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EFFECTS OF BETA-RECEPTOR BLOCKING DRUGS ON CARDIAC METABOLISM

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Abstract—The effects of the three β-receptor blocking drugs pronethalol, propranolol and dichlorisopropyl-noradrenaline on changes in fat and carbohydrate metabolism induced by adrenaline were studied in isolated perfused rat and guinea-pig hearts. The actions of adrenaline on all aspects of metabolism examined were at least partially blocked by β-receptor blocking drugs. Therefore the β-receptor appears to be involved to a considerable extent in the acceleration of cardiac metabolism produced by adrenaline.

There are differences in the action of β-receptor antagonists. Of the three drugs examined propranolol was the most effective antagonist of the actions of adrenaline on all aspects of metabolism except for the increase in glucose uptake, upon which its effects were minimal.

There was a relationship between the amplitude of contraction in response to these agents and the amount of endogenous triglyceride utilized; this also held for the β-receptor antagonists and adrenaline.

INTRODUCTION

AHLQUIST[1, 3] has classified the positive inotropic response of the heart to catecholamines as being mediated by activation of β-receptors. This classification is based on a consideration of the order of potency of a series of catecholamines, and in addition, by blockade of the positive inotropic responses by β-receptor blocking drugs.[2, 3]

Studies of the actions of catecholamines on fat and carbohydrate metabolism in a variety of *in vitro* tissue preparations and *in vivo* have shown that these actions are often partially antagonized by β-receptor blocking drugs,[5–7] and in some cases by α-receptor blocking drugs.[8, 9] However, β-receptor blocking drugs, are in general more effective in antagonizing the effects of catecholamines on metabolic processes than are α-receptor blocking drugs.[10, 11]

It was of interest to examine the actions of β-receptor blocking drugs on metabolism in the isolated, perfused heart, since a relationship between increased force of contraction and factors promoting increased carbohydrate metabolism has been emphasized by a number of authors.[5, 12, 13] Such an investigation might be expected to indicate what changes in metabolic processes in heart due to catecholamines are mediated via activation of the β-receptor.

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The suggestion has been made that adenyl cyclase, the enzyme catalyzing the formation of adenosine 3′,5′-monophosphate (cyclic 3′,5′-AMP), may be synonymous with the β-receptor[4]. Thus activation of this receptor would increase the formation of cyclic 3′,5′-AMP which in turn will promote the activation of glycogen phosphorylase, lipase and phosphofructokinase. Activation of these key enzymes will in turn cause glycogen breakdown, mobilization of free fatty acids (FFA) from trigylceride and increased utilization of glucose. This latter effect could be caused by activation of phosphofructokinase, which in turn decreases the level of glucose-6-phosphate, an inhibitor of hexokinase.

METHODS

The isolated, perfused hearts of rats of the Sprague–Dawley strain, and of guinea-pigs were used in this study. Animals were fed on a diet of Barostoc pellets.

Rats and guinea-pigs were anesthetized with a 50% CO_2–50% O_2 mixture in order to minimize hypoxia during dissection.[13] Hearts were rapidly removed from animals following incision of the thorax, and set up in a heart perfusion apparatus. The hearts were preperfused for 10 min with Krebs–Ringer bicarbonate solution[14] at 37° in order to remove blood and catecholamines released during preparation.

Williamson[13] reported that adrenaline recirculating through the heart may be rapidly inactivated. It was found however that perfusate containing 0·1 mM ascorbic acid allowed retention of adrenaline-potency when tested on fresh hearts after 40 min perfusion, the positive inotropic effect being of the same order on the second group of hearts as on the first. Therefore all perfusion media contained 0·1 mM ascorbic acid.

This solution was recirculated through the heart for 30 min using a gas (5% CO_2–95% O_2) lift to return perfusate to the upper reservoir where it was aerated with a stream of this gas. A sintered glass filter was included in the apparatus to remove debris. In order to maintain an enclosed system, recordings of amplitude of contraction were made by attaching a fine surgical thread to the apex of the ventrical with a silver hook and passing it through 2 polythene stoppers drilled with 0·1 mm. dia. holes, at each end of a tube containing silicone grease. Contractions were measured on a displacement transducer and chart recorder system (Both, Australia).

