Biochemical Pharmacology, Vol. 16, pp. 1429–1438. Pergamon Press Ltd. 1967. Printed in Great Britain

RELATIONSHIP BETWEEN METABOLIC AND MECHANIC ADRENERGIC STIMULATION OF THE HEART UNDER CONDITIONS OF SMALL DOSAGE, LOW CALCIUM, HYPOTHERMIA AND OUABAIN*

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(*Received* 28 *December* 1966; *accepted* 16 *February* 1967)

Abstract—Conditions facilitating a dissociation between the actions of noradrenaline (N) and isoprenaline (I) on phosphorylase activity (%-*a*) and isotonic contractions were studied in isolated perfused hearts. Hypothermia and ouabain pretreatment did not dissociate the rise in %-*a* from the inotropic effect of N in guinea-pig and rat hearts, but abolished the inotropic response in rabbit hearts. This is explained by high-calcium-inhibition of the contractile protein, caused by the stimulant actions of hypothermia, ouabain and N on calcium uptake. Low calcium perfusion inhibited the mechanical response of guinea pig hearts to I more markedly than its effect on %-*a*. Changes in calcium concentration are obviously more critical for contractile than for metabolic function. Small doses of N, which augmented contraction by 30–50 per cent did not raise %-*a*. In contrast, a 30 per cent increase in contractility, when evoked by I, was associated with a 57 per cent raise in %-*a*. It is proposed, that I increases 3′, 5′-AMP faster than N. With small doses of I (in contrast to N) a sufficient part of the same total amount of newly formed 3′, 5′-AMP could thus escape degradation before it reaches the cellular target, where %-*a* is augmented. The results agree with the theory that 3′, 5′-AMP initiates the positive inotropic effect of catecholamines as well as the rise in %-*a*, needed for the full development of the mechanical effect.

THE PRECISE role of phosphorylase activation in the mechanic response of the heart to catecholamines is still a matter of controversy. Whereas a number of studies have revealed a parallelism between both events under a variety of pharmacological conditions, there are reports, indicating that the mechanical and metabolic effects of these agents are not causally related. The evidence for both views has been reviewed by Haugaard and Hess.[1] In particular, Mayer *et al.*[2] failed to get a significant increase in active phosphorylase (%-*a*) in hearts of open chest dogs after small doses of 0·1 μg/kg adrenaline which raised contractile force by 34 per cent. Hess *et al.*[3] did not find a similar dissociation in rat heart (Langendorff), since 0·05 μg adrenaline, which augmented contractile force by only 10·4 per cent, increased %-*a* significantly from 27·4 to 31·0. On the other hand, Drummond *et al.*[4], probably by using a more rapid freezing technique which yielded lower control values for %-*a* confirmed the results obtained in dog hearts,[2] and also established a dissociation in isolated perfused rat hearts, where 0·025 and 0·04 μg adrenaline increased contractility by 23 and 43 per

* Some of the results have been presented at the 29th meeting of the German Pharmacological Society, held at Göttingen, Sept. 27–30, 1965 (*Arch. exp. Path. Pharmak.* **253**, 61, (1966).

cent, but did not raise %-*a*. Moreover, in hypothermic dogs (28–29°), pretreated with 50–75 μg/kg ouabain for 30–60 min, 10 μg/kg noradrenaline depressed cardiac contraction but raised %-*a* from 20 to 60.[2] A similar negative inotropic effect of adrenaline, associated with an increase in %-*a*, was found in hypothermic rats.[5] Finally, based on the finding that isopropylmethoxamine can inhibit certain metabolic responses to catecholamines in the dog, including activation of liver phosphorylase, without affecting the mechanical effects on the heart,[6, 7] it was postulated that the metabolic and the mechanical effects in the heart are mediated by separate types of receptor with different thresholds of sensitivity.

