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INDUCTION OF PHOSPHOENOLPYRUVATE CARBOXYKINASE BY SYMPATHETIC AGENTS IN PRIMARY CULTURES OF ADULT RAT HEPATOCYTES

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SUMMARY

Phosphoenolpyruvate carboxykinase was studied in primary adult rat hepatocyte cultures maintained for 48 h. Between 48 h and 52 h norepinephrine (10^{-5} mol/l) and epinephrine (10^{-6} mol/l) in the presence of dexamethasone (10^{-8} mol/l) and insulin (10^{-9} mol/l) increased the enzyme activity about fourfold. This increase was prevented by cycloheximide. The induction by norepinephrine and epinephrine could be inhibited almost completely by the ß-blocking agent propranolol, while that by glucagon remained unaffected. The concentration dependence of enzyme induction may indicate that epinephrine might act as a circulating hormone, while norepinephrine might be operative as a neurotransmitter requiring higher local concentrations. The results are in line with the proposal that hepatic nerves might directly control gene expression.

INTRODUCTION

Phosphoenolpyruvate carboxykinase (EC 4.1.1.49) is a key enzyme of hepatic gluconeogenesis (1). It was found to be heterogeneously distributed over the liver parenchyma with threefold higher activities in the periportal zone (2,3). The overall level is regulated by the nutritional conditions (4-6) and by the concomitant activity changes of the endocrine (4,5) and autonomic nervous system (6). Injection of glucagon (4) and of epinephrine (6) induced and insulin (4,7) deinduced the enzyme. Electrical stimulation of the ventromedial hypothalamus and thus of the sympathetic system led to an increase, while stimulation of the lateral hypothalamus and thus of the parasympathetic system resulted in a decrease of enzyme activity (8). The mechanisms of hormonal and nerval regulation are poorly understood, since in whole animals the interdependence of different hormonal systems and the dependence of the endocrine on the nervous system make it difficult if not impossible to isolate a single effect.

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Most of these problems can be overcome by working with adult liver cells in culture. Studies with this system have corroborated that glucagon is an inducer and insulin a deinducer of phosphoenolpyruvate carboxykinase (9, 10). They failed, however, to establish that epinephrine is a direct inducer (9) and pointed to the possibility that the injected catecholamines (6) and the electrically stimulated sympathetic system (8) acted via the release of pancreatic glucagon.

In the present investigation it was found that epinephrine and norepinephrine can induce phosphoenolpyruvate carboxykinase in adult rat hepatocyte cultures. From the concentration dependence of the effects it is concluded that epinephrine acted as a circulating hormone and norepinephrine as a neurotransmitter. Some of the results have been reported in a preliminary abstract (13).

MATERIAL AND METHODS

Rat liver parenchymal cells were isolated from fed male Wistar rats and cultured on floating collagen gels as described by Katz et al (14). Hormones, sympathetic agonists and antagonists, and cycloheximide were added as indicated in the legends to figures and tables.

For the assay of phosphoenolpyruvate carboxykinase gels were washed in 0.9% NaCl and then homogenized using an Ultra-Turrax (Janke and Kunkel KG, D-7813 Staufen) in Tris·HCl 0.5 mol/l pH 8.1 containing $MnCl_2$ 2.5 mmol/l and dithioerythritol 10 mmol/l. Phosphoenolpyruvate carboxykinase was assayed according to Seubert and Huth (15). DNA was estimated as described (9). Epinephrine and norepinephrine were determined fluorometrically (16).

The materials used were obtained as reported (14). Epinephrine and norepinephrine were supplied by Serva, D-6900 Heidelberg. Phenoxybenzamine and propranolol were purchased from Röhm-Pharma, D-6100 Darmstadt and Rhein-Pharma, D-6831 Plankstadt; BAL = 2,3-Dimercaptopropanol (1) was obtained from Merck AG, D-6100 Darmstadt.

RESULTS

In primary adult rat hepatocyte cultures maintained for 48 h epinephrine and norepinephrine increased phosphoenolpyruvate carboxykinase activity about fourfold (Fig. 1). This increase was almost linear with time reaching a maximum after about 4 h; it could be prevented by cycloheximide. The time dependence of the increase and its prevention by a protein synthesis inhibitor indicated that the effect was due to an induction of the enzyme.