Hearts were perfused with media containing a drug or combinations of drugs for 30 min. The preperfusion media also contained β-receptor blocking drugs where blockade of the effect of adrenaline was to be investigated. At the end of the 30 min perfusion period, samples of the perfusate were analyzed for glucose[15] and lactate[16] content. From comparison of glucose concentrations in the media prior to and after perfusion an estimate of glucose uptake was made. Hearts were removed from the cannula, cut into slices, mixed to insure a random distribution of tissue, and divided into 3 portions which were stored in liquid nitrogen for subsequent analyses of glycogen,[15] lactate,[16] and fats (FFA and triglyceride).[18, 19]

l-Adrenaline tartrate was obtained from Burroughs Wellcome and Co. Ltd. (Australia). Drug concentrations used were determined in preliminary experiments to be average effective doses.

RESULTS

Changes in fat, carbohydrate and lactate during perfusion

Williamson[13] reported that perfusion of rat hearts with a medium containing 5·6 mM glucose caused a decrease in the glycogen content. Randle, Newsholme and Garland[19]

reported that glycogen levels remained unchanged under these conditions. The results in Table 1 show that during a 40-min perfusion period with Krebs bicarbonate solution containing 5·6 mM glucose, a significant net synthesis of glycogen occurred. During this period 14·2 μM/g of glucose were utilized and the total lactate level (in both heart and perfusate) increased from 8·1 to 13·2 μM/g. Half the glucose utilized can be accounted for by an increase in glycogen and lactate.

TABLE 1. UTILIZATION OF FAT AND CARBOHYDRATE BY RAT HEART DURING 40-MIN PERFUSION

	Glycogen content (Glucose equiv.)	Glucose uptake from medium	Total lactate produced (heart and perfusate)	FFA	Triglyceride (FFA equiv.)
	μM/g	μM/g	μM/g	μM/g	μM/g
Fresh whole heart	16·7 ± 0·7 (7)		8·1 ± 0·5 (8)	3·43 ± 0·70 (6)	10·00 ± 1·82 (6)
Perfused	21 7 ± 1·7* (8)	14·2 ± 2·2 (10)	13·2 ± 1·2* (6)	1·05 ± 0·10* (6)	1·72 ± 0·16* (3)

Values given are mean ± S.E. (number of animals in the group in brackets).
* $P < 0·05$ against fresh whole heart.

Table 1 also shows that endogenous FFA decreased from 3·43 to 1·05 μM/g and endogenous triglyceride from 10·00 to 1·72 μM/g; no significant amounts of either were found in the perfusate. Thus considerable quantities of fat are used to sustain the perfused rat heart under these conditions. These observations are in agreement with a report of Bing[20] that the normal metabolism of the heart is largely dependent on fat utilization.

The effects of adrenaline on endogenous triglyceride, FFA and glycogen content of the heart, and on glucose utilization and lactate production

It is well known that adrenaline mobilizes FFA from triglyceride in adipose tissue[21] and heart.[17] It might be expected that increased utilization of FFA would be associated with the increased force of contraction in order to provide additional energy to the heart. Tables 2 and 3 show that adrenaline (0·5 and 1·0 μg/ml respectively) decreased the level of triglyceride in both rat and guinea-pig hearts. The level of FFA in rat heart was not decreased but increased, suggesting that although FFA was mobilized from triglyceride by adrenaline it was not used to sustain the positive inotropic effect. The increase in level of FFA in rat heart was greater than the decrease in the level of triglyceride. This is possibly due to release of FFA from phospholipid. FFA could not be detected in the perfused guinea-pig heart either in the presence or in the absence of adrenaline.

In rat heart, perfusion for 30 min (Table 3) with 0·5 μg/ml adrenaline almost halved the glycogen content and doubled the glucose uptake, concomittant with a 3-fold increase in lactate production. In guinea-pig heart (Table 2) perfusion for 30 min with 1·0 μg/ml adrenaline decreased the glycogen content by two thirds, doubled the glucose uptake and approximately trebled the lactate output.

