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INSULIN-INDUCED ENHANCEMENT OF UPTAKE OF NORADRENALINE IN ATRIAL STRIPS

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1 Addition of insulin to the organ bath increased the force of contraction of guinea-pig left atrial strips driven electrically at 1 Hz.

2 The positive inotropic response to insulin remained unaltered in atria depleted of catecholamine or when β-adrenoceptors were blocked by addition of propranolol to the organ bath.

3 The response of isolated atria to noradrenaline was significantly reduced in the presence of insulin.

4 Insulin affected neither the calcium accumulating abilities of the heart sarcolemma, mitochondria or microsomes, nor the cyclic adenosine 3′,5′-monophosphate (cyclic-AMP)-protein kinase-induced stimulation of microsomal calcium uptake.

5 Addition of insulin to the organ bath enhanced significantly the ability of the cardiac tissue to take up [^{3}H]-noradrenaline as well as [^{3}H]-metaraminol. The activities of monoamine oxidase and catechol-*O*-methyl transferase were not changed after addition of insulin to homogenates of the heart.

6 The ability of insulin to facilitate uptake of noradrenaline would be expected to cause a decrease in the amount of the amine reaching the receptors, thus leading to a diminished response to this amine. This may explain, at least in part, insulin-induced subsensitivity to noradrenaline.

7 This view is supported by the observation that after blockade of amine uptake by destruction of nerve terminals, insulin failed to reduce the positive inotropic response to noradrenaline.

Introduction

Insulin plays an important role in cardiac metabolism (Weissler, Altschule, Gibb, Pollack, & Kruger, 1973) but the consequences of its action on cardiac contractile performance have not been fully investigated. It has been shown that insulin and glucose improve cardiac output in shock (Weisul, O'Donnell, Stone & Clowes, 1975) and protect ventricular function from the consequences of experimental myocardial infarctin (Marako, Libby, Sobel, Bloor, Sybers, Sheel, Covell & Braunwald, 1972). Addition of insulin to solution perfusing anoxic isolated heart of rat improved ventricular performance (Weissler *et al.*, 1973). Several other studies have indicated that insulin exerts a positive inotropic effect in either intact heart (Downing, Lee & Riker, 1977) or isolated cardiac muscle (Lucchesi, Medina & Kniffen, 1972; Kones, 1974; Lee & Downing, 1976). The positive inotropic effect of insulin in the intact lamb was not prevented by β-receptor blockade or continuous glucose infusion to prevent hypoglycaemia (Downing *et al.*, 1977). This finding indicates that the positive inotropic effect of insulin is not mediated through an effect on the adrenoceptors and insulin may have a direct effect on the myocardium.

Whereas insulin exerts a positive inotropic effect, it antagonizes the positive inotropic response to adrenaline (Hiatt & Katz, 1969), noradrenaline (Lee & Downing, 1976) and cardiac glycoside (Kones, 1974). The response of an organ to catecholamines depends on at least three factors: (a) the amount of noradrenaline reaching the receptor site (this in turn is related to the efficiency of the mechanism responsible for inactivation of the amine); (b) the sensitivity of the adrenoceptors to noradrenaline; (c) the sequence of the events between the occupation of the adrenoceptors by the agonist and the contraction of the myofibrils. The main aim of the present study was to gain insight into the mechanism involved in insulin-induced subsensitivity to noradrenaline. For that purpose we determined the effect of insulin on the factors responsible for the inactivation of the amine. In addition, we investigated the effect of insulin on calcium accumulation by the heart, since calcium is a key factor in excitation-contraction coupling.

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Methods

Male guinea-pigs of 300 to 600 g body weight were stunned with a blow on the head and their hearts were rapidly removed. The left atrium was dissected from the heart and suspended in a modified Tyrode solution maintained at 34°C. It took approximately 10 min from the time of killing an animal to suspension of the atria in the organ bath. The solution used in all experiments had the following composition (mM): NaCl 154, KCl 5.63, $CaCl_2$ 2.16, $NaHCO_3$ 5.95 and glucose 11.1. It was aerated with 95% O_2 and 5% CO_2. The pH of the solution was maintained at approximately 7.4.

