Biochemical Pharmacology, 1966, Vol. 15, pp. 129–136. Pergamon Press Ltd., Printed in Great Britain.

HORMONAL CONTROL OF THE GLYCEROL-1-PHOSPHATE SYSTEM BY THYROID HORMONES

EXPERIMENTS WITH ISOLATED PERFUSED RAT LIVERS

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(*Received* 9 *September* 1965; *accepted* 7 *October* 1965)

Abstract—This paper describes the metabolic state of isolated perfused rat livers from hyper- and hypothyroid animals. Livers from hyperthyroid animals very rapidly take up lactate and pyruvate from the perfusion medium. On the other hand, in the state of hypothyreosis, lactate is released in small quantities from the liver into the perfusion medium.

In livers from hyperthyroid animals, the substrate content is generally reduced. In the state of hypothyreosis, substrates accumulate in the liver. Glycerol-1-phosphate is particularly affected by these substrate fluctuations; the ratio glycerol-1-phosphate/dihydroxyacetone phosphate (G/D)† also shifts from a normal 7 to 18 in hypothyreosis and to 3 in livers from hyperthyroid rats.

In experiments with livers from normal or thyroidectomized rats, the addition of triiodothyronin also affects particularly the level of glycerol-1-phosphate. In the state of hypothyreosis, TIT markedly reduces the high content of glycerol-1-phosphate, whereas in livers from normal animals a relative increase of this substrate after perfusion is observed.

IN ORDER to keep a rat alive at a normal basal metabolic rate only 0·7 μg thyroxin is required daily.[1, 2] This corresponds to 1 mμmole thyroxin or 6×10^{14} molecules daily. From the data of Allard and Estabrook[3] it can be estimated that the liver of a 200 g rat contains 3×10^{12} mitochondria. If the daily dose of thyroxin is used up only by the liver then 1 mitochondrion would require 200 molecules of thyroxin. The data of Estabrook further indicate that 1 mitochondrion contains about 1,000 molecules of cytochrome *a*, and accordingly approximately the same quantity of respiratory chains.

This calculation does not imply that the action of thyroxin is limited only to the

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† Abbreviations used in the text or tables:

ATP/ADP	Adenosintriphosphate/adenosindiphosphate
G/D	Glycerol-1-phosphate/dihydroxyacetonephosphate
L/P	Lactate/pyruvate
αGP	Glycerol-1-phosphate
DAP	Dihydroxyacetonephosphate
G6P	Glucose-6-phosphate
FDP	Fructose-1,6-diphosphate
GPox	Glycerol-1-phosphate oxidase (Meyerhof-Green-enzyme)
TIT	Tri-iodothyronine
nmoles	mμmoles

liver or to the mitochondria, and demonstrates only the relationship between the very small number of hormone molecules that are necessary and the great number of possible effector sites. Such calculation seems permissable for thyroxin because the turnover of this hormone is high. In rats the half life of thyroxin amounts to only 7 hr[4].

The contrast between the number of effector molecules and possible receptor sites, the multitude of all the known and varied effects of thyroxin such as changes in enzyme activities[5] would be explained. In consequence of our calculation, most of these observed effects of thyroxin are secondary. The problem therefore is to find the earliest and the specially important effects of the hormone in metabolism, from which further regulation of other metabolic chains is possible. It is very difficult if not impossible to find and characterize these essential control points in experiments *in vivo*. We cannot differentiate single effects of hormones *in vivo* without interference of other hormones or cross regulation, and isolated perfused rat liver was used to study the general questions of the regulation and co-ordination of the liver metabolism since the perfused organ remains comparable in many respects to the liver *in vivo*.[6]

MATERIALS AND METHODS

The experiments were performed with isolated perfused rat livers. The technique of perfusion is described[6] and the substrates were determined enzymatically.[7, 8] Male Wistar rats weighing 170–240 g were used (strain Brünger 46, Brünger, Halle/Westf., Germany). Hyperthyreosis was achieved by feeding rats with thyroid powder, 1% in standard rat food (Thyroid USP of the Nutrit. Biochem. Corp., Cleveland, Ohio). The animals were used in experiments after feeding over a period of 8–10 days. Hypothyreosis was reached by thyroidectomy and the animals were used experimentally 14–21 days after the operation. 3,3′5′-triiodothyronine was the product from Sigma (St. Louis, Missouri). Experiments *in vitro* with TIT were made by a single addition of TIT into the perfusion medium (after an equilibration period of the isolated organ of one hour, see reference 6).

