Biochemical Pharmacology, Vol. 22, pp. 997–1004. Pergamon Press, 1973. Printed in Great Britain.

EFFECT OF GLIBENCLAMIDE ON SUGAR TRANSPORT BY FED, STARVED AND DIABETIC RAT SMALL INTESTINE

JOSEPH D. TEALE* and ANDREW H. G. LOVE

Department of Medicine, The Queen's University of Belfast, Northern Ireland

(*Received* 14 *July* 1972; *accepted* 9 *November* 1972)

Abstract—Glucose transfer by jejunum from starved rats is not as susceptible to glibenclamide as is transfer by jejunum from fed or diabetic animals. Similarly, with fed rats, glucose transfer by ileum is not affected to the same extent by glibenclamide as is transfer by jejunum. Galactose transfer is less affected by glibenclamide than is glucose transfer, using jejunum from fed, starved or glucose-fed starved animals. Glibenclamide appears to deplete energy supplies in the intestinal cell by an uncoupling action. As a result, the transfer of a metabolized sugar, such as glucose, is markedly decreased because of an increase in metabolic rate. Consequently glucose transfer by intestinal tissue with a comparatively lower level of glycolysis is less affected. Similarly, the transfer of galactose is relatively unaffected compared with glucose transfer, since galactose is not involved in intracellular metabolism.

IN A previous study[1] it was shown that two sulphonylurea compounds, tolbutamide and glibenclamide, interfered with the glucose transport processes exhibited by everted sacs of rat jejunum.

Since glucose is both transported and metabolized by the intestine, the transport of a nonmetabolized sugar, galactose, was examined in the presence of glibenclamide in order to provide further evidence as to the possible mode of action of these drugs. In addition, the drug effects on transport by small intestine having different rates of glucose metabolism were investigated. In particular studies were carried out using tissue from fed, diabetic, starved and glucose-fed starved animals.

By being able to vary the type and level of metabolism within the absorbing intestinal cell, the drug effects under different metabolic conditions could be examined whilst the integrity of the gut wall was maintained and active sugar transfer could still occur.

METHODS

Experiments were carried out using the everted gut sac technique of Wilson and Wiseman.[2] Male albino rats (160–170 g) were used throughout the studies. Fed animals were allowed free access to water and commercial diet. Starved animals were given water for only 72 hr prior to sacrifice. For certain studies starved animals were given an isotonic glucose solution (5·4 g%) instead of water (glucose-fed starved animals). Animals were made diabetic by an intraperitoneal injection of streptozotocin in citrate buffer pH 4·5 (75 mg/g). This group was allowed free access to food and water for 4 weeks, after which time animals which had either lost weight or not gained weight at the normal rate were taken as being diabetic.[3]

The rats were sacrificed by a blow to the neck and the small intestine removed.

* Present address: Department of Biochemistry, University of Surrey, Guildford, Surrey.

After flushing out with Krebs–Ringer bicarbonate (KRB) buffer,[4] the gut was everted over a glass rod. Two 10-cm sacs, one acting as control to the other, were made from each piece of intestine (jejunum or ileum). Each sac was filled with 0·6 ml of KRB buffer, which will be referred to subsequently as serosal fluid. The sacs were incubated with gentle agitation in 5 ml of KRB buffer (mucosal fluid), contained in 50 ml conical flasks, and in equilibrium with a gas phase of 95% O_2–5% CO_2.

The sacs were weighed when empty and filled, before and after incubation to determine fluid uptake. Incubations were carried out at 37° for 1 hr.

After incubation sacs were removed from their bathing fluids, blotted lightly and drained. Sugar analysis was carried out on mucosal and serosal fluids. Glucose was determined by the method of Huggett and Nixon.[5] When galactose alone or glucose and galactose together were present total reducing sugar was estimated by the method of Nelson[6] as modified by Somogyi.[7] Galactose was estimated by the difference between total sugar and glucose.

From these results, calculations were made of the amount of sugar appearing in the serosal fluid (sugar transfer) and the amount disappearing from the mucosal fluid (sugar uptake). Where glucose was present initially in the mucosal fluid, the difference between uptake and transfer will be referred to as metabolism. Where glucose is present initially in the serosal fluid its disappearance will be called metabolism. Sodium estimations were carried out by flame photometer. Sodium transfer is the amount of sodium appearing in the serosal fluid.

Glibenclamide was used at concentrations of 50 and 100 μg/ml in the mucosal fluid and was kindly donated by Hoechst Pharmaceuticals.