The lower end of the atrium was tied to a plastic holder which contained punctate electrodes. The upper end was tied to a force transducer (Grass FT 0.03) and contractions were recorded on a Grass ink writing oscillograph. The atrium was electrically driven via platinum electrodes placed parallel to but not touching the tissue. Square-wave pulses of 5 ms duration were delivered at a frequency of 1 Hz and an above threshold voltage. Unless otherwise indicated, the resting tension on the atrium was 1 g. The atria were allowed to equilibrate for at least 1 h after being placed in the bath and were washed repeatedly after each addition of a drug. In the experiments in which noradrenaline was used, ethylenediaminetetraacetic acid (EDTA) was present at a bath concentration of 2.7×10^{-5} M during the periods of exposure to this amine, to retard oxidation. The concentration of EDTA had no effect on the force of contraction, the uptake, release, metabolism, or effectiveness of noradrenaline (Bhagat, Bovell & Robinson, 1967). Responsiveness to noradrenaline (1×10^{-7} M) was determined on the same tissue in the presence and in the absence of insulin at an interval of 60 min. Insulin was added about 40 min before the second response to the amine.

Estimation of catecholamine

The atria were homogenized in 25 ml of ice-cold 0.4 N perchloric acid. The protein-free supernatant obtained by centrifugation was absorbed on alumina at pH 8.6. The catecholamines were eluted with 3 ml of 0.04 N perchloric acid (Anton & Sayre, 1962). The noradrenaline in 0.2 ml of eluate was converted to its fluorescent trihydroxyindole derivative by oxidation with potassium ferricyanide at pH 6.5 according to the method of Von Euler & Lishajko (1961).

Estimation of labelled noradrenaline and metaraminol

In additional experiments, the accumulation of (±)-[^{3}H]-noradrenaline (8.72 Ci/mmol) or (±)-[^{3}H]-metaraminol (6.93 Ci/mmol) in isolated atria from guinea-pigs was determined. The isolated atria were incubated with 0.25 μCi/ml of either [^{3}H]-noradrenaline or [^{3}H]-metaraminol for 30 min at 34°C. Thereafter, atria were washed six times with the modified Tyrode solution at 2 min intervals at room temperature, blotted with filter paper and weighed.

The atria incubated with noradrenaline were homogenized in 2.0 ml ice-cold 0.4 N perchloric acid. The protein-free supernatant obtained by centrifugation was passed over alumina at pH 8.6 and eluted with 5 ml of 0.04 N perchloric acid. A 2.0 ml aliquot of the alumina eluate was added to 10 ml of Instagel and this was counted in a liquid scintillation spectrometer (Packard Instrument Co.).

The atria incubated with [^{3}H]-metaraminol were homogenized in 1.0 ml ice-cold 0.4 N perchloric acid. An aliquot of 0.3 ml of the protein-free supernatant obtained after centrifugation was added to 14.7 ml of Instagel and radioactivity was counted. The pellet (the perchloric acid precipitated protein) in both series of experiments was analysed according to the method of Lowry, Rosebrough, Farr & Randall (1951). All values for [^{3}H]-noradrenaline and [^{3}H]-

Estimation of monoamine oxidase and catechol-O-methyl transferase activity

Monoamine oxidase (MAO) was assayed by measuring the conversion of [^{14}C]-tryptamine to indoleacetic acid according to the method of Wurtman & Axelrod (1963). Catechol-*O*-methyl transferase (COMT) was assayed by measuring the formation of [^{3}H]-metanephrine on incubation with (−)-adrenaline and [^{3}H]-methyl-adenosylmethionine (specific activity 8.9 Ci/mmol, Amersham/Searle, Illinois) as described by Axelrod (1959).

Determination of calcium accumulation

The methods for the isolation and purification of sarcolemmal, mitochondrial, and microsomal (fragments of the sarcoplasmic reticulum) fractions from the guinea-pig ventricles were the same as those described by Tomlinson, Lee & Dhalla (1976). The calcium uptake by the microsomal fraction (0.02–0.03 mg protein/ml) was carried out at pH 6.8 in a medium containing 100 mM KCl, 10 mM $MgCl_2$, 20 mM Tris-HCl, 4 mM adenosine triphosphate (ATP), 0.1 mM $^{45}CaCl_2$ and 5 mM potassium oxalate in a total volume of 1 ml. The same incubation medium was employed for the mitochondrial (0.15–0.20 mg protein/ml) calcium uptake except that potassium oxalate was replaced by 4 mM KH_2PO_4 and 4 mM sodium succinate. The calcium

accumulation by the sarcolemmal fraction (0.15–0.20 mg protein/ml) was carried out at pH 6.8 in 100 mM KCl, 20 mM Tris-HCl, and 0.1 mM $^{45}CaCl_2$.