In some experiments, lactate (California Biochem. Corp.) was added to the perfusion medium by continuous infusion of 500 μmoles/hr/100 ml medium (the addition followed the one hour equilibration perfusion).

RESULTS

(a) *Blood substrates.* Perfusion experiments showed that the isolated liver of normal animals is capable of regulating and co-ordinating substrate levels like lactate and pyruvate inside and outside of the cell.[9] Neither in the state of hyper- or that of hypothyreosis is the isolated liver in a position to regulate and hold at a constant level the contents of lactate and pyruvate in the perfusion medium as livers from untreated rats do. In accordance with the opposite hyper- or hypothyroid metabolic state *in vivo*, different proportions of these substrates in the blood were also found *in vitro*.

The liver of a hyperthyroid animal absorbs lactate and pyruvate very rapidly from the external medium (Fig. 1). That means the turnover of these substrates is very high. The ratio lactate/pyruvate in the perfusion medium, which reflects the state of this system in the liver too,[9] decreases in the course of perfusion from high values (corresponding to values of hyperthyroid rats *in vivo*) to the normal ratio of 10. Therefore, the high reduction of the system lactate/pyruvate in livers of hyperthyroid

animals *in vivo* must be influenced by the whole body and not affected by the liver metabolism alone. In the case of hypothyreosis, lactate is even released from the liver into the perfusion medium and the proportion lactate/pyruvate is likely to rise during perfusion (Fig. 2). The contradictory relationship in liver metabolism between the liver of a hyper- and hypothyroid rat can also be seen in respect to the lactate uptake

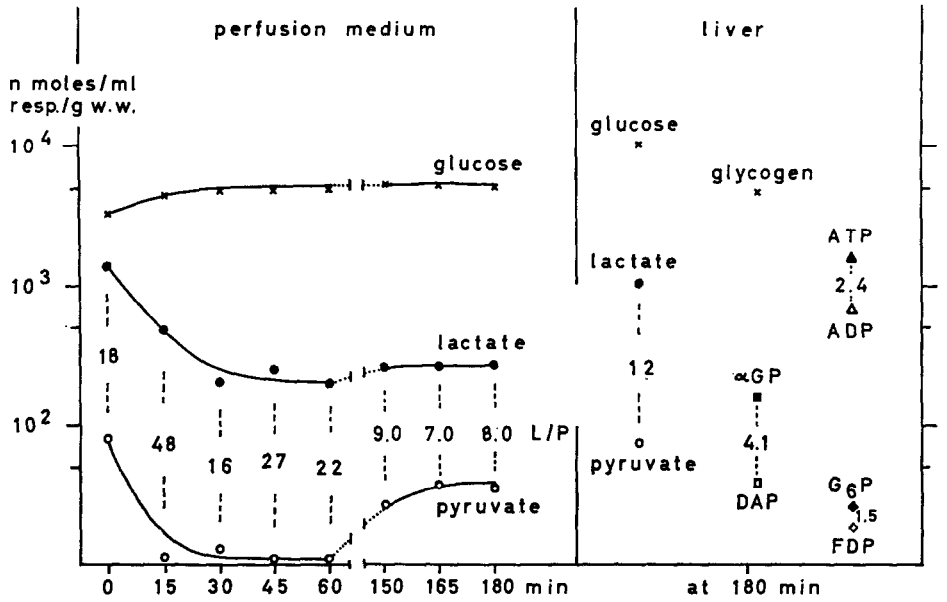

FIG. 1. Hyperthyreosis: Content and ratios of substrates in perfusion medium and in rat liver after perfusion.

