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DIFFERENCES IN DIHYDROERGOTAMINE ANTAGONISM OF GLUCOSE RELEASE BY CATECHOLAMINES, GLUCAGON AND ADENOSINE 3',5'-MONOPHOSPHATE IN RABBIT LIVER SLICES

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1 Quantitative studies were made on the glucose release from rabbit liver slices *in vitro* induced by a range of concentrations of (−)-adrenaline (Ad), (−)-isoprenaline (Iso), glucagon and adenosine 3',5'-monophosphate (cyclic AMP) in the presence and absence of several concentrations of dihydroergotamine (DHE).

2 DHE (3.16×10^{-6} M) shifted the Ad log concentration-response (LCR) curve to the right and also reduced the maximum response; at a higher concentration (3.16×10^{-5} M) it produced a greater shift to the right of the LCR curve and caused a reduction in the slope and a larger depression of the maximal responses. The LCR curve to Iso was similarly affected by this higher concentration of DHE.

3 DHE (1×10^{-5} M) produced no significant effect on the LCR curves of glucagon or cyclic AMP and even at 1×10^{-4} M DHE caused only a slight depression of the maximal responses to both agonists without any modification of the lower major portions of the curves.

4 These data indicate a selective antagonism by DHE at the rabbit liver adrenoceptor and, since the maximal responses to catecholamines were depressed by a lower concentration of DHE than was required to produce a slight depression of the responses to glucagon and cyclic AMP, the antagonism of DHE against catecholamines does not appear to be at a site beyond the formation of cyclic AMP, but rather at a site more intimately related to the adrenoceptor.

Introduction

Much of the uncertainty in the classification of the adrenoceptors involved in catecholamine-induced liver glycogenolysis is attributable to the fact that the ergot alkaloids were the first agents found to antagonize adrenaline hyperglycaemia (Miculicich, 1912) and, further, that the ergot derivatives may be effective antagonists in some species of animals when other α- and β-adrenoceptor antagonists are ineffective as inhibitors of adrenaline hyperglycaemia (Ellis, Anderson & Collins, 1953). Since it is generally believed that ergot and its dihydrogenated derivatives are specific α-adrenoceptor antagonists, the liver receptors have frequently been classified as α-adrenoceptors. There is also general agreement that the ergot alkaloids inhibit adrenaline-induced hyperglycaemia in all common laboratory animals, including the dog and cat in which the hepatic receptors have been found to be β-adrenoceptors (Ellis, Kennedy, Eusebi & Vincent, 1967). The species in which it has been most difficult to classify the adrenoceptors involved in activation of hepatic glycogenolysis are man, rat, mouse and rabbit (Ellis *et al.*, 1967).

Contrary to earlier findings in intact rabbits, our recent investigations of rabbit liver in slices demonstrated clearly that β-adrenoceptors are involved in activating glucose release in this tissue (Mühlbachová, Chan & Ellis, 1972). This conclusion was based on (a) the order of relative potencies of catecholamines ((−)-isoprenaline > (−)-adrenaline > (−)-noradrenaline); (b) the competitive antagonism of the effects of (−)-noradrenaline and (−)-isoprenaline by propranolol; and (c) the lack of antagonism of (−)-adrenaline-induced glucose production by phentolamine.

The glycogenolytic effects of adrenaline and glucagon in the liver are due to the activation of adenylate cyclase leading to an increased formation of adenosine 3',5'-monophosphate (cyclic AMP) which, through a complex cascade of kinases, activates the

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phosphorylase system (Sutherland, Robison & Butcher, 1968). The β-adrenoceptor is closely associated with the enzyme adenylate cyclase (Robison, Butcher & Sutherland, 1967) and cyclic AMP has been shown to induce glycogenolysis in rabbit liver slices *in vitro* (Sutherland, Øye & Butcher, 1965).

Dihydroergotamine (DHE) (Rothlin, 1947; Harvey, Wang & Nickerson, 1952; Ellis *et al.*, 1953; Wright, Jordan & Haight, 1958) and ergotamine (Miculicich, 1912; Pollak, 1922; Teschendorf, 1924; Rothlin, 1925; Harvey *et al.*, 1952) have been shown to antagonize adrenaline-induced hyperglycaemia in the rabbit. Furthermore, DHE selectively antagonized the hyperglycaemic response in the unanaesthetized rabbit and also the adrenaline-induced activation of glucose production in liver slices, without interfering with the same effects of glucagon (Ellis *et al.*, 1953). Similarly, in adenylate cyclase preparations from dog liver, DHE inhibited the stimulation of cyclic AMP production by adrenaline, but not that by glucagon (Sutherland *et al.*, 1965).