The membrane fractions were incubated for 3 min at 37°C in the absence or presence of insulin before starting the reaction by the addition of $^{45}CaCl_2$. Five min later the reaction was stopped by the millipore filtration. It should be pointed out that the calcium accumulating ability of the mitochondrial fraction was markedly inhibited by 5 mM sodium azide whereas the calcium accumulating ability of the sarcolemmal preparation was markedly depressed by Mg-ATP. These characteristics of the different heart membranes employed here are similar to those described elsewhere (Tomlinson *et al.*, 1976).

The following substances were used: bovine crystalline insulin (Eli Lilly and Co.), (−)-noradrenaline bitartate monohydrate, tyramine hydochloride, 6-hydroxydopamine hydrobromide and reserpine (Serpasil, Ciba). Reserpine (2.5 mg/kg) was injected intraperitoneally 24 h before the experiment. 6-Hydroxydopamine was dissolved in 0.001 N HCl immediately and injected intraperitoneally (200 mg/kg), 96 h before the experiment.

Insulin was obtained commercially and contained approximately 1.6% glycerine (w/v) and approximately 0.2% phenol (w/v) as a preservative. No effect of these preservatives was seen on the positive inotropic response to insulin or accumulation of [^{3}H]-noradrenaline by the tissue. While the concentrations of insulin used in the present study are 4–6 orders of magnitude higher than those that prevail physiologically, nevertheless they are the same as used by other investigators (Lee & Downing, 1976; Downing *et al.*, 1977).

Statistical evaluations (t test) were performed according to Snedecor (1956); $P < 0.05$ was regarded as significant.

Results

Effect of insulin on the contractility of guinea-pig isolated atrial strips, driven electrically

Since guinea-pig isolated atria were to be used in the present study, it was considered necessary to determine whether their response to insulin was similar to that observed for canine (Lee & Downing, 1976) and lamb (Downing *et al.*, 1977) cardiac muscle. Addition of insulin (1.0 u/ml) to the organ bath increased the force of contraction of left atrial strips, driven electrically at 1 Hz, within 2 min. The enhancement reached its maximum value within 10–20 min and the atria recovered completely within 5 min after washing.

The positive inotropic response to insulin remained unaltered when atria were depleted of catecholamine. Atria from reserpine-treated guinea-pigs, responded to insulin but the stimulatory response to tyramine was completely abolished. Similarly, the cardiostimulatory response to insulin was unaffected by blockade of β-adrenoceptors with propranolol (1.7 μM), although addition of noradrenaline now failed to elicit the usual response. Similar results have previously been described by others (Lucchesi *et al.*, 1972; Lee & Downing, 1976; Downing *et al.*, 1977); therefore, no details of the results are given.

Effect of insulin on the response to sympathomimetic amines of guinea-pig isolated atrial strips, driven electrically

The positive inotropic response to (−)-noradrenaline was determined in the absence and in the presence of insulin. There was an interval of about 60 min between the two additions of noradrenaline and insulin was added to the organ bath 40 min before the second addition. Insulin significantly reduced ($P < 0.05$) the sensitivity of the atria to noradrenaline (Table 1). Similarly, the response to tyramine was also significantly reduced in the presence of insulin (Table 1). Insulin-induced subsensitivity of dog and lamb myocardium to noradrenaline has previously been reported (Lee and Downing, 1976; Downing *et al.*, 1977).

When a tissue is repeatedly exposed to a pharmacologically active agent, it usually develops a tolerance to the subsequent effect of that particular agent (Snyder, 1979). To rule this out as an explanation for our findings, three successive responses to noradrenaline were determined at 60 min intervals in the absence of insulin. There was no significant ($P > 0.05$) change in the response of the atria to noradrenaline for up to 3 h.

Effect of insulin on the cardiac monoamine oxidase and catechol-O-methyl transferase activities

Reduced sensitivity to noradrenaline could occur if insulin increased the metabolic degradation of noradrenaline. The results in Table 2 show that the activities of enzymes responsible for degradation of noradrenaline did not change ($P > 0.05$) after the addition of insulin (1.0 u/ml) to the heart homogenates.