In a preceeding communication, evidence was presented showing that isopropylmethoxamine reduces the positive inotropic effects of three sympathomimetics in the isolated perfused guinea pig heart by about the same degree as their stimulatory actions on %-*a*.[8] These results do not support the assumption of different (mechanical and metabolic) adrenergic receptors within the cardiac tissue. The present experiments are further attempts to establish conditions under which a dissociation between the metabolic and the mechanic effects of catecholamines in the heart have been found.[2, 4, 5] For this purpose, the actions of noradrenaline on both parameters were investigated in isolated perfused hearts from three different species, perfused at 27° and pretreated with ouabain. Further, the changes in contractility and %-*a* were compared under minimum effective doses of noradrenaline and isoprenaline. Finally, the action of isoprenaline on %-*a* was studied during partial inhibition of the inotropic response by low-calcium-perfusion.

MATERIALS AND METHODS

Animals and preparation

Freshly excised hearts of stunned male and female guinea pigs, rabbits and rats were perfused on a Langendorff apparatus in the same manner as described earlier.[9] Isotonic contractions and rate were recorded on a smoked drum.

Phosphorylase determinations

Active and total phosphorylase were measured in 30–60 mg specimens of cardiac tissue, cut from the right ventricular wall during control periods or at the climax of drug action, in the same way as described elsewhere.[9]

Drugs

Noradrenaline (L-Arterenol bitartrate) was purchased from the Sterling-Winthrop Research Institute (Rensselaer, N.Y.), Isoprenaline (DL-*N*-Isopropylarterenol hydrochloride) from the California Corporation for Biochemical Research (Los Angeles), and Ouabain (*g*-Strophanthin) from Oesterreichische Heilmittelwerke (Vienna). Pronethalol (Alderlin) was generously supplied by Dr. J. W. Black, and Propranolol (Inderal) by Dr. G. V. McHattie (Imperial Chemical Industries Ltd, Cheshire, England).

Evaluation of results

Data for the calculation of mean values ± S.E.M. were only taken from experiments in which the entire procedure, as indicated by the sequence of columns in Fig. 1–3 and in Table 1. could be carried through in the same heart. Fig. 4 compiles data from

two groups of hearts (1/2 Ca^{2+} and 1/10 Ca^{2+},). This was possible, since usually 6–8 samples for phosphorylase determinations could be obtained from one heart without noticeable impairment of mechanical performance. Experiments, in which cardiac function was impaired by this procedure were discarded. Student's *t*-test was employed for the calculation of differences and $P < 0{\cdot}05$ was considered significant.

RESULTS

1. *Influence of ouabain pretreatment on the actions of noradrenaline in the hypothermic heart*

Guinea pig hearts were perfused for 15 min at 37°. A control biopsy for the determination of %-*a* was taken thereafter from the right ventricular wall and the perfusion temperature then lowered to 27°. This was followed by a 50 per cent reduction of the contractile amplitude ($P < 0{\cdot}01$) and rate ($P < 0{\cdot}001$) and a 40 per cent decrease of %-*a* ($P < 0{\cdot}02$; Fig. 1). Ouabain was then given in two consecutive doses of 50 and 100 μg. In both concentrations this substance raised %-*a* and contractility of the hypothermic heart significantly ($P < 0{\cdot}01$) to approximately the level of the euthermic controls but did not change the rate. The positive inotropic effect of the glycoside lasted for another 25–30 min at about half its original intensity. When noradrenaline in a fully active dose of 5 μg was given 8–10 min after a second application of ouabain, it produced strong and highly significant increases of both contractility and %-*a* ($P < 0{\cdot}001$), the latter effect being more pronounced (Fig. 1). There was also a significant ($P < 0{\cdot}05$), if small, increase in rate. About 5 min after the injection of noradrenaline, contractility and rate returned to hypothermic control levels, whereas %-*a* remained elevated for another few minutes.

