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STIMULATION OF GLYCOGENOLYSIS AND GLUCONEOGENESIS BY EPINEPHRINE INDEPENDENT OF ITS BETA-ADRENERGIC FUNCTION IN PERFUSED RAT LIVER

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Abstract—A study was made of the relationship between the alpha and beta-adrenergic function and the stimulation of glycogenolysis and gluconeogenesis by catecholamines. Glycogenolysis was activated by epinephrine, norepinephrine and isoproterenol, with this decreasing order of potency. The activations of glycogenolysis by epinephrine and isoproterenol and of gluconeogenesis by epinephrine were not blocked by phentolamine, propranolol or both, despite complete blockade by propranolol of the epinephrine- and isoproterenol-induced increases in tissue cyclic AMP levels. Theophylline increased tissue cyclic AMP but was without effect on glycogenolysis and gluconeogenesis in either the normal or epinephrine-treated liver. These results not only are in accord with the view that the adrenergic receptor in the liver cannot be readily classified into an alpha- or a beta-receptor, but also suggest that intracellular cyclic AMP is not necessarily the only factor involved in the activation of glycogenolysis and gluconeogenesis by epinephrine in perfused rat liver.

It has frequently been observed *in vivo* and *in vitro* that alterations of the activity of carbohydrate metabolism induced by various catecholamines in the liver are not only resistant to adrenergic blocking agents but also, in magnitude, at variance with the relative potency of the catecholamines as adrenergic alpha- or beta-stimulants. Based on these observations, it is now accepted that the adrenergic receptor involved in the regulation of hepatic carbohydrate metabolism cannot be classified with ease as either an alpha- or a beta-receptor (see Ref. 1 for review). It is probable that the metabolic response of the liver observed *in vivo* is modified by a variety of extraneous factors originating from extrahepatic tissues, thereby obscuring the real properties of the response. However, studies with isolated liver preparations, despite their advantage in eliminating interference by such extraneous factors, have also afforded inconsistent results e.g. epinephrine-induced activations of gluconeogenesis and phosphorylase were relatively insensitive to the adrenergic beta-blockade in rat liver cells [2] and in perfused rat liver [3], whereas the stimulation of glycogenolysis by catecholamines was competitively inhibited by propranolol, a beta-blocker, in rabbit liver slices [4].

The purpose of the present paper is to study the relation of the beta-receptor-mediated function to the strong activation of glycogenolysis and gluconeogenesis induced by epinephrine in perfused rat liver.

METHODS

Livers from Wistar rats weighing 100–130 g were perfused by the technique of Mortimore [5]. The perfusion apparatus and techniques are the same as those described by Exton and Park [6], except that their rotating oxygenation chamber was replaced by the spiral tube [7] in our apparatus. Livers from fed rats were perfused by recirculating the perfusion medium consisting of Krebs–Ringer bicarbonate solution containing 3% bovine serum albumin (Fraction V, Sigma) and 20% bovine erythrocytes [8].

Since the previous studies showed that the responses of glycogenolytic [7, 9] and gluconeogenic [10, 11] activities in the perfused liver to epinephrine (or glucagon) become very pronounced after their basal activities have subsided to low levels following a 1-hr recirculation of perfusate, all the experiments in the present paper have been carried out according to the following protocol. Livers from fed rats were first perfused with the perfusion medium containing no substrate of gluconeogenesis for 1 hr and then [^{14}C-U]lactate (10 μCi/liver to make a final concentration of 20 mM) was added, with or without catecholamine. Adrenergic blocking agents, if necessary, were added 20 min before epinephrine (or other mimetic drugs). Glucose [12] and [^{14}C]glucose [13] in the perfusate were determined on small amounts (0·1 to 0·2 ml) of perfusate withdrawn at intervals. In some experiments, portions of minor hepatic lobes were removed at intervals by the double-ligation technique and the excised liver was quickly frozen in a clamp precooled in liquid N_2. The frozen liver was analysed for phosphorylase [14], glycogen synthetase [15] and cyclic AMP [15].

