Biochimica et Biophysica Acta, 778 (1984) 618–622
Elsevier

BBA Report

BBA 70195

REGULATORY EFFECTS OF INSULIN AND EXPERIMENTAL DIABETES ON NEUTRAL AMINO ACID TRANSPORT IN THE PERFUSED RAT EXOCRINE PANCREAS

KINETICS OF UNIDIRECTIONAL L-SERINE INFLUX AND EFFLUX AT THE BASOLATERAL PLASMA MEMBRANE

G.E. MANN and P.S.R. NORMAN

Department of Physiology, Queen Elizabeth College, University of London, Campden Hill Road, London W8 7AH (U.K.)

(Recevied August 7th, 1984)

Key words: Amino acid transport; Serine transport; Insulin; Diabetes; (Rat exocrine pancreas)

Relatively little is known about the hormonal regulation of amino acid transport in the normal and diabetic exocrine pancreas. In this study unidirectional influx and tracer efflux of L-serine at the basolateral interface of the rat pancreatic epithelium was investigated in the perfused exocrine pancreas using a rapid (< 30 s) paired-tracer dilution technique. In the non-diabetic pancreas L-serine influx was saturable and stimulated by perfusion with exogenous bovine insulin (100 μU/ml). Transport of L-serine and methylaminoisobutyric acid was markedly elevated in pancreata isolated from streptozotocin diabetic rats and insulin partially reversed the stimulation of L-serine transport induced by experimental diabetes. These results suggest that insulin and diabetes modulate the epithelial transport activity for small neutral amino acids in the intact exocrine pancreas.

Pancreatic exocrine cells are known to accumulate extracellular amino acids rapidly against a concentration gradient and also exhibit the highest protein synthesizing capacity of any secretory organ [1]. In the rat the existence of an insulo-acinar portal axis [2] and the localization of high-affinity insulin receptors in the basolateral membrane of pancreatic acinar cells [3–5] suggests that insulin may regulate nutrient transport. Earlier studies with pancreatic fragments in vitro failed to demonstrate a significant effect of insulin on amino acid uptake [6,7]. More recent experiments with pancreatic acini isolated from normal and streptozotocin-diabetic rats have only established a stimulatory effect of insulin on protein synthesis [8], although activation of amino acid transport by insulin in other tissues is well documented [9]. Insulin appears to affect predominantly the transport rate (V_{max}) of a sodium-dependent System A, and in cultured hepatocytes has been reported to partially reverse the marked stimulation of amino acid transport induced by diabetes [10]. In the present study we investigated the regulatory role of a physiological concentration of insulin on the kinetics of L-serine transport, a naturally occurring neutral amino acid, in the perfused exocrine pancreas isolated from non-diabetic and streptozotocin-induced diabetic rats. A preliminary account of part of this work has been communicated in abstract form [11].

Male Sprague-Dawley rats were fasted 24 h before being anaesthetized with intraperitoneal sodium pentobarbitone (60 mg/kg). Experimental diabetes was induced by an intravenous injection of 65 mg/kg streptozotocin (Sigma Chemical Co., U.K.) dissolved in 0.1 M citrate buffer and con-

firmed by elevated plasma glucose levels (21–39 mM), increased urine volumes (> 150 ml/24 h) and markedly decreased insulin concentrations in the pancreatic venous effluent. The non-stimulated pancreas was perfused in situ at a constant flow rate (approx. 1 ml/min per g) via the aorta with a Krebs-Henseleit bicarbonate solution containing 10 g/l bovine serum albumin [11,12]. The perfusate was oxygenated at 38°C with 95% O_2/5% CO_2 to a pH between 7.3 and 7.4. The pancreas was maintained in a heated and humidified perfusion cabinet throughout an experiment and each preparation was perfused in randomized order with different concentrations (0.05–50 mM) of a given unlabelled amino acid. The radioactive molecules, L-[3-^{3}H]serine (29 Ci/mmol), α-[1-^{14}C]methylaminoisobutyric acid (48.4 mCi/mmol), D-[1-^{14}C]mannitol (45 mCi/mmol) and D-[1-^{3}H]mannitol (27 Ci/mmol) were purchased from either New England Nuclear Chemicals, Dreieich, F.R.G. or Amersham International, U.K.