In rat hearts, which received a smaller dose of 2 μg noradrenaline because of their

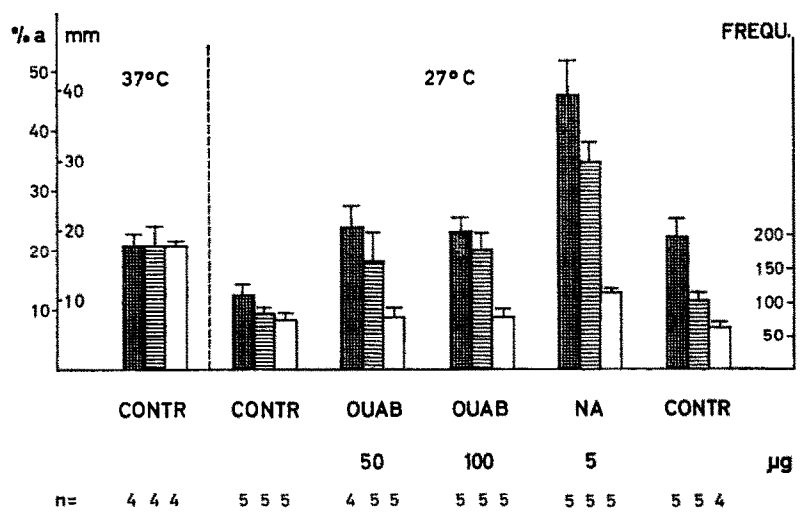

FIG. 1. Influence of hypothermia (27°) and ouabain-pretreatment on the rise in phosphorylase *a* (%-*a*; dotted columns) isotonic contractions (mm; shaded columns) and frequency (Frequ; open columns), produced by noradrenaline in isolated perfused guinea pig hearts. The scales for the three parameters were adapted to give columns of equal height for the control values, obtained at 37°. T-shaped bars are standard errors.

lower weight, but the same amounts of ouabain, since rat hearts are particularly resistant to the actions of cardiac glycosides,[10–13] hypothermia did not reduce %-*a* and amplitude of isotonic contractions (Table 1). Rate was not measured in these experiments. The higher dose of 100 μg ouabain caused a sharp and significant ($P < 0.05$) rise in contractility and %-*a* that was completely reversed after 3–4 min, whereas the smaller dose had no effect. Noradrenaline, when given after ouabain, sigficantly ($P < 0.01$) increased both %-*a* and contractility to about the same extent. In some of these experiments on rat hearts, 10 μg pronethalol were given. It was found, that this β-blocking agent not only completely inhibited all the actions of 2 μg noradrenaline, but also the effects of 100 μg ouabain.

TABLE 1. INFLUENCE OF HYPOTHERMIA (27°) AND OUABAIN ON METABOLIC (%-*a*) AND MECHANICAL (mm) STIMULATION OF ISOLATED HEARTS BY NORADRENALINE

Species		Control (37°)	Control (27°)	Ouabain (50 μg)	Ouabain (100 μg)	Control (27°)	Noradren. (2 μg)
Rat ($n = 5$)	%-*a*	30 ± 1·4	29 ± 0·8	32 ± 2·9	44 ± 5·9*	28 ± 0·7	51 ± 2·5*
	mm	31 ± 0·9	29 ± 3·8	30 ± 1·5	42 ± 3·3*	18 ± 5·8	47 ± 5·2*
				150 μg	300 μg		15 μg
Rabbit	%-*a*	25 ± 0·5	22 ± 0·7	33 ± 3·1*	25 ± 2·4	24 ± 3·2	37 ± 5·6*
($n = 4$)	mm	45 ± 3·1	37 ± 6·3	56 ± 5·9*	36 ± 5·2	37 ± 7·2	43 ± 6·9

* Significant with respect to aforegoing control ($P < 0.05$).

In rabbit hearts, hypothermia considerably reduced distolic relaxation, as indicated by an average elevation of the base line by 22 mm. A similar but small effect, was found in some of the guinea pig hearts, but not in rat hearts. The amplitude of isotonic contractions in rabbit hearts was slightly smaller at 27° than at 37°. There was also a minor reduction of %-*a* (Table 1). Ouabain was given in these experiments in two consecutive injections of 150 and 300 μg. These high doses of ouabain and of noradrenaline (15 μg) were selected because of the larger size of the rabbit heart. As shown in Table 1, the first dose of ouabain produced a 50 per cent increase in %-*a* and in amplitude of contraction ($P < 0.02$), whereas the subsequent higher dose had no effect. When noradrenaline was given 8 min later, it produced a substantial increase in %-*a* ($P < 0.001$) but no significant augmentation of the contractile amplitude.