Since the glycogenolytic effects of both glucagon and adrenaline are thought to be mediated by cyclic AMP, the basis for selective antagonism by DHE in the rabbit of adrenaline but not of glucagon (Ellis *et al.*, 1953) required further investigation. There has been no general agreement as to which receptor DHE acts on to produce glycogenolytic blockade. Some investigators (Furchgott, 1959; Nickerson, 1967) seemed to favour the idea that the blocking effects of DHE, particularly the effects on carbohydrate metabolism and on certain muscular organs, do not involve the α- or the β-adrenoceptors, but may be exerted on another type of receptor. The conflicting views concerning the site of the antagonistic effects of DHE on drug-induced glycogenolysis have been reviewed (Ellis, 1967; Himms-Hagen, 1967).

The present study was designed to investigate the manner in which DHE antagonizes drug-induced glucose release from rabbit liver slices by obtaining accurate log concentration-responses to adrenaline, isoprenaline, glucagon and cyclic AMP in both the absence and the presence of several concentrations of DHE. A preliminary account of this work has been published (Chan, Ellis & Mühlbachová, 1969).

Methods

New Zealand white rabbits of either sex, weighing 2 to 5 kg, were maintained on Purina Rabbit Chow and tap water *ad libitum*. The animal was killed by a blow on the head, exsanguinated, and the liver was removed rapidly and kept in ice-cold 0.9% w/v NaCl solution (saline). The incubation medium (Sutherland & Cori, 1948) consisted of 1 part 0.1 M potassium phosphate buffer pH 7.4 and 4 parts 0.154 M NaCl. The mixture was bubbled with air for 10 min and adjusted to pH 7.4. Liver slices, approximately 0.5 mm thick and weighing 50 to 100 mg, were made with a Stadie–Riggs tissue slicer kept at 4°C. They were trimmed and placed in ice-bathed 20 ml Pyrex beakers containing 2 ml of incubation medium with or without dihydroergotamine. The beakers were kept for 60 min on ice in the cold room before the addition of the glycogenolytic agents.

For each point on the concentration-response curve three separate slices from the same liver were run in each complete agonist–antagonist experiment and each complete study was repeated on at least seven different rabbit livers. Glucagon, (−)-adrenaline bitartrate, (−)-isoprenaline hydrochloride or cyclic AMP, dissolved in incubation medium, was added in a volume of 0.2 ml immediately before incubation. The incubation, lasting 1 h, was carried out in air at 37°C in a Dubnoff metabolic incubator shaking at 70–80 oscillations per min. The same procedures were used by Mühlbachová *et al.* (1972).

At the end of the incubation, the liver slices were blotted with filter paper and weighed. The incubation medium was centrifuged at approximately 1500 *g* for 15 min and the supernate was analyzed for glucose by the method of Hoffman (1937) modified for the Technicon Auto-Analyzer.

Generous supplies of dihydroergotamine methanesulphonate as a crystalline material and in ampoules containing 1 mg/ml solution of dihydroergotamine mesylate (D.H.E. 45) were furnished by Sandoz Pharmaceuticals, Hanover, New Jersey. (−)-Isoprenaline hydrochloride and (−)-adrenaline bitartrate were obtained through the kindness of Dr F. P. Luduena, Sterling–Winthrop Research Institute, Rensselaer, New York. Glucagon (crystalline) and adenosine 3′,5′-cyclic monophosphoric acid (cyclic AMP) (crystalline) were purchased from Sigma Chemical Company, St. Louis, Mo.

Results

Dihydroergotamine interactions with (−)-adrenaline and with (−)-isoprenaline

Log concentration-response (LCR) curves of adrenaline (Ad) in the absence and presence of dihydroergotamine (DHE) are shown in Figure 1. Since DHE alone decreased the control glucose release about 10 to 15%, the values for the DHE plus agonist curves have been calculated as the difference between glucose release with DHE alone and glucose release in the presence of DHE plus agonist.

DHE at 3.16×10^{-6} M was sufficient to shift the position of the LCR curve of Ad to the right and also to produce a small depression of the maximal

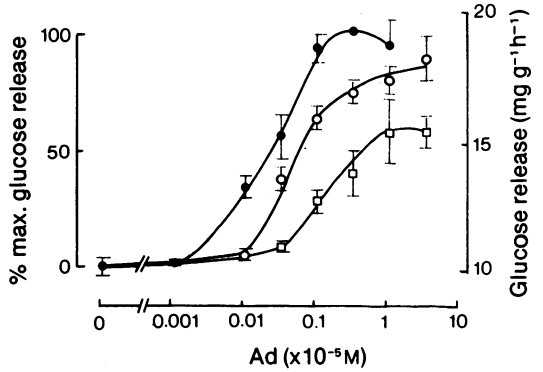

Figure 1 Interactions of adrenaline (Ad) with dihydroergotamine (DHE) on glucose release (as mg released per g wet wt. in 1 h) from rabbit liver slices *in vitro*. The liver slices were preincubated with or without DHE for 60 min in an ice-bath before addition of Ad. The incubation was carried out for 60 min at 37°C. (●) Ad alone; (○) Ad + DHE (3.16×10^{-6} M); (□) Ad + DHE (3.16×10^{-5} M). Vertical bars are standard errors (s.e.) of the mean value of 8 to 13 experiments.

