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HEPATIC GLYCOGEN AND BLOOD GLUCOSE CONTROL

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Abstract—The hyperglycaemic response to adrenaline and the hypoglycaemic response to insulin was measured in normal rats and in rats pretreated with either dihydroergotamine or propranolol under both fed and 24 hr starved conditions. Administration of propranolol to starved rats both impaired the hyperglycaemic response to adrenaline and increased sensitivity to the hypoglycaemic action of insulin. Although dihydroergotamine caused similar impairment of adrenaline hyperglycaemia in fed rats, there was no increase in insulin sensitivity. Potentiation of insulin hypoglycaemia may not be strictly related to blockade of adrenaline hyperglycaemia.

Starvation for 24 hr caused over 95 per cent depletion of liver glycogen in the rat and over 99 per cent depletion in the rabbit. Despite this, there was no impairment of the hyperglycaemic response to adrenaline in the rat and the rabbit showed a good hyperglycaemic response to glucagon. An adequate glycaemic response to pharmacological doses of these drugs does not depend upon hepatic glycogenolysis. Propranolol neither impaired the hyperglycaemic response to adrenaline nor increased sensitivity to insulin in the fed rat. It may be that propranolol will potentiate insulin hypoglycaemia only in the absence of liver glycogen.

THE LIVER is predominantly responsible for blood glucose production[1] and plays a major part in glucose metabolism. Experiments were carried out which help to define the importance of liver glycogen stores in blood glucose control. Glucose homeostasis is to some extent controlled by the sympatho-adrenal system and some effects of the adrenolytic agents propranolol and dihydroergotamine (DHE) are also described.

EXPERIMENTAL

Experiments were on 200–300 g male Wistar rats or 1·5–2·5 kg male rabbits of indeterminate strain.

Hyperglycaemic response to adrenaline. Adrenaline (as the hydrochloride) 0·1 mg/kg s.c. was given to fed rats, allowed free access to food until the experiment. One hour later they were stunned, the abdomen rapidly opened and duplicate samples of blood removed from the abdominal aorta of each rat for glucose estimation by glucose oxidase.[2] Other rats were given adrenaline 0·5 mg/kg or adrenaline 1·0 mg/kg and similar experiments were carried out on rats starved for 24 hr.

Blood glucose was also measured after these doses of adrenaline in fed rats pretreated 90 min beforehand with DHE 1 mg/kg s.c. or propranolol 20 mg/kg i.p. In control rats 0·9 g/100 ml sodium chloride solution replaced adrenaline.

Hyperglycaemic response to glucagon. Blood glucose was measured in 24 hr starved

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rabbits at intervals after 0·9 g/100 ml sodium chloride i.v. and several days later at intervals after glucagon (Eli Lilly & Co.) 0·1 mg/kg i.v., taking samples from a marginal ear vein.

Hypoglycaemic response to insulin. Fed rats were pretreated with either DHE 1 mg/kg s.c. or propranolol 20 mg/kg i.p. Some were killed 60 min later and the remainder, together with an equal number of weight matched controls, were given insulin 7·5 units/kg s.c. Blood glucose was measured at 90 min, 150 min or 300 min after insulin. In a similar experiment 24 hr starved rats were pretreated with propranolol 20 mg/kg i.p.

Liver glycogen. Liver glycogen was estimated in fed and 24 hr starved rabbits and rats.[3]

RESULTS

Hyperglycaemic response to adrenaline. (Figs. 1 and 2) Mean blood glucose of controls was subtracted from the blood glucose after adrenaline to obtain the mean increment for each dose of adrenaline. In fed rats, propranolol had no effect but DHE suppressed hyperglycaemia ($P<0·001$, Student t-test) at all doses of adrenaline. In starved rats