2. *Actions of small doses of noradrenaline and isoprenaline*

In the search for minimum effective doses of noradrenaline and isoprenaline that would elicit just a slight but reproducible mechanical stimulation, considerable variations in the sensitivity of different guinea pig hearts were found. For that reason a dose had to be found in each experiment, that caused a reproducible elevation of the contractile amplitude of about 30 per cent. This was the smallest positive inotropic effect clearly distinguishable from spontaneous changes in the course of the experiment. The doses of noradrenaline, necessary to evoke significant ($P < 0.05$) rises in contractility of 30 per cent ranged from 12·5–65 ng. In these amounts, noradrena-

line did not increase %-*a* or heart rate significantly (Fig. 2). With 25–80 ng noradrenaline rises of 50 per cent in contractile amplitude were produced but there were still no significant increases in %-*a* and heart rate. For comparison, a high dose of 169 ng (1nM) was given that highly significantly elevated %-*a* and contractility. There was also a small increase in heart rate.

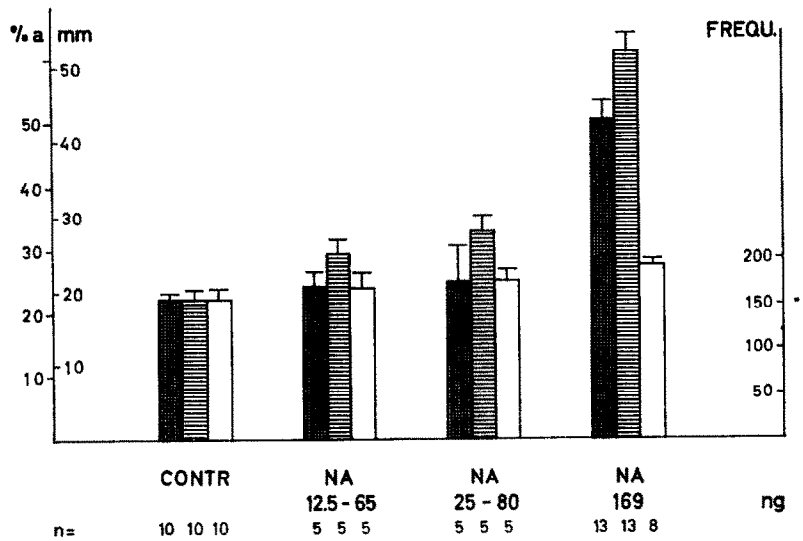

FIG. 2. Effects of small doses of noradrenaline in isolated guinea pig hearts. Columns as in Fig. 1.

When, however, the same procedure was carried out with isoprenaline it was found that doses of 0·5–2 ng which increased contractility by about 30 per cent produced a rise of 57 per cent in %-*a* ($P < 0{\cdot}001$), whereas heart rate was not clearly changed (Fig. 3). In larger doses of 5 and 100 ng, which were also given for comparison, isoprenaline produced the usual highly significant ($P < 0{\cdot}001$) increases in %-*a* contractility and heart rate.

It was noticed that after injections of small doses of isoprenaline the development of the inotropic response occurred faster than after noradrenaline. A 30 per cent increase in contractility, when caused by isoprenaline, was completed within 20·4 ± 2·8 sec ($n = 5$), whereas the same effect of noradrenaline required 31·1 ± 3·5 sec ($n = 5$). The difference is significant ($P < 0{\cdot}01$).