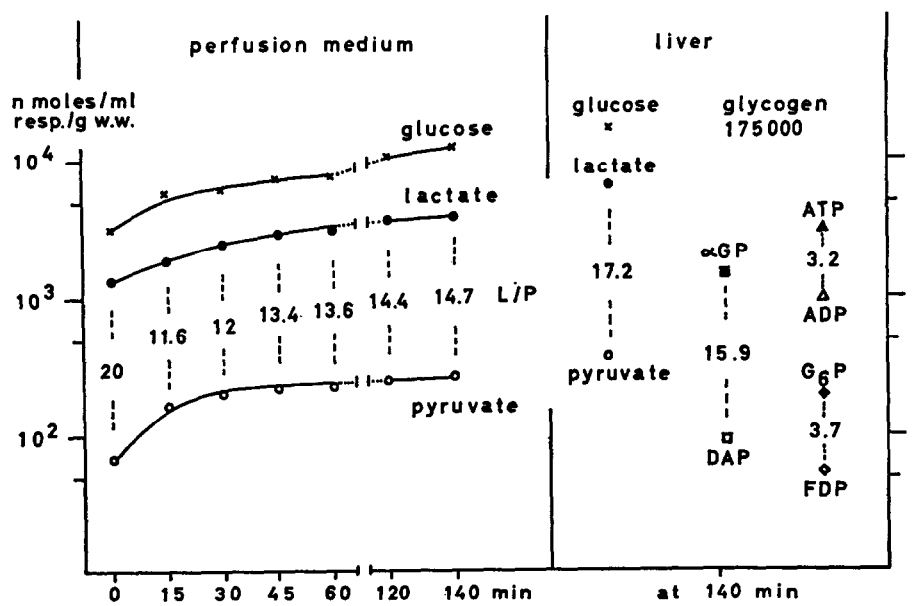

FIG. 2. Hypothyreosis: Content and ratios of substrates in perfusion medium and in liver after perfusion.

under continuous infusion of lactate. Under these experimental conditions the liver of a normal rat takes up 20 μmoles of lactate/g/hr,[10] whereas the liver of a hyperthyroid rat takes up three times more lactate from the perfusion medium (see Table 1). In the hypothyroid state the lactate uptake is decreased by 85% as compared with livers from untreated rats (Table 1). Glucose is not absorbed as rapidly as a suitable substrate by the liver in the state of hyperthyreosis, although the level of glycogen is extremely low in the livers of hyperthyroid animals they give up glucose into the perfusion medium (see Fig. 1 and Table 1).

TABLE 1. CHARACTERISTIC DATA OF DIFFERENT METABOLIC STATES IN THE ISOLATED PERFUSED RAT LIVER

	Control	Hyperthyreosis	Hypothyreosis
O_2-consumption:			
μ moles O_2/g/min	2·2	4·0 (+80%)	1·9 (−15%)
Substrate content:			
n moles/g wet wt.)			
Glycogen	185×10^3	$2{\cdot}6 \times 10^3$	160×10^3
Lactate	3400	970	6500
Pyruvate	345	93	360
Glycerol-1-P	450	145	1460
Dihydroxyacetone-P	67	41	80
Fructose-1.6-P	41	26	50
Glucose-6-P	200	120	124
Substrate uptake:			
Lactate (n moles/g/hr)	20×10^3	60×10^3	3×10^3
Ratios of substrates:			
Lactate/pyruvate	10·0	10·8	16
Glycerol-1-P/dihydroxyacetone-P	6·5	3·5	18
ATP/ADP	3·3	2·3	3·3

(b) *Substrate content in livers after perfusion.* The changes of the substrates in the perfusion medium reflect the substrate level in the perfused livers. In addition to the well-known effects on the glycogen levels, there is a state of hyperthyreosis, a general decrease of substrates and a substrate accumulation in the livers of hypothyroid animals (Table 1). The system glycerol-1-phosphate/dihydroxyacetone-phosphate (G/D) is particularly affected by these conditions. In livers of normal untreated rats the ratio G/D is 7, in the state of hypothyreosis 18, and in the hyperthyroid liver 3. This results from the fact that the content of glycerol-1-phosphate is particularly decreased under the effect of thyroxin and considerably increased in the case of hypothyreosis.