RESULTS

Effect on fed, starved and diabetic jejunum. Table 1 shows the results obtained. At the

TABLE 1. EFFECT OF GLIBENCLAMIDE ON SODIUM AND GLUCOSE TRANSPORT BY SACS OF EVERTED JEJUNUM FROM FED, STARVED AND STREPTOZOTOCIN-DIABETIC RATS

Glibenclamide concn (μg/ml)	No. exp.	Sodium transfer (μEq/hr/sac)	Glucose (μmole/hr/sac) Transfer	Uptake	Metabolism	Gut wet wt (mg/10 cm)
Fed						
0	16	60 ± 10	9·2 ± 1·5	64·5 ± 4·1	55·3 ± 3·9	1070 ± 85
50	8	38 ± 6	5·3 ± 0·8	71·8 ± 4·8	66·5 ± 5·1	
100	8	10 ± 3	2·3 ± 0·7	49·5 ± 3·3	47·2 ± 1·9	
Starved						
0	22	42 ± 7	19·0 ± 3·2	54·0 ± 5·4	35·0 ± 4·4	745 ± 75
50	11	29 ± 6	10·0 ± 1·1	59·3 ± 3·9	49·3 ± 4·5	
100	11	28 ± 5	8·3 ± 0·6	29·5 ± 2·5	21·2 ± 1·8	
Diabetic						
0	24	121 ± 15	29·7 ± 7·2	116·7 ± 9·0	87·0 ± 5·7	1190 ± 125
50	12	92 ± 12	17·6 ± 4·5	99·0 ± 6·2	81·4 ± 5·1	
100	11	47 ± 7	4·2 ± 0·9	66·8 ± 5·6	62·6 ± 5·5	

Sacs, each 10 cm in length, were made from the everted jejunum of fed, 72-hr starved and streptozotocin-diabetic rats. Each sac contained 0·6 ml KRB buffer and was incubated in 5 ml KRB buffer containing 27·8 mM glucose. Glibenclamide, where present, was contained in the mucosal fluid. Incubations were carried out at 37° for 1 hr.

low (50 μg/ml) drug concentration glucose transfer is decreased markedly in the fed and diabetic gut but transfer by starved tissue is not affected to the same extent. Glucose metabolism is increased in fed and starved tissue by 50 μg/ml glibenclamide. Consequently glucose uptake shows a concomitant increase under these conditions. At the higher drug concentration a general depression in activity is observed. Gut weight varies, being decreased markedly after starvation and showing a slight increase in the diabetic animals.

Effect on fed and starved ileum. Table 2 shows the results obtained. In the case of the

TABLE 2. EFFECT OF GLIBENCLAMIDE ON SODIUM AND GLUCOSE TRANSPORT BY SACS OF EVERTED ILEUM FROM FED AND STARVED RATS

Glibenclamide concn (μg/ml)	No. exp.	Sodium transfer (μEq/hr/sac)	Glucose (μmole/hr/sac) Transfer	Uptake	Metabolism
Fed					
0	16	32 ± 5	7·0 ± 1·7	35·0 ± 5·8	28·0 ± 5·6
50	8	26 ± 8	6·9 ± 0·8	39·5 ± 4·4	32·6 ± 3·3
100	8	24 ± 7	5·9 ± 2·0	26·2 ± 3·4	20·3 ± 3·0
Starved					
0	22	31 ± 7	11·1 ± 2·2	19·5 ± 2·8	8·4 ± 2·1
50	11	24 ± 6	7·9 ± 1·1	30·6 ± 4·4	22·7 ± 3·2
100	11	24 ± 3	7·7 ± 0·4	7·8 ± 1·9	0·1 ± 0·3

Sacs, each 10 cm in length, were made from the everted ileum of fed and 72-hr starved rats. Each sac contained 0·6 ml KRB buffer and was incubated in 5 ml KRB buffer containing 27·8 mM glucose. Glibenclamide, where present, was contained in the mucosal fluid. Incubations were carried out at 37° for 1 hr.

fed ileum, although in general the values are lower, glucose uptake and metabolism are affected in a similar manner to the effect on jejunum (Table 1). However, glucose transfer is virtually unaffected, even by 100 μg/ml glibenclamide. In the starved state, glucose uptake and metabolism are increased and decreased dramatically by low and high drug concentrations respectively. Glucose transfer, however, is maintained almost unaffected.