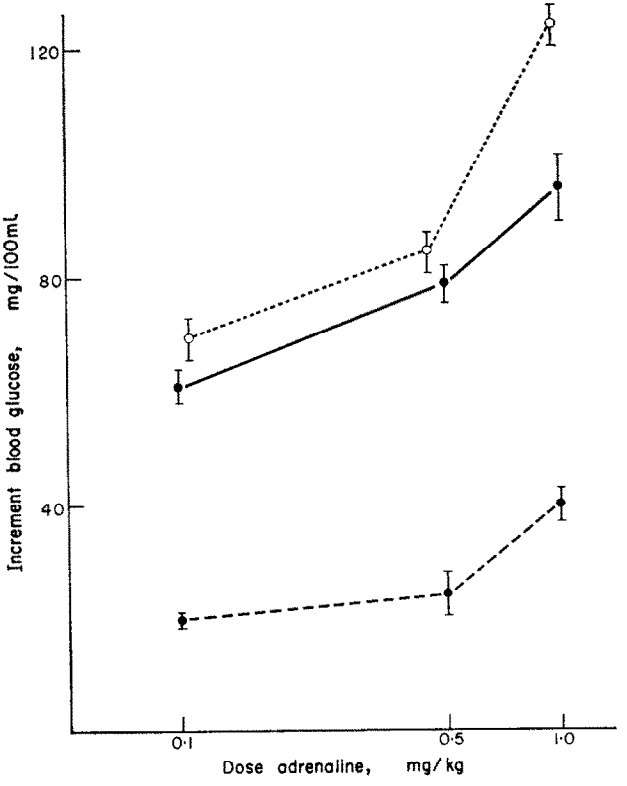

FIG. 1. Increase in blood glucose ("increment") following adrenaline in fed rats (mean of 5 ± S.E.). Normal controls: ●——●, DHE pretreated: ●– – –●, Propranolol pretreated: ○ · · · ○.

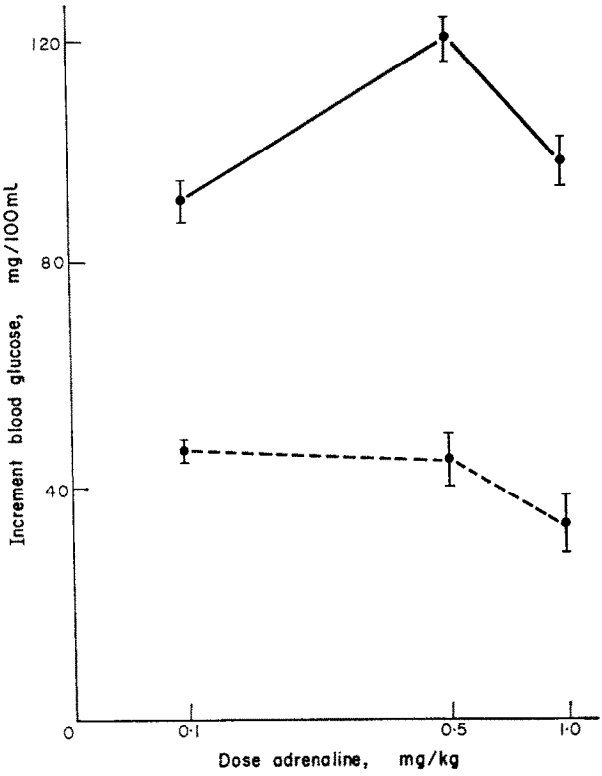

FIG. 2. Increase in blood glucose ("increment") following adrenaline in 24 hr starved rats (mean of 5 ± S.E.). Normalcontrols: ●——●, Propranololpretreated: ● - - ●.

TABLE 1. BLOOD GLUCOSE (MEAN ± S.E.), RESTING AND AT INTERVALS AFTER INSULIN

Starvation	Pretreatment (mg/kg)	Blood glucose (mg/100 ml)			
		resting	90 min	150 min	300 min
fed	DHE 1	83·0 ± 1·5 (5)	28·6 ± 1·8 (10)	31·0 ± 4·2 (5)	39·3 ± 8·0 (5)
fed	none	92·3 ± 2·2 (5)	30·5 ± 1·8 (10)	26·5 ± 2·1 (5)	45·6 ± 6·0 (5)
fed	propranolol 20	78·3 ± 2·1 (6)	28·3 ± 2·7 (5)	25·8 ± 2·1 (5)	24·0 ± 3·2 (10)
fed	none	82·4 ± 2·2 (6)	26·7 ± 1·2 (5)	25·5 ± 1·9 (5)	19·2 ± 2·9 (10)
24 hr	propranolol 20	64·1 ± 2·7 (5)		20·7 ± 4·8 (6)	6 rats died (10)
24 hr	none	61·3 ± 3·2 (5)		18·2 ± 5·2 (6)	18·9 ± 2·5 (10)

Figures in brackets are the number of rats killed at that time to determine the mean.

propranolol suppressed hyperglycaemia at all doses of adrenaline ($P<0{\cdot}001$). There was no significant difference between DHE suppression of hyperglycaemia in fed rats and propranolol suppression of hyperglycaemia in starved rats. The hyperglycaemic response to adrenaline 0·1 mg/kg and adrenaline 0·5 mg/kg was greater in normal starved rats than in normal fed rats ($0{\cdot}001<P<0{\cdot}01$).