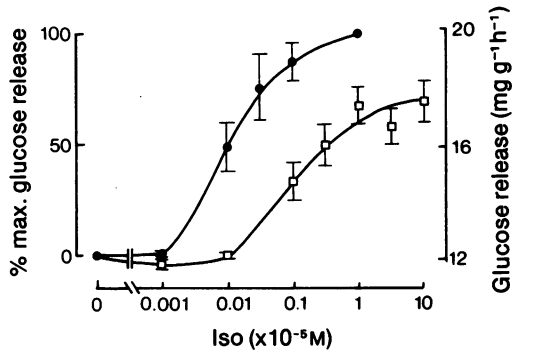

Figure 2 Interactions of isoprenaline (Iso) with dihydroergotamine (DHE) on glucose release (as mg released per g wet wt. in 1 h) from rabbit liver slices *in vitro*. The liver slices were preincubated with or without DHE for 60 min in an ice-bath before addition of Iso. (●) Iso alone; (□) Iso + DHE (3.16×10^{-5} M). The incubation was carried out for 60 min at 37°C. Vertical bars are standard errors (s.e.) of the mean value of 7 experiments.

response. The higher concentration of DHE (3.16×10^{-5} M) shifted the Ad curve further to the right and greatly depressed the maximal response.

DHE (3.16×10^{-5} M) also produced a shift to the right of the isoprenaline (Iso) LCR curve and depressed its maximal response to approximately the same extent as it had altered the Ad responses (Figure 2).

Dihydroergotamine interactions with glucagon and cyclic AMP

Higher concentrations of DHE were used in these experiments (Figure 3). First, it is evident that 1×10^{-5} M DHE had no significant effects on glucagon-induced glucose release, in contrast to the antagonism of Ad and Iso which had been observed with 3.16×10^{-6} M DHE. Even at 1×10^{-4} M, DHE caused only a small, insignificant depression of the maximal responses to glucagon but produced no real change in the lower portion of the LDR curve (Figure 3).

The results with DHE and cyclic AMP are shown in Figure 4. It is quite evident that DHE at 1×10^{-5} M did not significantly modify the position of the LDR curve of cyclic AMP or the maximum response to cyclic AMP and even at the highest concentration (1×10^{-4} M) the only significant effect on the response to cyclic AMP was a reduction of the maximum response.

Discussion

These quantitative experiments on rabbit liver slices support and extend previous qualitative studies which showed that DHE selectively antagonized the effect of Ad on rabbit blood glucose *in vivo* and on glucose release from rabbit liver slices without inhibiting the effect of glucagon (Ellis *et al.*, 1953). A similar selectivity of ergotamine was found in cat and dog liver preparations by Berthet, Sutherland & Rall (1957). Furthermore, it is evident from the present studies with cyclic AMP that low concentrations of DHE, which markedly inhibited Ad- or Iso-stimulated glucose release, produced little effect on the cyclic AMP-stimulated release of glucose.

In addition to these facts the type of antagonism produced by high concentrations of DHE against glucagon and cyclic AMP appears quite different from that against the catecholamines. The interaction of DHE with catecholamines was characterized by a shift to the right of the concentration-effect curve with a reduction of the maximal effect, the latter becoming more prominent at higher concentrations of DHE. Thus marked changes occurred in the responses to low effective concentrations of catecholamines. In contrast, the effect of DHE on both glucagon and cyclic AMP was to reduce only the levels of the maximal effects without significant changes in the lower portions of the concentration-response curves. Consequently, DHE had little influence even on moder-

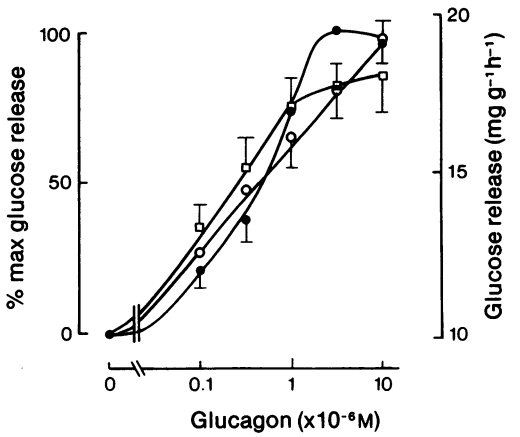

Figure 3 Interactions of glucagon with dihydroergotamine (DHE) on glucose release (as mg released per g wet wt. in 1 h) from rabbit liver slices *in vitro*. The liver slices were preincubated with or without DHE for 60 min in an ice-bath before addition of glucagon. (●) Glucagon alone; (○) glucagon + DHE (1×10^{-5} M); (□) glucagon + DHE (1×10^{-4} M). The incubation was carried out for 60 min at 37°C. Vertical bars are standard errors (s.e.) of the mean value for 8 experiments.