Effect on galactose movement by gut from animals maintained on different diets. In these experiments, the movement of galactose from the mucosal fluid was examined either when no exogenous energy supply was available or when energy (in the form of glucose) was supplied either from the serosal fluid or from the mucosal fluid. Tables 3–5 show the results obtained with fed, starved and glucose-fed starved animals, respectively. These results indicate primarily that glibenclamide did not affect galactose transfer to the same degree as it affected glucose transfer. Also, when glucose was supplied in the serosal fluid, glibenclamide produced no obvious change in the metabolic rate exhibited by any of the systems, but the metabolism of glucose from the mucosal fluid varied in a similar manner to that observed in previous experiments.

DISCUSSION

Sulphonylureas have been shown to decrease energy supplies in the form of ATP, in

several tissues from the rat, including pancreatic β-cells,[8] liver,[9] brown fat cells[10] and other tissues.[11] The present results and the previous study[1] indicate that intestinal tissue may be affected in a similar manner.

Active transport by the everted gut sac is particularly susceptible to any interruption of oxidative metabolic activity. It has been shown[12] that both eversion of the gut and incubation in an oxygen atmosphere are necessary for the occurrence of active transport, i.e. accumulation of solute against a concentration gradient. At the same time transfer into the serosal fluid requires solute to cross several layers of smooth muscle. Since this latter process is postulated as being simple diffusion down a concentration gradient from intracellular fluid to serosal fluid,[13] it is necessary in the everted sac for a substantially greater intracellular accumulation of solute to occur before any appreciable amounts appear in the serosal fluid. In addition, the observed high rate of glycolysis by intestinal epithelial cells[14] is a large drain on intracellular glucose. These facts are borne out by the observation that only 14 per cent of the glucose absorbed at the mucosal surface is transferred to the serosal fluid in a normal system (Table 1).

It has been reported[15] that 2,4-dinitrophenol, a substance known to uncouple oxidative phosphorylation and activate mitochondrial ATPase, stimulates aerobic glycolysis by small intestinal mucosa. The low concentration of glibenclamide may have a similar effect on the mucosal cell mitochondria. This would provoke an increase in metabolism resulting in an immediate drain on the glucose available for transfer and also an increase in uptake to feed the metabolic pool. Enough energy would be available from aerobic glycolysis to promote mucosal transport. Part of the increase in metabolism may be due to an increase in glycogen synthesis. However, since intestinal tissue normally exhibits very low levels of glycogen synthesis[16] compared with very high levels of glycolysis,[14] this consideration may be insignificant. At the higher drug concentration energy supplies are so diminished that the mucosal transport system is affected, thereby decreasing glucose uptake into the metabolic pool.

The metabolic rate also depends on the type of tissue used. When glucose was freely available from the serosal fluid, metabolism by fed tissue was lower than that by either of the starved groups (Tables 3–5). This may be due to a decrease in muscle thickness during starvation, allowing an increase in glucose diffusion (a process not requiring ATP) from the serosal fluid into the cell.[17] When glucose was supplied in the mucosal fluid and required energy for transport into the metabolic pool, starvation reduced uptake through a reduction in metabolism.[14] The combination of a reduction in wasteful metabolism and thinning of the muscle wall resulted in a higher percentage of the glucose absorbed at the mucosal surface being transferred to the serosal fluid. Consequently, although 50 μg/ml glibenclamide produced a greater increase in glucose metabolism by starved tissue compared with fed tissue, transfer into the serosal fluid was not affected to the same extent (Tables 1, 3 and 4). Feeding glucose during starvation prevented the depression of glycolysis and maintained the rate of uptake (Table 5).

Similarly, in a comparison of jejunum and ileum, starved jejunum shows the same pattern of response to glibenclamide as does fed ileum, which like starved jejunum, shows a much lower rate of glycolysis than fed jejunum.[18] This effect is much more marked using starved ileum (Tables 1 and 2).

Diabetic tissue (Table 1) shows the same pattern of response to glibenclamide as does normal fed tissue, although the general metabolic activity is markedly increased