Sources of the reagents are: epinephrine tartrate, Merck; isoproterenol and norepinephrine, Sigma; propranolol, a kind gift from Ohtsuka Pharmaceutical Co., Tokushima, Japan; and phentolamine, Ciba-Geigy, Japan.

RESULTS

After a 1-hr pre-perfusion of the liver from the fed rat with the perfusion medium containing no lactate,

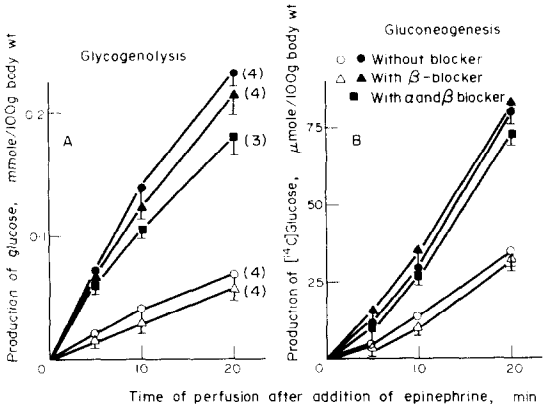

Fig. 1. Effect of epinephrine on glucose production (panel A) and gluconeogenesis (panel B) in the presence or absence of adrenergic blocking agents. The livers of fed rats were perfused for 1 hr in the medium without substrate and then [^{14}C-U]lactate (20 mM) with epinephrine, 5×10^{-6} M (solid symbols), or with saline (open symbols), was added. Zero time shows the time of addition of epinephrine or saline. Propranolol (5×10^{-5} M), phentolamine (5×10^{-5} M) or both were added 20 min before epinephrine or saline. Glucose production is expressed on the basis of 100 g body weight. The number of observations for each point in both panels is shown in parentheses in panel A.

[^{14}C]lactate was added to initiate gluconeogenesis from lactate (Fig. 1). Figure 1A shows the net liberation of glucose into the perfusate, while Fig. 1B shows the conversion of [^{14}C]lactate to perfusate glucose. Since livers from fed rats contained large amounts of glycogen (25–40 mg/g wet liver), most (roughly three-fourths) of the glucose liberated had originated from liver glycogen, as revealed by a comparison of Fig. 1A with 1B. (Hence, glucose production in panel A is referred to as 'glycogenolysis'; the same expression holds for the other figures.) As had been previously reported [10, 11], both glycogenolytic and gluconeogenic activities had subsided to a very low level during the 1-hr pre-perfusion and were maintained at this low level for further duration of perfusion. The addition of 5×10^{-6} M epinephrine after 1 hr (shown in figures as zero time) produced a 4- to 5-fold increase of glycogenolytic activity and about a 2·5-fold increase of gluconeogenesis. When 5×10^{-5} M propranolol, an adrenergic beta-receptor blocking agent, was added 20 min before epinephrine, there was essentially no change in the basal glycogenolytic and gluconeogenic activities nor was the epinephrine-induced activation of both pathways affected significantly.

It is known that epinephrine-induced hyperglycemia is completely abolished by the administration of both alpha- and beta-blockers but is only partially blocked by a beta-blocker alone [16, 17]. It would appear, therefore, that the effect of epinephrine on carbohydrate metabolism is mediated by an alpha-action. Therefore, the effect of phentolamine, combined with propranolol, on the epinephrine activation of glycogenolysis and gluconeogenesis was also studied in Fig. 1. Glycogenolysis activated by epinephrine was very slightly inhibited, but gluconeogenesis was not affected, by a simultaneous addition of phentolamine and propranolol. The liver perfused under the same condition as in Fig. 1A and 1B was excised 20 min after epinephrine and assayed for phosphorylase activity and the per cent of glycogen synthetase in the I-form (Table 1). Previous studies [7] showed that the rapid increase of glucose production caused by epinephrine in the perfused liver was associated with the increase in phosphorylase activity and with the I to D conversion of glycogen synthetase which occurred within 10 min after epinephrine addition. In accord with this, Table 1 shows that phosphorylase activity was higher and the per cent of glycogen synthetase in the I-form was lower 20 min after epinephrine than they were in the control liver. These epinephrine-induced changes in enzyme activities were not reduced by the combined addition of phentolamine and propranolol.

