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On sympathetic regulation of carbohydrate metabolism in the liver

E. S. VIZI,* G. POGÁTSA† AND A. KÁLDOR†

Glycogenolysis in the liver produced by isoprenaline was inhibited by β-receptor blocking agents using the perfusion technique. Dichloroisoprenaline also inhibited spontaneous glucose release, as did α-receptor stimulation by noradrenaline.

REGULATION of glucose level, the main energy source of the organism, is under the influence of the sympathetic nervous system. Dale, in 1906, suggested that sympathetic nervous activity was mediated through two different receptors, thus explaining the modified response to adrenaline after the administration of ergot alkaloids. Ahlquist (1948) demonstrated that sympathomimetic agents exerted their effect on two different receptors, which he called alpha and beta. Since then several authors have examined the mode of action of sympathomimetic agents on the receptors and their effect on carbohydrate metabolism. Thus, van der Pool (1956) found both kinds of receptors were involved in the development of hyperglycaemia, since the α-receptor stimulator noradrenaline, given together with isoprenaline (a β-receptor stimulator), mutually increased the hyperglycaemic effect. Regulation of the glycogenolysis of the striated musculature is considered to be a β-receptor function (Ellis, Davis & Anderson, 1955; Vrij, Gho, de Groot & Weber, 1956; Furchgott, 1959), but in liver glycogenolysis an important role is attributed to the α-receptors. Thus, Vrij & others (1956) demonstrated *in vivo* that noradrenaline decreases the glycogen content of the rat liver, whereas isoprenaline given in similar doses does not. Again, Ellis (1951) and van Roy & Schulhof (1961) could not find any effect of isoprenaline on the glycogen content of liver slices. The experiments of Sutherland & Cori (1948) showed that adrenaline increased glucose release from liver slices more than did noradrenaline or isoprenaline. The present text is concerned with a re-examination of the problem using the perfused isolated liver of the rat.

METHODS

Albino rats of either sex, of the same stock, weighing 150–220 g and kept on standard diet, were used. The animals were bled and an isolated liver perfusion prepared (Issekutz, 1924). Cannulae were introduced into the portal vein and after washing through with Tyrode fluid for 15 min they were perfused with 100 ml of glucose-free Tyrode solution or with Tyrode solution containing 100 mg % glucose. Phenoxybenzamine, 10 mg % or dichloroisoprenaline, 0·7 mg %, was added to the perfusion fluid. Concentrations of pronethalol, 0·7 mg%, noradrenaline 0·05 mg% or (–)-isoprenaline, 0·002 mg %, were used. The liver was perfused at

*From the Department of Pharmacology, University Medical School, Budapest, Hungary, and

†Second Department of Medicine, University Medical School, Budapest, Hungary.

4 ml/min with a total volume of 100 ml Tyrode solution at 37° thermostatically controlled and also with constant oxygenation of the perfusion fluid. The glucose content of the fluid was determined every 30 min (Hagedorn & Jenson, 1923) for 2 hr, amino-acid nitrogen determinations were made 30 min after the 15 min preliminary period and after 2 hr (see Danielson, 1933). In the isolated organ-bath, according to Kline (1949) and Kaufmann & Wertheimer (1957), the increased nitrogen which is released secondary to cellular damage becomes negligible after the first 30 min, and the release of nitrogen which follows is a true indicator of protein metabolism. At the beginning and the end of the experiment the glycogen content of the liver was determined by the Good-Kramer-Somogyi method (1933). Each group, containing 10 livers, was analysed by the Student "*t*" test.

Results

Glucose released from the isolated perfused liver increased significantly during perfusion with glucose-free Tyrode solution and the decrease in glycogen content was accelerated significantly. Dichloroisoprenaline (Powell & Slater, 1958), a β-receptor blocking agent (Moran & Perkins, 1958), practically abolished the release of glucose. On the other hand, phenoxybenzamine, an α-receptor blocking agent, proved to be ineffective (Table 1). β-Receptor blockade also entirely inhibited the spontaneous release of glucose after perfusion with normal Tyrode solution containing glucose. β-Receptor blockade also increased the amino-acid nitrogen released from the liver, and this can perhaps be considered as an indicator of glyconeogenesis. In this respect the effect of dichloroisoprenaline was similar to that of the oral hypoglycaemic agent chlorpropamide (Pogátsa & Káldor, 1965).