The main differences between the adrenaline responses in the two species is that the net lactate production in the rat heart is more than twice that for guinea-pig heart, both

TABLE 2. EFFECTS OF PRONETHALOL, PROPRANOLOL AND D.C.I. ALONE AND IN COMBINATION WITH ADRENALINE IN PERFUSED GUINEA-PIG HEARTS

	Glycogen content (glucose equiv.) μM/g	Glucose uptake from medium μM/g	Lactate		Triglyceride (FFA equiv.) μM/g	Size of contraction 10 min after drug
			Total heart + perfusate	Percent of total present in heart		Size of contraction without drug
Control perfused	23·7 ± 2·8 (8)	17·8 ± 3·8 (8)	6·9 ± 1·6 (8)	74·5	1·78 ± 0·12 (8)	
1-Adrenaline tartrate 1·0 μg/ml	8·0 ± 1·7*†‡§ (9)	33·9 ± 3·3*†‡§ (9)	18·3 ± 2·7*†‡§ (12)	33·3	1·30 ± 0·13*†‡ (6)	2·40 ± 0·31 (6)
Pronethalol hydrochloride 5·0 μg/ml	19·9 ± 2·0 (7)	20·5 ± 2·8 (7)	5·9 ± 0·7† (7)		4·93 ± 0·46*† (6)	0·43 ± 0·06 (4)
Propranolol hydrochloride 1·0 μg/ml	13·6 ± 2·0*† (7)	16·1 ± 2·2† (7)	12·3 ± 1·0* (7)		3·06 ± 0·19*† (7)	0·55 ± 0·05 (5)
D.C.I. hydrochloride 10·0 μg/ml	22·7 ± 2·3 (8)	19·5 ± 3·2 (8)	11·3 ± 1·2* (8)		3·29 ± 0·24 (4)	0·69 ± 0·08 (6)
Adrenaline + pronethanol	16·4 ± 1·7 (7)	19·7 ± 2·7 (8)	10·8 ± 1·6 (7)		1·85 ± 0·17 (8)	1·41 ± 0·28 (4)
Adrenaline + propranolol	19·5 ± 1·7 (9)	25·8 ± 2·8 (9)	8·0 ± 1·3 (9)		1·99 ± 0·23 (6)	1·05 ± 0·16 (5)
Adrenaline D.C.I.	17·3 ± 1·3 (9)	15·1 ± 2·0 (9)	11·6 ± 0·6 (9)		2·17 ± 0·47 (4)	1·10 ± 0·10 (5)

Values given are mean ± S.E. (number of animals in the group in brackets).
Concentrations of drugs in combination are the same as individual concentrations.

* $P < 0.05$ compared to control.

† $P < 0.05$ compared to adrenaline + pronethalol.

‡ $P < 0.05$ compared to adrenaline + propranolol.

§ $P < 0.05$ compared to adrenaline + D.C.I.

TABLE 3. ANTAGONISM BY BETA-RECEPTOR BLOCKING DRUGS OF ADRENALINE EFFECTS IN PERFUSED RAT HEART

	Glycogen content (glucose equiv.) μM/g	Glucose uptake from medium μM/g	Lactate		Triglyceride (FFA equiv.) μM/g		Size of contraction 10 min after drug
			Total (heart + perfusate)	Percent of total present in heart		FFA μM/g	Size of contraction without drug
Control perfused	21·2 ± 1·2 (10)	14·4 ± 2·2 (10)	14·3 ± 0·9 (10)	40·8	1·72 ± 0·15 (3)	1·14 ± 0·07 (5)	
1-adrenaline tartrate 0·5 μg/ml	11·3 ± 1·3* (23)	28·9 ± 3·7* (24)	41·6 ± 4·5 (11)	14·2	1·55 ± 0·14 (8)	2·06 ± 0·33* (8)	
1-adrenaline tartrate 0·5 μg/ml pronethalol hydrochloride 5·0 μg/ml	12·1 ± 2·4 (4)	21·6 ± 3·3 (4)	43·4 ± 8·6 (4)		1·88 ± 0·60 (4)	0·39 ± 0·10† (4)	1·30 ± 0·22 (5)
1-adrenaline tartrate 0·5 μg/ml propranolol hydrochloride 1·0 μg/ml	17·3 ± 2·6† (8)	31·1 ± 3·9 (8)	26·8 ± 6·2† (7)		1·90 ± 0·19 (8)	0·51 ± 0·18† (7)	1·03 ± 0·18 (5)
1-adrenaline tartrate 0·5μg/ml D.C.I. hydrochloride 10·0μg/ml	15·8 ± 2·6 (4)	26·1 ± 3·9 (4)	43·1 ± 4·7 (4)		2·36 ± 0·38 (4)	0·49 ± 0·06† (4)	1·10 ± 0·10 (5)

Values given are mean ± S.E. (number of animals in the group in brackets).

* $P < 0.05$ compared to control.