Figure 2A shows the dose-dependent activation of glycogenolysis by epinephrine. It is seen that the activation of glycogenolysis at 20 min is dose-dependent between 5×10^{-8} M and 2×10^{-6} M. Since metabolic changes induced by the non-saturating concentration of epinephrine are likely to be more readily attenuated by inhibitors or blockers than those induced by its 'over-saturation' concentration, we studied the effect of propranolol and phentolamine on the activation of glycogenolysis induced by 5×10^{-7} M epinephrine. The results shown in Fig.

Table 1. Effect of epinephrine on phosphorylase and glycogen synthetase I activity in the presence or absence of adrenergic alpha- and beta-blockers in perfused liver*

Addition	Phosphorylase (mmoles Pi liberated g tissue 15 min)	Glycogen synthetase (I-form, % of total)
Saline	0·11 ± 0·01 (4)	41·2 ± 3·1 (4)
Epinephrine	0·18 ± 0·02 (4)†	32·6 ± 2·9 (4)‡
Epinephrine, phentolamine, propranolol	0·17 ± 0·02 (3§	29 ± 2·7 (3§

* A portion of the rat liver, perfused under the same condition as in Fig. 1, was excised 20 min after the addition of saline or epinephrine and quickly frozen in liquid N_2. Frozen tissue was analyzed for enzyme activities, which are shown as mean ± S.E.M. (number of observations).

† Significantly different from saline (< 0·01).

‡ Significantly different from saline (< 0·05).

§ Not different from epinephrine (> 0·05).

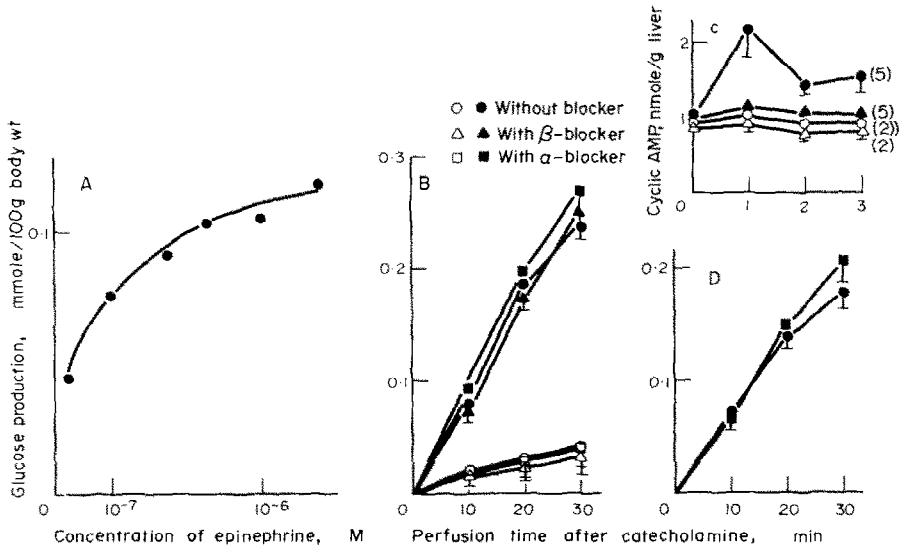

Fig. 2. Effects of catecholamines on glycogenolysis and cyclic AMP levels in the perfused rat liver. Livers from fed rats were perfused as in Fig. 1. In panel A, the difference of glucose production between the control and epinephrine-treated livers (epinephrine treatment, 20 min) is plotted against the concentration of epinephrine. In pabel B, 5×10^{-7} M epinephrine (solid symbols) or saline (open symbols) was added at zero time, with or without the addition of phentolamine or propranolol 20 min earlier. In panel C, 5×10^{-6} M epinephrine was used. In panel D, 5×10^{-6} M norepinephrine was used. The concentration of blockers is 10-fold higher than epinephrine or norepinephrine. Number of observations: four for each point in panels A, B and D, and as indicated in panel C.