Uptake of L-serine and the non-metabolized System A analogue methylaminoisobutyric acid by the exocrine pancreatic epithelium was measured using a rapid (< 30 s) paired-tracer dilution technique previously described in detail [13]. For example, following a bolus intra-arterial injection (100 μl in 2 s) of L-[^{3}H]serine and D-[^{14}C]mannitol (extracellular reference tracer) thirty 60-μl samples of effluent were collected sequentially from the cannulated portal vein in 45–60 s, and a final venous sample was accumulated for a further 4 min to assess tracer efflux from the pancreatic epithelium. Tracer amino acid uptake (U) in successive venous samples was quantified: $U = 1 - [[^3\text{H}]\text{serine}]/[\text{D-}[^{14}\text{C}]\text{mannitol}]$, and the unidirectional influx (v) was estimated from the maximal fractional tracer uptake (U_{max}), the perfusion rate (F, ml/min per g) and the perfusate concentration of unlabelled L-serine (C_a, mM): $v = -F \cdot \ln(1 - U_{max}) \cdot C_a$ [13,14]. Tracer efflux was estimated from $(1 - (U_T/U_{max})) \times 100$, where the overall uptake of L-[^{3}H]serine (U_T) was calculated from the integrated tracer recoveries starting from the time of maximal uptake and including the final 4-min sample [15].

Transport of L-serine was initially investigated in the non-diabetic pancreas perfused with a wide range of unlabelled L-serine concentrations, and Fig. 1 illustrates the significant self-inhibition observed. In the absence of insulin unidirectional L-serine influx in the normal pancreas was saturable with an apparent $K_t = 16 \pm 3$ mM and $V_{max} = 10.7 \pm 0.9$ μmol/min per g. When in separate experiments the normal pancreas was perfused for 20–30 min with bovine insulin (100 μU/ml, Wellcome, U.K.), the transport affinity ($K_t = 26 \pm 2$ mM) was decreased, whereas the transport rate ($V_{max} = 22.6 \pm 0.6$ μmol/min per g) significantly increased (Fig. 2). In other tissues the mechanism by which insulin stimulates uptake appears to be independent of cyclic AMP-mediated processes and is influenced by the sodium electrochemical gradient [9]. As previous studies in the pancreas have shown that small neutral analogues such as methylaminoisobutyric acid [14] and L-alanine [16,17] are co-transported with sodium, it is possible that an insulin-induced membrane hyperpolarization demonstrated in a variety of tissues [18], could in the pancreas also provide the energy source for stimulation of System A activity. However, it is also conceivable that the elevated transport activity observed was secondary to insulin's effect on the driving forces for amino acid uptake and/or protein synthesis. In cultured hepatocytes evidence is available that insulin stimulates the synthesis of a high-affinity compo-

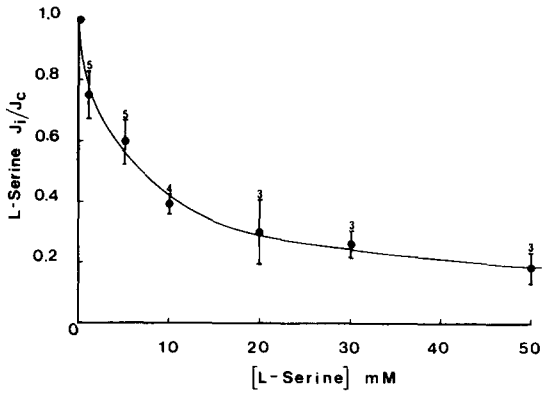

Fig. 1. Self-inhibition of unidirectional L-serine influx at the basolateral plasma membrane of the exocrine pancreatic epithelium. Inhibition of L-[^{3}H]serine influx was measured at a constant perfusate concentration of 0.05 mM in the absence (J_c) or presence (J_i) of increasing concentrations of unlabelled L-serine. Values are the means ± S.E. of n measurements made in a total of nine rats.

nent of System A transport [19] which is suppressed following microtubule disruption [20]. The ineffectiveness of insulin in enhancing amino acid uptake in pancreatic fragments [6,7] or isolated acini [8] may have occurred due to the loss of hormone sensitivity and/or membrane polarity [21].