Hyperglycaemic response to glucagon. (Fig. 3) There was a rapid and sustained hyperglycaemic response to glucagon in starved rabbits at all time intervals ($P<0{\cdot}001$).

Hypoglycaemic response to insulin. (Table 1) In fed rats, blood glucose of controls was not significantly different from that of DHE or propranolol pretreated animals. Of ten propranolol pretreated starved rats to be sacrificed at 300 min after insulin, six died before this time, following convulsions and with a blood glucose in each case of

TABLE 2. LIVER GLYCOGEN UNDER FED AND 24 hr STARVED CONDITIONS (MEAN OF 5 ± S.E.)

	Liver glycogen (mg/100 mg wet tissue)	
	fed	starved
Rats	1·17 ± 0·08	0·055 ± 0·020
Rabbits	9·0 ± 2·0	0·040 ± 0·018

less than 6 mg/100 ml. No controls died and this is a significant difference in mortality (Fisher exact probability test $P<0{\cdot}025$).

Liver glycogen. (Table 2) Starvation for 24 hr resulted in over 95 per cent depletion of hepatic glycogen in the rat and over 99 per cent depletion in the rabbit.

DISCUSSION

The hyperglycaemic response to adrenaline and to glucagon is usually interpreted as an index of hepatic glycogenolysis.[4] However, there was a sustained hyperglycaemic response to glucagon in the rabbit (Fig. 3), despite the virtual absence of liver glycogen. The gluconeogenic action of glucagon[5,6] was most likely responsible for the glycaemia. Glucagon was studied in the rabbit since the hyperglycaemic response in rats is inconstant.[7] Similarly, adrenaline hyperglycaemia was normal in the starved rat (Fig. 2) and could result from muscle glycogenolysis, lipolysis with glucose production from glycerol, or from inhibition of peripheral glucose uptake which itself may be secondary to increased levels of free fatty acids.[8] Hepatic glycogenolysis is certainly not essential for a normal glycaemic response to adrenaline or to glucagon, at least when these are given in pharmacological doses.

In the rat, hepatic glycogenolysis is thought to be mediated by way of alpha-adrenergic receptors, as opposed to muscle glycogenolysis,[9,10] inhibition of peripheral glucose uptake,[11] and lipolysis[12] which are by way of beta-receptors. Rats starved for 24 hr and treated with propranolol not only have virtually absent hepatic glycogenolysis, but also impairment of muscle glycogenolysis and lipolysis, together with less reduction of peripheral glucose uptake in response to adrenaline. Blockade of adrenaline hyperglycaemia under these circumstances (Fig. 2) is not unexpected.