2B reveal that neither propranolol nor phentolamine modified epinephrine action. Norepinephrine at a concentration of 5×10^{-6} M was also effective in increasing glycogenolysis, though to a lesser extent than epinephrine at the same molar concentration (Fig. 2D). This action of norepinephrine was not influenced by the addition of phentolamine at a 10-fold higher concentration. The level of cyclic AMP in the perfused liver increased very promptly in response to 5×10^{-6} M epinephrine with a peak increase (2-fold) at 1 min (Fig. 2C). This action of epinephrine was completely blocked by propranolol, which failed to block the stimulatory action of epinephrine on glycogenolysis and gluconeogenesis.

The effect of isopreoterenol on glycogenolysis and gluconeogenesis is shown in Fig. 3. The addition of isoproterenol, 5×10^{-6} M, to the perfusate caused an activation of glycogenolysis (panel A), but in a much smaller degree than epinephrine or norepinephrine, despite a greater increase by isoproterenol in the level of cyclic AMP (inset of panel B). The increase of cyclic AMP induced by isoproterenol was, but the activation of glycogenolysis was not, blocked by propranolol. Gluconeogenesis was not enhanced by isoproterenol as shown in panel B.

In order further to examine the apparent discrepancy thus far observed between metabolic alterations and tissue levels of cyclic AMP as regards the responses to catecholamines or blockers, theophylline was added to the perfusate 5 min before epinephrine and its effect on the activities of glycogenolysis and gluconeogenesis and the tissue level of cyclic AMP was studied (Fig. 4). Theophylline not only caused a slight but significant increase of tissue cyclic AMP level by itself, but also enhanced the epinephrine-induced increase of cyclic AMP (panel C). Nevertheless, the activation of glycogenolysis and gluconeogenesis caused by epinephrine was not affected by the pretreatment with theophylline. Though theophylline alone tended to increase glycogenolysis, this effect, along with the effect on gluconeogenesis, was not statistically significant.

DISCUSSION

The results in the present paper show that the order of potency of catecholamines was epinephrine >

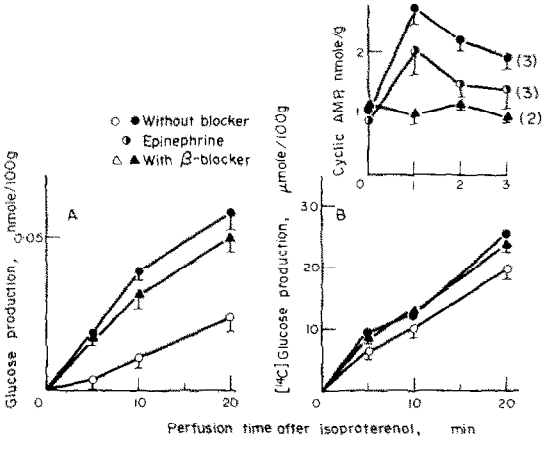

Fig. 3. Effect of isoproterenol on glucose production, gluconeogenesis and tissue cyclic AMP. Isoproterenol (5×10^{-6} M, solid symbols) was added at zero time. The effect of epinephrine is also recorded in the inset of panel B for comparison. Open symbols: neither isoproterenol nor epinephrine was added. The number of observations is four for each point in panels A and B, and as indicated in inset.

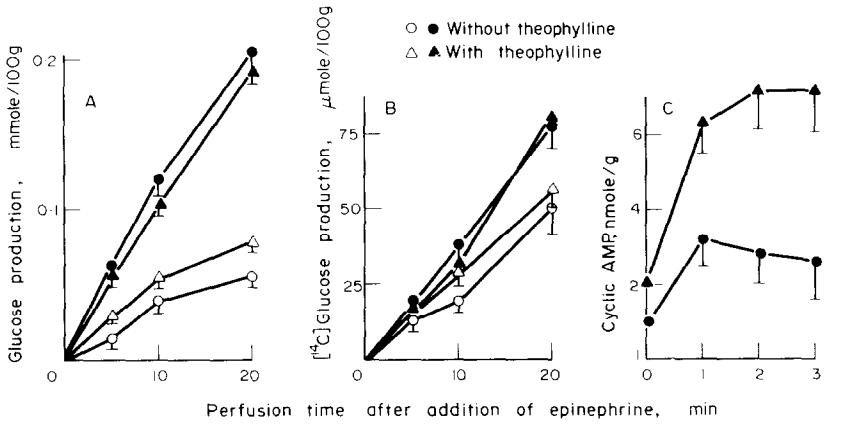

Fig. 4. Effect of epinephrine on glucose production, gluconeogenesis and tissue levels of cyclic AMP in the presence or absence of theophylline. Epinephrine (5×10^{-6} M, solid symbols) or saline (open symbols) was added at zero time and $1{\cdot}25 \times 10^{-3}$ M theophylline was added 5 min earlier. The number of observations is four.

