc.* 30:632, Abstr.
84. Spilker, B. 1970. *J. Pharmacol. Exp. Ther.* 175:361–67
85. Lucchesi, B. R. 1968. *Circ. Res.* 22: 777–87
86. Entman, M. L., Levey, G. S., Epstein, S. E. 1969. *Circ. Res.* 25:429–38
87. Tuttle, R. R. 1970. *Fed. Proc.* 29:611 Abstr.
88. Spilker, B. 1970. *Brit. J. Pharmacol.* 40:382–95
89. Wilbrandt, W., Koller, H. 1948. *Helv. Physiol. Pharmacol. Acta* 6:208–21
90. Lüttgau, H. C., Niedergerke, R. 1958. *J. Physiol.* 143:486–505