† $P < 0.05$ compared to adrenaline.

in the presence and absence of adrenaline. The increased lactate production (converted to glucose equivalents) in guinea-pig hearts in response to adrenaline accounted for only 18 per cent of the glycogen and glucose utilized. However the increased lactate production in rat heart accounted for 56 per cent. This may mean that more lactate is utilized by the guinea-pig heart.

A further species difference in net lactate production can be seen in Tables 2 and 3. A comparison of the proportions of lactate in the heart and perfusate reveals that the guinea-pig heart retained almost twice as much lactate as rat heart. Further, in guinea-pig heart the amount retained in the tissue increased in response to adrenaline. This emphasizes the possible error introduced by equating the amount of carbohydrate utilized with the amount of lactate released to the perfusate. Hirche and Lockner[22] have observed that infusion of lactate decreased the oxidation of FFA. It is possible that in perfused guinea-pig hearts the small amount of lactate released into the perfusate enables more FFA to be oxidized and thus accounts for the low level of FFA observed in guinea-pig hearts in our experiments. This of course assumes some compartmentation of lactate within the heart.

Effect of β-receptor blocking drugs on metabolism and contraction in heart

Pronethalol. Recent experiments[23] have shown that pronethalol possesses inherent sympathomimetic activity in its actions on the heart *per se*. Studies of the actions of pronethalol on metabolic processes in perfused guinea-pig heart (Table 2) reveal that pronethalol at a concentration of 5·0 μg/ml causes a mean increase of 16 per cent in the uptake of glucose from the medium. Although the effects of pronethalol on glycogen and glucose metabolism are not statistically significant, the sum effect suggests that pronethalol does in fact cause increased utilization of carbohydrate. However, pronethalol failed to cause an increase in lactate production in our experiments.

In perfused guinea-pig heart, the decrease in glycogen content, the increased glucose utilization and the increased lactate production in response to adrenaline (1 μg/ml) were partially antagonized by pronethalol. In contrast to these observations, in rat heart (Table 3) the same concentrations of pronethalol had little effect on the glycogen depletion caused by 0·5 μg/ml adrenaline. The increased glucose uptake due to adrenaline was reduced by 50 per cent; no antagonism of the increased lactate production was observed. Pronethalol would appear to be a less effective adrenaline antagonist in rat than in guinea-pig heart with respect to these metabolic parameters.

Table 2 shows that pronethalol failed to deplete the triglyceride content of the guinea-pig heart. The results are contrary to the sympathomimetic effects of the drug on carbohydrate metabolism, since the level of triglyceride in pronethalol-treated hearts was 2·8 times greater than the mean value in control perfused hearts. It would appear therefore, that the normal utilization of triglyceride by control perfused hearts was inhibited by the presence of pronethalol.

The inhibition of the mobilization of triglyceride by pronethalol is further reflected by the observations (Tables 2 and 3) that in both species adrenaline-induced mobilization of triglyceride is more than completely antagonized by the presence of pronethalol. It could more properly be stated that adrenaline partially antagonizes the inhibition of the utilization of triglyceride due to pronethalol.

Propranolol. Despite reports that propranolol has no sympathomimetic activity,[23, 24] in our experiments propranolol at a concentration of 1·0 μg/ml caused a mean depletion of 43 per cent in the glycogen content of guinea-pig heart (Table 2). Glucose uptake however was not altered, while a mean increase of 78 per cent in lactate production occurred. In this species the combination of 1·0 μg/ml adrenaline with propranolol, inhibited the decrease in glycogen content due to adrenaline by 74 per cent, the increased glucose uptake by 52 per cent and the increased lactate production by 90 per cent.

Propranolol was less effective in antagonizing adrenaline-accelerated carbohydrate metabolism in rat heart than in guinea-pig heart. In the rat heart, although propranolol partially antagonized adrenaline-induced changes in glycogen and lactate levels, it had no effect on the increased uptake of glucose due to adrenaline (Table 3).

Studies of the actions of propranolol on the concentration of triglyceride in heart reveal that this drug has an action similar to that of pronethalol, since it also spares the utilization of triglyceride. Table 2 shows that in guinea-pig heart propranolol (1·0 μg/ml) caused a 70 per cent increase in triglyceride. However, in the presence of 1·0 μg/ml adrenaline this increase was limited to 12 per cent. In the rat heart adrenaline caused a similar inhibition of the propranolol-induced increase in triglyceride (Table 3).

