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THE INFLUENCE OF THE β-SYMPATHICOLYTIC AGENT PROPRANOLOL ON GLYCOGENOLYSIS AND GLYCOLYSIS IN MUSCLE, BRAIN AND LIVER OF WHITE MICE

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Abstract—The influence of the β-sympathicolytic agent propranolol on the carbohydrate metabolism of liver, brain and skeletal muscle has been investigated in white mice. 20 mg/kg propranolol raised the glycogen contents of muscle and brain 30–60 min after injection, probably by decreasing phosphorylase activity. Simultaneously the pyruvate and lactate contents fell as evidence of reduced glycolytic carbohydrate metabolism. But in the liver, the glycogen content was not increased but somewhat lowered after 30 min. At the same time the levels of glucose, glucose-6-phosphate, fructosediphosphate, dihydroxyacetone-phosphate and pyruvate in the liver and of glucose and pyruvate in the blood rose. In the liver, propranolol obviously stimulates glycogenolysis and glycolysis thus acting in some direct or indirect manner like a sympathicomimetic, whereas in muscle and brain its effect on the carbohydrate metabolism is in line with its sympathicolytic properties.

PHOSPHORYLASE, the enzyme catalysing the enzymatic breakdown of glycogen is under hormonal control, especially under that of the catecholamines.[1–3] In muscle its activation by catecholamines is supposed to be mediated by the adrenergic β-receptors. The type of receptor involved in the activation of phosphorylase in the liver could not yet be clearly defined.[4–7] In both liver and skeletal muscle, however, it is possible to inhibit by help of β-sympathicolytics the glycogenolytic effect of catecholamines released from the adrenals or supplied exogenously as well as the glycogenolysis induced by higher activity of the sympathetic nervous system.[1, 8, 9] Experiments with β-sympathicolytics led to the presumption that these drugs exercised also a certain influence on the carbohydrate metabolism of animals which were not exposed to stress increasing the tone of the sympathetic nervous system or causing release of catecholamines from the adrenal medulla.[9,10] Therefore, in the experiments presented in this paper we investigated the actual influence of the β-sympathicolytic drug propranolol* (Dociton, Inderal) on the glycolytic carbohydrate metabolism of liver, skeletal muscle and brain of untreated mice and determined for this purpose the glycogen, glucose, glucose-6-phosphate, fructose-6-phosphate, fructose-1·6-diphosphate, dihydroxyacetone-phosphate, pyruvate and lactate contents in these three organs.

METHODS

All experiments were performed on female NMRI-mice which had free access to standard diet (Altromin†) and tap-water before and during the experiments and which

* Propranolol was kindly supplied by Rhein-Pharma Arzneimittel GmbH, Heidelberg, Germany.
† Manufactured by Altromin GmbH, Lage/Lippe, Germany.

were individually caged at 28° environmental temperature. At the beginning of the experiment the animals received 20 mg/kg propranolol i.p. or saline respectively. 30 or 60 min. later they were either decapitated and exsanguinated for collecting blood samples or killed by freezing in liquid air for the estimation of the metabolites in the organs. Liver, brain and muscular tissues from the hind legs were prepared while still being frozen and extracted in the cold. Analyses were made according to the following methods: glycogen according to Kemp and Kits van Heijningen,[11] glucose according to Huggett and Nixon[12]* (interfering substances were removed previously with charcoal as described by Kemp and Kits van Heijningen[11]), glucose-6-phosphate and fructose-6-phosphate according to Hohorst,[13] fructose-1·6-diphosphate and dihydroxyacetone-phosphate according to Bücher and Hohorst,[14] pyruvate according to Bücher,[15]* and lactate in accordance with the principle of Scholz and co-workers.[16]*

In order to test the psychomotor activity we tested the holding reflex according to Julou,[17] the motor coordination, the curiosity reaction and the ability of the mice to climb up a polished metal plate declined at an angle of 45° to the horizontal.

The values presented in the figures are means and their standard errors calculated from 7–30 single determinations. The results were checked by means of Student's *t*-test and differences between two values were regarded as significant if $P \leqq 0{\cdot}05$.

RESULTS AND DISCUSSION

The changes in the metabolite contents of the brain produced by propranolol are depicted in Fig. 1.