In the diabetic pancreas transport of L-serine was increased well above the insulin-stimulated control preparations (Fig. 2) and the kinetic constants estimated were $K_t = 33 \pm 6$ mM and $V_{max} = 34 \pm 4$ μmol/min per g. In contrast to L-serine, saturation of methylaminoisobutyric acid influx occurred at much lower concentrations in the non-diabetic pancreas ($K_t = 1.8 \pm 0.3$ mM; $V_{max} = 0.54 \pm 0.04$ μmol/min per g; $n = 6$ rats), and appeared to be enhanced in the diabetic pancreas ($K_t = 4.1 \pm 1.9$ mM, $V_{max} = 0.98 \pm 0.30$ μmol/min per g). Interestingly, the diabetes-induced stimulation of transport observed in our experiments appears to be paralleled by a decrease in amylase and mRNA content in isolated diabetic acini [22]. When we perfused the diabetic pancreas with 100 μU/ml insulin, both the K_t(25 ± 2 mM) and V_{max} (21.3 ± 0.7 μmol/min per g) for L-serine decreased to values observed in the insulin-stimulated non-diabetic preparation (Fig. 2). Our findings demonstrate that insulin acts directly on the exocrine pancreatic epithelium to reduce the effect of diabetes on neutral amino acid transport. Similarly, administration of insulin to diabetic rats in vivo has been shown to reverse diabetes-stimulated amino acid transport in hepatocytes [10] and R3230AC mammary adenocarcinoma cells [23]. In hepatocytes a possible regulatory role for glucagon was inferred from the observation that diabetes is generally characterized by elevated plasma glucagon levels [24] and that glucagon itself stimulates neutral amino acid transport in the liver and other tissues [9,10]. A prolonged exposure in vivo to high levels of glucagon could induce System A activity. Although an elevated secretion of glucagon from diabetic pancreatic islets has been reported [25], the levels of glucagon in the venous effluent of the perfused pancreas isolated from normal and streptozotocin-diabetic rats do not appear to differ [26]. The precise mechanism by which insulin reverses the diabetes-induced stimulation of amino acid transport remains to be determined.

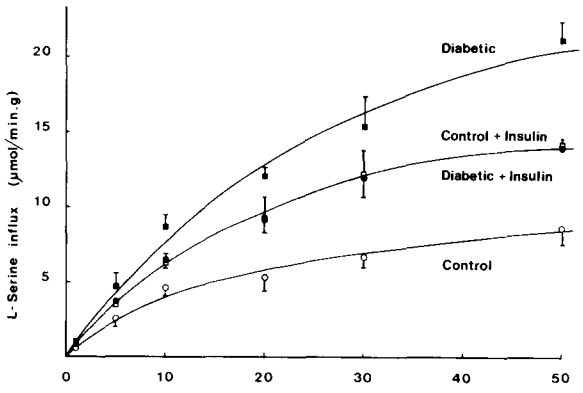

Fig. 2. Effects of insulin and experimental diabetes on the kinetics of L-serine influx across the pancreatic exocrine epithelium. The regulatory effect of 100 μU/ml bovine insulin (Wellcome, U.K.) on L-serine transport was studied in pancreata isolated from control non-diabetic (− insulin: ○———○, $n = 9$; + insulin: □———□, $n = 7$) and streptozotocin-treated diabetic (− insulin: ■———■, $n = 3$; + insulin: ●———●, $n = 3$) rats. In each perfused pancreas influx was measured successively at different unlabelled L-serine concentrations (0.05–50 mM), and the solid curves represent single rectangular hyperbolas obtained by a direct fit to each of the four sets of mean influx values. For each curve the mean influx values were weighted for the reciprocal of their respective standard errors and the vertical lines denote the S.E. for at least three measurements. The calculated curves for insulin-treated non-diabetic and diabetic pancreata were virtually superimposed and hence only one curve is shown.