Propranolol (1·0 μg/ml), given together with adrenaline (0·5 μg/ml) decreased the level of FFA in rat heart to 45 per cent of that found after perfusion in the absence of drugs (Table 3). This result is similar to that obtained with pronethalol, and although these two β-receptor blocking drugs may promote triglyceride synthesis from FFA, another possible explanation is that these drugs are inhibiting the effects of endogenous catecholamines on FFA mobilization.

Dichlorisopropylnoradrenaline (D.C.I.). D.C.I., regarded as having the most powerful sympathomimetic action of all the β-receptor blocking drugs, appeared to cause only a small degree of carbohydrate utilization (Table 2). Glycogen levels in the guinea-pig heart were unchanged by perfusion with 10 μg/ml D.C.I., while glucose uptake was increased by approximately 2 μM/g. The level of lactic acid increased about 4 μM/g which is consistent with the increased glucose uptake.

In guinea-pig heart D.C.I. completely prevented the accelerated glucose uptake due to adrenaline (Table 2) while the decrease in glycogen content and increase in lactate production were only partially blocked.

Rat heart was much more resistant to blockade of adrenaline-stimulated metabolism by D.C.I., glycogen depletion was inhibited by 45 per cent, glucose utilization by 19 per cent, and no antagonism of the increased lactate production could be observed.

The suppression of the normal utilization of triglyceride in the perfused guinea-pig heart by pronethalol and propranolol also occurs in response to D.C.I. This drug increased the level of triglyceride in guinea-pig heart to 80 per cent above the control value. Adrenaline reduced this increase to 21 per cent above the control value (Table 2); in rat heart (Table 3) this increase was reduced to 37 per cent. The combination of D.C.I. and adrenaline in rat heart depleted the FFA content to 43 per cent of the level observed in hearts perfused with drug-free media. This action is similar to that seen with pronethalol and propranolol in this preparation.

Changes in amplitude of contraction in response to β-receptor blocking drugs and their combination with adrenaline

Of the three β-receptor blocking drugs examined, propranolol and D.C.I. initially caused an increase in the amplitude of contraction in perfused guinea-pig hearts. This result was not observed with pronethalol. All three drugs subsequently depressed the amplitude of contraction (Table 2). In both species pronethalol caused only a partial antagonism of the increased amplitude of contraction due to adrenaline, whereas D.C.I. and propranolol almost completely antagonized this effect (Tables 2, 3).

The effects on metabolism and amplitude of contraction in heart of the three β-receptor blocking drugs, both alone and in combination with adrenaline, are correlated in Fig. 1. All measurements of amplitude of contraction cited in the diagram are represented as percentage changes, comparing contraction size prior to and 10 min after drug administration. This figure demonstrates that a relationship exists between depression of the utilization of triglyceride and the decrease in the amplitude of contraction in both species. Thus where the amplitude of contraction is depressed triglyceride utilization is spared and vice versa.