3. *Influence of low-calcium-perfusion on the mechanical and metabolic effects of isoprenaline*

Since it has been argued that the increase in %-*a* and in the concentration of 3′,5′-AMP might be a consequence rather than the cause of the positive inotropic action of catecholamines,[14] two groups of guinea pig hearts were perfused with Locke-solution, containing either 1/2 (0·008 per cent; 0·72 mM) or 1/10 (0·0016 per cent; 0·144 mM) of normal concentration of anhydrous $CaCl_2$ (0·016 per cent; 1·44 mM).[9] The effect of submaximal doses of 5 ng isoprenaline on contractility and %-*a* were then compared in the same heart under conditions of normal and diminished concentrations of extracellular calcium. The results obtained are summarized in Fig. 4. During the control

period, 5 ng of isoprenaline produced the usual increases in %-*a*, contractile amplitude and rate. Switching to a Locke-solution containing 50 per cent of the normal calcium concentration (1/2 Ca^{2+}) was followed by a 60 per cent reduction of amplitude ($P < 0.01$) in the control period, whereas %-*a* and rate were not changed significantly. Isoprenaline elicited the same rise in %-*a* as seen under normal conditions, but its

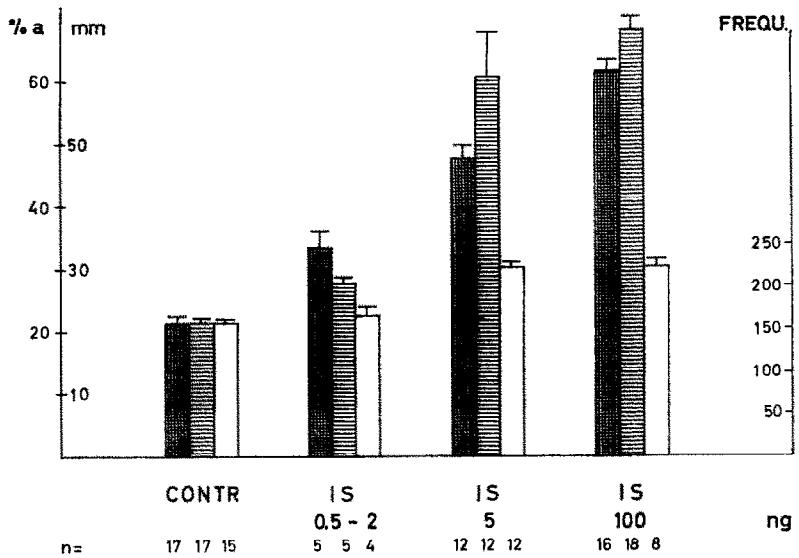

FIG. 3. Effects of various doses of isoprenaline in isolated guinea pig hearts. Columns as in Fig. 1.

mechanical effects, especially on contractility, were greatly reduced. Return to a normal calcium concentration in the perfusion fluid rapidly re-established the initial amplitude of spontaneous contractions and a normal mechanical response to isoprenaline, with a slight overshoot in the positive inotropic effect (Fig. 4; 5th group of columns).

When the calcium concentration in the perfusion fluid was lowered to 10 per cent of normal (1/10 Ca^{2+}; Fig. 4), the amplitude of isotonic contractions was drastically reduced from 22·4 ± 1·2 mm to almost zero (1·5 ± 0·2 mm). Heart rate could not be measured exactly under these conditions, but %-*a* remained normal. Isoprenaline (5 ng) raised very slightly, but significantly ($P < 0.05$) the contractile amplitude to 4·6 ± 0·8 mm (Fig. 4), which is from 6·7 to 20·5 per cent of the control amplitude. At the same time, isoprenaline increased the %-*a* by 50 per cent above normal ($P < 0.01$). This activation of phosphorylase, although considerably smaller than with normal calcium concentrations by far exceeded the positive inotropic effect. In a few experiments, 50 μg pronethalol or 30 μg propranolol were injected and isoprenaline was given subsequently. Both β-adrenergic blocking agents completely inhibited the effects of isoprenaline during low calcium perfusion. When perfusion with normal Locke solution was resumed the heart quickly regained its initial activity and responded fully to isoprenaline with increases in %-*a* and rate and a slightly higher rise in contractility than before (Fig. 4, last group of columns).