The influence of propranolol on the glycogen contents of various organs differs markedly. The increase of the glycogen content in the brain and in the skeletal muscle points to an imbalance between biosynthesis and breakdown of glycogen, which is probably caused by a decrease of the activity of the glycogen splitting enzyme phosphorylase, since the activation of glycogen phosphorylase is dependent upon the catecholamines which enhance the synthesis of cyclic 3′,5′-adenosine-monophosphate, which in turn promotes the conversion of phosphorylase *b* into the more active phosphorylase *a*.[1–3] Probably the β-sympathicolytic action of propranolol does not only diminish the effect of catecholamines supplied exogenously or secreted from the adrenals in stress-like situations but also the effect of the catecholamines circulating in the bloodstream or released from the sympathetic nerve endings under normal conditions. This means a reduction of "basal sympathetic tone" at the phosphorylase and accordingly a decreased breakdown of glycogen.

The glycogen content in the liver is—in contrast to that in muscle and brain—not increased but even somewhat reduced, at least after 30 min. Simultaneously with the decrease of the glycogen content glucose, fructosediphosphate, dihydroxyacetonephosphate and pyruvate, i.e. metabolites which certainly derive from the breakdown of glycogen, increase. This gives evidence of a stimulation of glycogenolysis in the liver with enhanced formation of glucose and pyruvate, which are partly delivered to the blood as shown by the elevated blood levels.

The enhancement of glycogenolysis shows that in some direct or indirect manner propranolol acts on the carbohydrate metabolism of the liver like a sympathomimetic

* Biochemica-Test-Combinations (C. F. Boehringer & Soehne, Mannheim, Germany) were used.

agent. The same holds true, perhaps, of other β-sympathicolytic drugs as dichloroisoproterenol (DCI) or Kö 592 which rise the blood glucose level, too.[18, 19] This does not, however, exclude the possibility, that these drugs are able to prevent the hyperglycemic action of catecholamines and thus act as sympathicolytics.[7] The increased glycogenolysis, which can be observed in the liver after propranolol is only transitory,