Effect of insulin on accumulation of [^{3}H]-noradrenaline and [^{3}H]-metaraminol in atria

Reduced responsiveness of atrial tissue from guinea-pigs to noradrenaline could be explained if insulin

Table 1 Effect of insulin on the response to sympathomimetic amines of guinea-pig isolated atria, driven electrically

	Inotropic response (% increase in tension) Mean ± s.e.mean	
Atria from untreated guinea-pig		
Noradrenaline (1×10^{-7} M)		
Control	99 ± 5.0 (6)	$P < 0.01$
Insulin	50 × 4.8 (6)	
Tyramine (5×10^{-5} M)		
Control	88 ± 6.2 (6)	$P < 0.01$
Insulin	43 ± 4.2 (6)	
Atria from 6-OHDA-treated guinea-pig		
Noradrenaline (0.6×10^{-7} M)		
Control	92 ± 4.3 (8)	$P > 0.05$
Insulin	87 ± 5.2	

Responses of guinea-pig isolated left atrial strips, driven electrically at 1 Hz in bathing solution at 34°C, to noradrenaline or tyramine. There was an interval of 60 min between each addition of the amine; insulin (1 u/ml) was added to the organ bath 40 min before the second addition of the amine. The results are expressed as % increase in tension before and after addition of insulin. In certain experiments, guinea-pigs were injected intraperitoneally with 6-hydroxydopamine (6-OHDA) 200 mg/kg 96 h before the experiment.

caused an increase in the uptake of noradrenaline. This hypothesis was tested in the following experiments. Isolated atria were exposed to 0.25 μCi/ml of (±)-[^{3}H]-noradrenaline in the absence and presence of insulin (1.0 u/ml). Thirty min later they were analysed for [^{3}H]-noradrenaline. The results in Table 3 indicate that insulin significantly ($P < 0.01$) enhanced the accumulation of [^{3}H]-noradrenaline. In another series of experiments, the effect of insulin on the accumulation of [^{3}H]-metaraminol in atrial strips was determined. The results in Table 3 indicate that insulin significantly ($P < 0.01$) enhanced the accumulation of metaraminol. These results confirm the effect of insulin on an uptake process for sympathomimetic amines.

Effect of insulin on the responsiveness of isolated atrial muscle from guinea-pigs pretreated with 6-hydroxydopamine

Atrial preparations obtained from 6-hydroxydopamine-treated and untreated animals were examined for catecholamine content, uptake of [^{3}H]-noradrenaline and responsiveness to noradrenaline. The mean catecholamine concentration in atria of 6 such pretreated animals was 0.08 (s.e. ± 0.04) μg/g whereas that obtained for a group of 6 untreated animals was 1.32 (s.e. ± 0.06) μg/g. The uptake of [^{3}H]-noradrenaline by the atrial strips of pretreated animals was 10.2 ± 1.6% ($n = 6$) of the controls. Responses to noradrenaline on such atrial strips were determined in the presence and absence of insulin (1u/ml). Insulin failed to alter significantly ($P > 0.05$) the positive inotropic response to noradrenaline (Table 1).

Effect of insulin on calcium accumulation by heart membranes

Different membranes such as sarcolemma, sarcotubular system (microsomes) and mitochondria, by virtue of their abilities to accumulate calcium, are

Table 2 Effect of insulin on monoamine oxidase (MAO) and catechol-*O*-methyl transferase (COMT) activity in the guinea-pig heart

Treatment	*MAO activity*	*COMT activity*
Control	2548 ± 126	0.159 ± 0.04
Insulin	2333 ± 105*	0.156 ± 0.05*

The activity of MAO and COMT are expressed as nmol of product formed per g of tissue during 1 h of incubation at 37°C. Product refers to [^{14}C]-indoleacetic acid or [^{14}C]-normetanephrine. The results are the mean ± s.e.mean, $n = 6$. *$P > 0.05$.

Table 3 The effect of insulin on accumulation of [^{3}H]-noradrenaline and [^{3}H]-metaraminol

	[^{3}H]-noradrenaline	*[^{3}H]-metaraminol*
Control	12.57 ± 1.47	51.90 ± 3.59
Insulin	23.78 ± 1.81*	88.07 ± 5.44*

Isolated atria from guinea-pig were exposed to 0.25 μCi/ml of either (±)-[^{3}H]-noradrenaline or (±)-[^{3}H]-metaraminol in the absence and in the presence of insulin (1u/ml). Thirty min later they were washed and analysed for [^{3}H]-noradrenaline or [^{3}H]-metaraminol. Results are expressed as $d\,min^{-1}\,\mu g^{-1}$ protein and show the mean ± s.e.mean, $n = 6$. *$P < 0.01$.

believed to be intimately involved in the regulation of cardiac contractile force (Dhalla, Ziegelehoffer & Harrow, 1977). It has been suggested that agents favouring an increase in calcium-accumulating abilities of one or more membranes make more calcium available for release upon excitation and thus produce a positive inotropic effect. In order to gain some information on this aspect, the calcium-accumulating abilities of heart sarcolemma, microsomes, and mitochondria were studied in the presence of different concentrations of insulin (0.001–1.0 u/ml). The results in Table 4 indicate that insulin did not increase calcium-accumulating abilities of heart membranes significantly ($P > 0.05$). It should be pointed out that the values for calcium-accumulating ability of different heart membranes are comparable to those reported elsewhere.