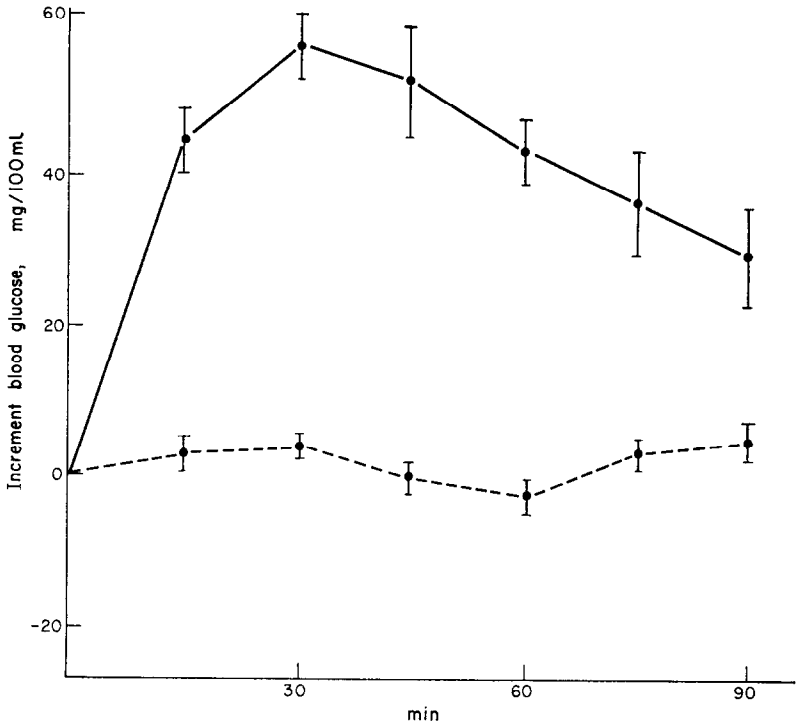

FIG. 3. Increase in blood glucose ("increment") following administration of glucagon 0·1 mg/kg or saline to 24 hr starved rabbits (mean of 5± S.E.). Glucagon: ●——●, Saline: ● - - ●.

Propranolol did not impair adrenaline hyperglycaemia in fed rats (Fig. 1). Blockade of the beta-mediated hyperglycaemic actions of adrenaline was presumably masked by massive alpha-mediated hepatic glycogenolysis. Similarly propranolol did not increase insulin hypoglycaemia in the fed rat (Table 1) in which unimpaired hepatic glycogenolysis appeared adequate to compensate for blockade of beta-mediated hyperglycaemic mechanisms.

Only when hepatic glycogen was depleted did propranolol impair adrenaline hyperglycaemia and increase insulin sensitivity. In a report of increased sensitivity to insulin following propranolol treatment,[13] rats were starved for 14 hr and given 3 ml corn oil before insulin. Liver glycogen in these animals was presumably decreased. Since liver glycogen is normal in clinical as opposed to experimental diabetes,[14] propranolol should not potentiate insulin in clinical use. This may explain the lack of reports of propranolol precipitated hypoglycaemia in clinical diabetes.

There are reports however of increased sensitivity to insulin in non-diabetic patients given propranolol,[15,16] despite the findings that beta-blockade does not interfere with the hyperglycaemic response to adrenaline in man.[17] Potentiation of insulin hypoglycaemia may not therefore be strictly related to blockade of adrenaline hyperglycaemia. This is supported by the present finding that DHE, predominantly an alpha-blocking agent,[18] caused blockade of adrenaline hyperglycaemia but had no effect on insulin sensitivity tested under the same conditions.

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REFERENCES

1. P. K. BONDY, *Am. J. Med.* **24**, 428 (1958).
2. V. MARKS, *Clin. chim. Acta* **4**, 395 (1959).
3. C. R. KRISMAN, *Analyt. Biochem.* **4**, 17 (1962).
4. E. SAMOLS and D. HOLDSWORTH, *Carbohydrate Metabolism and its Disorders*, Volume 2, Academic Press (1968).
5. J. M. SALTER, I. W. F. DAVIDSON and C. H. BEST, *Diabetes* **6**, 248 (1957).
6. L. L. MILLER, *Fedn Proc.* **24**, 737 (1965).
7. C. CAVELLERO and B. MALANDRA, *Acta endocr., Copenh.* **13**, 579 (1953).
8. J. HIMMS-HAGEN, *Pharmac. Rev.* **19**, 367 (1967).
9. B. L. KENNEDY and S. ELLIS, *Fedn Proc.* **22**, 449 (1963).
10. W. W. FLEMING and A. D. KENNY, *Br. J. Pharmac. Chemother.* **22**, 267 (1964).
11. E. A. ABRAMSON and R. A. ARKY, *Diabetes* **17**, 141 (1968).
12. J. J. BURNS, K. I. COLVILLE, L. A. LINDSAY and R. A. SALVADOR, *J. Pharmac exp. Ther.* **144**, 163 (1964).
13. S. O. BYERS and M. FRIEDMAN, *Proc. Soc. exp. biol. Med.* **122**, 114 (1966).
14. J. A. HILDES, S. SHERLOCK and V. WALSH, *Clin. Sci.* **7**, 287 (1949).
15. E. A. ABRAMSON, R. A. ARKY and K. A. WOEBER, *Lancet* **2**, 1386 (1966).
16. M. N. KOTLER, L. BERMAN and A. H. RUBENSTEIN, *Lancet* **2**, 1389 (1966).
17. T. R. E. PILKINGTON, B. F. ROBINSON and E. TITTERINGTON, *Lancet* **2**, 316 (1962).
18. B. LEVY and R. P. AHLQUIST, *J. Pharmac. exp. Ther.* **133**, 202 (1961).