norepinephrine > isoproterenol for the activation of glycogenolysis in the perfused rat liver. Epinephrine was much more effective than isoproterenol in stimulating gluconeogenesis as well. Isoproterenol was a more potent beta-adrenergic stimulant than epinephrine, as shown by the relative response of tissue cyclic AMP to these catecholamines. Although unlikely, the possibility cannot fully be excluded that isoproterenol has been converted to an apparently inhibitory metabolite during perfusion [18, 19], because the time course of glucose production in the presence of isoproterenol was not consistent with the typical pattern of transient activation followed by its rapid or gradual decrease (Fig. 3). Thus, the order of potency for the metabolic responses observed for catecholamines is at variance with their relative potencies as either a beta- or alpha-stimulant. Moreover, epinephrine-induced activation of glycogenolysis and gluconeogenesis was not blocked by propranolol, phentolamine, or both. Nor was the activation of glycogenolysis by norepinephrine and isoproterenol affected by phentolamine and propranolol respectively. These results obtained in perfused liver are in good agreement with the previous findings in liver free cells [2] and lend a strong support to the view [1] that the adrenergic receptor involved in the regulation of carbohydrate metabolism in rat liver cannot be readily classified into an alpha- or a beta-type.

It is widely accepted that epinephrine (and other beta-stimulants) activates adenylate cyclase, thereby increasing cellular cyclic AMP, which, in turn, leads to the activation of metabolic pathways such as glycogenolysis and gluconeogenesis through the phosphorylation of enzyme protein by cyclic AMP-dependent protein kinase. In the present paper, however, there are several cases in which metabolic pathways are activated by catecholamines without a detectable increase in tissue cyclic AMP, i.e. the activations of glycogenolysis by epinephrine and isoproterenol and of gluconeogenesis by epinephrine in the presence of a beta-adrenergic blocker. In addition, recent studies from this laboratory have shown that 3×10^{-8} M epinephrine failed to increase the level of cyclic AMP but activated phosphorylase during perfusion of the liver from hydrocortisone-treated rats [7]. Likewise, it was reported by Tolbert *et al.* [2] that 5×10^{-7} M epinephrine stimulated gluconeogenesis despite a lack of increase of cyclic AMP in isolated rat liver cells. On the other hand, there are also several cases in which the increase in tissue cyclic AMP does not lead to an increase in metabolic activities e.g. the addition of theophylline in the presence or absence of epinephrine produced essentially no increase in glucose production from either glycogen or lactate, despite a significant rise of tissue cyclic AMP. Similarly, there was no increase in gluconeogenesis and only a small increase in glycogenolysis after the addition of isoproterenol in such a concentration as to cause a marked rise of tissue cyclic AMP level.

Activation of metabolic pathways without a preceding increase of tissue cyclic AMP could be explained by the shift in intracellular cyclic AMP, or alternatively by assuming that there may be a cyclic AMP-independent mechanism in addition to a cyclic AMP-dependent one, as suggested by Tolbert *et al.* [2]. However, the inability of increased cyclic AMP to activate glycogenolysis, observed in the present study, is hardly accounted for by this assumption alone. Instead, it might be possible to speculate that not only the formation of cyclic AMP but also the action of cyclic AMP formed in the cell is favored by epinephrine (or by other catecholamines to a lesser extent). AMP formation is via the beta-adrenergic receptor but epinephrine activation of cyclic AMP action is independent of beta-adrenergic function. In accord with this speculation, modification of the action of cyclic AMP by calcium [20], glucocorticoids [21] and shifts of bodily pH [15, 22] has been reported. Work along this line is now in progress in our laboratory.

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