DISCUSSION

The results obtained in the first section of this study (Fig. 1, Table 1) have shown that in isolated perfused hearts of guinea pigs and rats induction of hypothermia (27°) and pretreatment with two different doses of ouabain did not reverse the positive inotropic effect of noradrenaline. Although contractility was comparatively less augmented than %-*a* under these circumstances, the results differ from those obtained

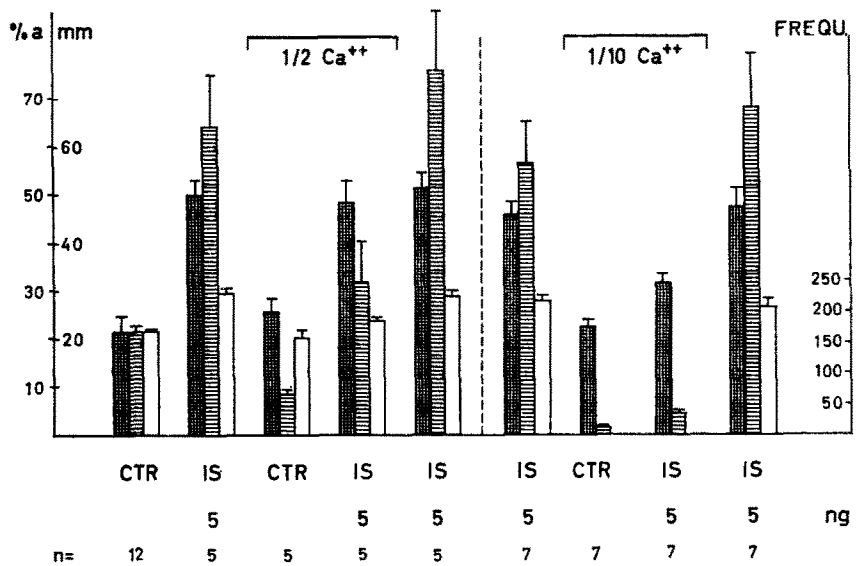

FIG. 4. Influences of low calcium perfusion on the actions of isoprenaline in isolated perfused guinea pig hearts. Columns as in Fig. 1.

in hearts of open-chest-dogs under comparable conditions.[2, 5] When similar experiments were performed with rabbit hearts, however, the inotropic action of noradrenaline was inhibited, while the rise in %-*a* remained unchanged. Hypothermia, ouabain and noradrenaline, have in common that although by different mechanisms, they make available more calcium to the contractile system of the myocardial cell[15–20]. This is usually associated with a positive inotropic effect. When the myofibrils are saturated, a further increase in calcium uptake becomes ineffective and eventually depresses contractility.[16, 23] This explains why hypothermia can abolish the inotropic action of ouabain[15] and noradrenaline,[5, 21] why high external calcium concentrations prevent or reverse the inotropic effect of cold[16] and are even able by themselves to depress contractility.[22, 23] The failure of noradrenaline to stimulate contraction in hypothermic and ouabain-pretreated rabbit hearts, and probably also its negative inotropic action seen in dog hearts under similar conditions,[2, 5] could thus be due to a more or less pronounced direct depression of the myofibrils by excess calcium. Rabbit hearts are probably more sensitive to cold-induced augmentation of calcium uptake than hearts of rats and guinea pigs, since they responded to hypothermia with diminished diastolic relaxation, and were not stimulated by a second dose of ouabain. However the latter effect could also be due to tachyphylaxis, if the first dose of ouabain had acted by a release of noradrenaline as found in isolated papillary muscles

of cats.[24] Such a mechanism is further indicated by the present results in rat hearts showing that the action of ouabain on contractility and %-*a* could be blocked by pronethalol. Partial inhibition of ouabain-induced increases in contractile force by pronethalol was also found in frog ventricles.[25]