TABLE 1. PERFUSION OF THE ISOLATED LIVER OF THE RAT. ALTERATIONS OF GLUCOSE RELEASED, GLYCOGEN CONCENTRATION AND AMINO-ACID NITROGEN RELEASED INTO NORMAL TYRODE SOLUTION OR INTO GLUCOSE-FREE TYRODE SOLUTION, AFTER THE ADMINISTRATION OF PHENOXYBENZAMINE OR DICHLOROISOPRENALINE IN GLUCOSE-FREE SOLUTION

	mg glucose/g liver/hr (mean ± s.e.) at times (min)				mg amino-acid nitrogen/g liver/hr (mean ± s.e.) at times (min)		Change of glycogen content %
	30	60	90	120	30	120	
Glucose-free Tyrode solution	18·4 ± 1·4*	6·0 ± 0·81	7·0 ± 0·87*	7·3 ± 0·89*	1·54 ± 0·22	0·05 ± 0·02	−77 ± 4*
Tyrode solution	10·2 ± 1·5	4·8 ± 2·5	4·3 ± 1·4	4·6 ± 1·8			−49 ± 9
Phenoxybenzamine	17·9 ± 1·4	10·1 ± 1·05*	9·0 ± 1·0	6·0 ± 0·8	1·56 ± 0·11	0·07 ± 0·02	−67 ± 6
Dichloro-isoprenaline	3·0 ± 0·5*	0·3 ± 0·17*	0·2 ± 0·14*	1·1 ± 0·34*	1·41 ± 0·12	0·25 ± 0·05*	−36 ± 6*

Phenoxybenzamine 10 mg/100 ml, and dichloroisoprenaline 0·7 mg/100 ml were each given at the beginning of perfusion. Significance* between groups 1 and 2: $P < 0.001$; 3 and 1: $P < 0.01$; 4 and 1: $P < 0.001$.

The release of glucose from livers perfused with normal Tyrode solution containing isoprenaline (0·002 mg %) was increased significantly. Noradrenaline, in concentrations which produced selective α-receptor stimulation, inhibited the release of glucose. Dichloroisoprenaline inhibited

the increased release of glucose produced by isoprenaline and also inhibited the decrease of glycogen. Pronethalol (0·7 mg %) did not inhibit the spontaneous release of glucose (dichloroisoprenaline proved to be effective here) but abolished the effect of isoprenaline in releasing glucose (Table 2). The β-receptor blocking activity of pronethalol proved to be ten times more powerful than that of dichloroisoprenaline using the method of Smith (1963) on the nictitating membrane; the effect of pronethalol on the inhibition of glycogenolysis produced by isoprenaline is also stronger and more lasting.

TABLE 2. PERFUSION OF THE ISOLATED LIVER OF THE RAT. ALTERATIONS OF GLUCOSE RELEASED AND GLYCOGEN CONCENTRATION AFTER THE ADMINISTRATION OF NORADRENALINE, ISOPRENALINE, DICHLOROISOPRENALINE OR PRONETHALOL.

	mg glucose/g liver/hr (mean ± s.e.) at times (min)				Change of glycogen content %
	30	60	90	120	
Tyrode solution	10·2±1·5	4·8±2·5	4·3±1·4	3·6±1·8	−49±9
Noradrenaline	9·9±0·9	−2·1±0·5	−2·6±0·5*	−5·1±1·2*	−64±7
Isoprenaline	11·9±1·8	16·0±2·9*	10·6±3·0	6·1±3·7	−89±3*
Dichloroisoprenaline + isoprenaline	3·6±1·7*	−3·8±1·7*	1·4±2·6*	−1·4±2·2	−29±10*
Pronethalol + isoprenaline ..	8·2±3·4	0·1±1·3*	0·7±2·5*	−6·3±1·4*	−54±35*

Dichloroisoprenaline 0·7 mg/100 ml and pronethalol 0·7 mg/100 ml were each given at the beginning of the perfusion and noradrenaline 0·05 mg/100 ml, isoprenaline 0·002 mg/100 ml after 30 min. Each group contained 10 livers. Significance (*) between groups 2 and 1: $P<0·01$; 3 and 1: $P<0·001$; 4 and 3: $P<0·001$; 5 and 3: $P<0·001$.

Discussion

The results of these experiments are in line with the observations of Sutherland & Rall (1960) which demonstrated that phosphorylase activation was most effective with isoprenaline, and this activation was inhibited by β-receptor blocking agents (Hornbrook & Brody, 1963). Furthermore, dichloroisoprenaline and pronethalol prevented the action of adrenaline or isoprenaline on carbohydrate metabolism in the heart and skeletal muscle (Mayer, Moran & Fain, 1961; Murad, Chi, Hall & Sutherland, 1962). Comparing our results on the liver and the observations of other authors on the muscle, it seems that receptors sensitive to isoprenaline distributed throughout the organism have a physiological function in carbohydrate metabolism, and receptors that are stimulated by isoprenaline may have a regulatory function in these metabolic processes.

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