TABLE 3. EFFECT OF GLIBENCLAMIDE ON SODIUM, GLUCOSE AND GALACTOSE UTILIZATION BY SACS OF EVERTED JEJUNUM FROM FED RATS

Initial glucose concn (mM)		No. exp.	Glibenclamide concn (μg/ml)	Sodium transfer (μEq/hr/sac)	Galactose (μmole/hr/sac)		Glucose (μmole/hr/sac)		
Mucosal	Serosal				Transfer	Uptake	Transfer	Uptake	Metabolism
		24	0	35 ± 5	14·4 ± 2·4	25·0 ± 4·2			
		12	50	26 ± 4	12·7 ± 1·8	23·4 ± 3·9			
		12	100	24 ± 2	10·2 ± 1·7	18·9 ± 3·6			
	210	24	0	49 ± 8	22·6 ± 6·0	29·0 ± 6·7			54·5 ± 6·7
	210	12	50	45 ± 7	18·3 ± 3·6	34·5 ± 5·0			51·7 ± 5·0
	210	12	100	35 ± 4	13·8 ± 4·1	24·5 ± 4·2			51·7 ± 7·2
28		24	0	67 ± 7	14·1 ± 2·1	31·6 ± 3·6	9·9 ± 2·5	79·0 ± 5·0	69·1 ± 4·2
28		12	50	51 ± 4	10·4 ± 1·4	31·1 ± 5·0	4·4 ± 0·9	66·0 ± 4·7	61·6 ± 4·2
28		12	100	33 ± 3	5·8 ± 0·7	22·2 ± 3·9	1·1 ± 0·4	47·8 ± 3·6	46·7 ± 3·9

Ten-cm sacs of everted fed jejunum, containing 0·6 ml KRB buffer with or without 210 mM glucose, were incubated in 5 ml KRB buffer containing 27·8 mM galactose in all cases and, in addition, 27·8 mM glucose where indicated. Glibenclamide, when present, was contained in the mucosal fluid. Incubations were carried out at 37° for 1 hr.

TABLE 4. EFFECT OF GLIBENCLAMIDE ON SODIUM, GLUCOSE AND GALACTOSE UTILIZATION BY SACS OF EVERTED JEJUNUM FROM STARVED RATS

Initial glucose concn (mM)		No.	Glibenclamide concn	Sodium transfer	Galactose (μmole/hr/sac)		Glucose (μmole/hr/sac)		
Mucosal	Serosal	exp.	(μg/ml)	(μEq/hr/sac)	Transfer	Uptake	Transfer	Uptake	Metabolism
		24	0	24 ± 3	19·4 ± 2·4	26·6 ± 6·1			
		12	50	20 ± 4	18·3 ± 2·4	30·5 ± 4·4			
		12	100	15 ± 2	13·3 ± 1·6	24·0 ± 3·6			
	210	24	0	68 ± 9	33·1 ± 6·0	40·5 ± 5·6			60·0 ± 5·6
	210	12	50	70 ± 9	28·6 ± 8·5	44·5 ± 4·7			65·7 ± 6·1
	210	12	100	64 ± 9	27·1 ± 6·9	44·5 ± 3·6			64·5 ± 4·2
28		23	0	55 ± 8	13·2 ± 1·9	30·0 ± 4·2	19·6 ± 3·3	67·2 ± 6·7	47·6 ± 5·6
28		12	50	59 ± 9	12·8 ± 2·0	27·2 ± 5·8	16·0 ± 3·2	86·1 ± 9·7	70·1 ± 9·2
28		12	100	56 ± 8	11·2 ± 0·9	28·4 ± 5·3	10·9 ± 2·8	62·1 ± 7·5	51·2 ± 6·4

Ten-cm sacs of everted starved jejunum, containing 0·6 ml KRB buffer with or without 210 mM glucose, were incubated in 5 ml KRB buffer containing 27·8 mM galactose in all cases and, in addition, 27·8 mM glucose where indicated. Glibenclamide, when present, was contained in the mucosal fluid. Incubations were carried out at 37° for 1 hr.

TABLE 5. EFFECT OF GLIBENCLAMIDE ON SODIUM, GLUCOSE AND GALACTOSE UTILIZATION BY SACS OF EVERTED JEJUNUM FROM RATS WHICH WERE STARVED BUT FED GLUCOSE