Enzyme induction by epinephrine (10^{-6} mol/l) was inhibited by equimolar concentrations of the ß-blocking agent propranolol; it remained unaffected by the α-blocking agent phenoxybenzamine (Fig. 2).

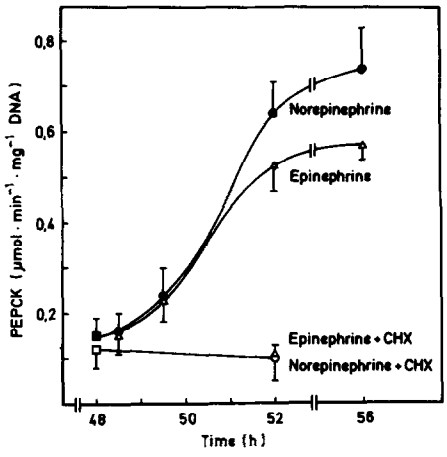

Fig. 1. Induction of phosphoenolpyruvate carboxykinase in primary cultures of hepatocytes by sympathetic agents: Liver parenchymal cells were cultured under standard conditions in the presence of insulin (10^{-9} mol/l) and dexamethasone (10^{-8} mol/l). 4 h, 24 h and 48 h after plating the medium was changed. At 48 h the following additions were made as indicated: norepinephrine (10^{-5} mol/l) or epinephrine (10^{-6} mol/l) ± cycloheximide (CHX, 10 mg/ml). Values are means ± SEM of 6 cultures from 2 representative experiments.

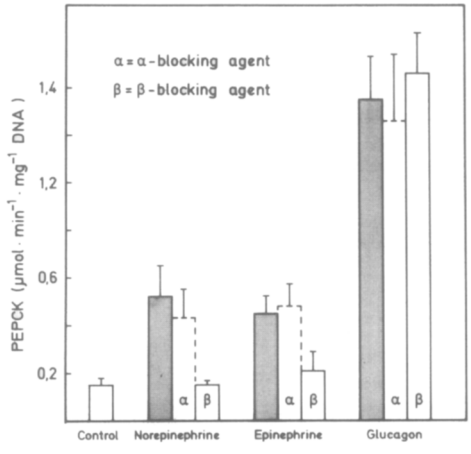

Fig. 2. Induction of phosphoenolpyruvate carboxykinase in cultured hepatocytes by catecholamines via ß-receptors. Liver parenchymal cells were cultured under standard conditions in the presence of insulin (10^{-9} mol/l) and dexamethasone (10^{-8} mol/l). 4 h, 24 h and 48 h after plating the medium was changed. At 48 h the following additions were made as indicated: Norepinephrine (10^{-5} mol/l) ± phenoxybenzamine (10^{-5} mol/l; α-blocking agent = α) or propranolol (10^{-5} mol/l; ß-blocking agent = ß); epinephrine (10^{-6} mol/l) ± phenoxybenzamine (10^{-6} mol/l) or propranolol (10^{-6} mol/l); glucagon (10^{-8} mol/l) ± phenoxybenzamine (10^{-6} mol/l) or propranolol (10^{-6} mol/l). Enzyme activity was measured at 52 h. Values are means ± SEM of 6 cultures from 2 representative experiments.

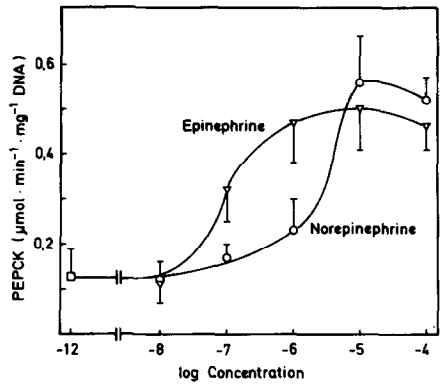

Fig. 3. Dependence of phosphoenolpyruvate carboxykinase induction on the concentration of norepinephrine or epinephrine. Liver parenchymal cells were cultured as described in figure 2. Sympathetic agents were added at 48 h. Enzyme activity was measured at 52 h. Values are means ± SEM of 6 cultures from 2 representative experiments. The catecholamine concentrations added at the beginning of induction are indicated on the abscissa; the average concentrations during the induction period were about 20% lower due to catecholamine degradation (Table 1).