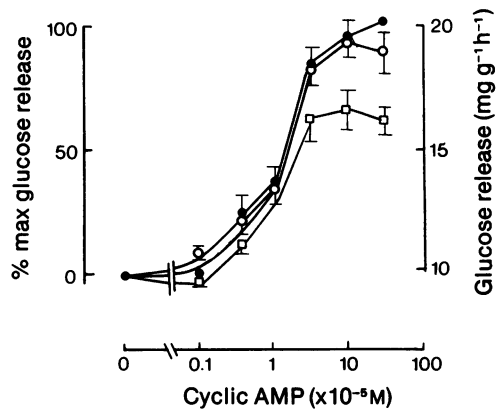

Figure 4 Interactions of cyclic AMP with dihydroergotamine (DHE) on glucose release (as mg released per g wet wt. in 1 h) from rabbit liver slices *in vitro*. The liver slices were preincubated with or without DHE for 60 min in an ice-bath before addition of cyclic AMP. (●) Cyclic AMP alone; (○) cyclic AMP + DHE (1×10^{-5} M); (□) cyclic AMP + DHE (1×10^{-4} M). The incubation was carried out for 60 min at 37°C. Vertical bars are standard error (s.e.) of the mean value of 8 experiments.

ately-effective concentrations of glucagon and cyclic AMP.

These findings could be interpreted as an action of DHE at a site close to the adrenoceptor. Such a hypothesis was put forward earlier to account for the fact that DHE selectively antagonizes catecholamine effects on the liver and blood sugar in rabbits without influencing those effects of glucagon (Ellis *et al.*, 1953; Berthet *et al.*, 1957). At higher concentrations of DHE there may also be an inhibition at a site beyond the formation of cyclic AMP thereby causing the reduction in the maximal responses to cyclic AMP and glucagon.

Contrary to this interpretation of the site of action of DHE are experimental findings on rat blood glucose (Northrop & Parks, 1964) and on perfused rat liver (Northrop, 1968) in which the responses to cyclic AMP are antagonized by the same dose of DHE as required to inhibit the response to Ad. It is known, as was indicated by Northrop & Parks, that the rat liver response to Ad is less sensitive to inhibition by DHE than is the rabbit liver. This might lead to the confusing situation in which the concentration of DHE required for influencing the adrenoceptor is high enough to suppress also the subsequent effect of cyclic AMP. At least such an interpretation is consistent with all the data, whereas an action of DHE solely on a step beyond the formation of cyclic AMP would account only for the observations in the rat liver. Moreover, some newer findings from this laboratory (Gothelf & Ellis, 1972) appear to conflict with the findings of Northrop & Parks since in these studies in rats the dose of DHE which inhibited Ad-induced liver glycogen depletion did not modify the glycogen-depleting effects of either cyclic AMP or glucagon.

Some reports of the use of ergot alkaloids on subcellular preparations are in keeping with DHE having a site of action close to the adrenoceptor and not on the phosphorylase-activating system subsequently influenced by cyclic AMP. Thus, Murad, Chi, Rall & Sutherland (1962) found that ergotamine, which did not directly depress the adenyl cyclase system from the dog liver, antagonized the stimulation of cyclic AMP formation by Ad. Furthermore, in the cyclic AMP assay system involving the complete system for the activation of phosphorylase, DHE did not produce any antagonism to added cyclic AMP (Greengard, Robison & Sutherland, 1968).

The characteristics of the antagonistic effect of DHE against the actions of Ad and Iso indicate a complex mode of action of DHE. The shift to the right of the lower portion of the LCR curves of the catecholamines and the depression of the maximal response suggest a combination of competitive and non-competitive antagonism. The antagonistic effect is most probably at the β-adrenoceptor, a site also suggested by Levy & Ahlquist (1961) based on their

observations of the antagonistic effects of DHE on the responses of the heart and intestine of the dog.

The effect of DHE on the responses to glucagon and to cyclic AMP has an unusual feature. The lower portions of the log concentration-response curves appear essentially unchanged from the control responses and only at the very high concentration of 1×10^{-4} M does DHE depress the maximal responses. This type of inhibitory effect has no simple explanation; but, since *in vivo* studies seldom involve maximal agonist effects, it is unlikely that this inhibitory effect of DHE against glucagon and cyclic AMP would be evident in intact animal studies.

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