We also investigated the possible regulatory effects of insulin and diabetes on the cellular efflux of transported L-serine, and Fig. 3 illustrates the concentration dependence of L-serine efflux from the epithelium. Insulin had no effect on efflux in either the normal (Fig. 3) or diabetic (data not shown) pancreas. Saturation of L-serine influx in all the present experiments usually occurred at a perfusate concentration around 30–40 mM (well above L-serine's concentration in rat plasma), and yet tracer efflux reached a maximum value at 10 mM (Fig. 3). The differential sensitivity of influx and efflux to the circulating concentration of unlabelled L-serine together with the observation that at 0.05 mM L-serine influx remained constant over a flow range of 0.4–1.3 ml/min per g argues against the presence of a diffusive resistence to overall L-serine transport. In an attempt to partially resolve the actions of insulin we also investigated the effects of *N*-ethyl-

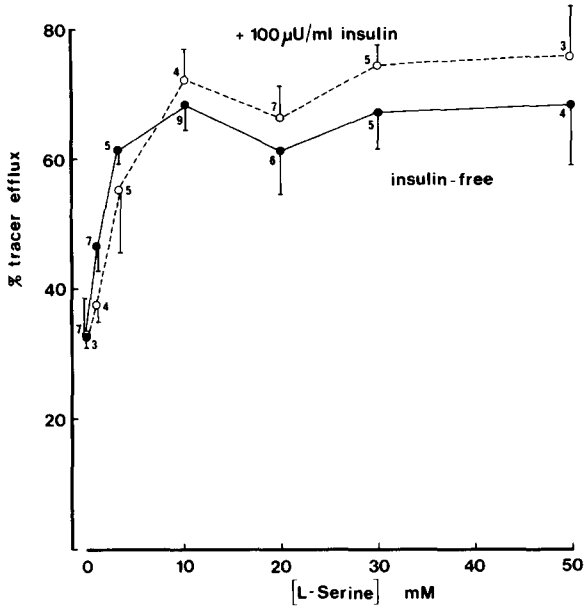

Fig. 3. Effect of insulin on the concentration dependent efflux of transported L-serine from the epithelium in the non-diabetic pancreas. Tracer efflux was calculated in all of the kinetic influx experiments. A thin-layer chromatographic analysis [15] of the pancreatic venous effluent indicated that 5-min after the intra-arterial injection of L-[^{3}H]serine the ^{3}H activity was still associated with L-serine. In the absence of insulin and at low concentrations of L-serine (0.05 mM) the efflux was $32 \pm 2\%$ ($n = 7$), and at concentrations above 5 mM efflux increased to an average value of $65 \pm 2\%$ ($n = 29$). When preparations isolated from non-diabetic and diabetic (data not shown) rats were perfuxed with 100 μU/ml insulin the pattern of efflux remained unchanged. These data indicate the insulin selectively regulates unidirectional amino acid influx without altering its efflux.

maleimide, a sulfhydryl blocker. Experiments were performed in the normal pancreas and influx of L-serine was measured at a perfusate concentration of 10 mM. In the absence of insulin perfusion with 1 mM *N*-ethylmaleimide for up to 20 min had no effect on L-serine influx or tracer efflux. Moreover, the insulin-induced stimulation of transport observed in the non-diabetic pancreas (Fig. 2) could not be reversed by subsequent perfusion with 1 mM *N*-ethylmaleimide. Contrary to our findings, Czech [27] has reviewed the inhibitory and/or stimulatory effects of *N*-ethylmaleimide on transport and concluded that sulfhydryl-disulfide interchanges may be involved in the activation and deactivation of insulin's effects.

The rapid tracer technique [13] applied in this study has enabled us to characterize the regulatory effects of insulin on pancreatic amino acid influx independently of its actions on cellular metabolism and protein synthesis. Our results suggest that exogenous insulin and experimental diabetes modulate the transport activity for neutral amino acids at the basolateral interface of the pancreatic exocrine epithelium. Although L-serine influx may represent the overall uptake by several membrane carriers, including the hormone-sensitive System A [9], the insulin- and diabetes-induced alterations in transport are of relevance to clinical pancreatic function tests.