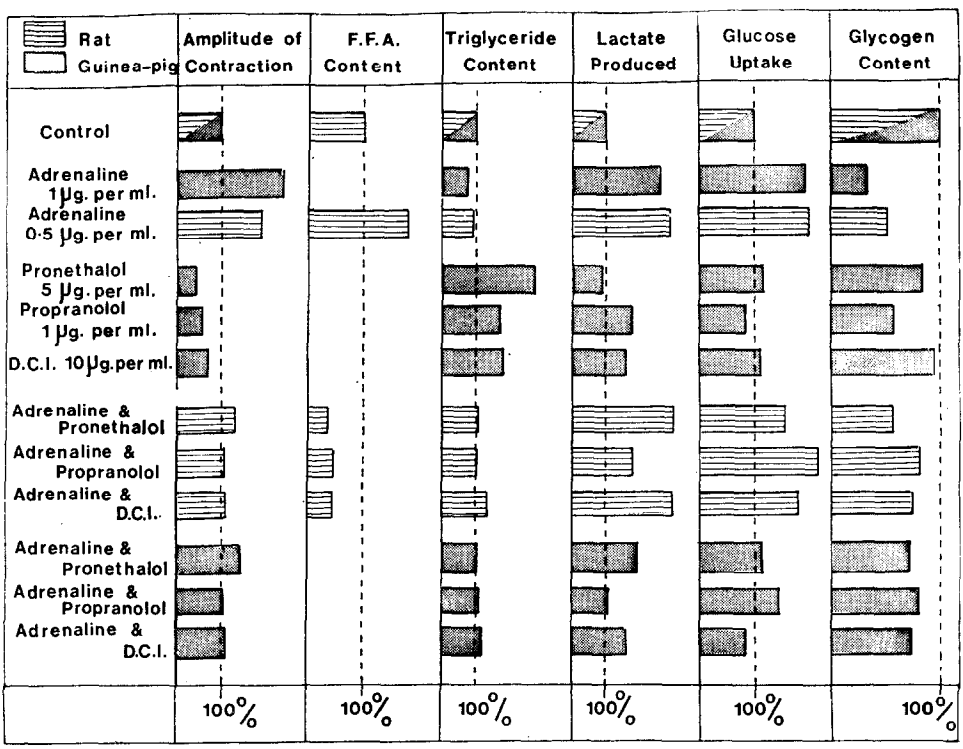

FIG. 1. A summary of the effects of β-receptor blocking drugs both alone and in combination with adrenaline on metabolism and contraction in perfused rat and guinea-pig hearts.

DISCUSSION

Studies of the isolated, perfused rat heart have shown that it utilizes considerable quantities of endogenous triglyceride and FFA, though relatively little carbohydrate. However, the increased force of contraction in response to adrenaline would appear to be energetically sustained largely by carbohydrate catabolism.

The effects of adrenaline on the aspects of cardiac metabolism which were studied were at least partially blocked by β-receptor blocking drugs and therefore the β-receptor would appear to play a large part in the mediation of the metabolic actions of adrenaline in the tissue. This is in agreement with the proposal[4] that the enzyme catalyzing the formation of cyclic 3′,5′-AMP may be synonymous with the β-receptor, since cyclic3′,5′,-AMP is known to promote increased utilization of carbohydrate by muscle and mobilization of FFA from adipose tissue. Levine and Vogel[25] have shown that cyclic3′,5′-AMP, when administered to the right atrium of unanaesthetized dogs, causes an increase in heart rate and cardiac output. Although this suggests that the cyclic nucleotide mediates the effects of catecholamines on both metabolism and contraction in heart, other workers have failed to observe an effect of cyclic 3′,5′-AMP on contraction.[7]

Of the three β-receptor blocking drugs, propranolol, pronethalol and D.C.I., the first caused the greatest depletion of glycogen and was also the most effective antagonist of the adrenaline-induced depletion of glycogen. Further, propranolol caused the smallest increase in glucose uptake and was the weakest antagonist of the adrenaline-induced increase in glucose uptake (Fig. 1). These effects seen in both species would suggest a difference in the mode of action of propranolol and the other two β-receptor blocking drugs. Bray[26] has shown that propranolol blocks adrenaline-induced lipolysis but not adrenaline-induced glucose oxidation in adipose tissue. He concluded that the lipolytic effects of adrenaline are dissociated from its effects on glucose oxidation. It is more likely however, that this result is solely due to the inability of this β-receptor blocking drug to block adrenaline-accelerated glucose uptake.

The initial increase in amplitude of contraction and the increased lactic acid production due to D.C.I. may be explained by the well known sympathomimetic actions of this drug. It was of interest to note that propranolol also exerted both these effects, and also caused depletion of glycogen despite reports that it lacks sympathomimetic activity.

It is not clear as to why hearts treated with β-receptor blocking drugs utilize less endogenous triglyceride. One explanation is that the amplitude of contraction is decreased under these conditions and that less triglyceride would be required to maintain a reduced supply of energy. This is emphasized by the relationship between the decreased amplitude of contraction and the decreased utilization of triglyceride, and vice versa in response to β-receptor blocking drugs and their combination with adrenaline. Alternatively, adrenaline causes mobilization of FFA from triglyceride, and antagonism of the actions of endogenous catecholamines by β-receptor blocking drugs might cause a decreased utilization of triglyceride.

The concept of the identification of the β-receptor with adenyl cyclase raises certain problems when one considers the various effects of the three β-receptor blocking agents. If these drugs have the same site of action then one would expect their effects to vary quantitatively but not qualitatively, depending upon the extent to which they are antagonists or partial agonists of the action of adrenaline. Qualitative differences, such as the failure of propranolol to antagonize the adrenaline-induced increase in glucose uptake, speak against such a unifying concept. While a final decision as to the metabolic point of attack of adrenaline and its antagonists must await a full elucidation of control points in glycolysis, and their relation to lipolysis, one may, on the present evidence, suggest that the β-receptor may be heterogeneous.

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