Effect of insulin on cyclic AMP-protein kinase-induced stimulation of microsomal calcium uptake

The microsomal fraction (20–50 μg/ml) was incubated with or without 50 μg/ml protein kinase and 10^{-6} M cyclic AMP for 2 min in the absence or presence of insulin (1u/ml). The calcium uptake was determined by employing 10^{-6} M calcium for 1 min according to the procedure described elsewhere (Dhalla, Varley & Harrow, 1974). The control calcium uptake values in the absence and presence of insulin were 10.3 ± 0.7 and 9.6 ± 0.8 nmol Ca^{2+}/mg protein respectively, while the corresponding values with cyclic AMP-protein kinase present were 17.1 ± 1.3 and 17.3 ± 1.4 nmol Ca^{2+}/mg protein respectively. These results do not indicate any effect of insulin on the cyclic AMP-protein kinase-induced increase in calcium uptake activity of heart microsomes.

Discussion

Insulin has long been known to have a number of effects on the myocardium. Lee & Downing (1976) have shown that insulin significantly attenuates the inotropic action of noradrenaline on mammalian isolated cardiac muscle. In the present study, insulin reduced the positive inotropic response to noradrenaline of guinea-pig isolated atria. Similarly, the response to tyramine, an amine which stimulates the cardiac tissue indirectly (Bhagat & Gilliam, 1965) through the release of noradrenaline, was also reduced in the presence of insulin.

The response of an organ to noradrenaline depends on at least three factors (see Introduction). The amount of noradrenaline reaching the adrenoceptors of the effector organ is related to the efficiency of the mechanism responsible for inactivation of the amine (Bhagat, 1967; Bhagat *et al.*, 1967). When noradrenaline is added to the organ bath, a proportion of the amine is destroyed enzymatically, while another fraction is taken up by the nerve terminals after which it may be destroyed or stored in the granules so that only relatively small amounts reach the receptors. Thus, an increase in the capacity of the

Table 4 Effect of insulin on the calcium-accumulating abilities of guinea-pig heart membranes

Additions	*Calcium accumulation* (nmol mg^{-1} protein 5 min^{-1}) *Sarcolemma*	*Microsomes*	*Mitochondria*
Control	67 ± 9	1876 ± 133	365 ± 31
Insulin (0.001–0.1 u/ml)	78 ± 6*	1926 ± 117*	382 ± 28*

Each value is a mean ± s.e.mean, $n = 6$. For each preparation four concentrations (0.001, 0.01, 0.10 and 1.0 u/ml) of insulin were tested. Results in the presence of insulin are grouped together since the values with each concentration were overlapping. *$P > 0.05$.

tissue to take up noradrenaline and/or enhancement of its enzymatic degradation would reduce the amount of the amine reaching the receptors, resulting in a diminution of the response to this amine. Since insulin did not affect the activities of monoamine oxidase and catechol-*O*-methyl transferase, enzymes responsible for degradation or noradrenaline, it is unlikely that enzyme alterations are responsible for the insulin-induced subsensitivity to noradrenaline.

Accumulation of [^{3}H]-noradrenaline in atrial strips was significantly enhanced in the presence of insulin. The effect of insulin was not selective for noradrenaline, since the accumulation was also enhanced of [^{3}H]-metaraminol, a compound structurally related to noradrenaline but not metabolized by monoamine oxidase or catechol-*O*-methyl transferase. The neuronal uptake of noradrenaline is mediated by a carrier system located in the neuronal membrane (Dengler, Spiegal & Titus, 1963; Bogdanski & Brodie, 1969). The carrier transports both Na^+ and noradrenaline across the neuronal membrane into the cytoplasm, where the affinity of the carrier for noradrenaline is reduced by the low Na^+ and the high K^+ (which antagonizes Na^+) thereby causing the noradrenaline to be released intracellularly. The transported Na^+ is then removed from the cell by a Na^+ pump which is linked to a Na^+-K^+-stimulated ATPase (Dengler *et al.*, 1963). This mechanism for the uptake of amine is similar to that for sugars and amino acids (Crane, 1965) and it should be noted that insulin is known to facilitate the uptake of glucose and amino acids by various tissues (Morgan, Henderson, Regen & Park, 1961). It is therefore not surprising that, in the present study, insulin facilitated the uptake of noradrenaline and metaraminol by the guinea-pig isolated atria. The consequence of this increased uptake would be to reduce the concentration of noradrenaline reaching adrenoceptors resulting in a diminished response. This may explain the observed insulin-induced subsensitivity of atria to noradrenaline.