Induction by norepinephrine (10^{-5} mol/l) could also be blocked by equimolar levels of propranolol; it was only slightly diminished by phenoxybenzamine. Induction by glucagon (10^{-8} mol/l) could not be influenced even by a 100fold molar excess of either blocking agent. It could therefore be concluded that the induction by catecholamines was effected via ß-receptors.

Induction by epinephrine and norepinephrine was different as to its concentration dependence (Fig. 3). The degradation of catecholamines during the experimental period was in the range of 40% to 50% (Table 1). Thus the average concentrations during the induction were about 25% below the initial concentrations. With epinephrine induction increased from 10^{-8} mol/l to a plateau at 10^{-6} and 10^{-5} mol/l. With norepinephrine it was low at 10^{-7} mol/l; it then rose sharply to a plateau at 10^{-5} and 10^{-4} mol/l. The concentration dependence just reached the physiological range of blood levels (17) with epinephrine, while it remained far outside that range with norepinephrine.

DISCUSSION

In this communication it was shown that phosphoenolpyruvate carboxykinase could be induced in primary cultures of adult rat

Table 1 Degradation of norepinephrine and epinephrine by adult rat hepatocytes in primary culture

Time after addition	Norepinephrine		Epinephrine	
h	µmol/l	µmol/l	µmol/l	µmol/l
0	9.9 ± 0.16	1.0 ± 0.04	1.0 ± 0.01	0.1 ± 0.001
0.5	8.2 ± 0.39	—	0.81 ± 0.02	—
1.5	7.6 ± 0.51	—	0.67 ± 0.02	—
3	5.9 ± 0.19	—	—	—
4	—	0.69 ± 0.04	0.47 ± 0.01	0.05 ± 0.002
5	5.3 ± 0.44	—	—	—

The experimental conditions were the same as described in fig. 1. Catecholamine concentration was determined fluorometrically (see METHODS). Values are means ± SEM of 6 cultures from 2 representative experiments.

hepatocytes by epinephrine and norepinephrine (Fig. 1) via ß-receptors (Fig. 2). Previously the enzyme was reported not to be induced by epinephrine in adult rat hepatocyte cultures under conditions that allowed induction by glucagon (9); norepinephrine was not used in that study. The advantage of the present culture system may perhaps be due mainly to the different hormone composition of the media used; in this investigation 100fold lower insulin concentrations and constant levels of dexamethasone were applied. Apparently there was no significant difference in the induction of phosphoenopyruvate carboxykinase by epinephrine in cultures of adult (this study) and fetal hepatocytes (18, 19); norepinephrine was not investigated in the fetal liver cell culture.

Induction of the enzyme in the cell cultures was possible with epinephrine concentrations that just reached the physiological range of blood levels (Fig. 3). It may therefore be concluded that circulating epinephrine released from the renal medulla or injected into adult (6) or fetal (20) animals could act as an inducer directly on the liver cell rather than indirectly via a stimulated release of pancreatic glucagon.

With norepinephrine enzyme induction could be effected only by concentrations that were outside the physiological range of blood levels (Fig. 3). If it is assumed that the sensitivity of the norepinephrine receptors on the cultured hepatocytes was similar to that in the intact animal, two conclucions may be

drawn: 1. Circulating norepinephrine released from the renal medulla and as overflow from sympathetic nerve endings (17) should not function as a direct inducer. The situation may be different during injection of norepinephrine (6, 20), if unphysiologically high blood levels are reached. 2. Since hepatocytes appear to be innervated both by the sympathetic and parasympathetic nervous system (12), the "low" sensitivity norepinephrine receptor may function within a synapse as the target of the neurotransmitter norepinephrine rather than within the sinusoids as the target of the circulating hormone norepinephrine. Within a synapse neurotransmitter concentrations appear to be in the range of 10^{-4}-10^{-3} mol/l (21) allowing for "low" sensitivity receptors.

Liver metabolism may thus be under direct neural control both with the short-term regulation of a particular pathway and with the long-term regulation of the cellular enzyme pattern. Evidence for an acute direct neural regulation is already available; glycogenolysis could be enhanced by electrical stimulation of perivascular nerve bundles of mouse liver perfused in situ (22). Evidence for the direct neural control of gene expression in liver is still missing.

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