The marked reduction of the content of glycerol-1-phosphate in the state of hyperthyreosis is better demonstrable in experiments with a continuous infusion of lactate. Normally, in the livers of untreated rats, the substrate content, including glycerol-1-phosphate, rises slightly under the continuous infusion of 500 μmoles L-lactate (Table 2). Also the ratio lactate/pyruvate (L/P) and G/D are a little higher compared with control experiments without addition of lactate (see also reference 10). In perfusion experiments with livers of hyperthyroid rats the continuous substitution of lactate prevents the reduction of the lactate and pyruvate levels. Nevertheless the

content of glycerol-1-phosphate is very low and also the ratio G/D is low, especially in relation to the L/P (Table 2). The system L/P does not change in the same manner as the system G/D. These two systems are at least functionally different and not strictly co-ordinated as mentioned by others.[7, 11]

TABLE 2. CONTENT AND RATIOS OF SOME SUBSTRATES IN PERFUSED RAT LIVERS AFTER CONTINUOUS INFUSION OF 500 μMOLES L-LACTATE PER HOUR INTO PERFUSION MEDIUM (EXPERIMENTAL DETAILS SEE 'METHODS'). DETERMINATION OF LIVER SUBSTRATES WERE MADE 2 HR AFTER BEGINNING LACTATE INFUSION

Experimental conditions	Substrates (mμmoles/g wet wt.) Lactate	Glycerol-1-phosphate	L/P	G/D	ATP/ADP
Untreated rats	4620	690	12	9	3·5
Hyperthyreosis	1550	135	12	4	2·6

Effects of triiodothyronin

(a) *Pre-treatment* in vivo. Thyroidectomized rats received intraperitoneally an injection of 35 μg triiodothyronin (TIT) 48 hr before the experiment. In the livers of these pretreated animals no substrate accumulation during perfusion was found as occurs in the hypothyroid rats (Table 3). The substrate levels are markedly reduced and the metabolic state is shifted nearly to that of normal livers from control rats.

TABLE 3. COMPARISON OF CONTENTS AND RATIOS OF SOME SUBSTRATES IN ISOLATED PERFUSED RAT LIVERS IN THE STATE OF HYPOTHYREOSIS AND AFTER PRETREATMENT BY A SINGLE INJECTION OF 35 μg TIT I.P., 48 HR BEFORE PERFUSION. ANALYSES MADE AFTER 2 HR OF PERFUSION

Experimental conditions	Substrates (mμmoles/g wet wt.) Lactate	Glycerol-1-phosphate	L/P	G/D	ATP/ADP
Hypothyreosis	6500	1460	16	18	3·3
Hypothyreosis + TIT (i.p.)	1800	350	12	8	3·2

(b) *Direct effects of TIT* in vitro. TIT added to the perfusion medium in the concentration of 4×10^{-6} M did not produce an increase in oxygen consumption or an uncoupling effect in isolated livers from normal or hypothyroid animals. However, the substrate levels showed changes which again particularly affected the glycerol-1-phosphate system.

In Table 4 is compared the content of the two end products of the glycolytic chain, lactate and glycerol-1-phosphate, under the direct influence of TIT. Whereas in the perfusion with TIT the levels of lactate are diminished in livers of untreated and of hypothyroid animals, the effects of TIT on the content of glycerol-1-phosphate are not always the same. They vary with the metabolic state of the isolated system.