Initial glucose concn (mM)		No. exp.	Glibenclamide concn (μg/ml)	Sodium transfer (μEq/hr/sac)	Galactose (μmole/hr/sac)		Glucose (μmole/hr/sac)		
Mucosal	Serosal				Transfer	Uptake	Transfer	Uptake	Metabolism
		24	0	24 ± 4	15·4 ± 1·9	40·0 ± 7·5			
		12	50	18 ± 3	12·1 ± 1·9	39·0 ± 7·8			
		12	100	13 ± 3	11·9 ± 1·4	34·0 ± 5·6			
	210	24	0	61 ± 8	25·9 ± 6·1	46·2 ± 7·5			61·1 ± 4·7
	210	12	50	68 ± 8	29·0 ± 7·5	54·5 ± 8·1			64·5 ± 4·5
	210	12	100	49 ± 8	24·4 ± 7·0	34·0 ± 5·6			61·1 ± 4·5
28		24	0	66 ± 5	15·6 ± 1·6	36·7 ± 3·3	16·6 ± 2·1	77·2 ± 5·6	60·6 ± 6·7
28		12	50	55 ± 8	13·1 ± 1·6	32·3 ± 7·5	9·0 ± 1·9	62·8 ± 6·7	53·8 ± 5·8
28		12	100	28 ± 4	9·6 ± 1·4	26·6 ± 0·6	2·5 ± 5·3	41·1 ± 4·7	38·6 ± 4·7

Ten-cm sacs of everted glucose-fed starved jejunum, containing 0·6 ml KRB buffer with or without 210 mM glucose, were incubated in 5 ml KRB buffer containing 27·8 mM galactose in all cases and, in addition, 27·8 mM glucose where indicated. Glibenclamide, when present, was contained in the mucosal fluid. Incubations were carried out at 37° for 1 hr.

in diabetes. This increase cannot be explained in terms of an increase in mucosal cell population.[19]

Finally, from Tables 3–5, it appears that galactose transfer is not as sensitive to glibenclamide as glucose transfer. Since galactose is transported but not metabolized by intestinal cells this observation provides further evidence that the drug affects glucose transfer by an alteration in the metabolic rate through changes in ATP levels. Since mucosal uptake requires energy,[20] glucose and galactose uptake are seen to vary approximately with glucose metabolism. Galactose transfer, being partially dependent on the serosal Na-pump as well as mucosal uptake, varies with sodium transfer which in turn depends on oxidative metabolism.[21]

Acknowledgements—We are indebted to the British Diabetic Association for financial support. We also wish to thank Mr. J. van Helmond and Mr. G. Harper for technical assistance.

REFERENCES

1. J. D. TEALE and A. H. G. LOVE, *Biochem. Pharmac.* **21,** 1839 (1972).
2. T. H. WILSON and G. WISEMAN, *J. Physiol.* **123,** 116 (1954).
3. A. JUNOD, A. E. LAMBERT, W. STAUFFACHER and A. E. RENOLD, *J. clin. Invest.* **48,** 2129 (1969).
4. H. A. KREBS and K. HENSELEIT, *Hoppe-Seyler's Z. physiol. Chem.* **210,** 33 (1932).
5. A. ST. G. HUGGET and D. A. NIXON, *Lancet* **2,** 368 (1957).
6. N. NELSON, *J. biol. Chem.* **153,** 375 (1944).
7. M. SOMOGYI, *J. biol. Chem.* **160,** 61 (1945).
8. B. HELLMAN, L.-A. IDAHL, H. TJALVES and A. DANIELLSON, *Diabetes* **18,** 509 (1969).
9. A. B. FALCONE, R. L. MAO and E. SHRAGO, *J. biol. Chem.* **237,** 904 (1962).
10. S. S. CHAN and J. N. FAIN, *Molec. Pharmac.* **6,** 513 (1970).
11. P. J. DE SCHEPPER, *Biochem. Pharmac.* **16,** 2337 (1967).
12. T. H. WILSON and G. WISEMAN, J. *Physiol.* **123,** 126 (1954).
13. R. K. CRANE, *Fedn Proc.* **21,** 891 (1962).
14. L. M. SRIVASTAVA and G. HUBSCHER, *Biochem. J.* **100,** 458 (1966).
15. W. G. J. IEMHOFF, J. W. O. VAN DEN BERG, A. M. DE PIJPER and W. C. HULSMAN, *Biochim biophys. Acta* **215,** 229 (1970).
16. H. J. LEESE and K. R. L. MANSFORD, *J. Physiol.* **212,** 819 (1971).
17. H. NEWEY, P. A. SANFORD and D. H. SMYTH, *J. Physiol.* **208,** 705 (1970).
18. H. S. A. SHERRATT, *Comp. Biochem. Physiol.* **24,** 745 (1968).
19. W. A. OLSEN and I. H. ROSENBERG, *J. clin. Invest.* **49,** 96 (1970).
20. G. A. KIMMICH, *Hoppe-Seyler's Z. physiol. Chem.* **353,** 3 (1972).
21. S. G. SCHULTZ and R. ZALUSKY, *J. gen. Physiol.* **47,** 1043 (1964).