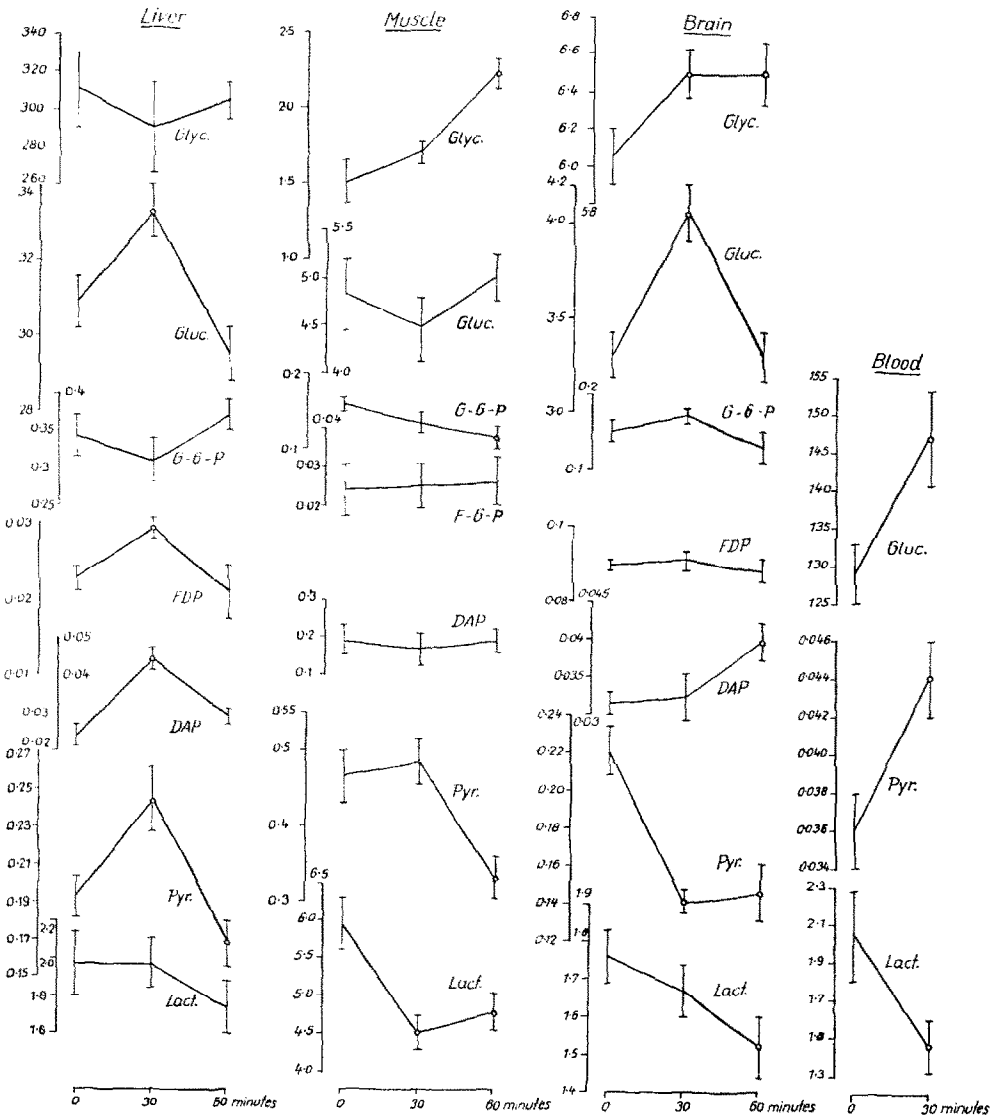

FIG. 1. The influence of 20 mg/kg propranolol i.p. on the glycogen (Glyc.), glucose (Gluc.), glucose-6-phosphate (G-6-P), fructose-6-phosphate (F-6-P), fructose-1·6-diphosphate (FDP), dihydroxyacetone-phosphate (DAP), pyruvate (Pyr.) and lactate (Lact.) contents of liver, skeletal muscle, brain and blood of white mice. The contents are given in μmoles/g wet wt. or μmoles/ml blood respectively, blood glucose in mg per cent.

The values denote means ± S.E.M. O = Value significantly different from the control value, $P \leqq 0{\cdot}05$.

since after 60 min the glycogen content has somewhat increased already, fructose-diphosphate and dihydroxyacetone-phosphate levels are no longer raised and the pyruvate content has now significantly decreased.

The anaerobic carbohydrate metabolism is not stimulated by propranolol in brain and muscle in contrast to that in the liver. For together with the rise of the glycogen content a fall of the pyruvate and lactate contents becomes manifest as soon as 30 min after the propranolol injection. A decrease of the final products of glycolysis is to be expected if they are formed to a smaller amount or are oxidized to a higher degree via tricarbonic acid cycle and respiratory chain. There is no evidence of the latter alternative, because the oxygen consumption and the oxidative metabolism of the animals is not raised by propranolol.[9, 20] More likely glycolysis is reduced, as is also supposed by Ehringer and Moser.[10] The impaired production of lactate shows itself in the decrease of the lactate level of the blood, too.

Several reasons need to be discussed which might account for the reduction of glycolytic carbohydrate metabolism in brain and muscle under the influence of propranolol. The impairment of glycolysis cannot be explained only by lack of substrate, resulting from decreased breakdown of glycogen, because the organs are fairly well supplied with substrate by the blood. For the blood-level of glucose is elevated 30 min after the administration of propranolol, and glucose is taken up by the cells probably even to a higher degree. This can be shown for the brain at least by calculation of the intracellular glucose content according to Hohorst and co-workers.[21] Thus the impaired breakdown of glycogen could have been compensated by an increased catabolism of glucose.

Therefore, we suspected propranolol to have a direct inhibitory effect on the glycolytic enzyme system. At first we expected that propranolol might act at the phosphofructokinase reaction, since this enzyme is rate-limiting for glycolysis and is activated by cyclic 3′,5′-AMP as well as phosphorylase if only by higher concentrations.[22–27] The estimation of the intermediary products of glycolysis, however, provided no evidence for this conjecture. More likely the stop is to be localized between dihydroxyacetone-phosphate and pyruvate.

Furthermore, it is to be considered that propranolol might reduce energy requirements and carbohydrate metabolism by blocking β-receptors and therewith the activating effects of endogenous catecholamines on function and metabolism of brain and muscle. According to Leszkovsky and Tardos[28] propranolol has CNS depressant effects as well as several other drugs with sympathicolytic action.[29, 30] On the other hand, no severe disorder of the psychomotor performance of our experimental animals could be observed.

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