Catecholamines increase the formation of cyclic-3',5'-AMP in the heart,[26–28] most probably in the membrane[29] of the myocardial cell, or a closely associated structure.[30] It has been proposed that 3',5'-AMP enhances the transmembranal movement of calcium.[9, 31] This should raise the calcium concentration at the contractile protein, resulting in stimulation. At the same time, 3',5'-AMP (as the 'second messenger' of adrenergic stimulation[30]) is released into the cytoplasm where it activates phosphorylase-*b*-kinase[27] and thus raises the formation of phosphorylase a. Active phosphorylase accelerates glycogenolysis and supplies additional energy, probably needed for an adequate inotropic response.[32] If hypothermia, either alone[5] or in combination with ouabain pretreatment[2] had elevated the concentration of calcium at the myofibrils to a level, where a further increase by cyclic AMP became ineffective or caused depression, this nucleotide could still activate phosphorylase. Although the latter reaction is also sensitive to changes in the concentration of calcium, this ion being needed for the activation of phosphorylase-*b*-kinase,[27] this kinase is not depressed, but stimulated by amounts of calcium high enough to inhibit contraction. This has been shown directly in guinea pig auricles.[22] If, on the other hand, the concentration of calcium was decreased below normal, the inotropic response to isoprenaline was considerably more diminished than the rise in %-*a* (Fig. 4). Hence, it appears that the range of calcium concentrations that will permit a full positive inotropic reaction to catecholamines is much smaller than that allowing an unrestricted metabolic response.

A clear dissociation was found in guinea pig hearts between the mechanical and metabolic actions of small doses of noradrenaline. Rises in contractile amplitude of 30 per cent and 50 per cent could be produced without significant changes in %-*a* (Fig. 2). Similar results, obtained in rat hearts, were reported recently,[33] indicating that low doses of noradrenaline augmented contractile force by 20 per cent, but did not affect %-*a*. When however, isoprenaline was used instead of noradrenaline in the present experiments, the results were quite different. Small doses that increased contraction by an average of 30 per cent augmented phophorylase *a* by 57 per cent (Fig. 3). We have not tested small doses of adrenaline, but conflicting results, as discussed in the introduction, have been reported from other laboratories.[2–4] An additional finding in working heart preparations of rats,[26] where 40 ng/ml adrenaline elevated aortic pressure by 30 per cent and simultaneously increased 3',5'-AMP by 120 per cent and phosphorylase *a* by 200 per cent supports the results of Hess *et al.*[3] All these findings could be explained, if we assume, that noradrenaline, adrenaline and isoprenaline, although producing similar total increases in 3',5'-AMP in mechanically equieffective doses, initiate this effect at different speeds. This is suggested by the present observation, that a 30 per cent rise in contractile amplitude, when initiated by noradrenaline, required an average of 31·4 sec, whereas the same effect, if produced by isoprenaline, was completed within 20·4 sec. Within the cell membrane, where the cyclic nucleotide is thought to elicit the inotropic response[9, 31] such a difference is probably less critical. If, however, 3',5'-AMP is released into the cytoplasm at different rates as a consequence of different speeds of production, the metabolic effects should be affected, since 3',5'-AMP is extremely susceptible[28] to hydrolysis by phosphodiesterase.[34]

This enzyme, although found in particulate as well as in supernatant fractions of heart homogenates, is thought to act primarily in the cytoplasm.[30] The chance of 3′,5′-AMP reaching its target, phosphorylase-*b*-kinase, could thus depend on the rate of release from the membrane. Noradrenaline should be the least –, isoprenaline the most potent accelerator of 3′,5′-AMP-production, whereas adrenaline stands in between. If these assumptions are correct, small doses of noradrenaline, in contrast to isoprenaline, may not be able to increase the speed of 3′,5′-AMP-production sufficiently to initiate a significant metabolic response. The potency of small doses of adrenaline to accelerate the formation of 3′,5′-AMP should then be in the vicinity of the threshold rate, required for a significant rise in %-*a*. In this way, minor variations of experimental technique could easily be responsible for the opposing results, obtained with small doses of adrenaline in different laboratories.[2–4, 26]

The present findings are compatible with the view, that 3′, 5′-AMP is the 'second messenger' initiating the inotropic and the metabolic response of the heart to catecholamines. Likewise, our results do not exclude the possibility, that activitation of phosphorylase is necessary for the full development of the mechanical effects of these substances, especially when higher doses are used.

Acknowledgement—The excellent technical assistance of Mrs. Vera Bauer is gratefully appreciated by the authors.

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