This work was supported by a grant to G.E.M. from MRC, U.K. We acknowledge the Wellcome Trust, U.K., for a Summer Vacation Scholarship to P.S.R. N. We thank Dr. R.G. Price for supplying the diabetic rats, Dr. S.L. Howell's research group for assistance with insulin RIA and plasma glucose determinations, and Professor D.L. Yudilevich for his helpful comments on the manuscript.

References

1 Case, R.M. (1978) Biol. Rev. 53, 211–354
2 Ohtani, O., Kukita, A., Ohtsuka, A., Taguchi, T. and Murakami, T. (1983) Arch. Histol. Jpn. 46, 1–42
3 Bergeron, J.J.M., Rachubinski, R., Searle, N., Sikstrom, R., Borts, D., Bastian, P. and Posner, B.I. (1980) Endocrinology 107, 1069–1080
4 Sankaran, H., Iwamoto, Y., Korc, M., Williams, J.A. and Goldfine (1981) Am. J. Physiol. 240, G63–G68
5 Goldfine, I.D., Kriz, B.M., Wong, K.Y., Hradek, G., Jones, A.L. and Williams, J.A. (1981) Am. J. Physiol. 240, G69–G75
6 Daneilsson, A. and Sehlin, J. (1974) Acta Physiol. Scand. 91, 557–565
7 Bieger, W., Weicker, H. and Haymovits, A. (1979) Horm. Metab. Res. 11, 352–358
8 Korc, M., Iwamoto, Y., Sankaran, H., Williams, J.A. and Goldfine, I.D. (1981) Am. J. Physiol. 240, G56–G62
9 Guidotti, G.G., Borghetti, A.F. and Gazzola, G.C. (1978) Biochim. Biophys. Acta 515, 329–366
10 Barber, E.F., Handlogten, M.E., Vida, T.A. and Kilberg, M.S. (1982) J. Biol. Chem. 257, 14960–14967
11 Mann, G.E. and Norman, P.S.R. (1984) J. Physiol. 354, 53P
12 Mann, G.E., Peran, S. and Yudilevich, D.L. (1983) Scand. J. Gastroenterol. 18, Suppl. 87, 114–115
13 Yudilevich, D.L. and Mann, G.E. (1982) Fed. Proc. 41, 3045–3053
14 Mann, G.E. and Yudilevich, D.L. (1984) in Secretion: Mechanisms and Control (Case, R.M., Lingard, J.M. and

Young, J.A., eds.), pp. 133–136, Manchester University Press, Manchester
15 Mann, G.E., Tradatti, C.E. and Yudilevich, D.L. (1982) Biochim. Biophys. Acta 692, 157–160
16 Tyrakowski, T., Milutinovic, J. and Schulz, I. (1978) J. Membrane Biol. 38, 333–346
17 Iwatsuki, N. and Petersen, O.H. (1980) Nature 283, 492–494
18 Moore, R.D. (1983) Biochim. Biophys. Acta 737, 1–49
19 Fehlmann, M., Le Cam, A. and Freychet, P.J. (1979) J. Biol. Chem. 254, 10431–10437
20 Prentki, M., Crettaz, M. and Jeanrenaud, B. (1981) J. Biol. Chem. 256, 4336–4340
21 Christensen, H.N. (1982) Physiol. Rev. 62, 1193–1233
22 Otsuki, M. and Williams, J.A. (1982) J. Clin. Invest. 70, 148–156
23 Hissin, P.J. and Hill, R. (1978) Cancer Res. 38, 36646–36655
24 Ungar, R.H. (1978) Metab. Clin. Exp. 27, 1691–1706
25 Buchanan, K.D. and Mawhinney, W.A.A. (1973) Diabetes 22, 797–800
26 Goto, Y., Berelowitz, M. and Frohman, L.A. (1981) Diabetologia 20, 66–71
27 Czech, M.P. (1977) Annu. Rev. Biochem. 46, 359–384