Administration of 6-hydroxydopamine causes complete destruction of adrenergic nerve terminals, and thereby causes marked depletion of catecholamine in the tissues (Jonsson & Sachs, 1972). The terminal parts of the adrenergic neurones selectively degenerate leaving the cell body and other tissue structures (as well as cholinergic nerve endings) morphologically unaffected. Since nerve terminals are destroyed, the uptake of noradrenaline is markedly impaired. If the reduced responsiveness of atrial tissue to noradrenaline in the presence of insulin is related to its ability to facilitate the uptake of the amine by the nerve terminals, then after loss of the uptake mechanism by destruction of nerve terminals, the response of the tissue to noradrenaline should not be affected by insulin. This concept is consistent with the results of the present study because insulin failed to reduce the response to noradrenaline in atrial strips from guinea-pigs pretreated with 6-hydroxydopamine. Reduced sensitivity to noradrenaline by insulin could also be due to its effect on the sensitivity of adrenoceptors, and/or the sequential steps subsequent to receptor activation which lead to the cellular response. Although no information concerning the sensitivity of adrenoceptors is available at present, we have shown that insulin did not interfere with cyclic AMP-protein kinase-induced increase in microsomal calcium uptake. In spite of the fact that this mechanism has been recognized as important in mediating the positive inotropic effect of catecholamines (Dhalla *et al.*, 1974), the interaction of insulin with noradrenaline or cyclic AMP at other sites in the myocardium cannot be ruled out at the present time.

Several studies have shown that insulin exerts a positive inotropic effect on mammalian isolated cardiac muscle (Lee & Downing, 1976; Downing *et al.*, 1977). This response to insulin is not mediated through the release of catecholamine or through the stimulation of adrenoceptor by insulin (Lee & Downing, 1976; and present study) and is not dependent upon the ability of the hormone to facilitate glucose transport across the myocardial cell (Lucchesi *et al.*, 1972).

The positive inotropic effect of insulin may be caused by changes in the permeability of plasma membrane to various cations such as Na^+, K^+ and Ca^{2+} (Kones, 1974). Although insulin has been shown to influence the Na^+ and K^+ fluxes in muscle, the exact site of this action is not understood because this hormone did not affect the Na^+-K^+ ATPase (Manery, Dryden, Still & Madapilli-Mattam, 1977). Likewise, insulin has been reported to stimulate the fluxes of ^{45}Ca from skeletal muscle (Clausen & Martin, 1977; Schudt, Gaertner & Pette, 1976). However, in the present study we failed to detect any change in the calcium accumulating abilities of heart sarcolemma, microsomes and mitochondria. It should be mentioned that some investigators (McDonald, Burns & Jarett, 1976) have shown a stimulatory effect of insulin on the nonspecific calcium binding by adipocyte plasma membranes while others have reported a depressant action by employing liver plasma membranes. Whether this discrepancy is due to tissue specificity or some other reason is not clear at this time. However, our experiments do not rule out the possibility that insulin may induce calcium release from the subcellular organelles directly or indirectly as well as augment calcium influx through heart sarcolemma by some regulatory mechanisms. Furthermore, the action of insulin on other functions of heart membrane systems cannot be ignored since this hormone has been reported to

stimulate adipocyte plasma membrane Mg^{2+}-stimulated ATPase (Jarett & Smith, 1974). Thus, the exact events leading to an increase in the intracellular concentration of free calcium and subsequent positive inotropic action of insulin remain to be established.

In conclusion, this study has shown that insulin exerted a positive inotropic effect on guinea-pig isolated atria which was not dependent on any change in Ca^{2+}-accumulating abilities of the heart-sarcolemma, mitochondria or microsomes. This study also offers an explanation for the mechanism involved in insulin-induced subsensitivity of noradrenaline. It has shown that insulin-induced subsensitivity to noradrenaline is related, at least in part, to insulin's ability to facilitate uptake of amine by the nerve terminals, since after blockade of uptake by destruction of nerve terminals, the response of the tissue to noradrenaline was not affected by insulin.

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