TIT effects a marked reduction of the glycerol-1-phosphate level in the livers of hypothyroid animals. On the other hand, in livers from untreated rats we find a relative elevation of glycerol-1-phosphate after perfusion with TIT. The ratio ATP/ADP remains at about 3·0 in all experiments, which means that there is no uncoupling effect.

TABLE 4. DIRECT EFFECTS OF TIT ON THE CONTENT OF LACTATE AND GLYCEROL-1-PHOSPHATE IN LIVERS OF NORMAL AND HYPOTHYROID RATS AFTER PERFUSION WITH TIT (4×10^{-6} M) FOR 2 HR

Experimental conditions	Substrates (mμmoles/g wet wt.) Lactate	Glycerol-1-phosphate	ATP/ADP
Hypothyreosis	6500	1460	3·3
Hypothyreosis + TIT	4300	700	3·0
Untreated rats	3400	450	3·3
Untreated rats + TIT	2300	450	3·0

DISCUSSION

The simplest explanation of the differences in the level of substrates in the state of hyper- and hypothyreosis might be provided by the change in the oxygen consumption, which is, as is well known, above normal in hyperthyreosis and below normal in hypothyreosis. However, a higher uptake of lactate and a lower substrate content can also be found in perfusion experiments in the livers of rats after starvation for 16–20 hr.[10]

Nevertheless the change which takes place in the content of glycerol-1-phosphate, and thereby in the ratio G/D without being accompanied by a simultaneous change in the system L/P may be considered of importance and particular interest.

A possible explanation of these findings is offered by the results of the work of Lardy and co-workers,[12, 13] who have described a large increase of the mitochondrial enzyme glycerol-1-phosphate oxidase under the influence of thyroxin. Contrary to this, the hypothyroid state displays hardly any detectable activity of this enzyme in the liver (see also reference 14). The formulation of a connection between the two parameters—enzyme activity and substrate level—is, however, likely to require further evidence because, as was mentioned at the beginning of our presentation, changes of enzyme activities in the state of hypo- and hyperthyreosis have been recorded for many enzymes. Nevertheless, the enzyme glycerol-1-phosphate oxidase seems to hold a special position with regard to thyroid hormones. No other enzyme activity exhibits, in the state of hyperthyreosis, such comprehensive changes as that of glycerol-1-phosphate oxidase. Furthermore, no other hormone is known to stimulate the activity of glycerol-1-phosphate oxidase to the same extent as that of the thyroid gland. The most specific effect of thyroxin, apart from the change in the basal metabolic rate, therefore seems to be the change produced in the activity of glycerol-1-phosphate oxidase (and these with that combined substrate effects).

Comparing Lardy's findings on the different activities of glycerol-1-phosphate oxidase with our own data shows that the change in the glycerol-1-phosphate level corresponds to those which might have been predicted on the basis of the change in the enzyme activity. But there is no constant equilibrium between the G/D system and

other cytoplasmatic DPN/DPNH dependent systems like L/P, as described.[7, 11] Similar evidence and conclusions, viz. that the G/D system is an independent one, are also given by Hoberman.[15] The described direct effects of TIT *in vitro* are chronologically among the most rapidly appearing effects of the thyroid hormones[16]. The effects of the thyroid hormones on the glycerol-1-phosphate system (changes in enzyme activities and substrate levels) appear more specific than the stimulating effect on protein synthesis.[16] One of the functions of the glycerol-1-phosphate system[17, 18, 19] is hydrogen transport to the respiratory chain via the glycerol-1-phosphate. Accordingly, glycerol-1-phosphate is a hydrogenated end product of the Embden–Meyerhof chain with a close connection to the respiratory chain. Another important role of the system is its connection with lipid metabolism and coupling substrate with other metabolic chains. At present we do not know the complete significance of the glycerol-1-phosphate system. It may be one of the important metabolic 'switches' (like the role of G6P-FDP[20–22]).

Acknowledgement—This research was supported by a grant from the Deutsche Forschungsgemeinschaft.

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