Biochimica et Biophysica Acta, 943 (1988) 541–546
Elsevier

BBA 70576

BBA Report

Secretagogue-induced changes in system A amino acid transport in the rat exocrine pancreas: stimulation of 2-methylaminoisobutyric acid efflux by carbachol

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(Received 7 March 1988)
(Revised manuscript received 1 July 1988)

Key words: Amino acid transport; Amino acid efflux; System A; Sodium ion dependence; 2-Methylaminoisobutyric acid; Carbachol; Pancreatic secretion; (Rat pancreas)

Secretagogue-induced changes in exocrine pancreatic amino acid transport are poorly understood. In this study uptake of the specific non-metabolized System A amino acid analogue 2-methylaminoisobutyric acid (2-MeAIB) was measured in the isolated perfused rat pancreas during 60 min loading with D-[^{3}H]mannitol (extracellular tracer) and 2-[^{14}C]MeAIB. Tracer 2-MeAIB reached a maximal uptake of $37 \pm 4\%$ ($n = 4$) after 3 min of loading and gradually decreased to a steady-state uptake of $13 \pm 1\%$. Infusion of carbachol ($3 \cdot 10^{-7}$ M) during the tracer loading period abolished net tracer 2-MeAIB uptake, and reperfusion in the absence of carbachol restored net uptake to the prestimulus value. Less than 41% of the arterial 2-[^{14}C]MeAIB or D-[^{3}H]mannitol activity appeared in the basal pancreatic secretion. Carbachol evoked a 4.8-fold increase in pancreatic juice flow and appeared to reduce the activity of both tracers in the exocrine secretion. During washout of the pancreas with an isotope-free medium 2-[^{14}C]MeAIB cleared from a rapidly exchanging pool with a time constant (τ_1) of 1.4 ± 0.3 min ($n = 4$) and a more slowly exchanging pool with a time constant (τ_2) of 20.7 ± 1.1 min. Carbachol accelerated efflux of 2-[^{14}C]MeAIB from the epithelium but had no effect on the slow phase of D-[^{3}H]mannitol washout. Our findings suggest that activation of cholinergic receptors modifies Na^+-dependent System A amino acid transport in the basolateral membrane of the exocrine pancreatic epithelium.

Little is known concerning the mechanisms by which secretagogues modulate amino acid transport in the exocrine pancreas. Although earlier work in pancreatic fragments failed to identify a regulatory role for secretagogues [1,2], more recent studies in isolated mouse pancreatic acini demonstrated that caerulein, cholecystokinin and carbachol stimulated facilitated D-glucose transport [3] but inhibited Na^+-dependent aminoisobutyric acid (2-AIB) accumulation [4]. Caerulein was shown to simultaneously reduce influx and stimulate efflux of 2-AIB. Inhibition of 2-AIB accumulation appeared to be mediated by intracellular Ca^{2+}, however, indirect effects of changes in the membrane potential and Na^+-gradient could not excluded [4].

Our previous studies of methylaminoisobutyric acid (2-MeAIB) transport in the isolated perfused rat pancreas revealed that influx of this non-

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metabolized System A analogue was saturable (K_t = 1.7 mM, V_{max} = 0.49 μmol/min per g), critically dependent upon the Na^+ gradient, inhibited by ouabain and sensitive to trans-inhibition [5,6]. Electrogenic Na^+/alanine cotransport has been described in pancreatic membrane vesicles [7], and in pancreatic acinar cells L-alanine evokes a marked basolateral membrane depolarization [8,9]. Recent whole-cell patch clamp studies with mouse pancreatic acinar cells have established that this cotransporter exhibits sensitivity to 2-MeAIB, transport symmetry, pH dependence and a sodium/alanine stoichiometry of 1 : 1 [10]. These findings together with our observations [5,6] are consistent with transport occurring via the hormone sensitive System A [11–13]. Moreover, K_t values for alanine transport in patch clamp studies (K_t = 2.9 mM, [10]) and previous microelectrodes studies (K_t = 1.2–1.6 mM, [9,10]) are similar to the K_t we measured for 2-MeAIB in the perfused rat pancreas [5].

In the present study we have investigated the effects of carbachol on 2-[^{14}C]MeAIB uptake and efflux in the isolated perfused rat pancreas [14–16]. Transfer of an extracellular tracer and 2-[^{14}C]-MeAIB into the pancreatic secretion was determined simultaneously. A dual isotope loading and washout technique [17] was used to assess 2-[^{14}C]MeAIB transport at the basolateral membrane of the exocrine epithelium. Simultaneous measurements of uptake and release of D-[^{3}H]mannitol were used to correct transport for the extracellular component. The protocol for a tracer loading and washout experiment is il-

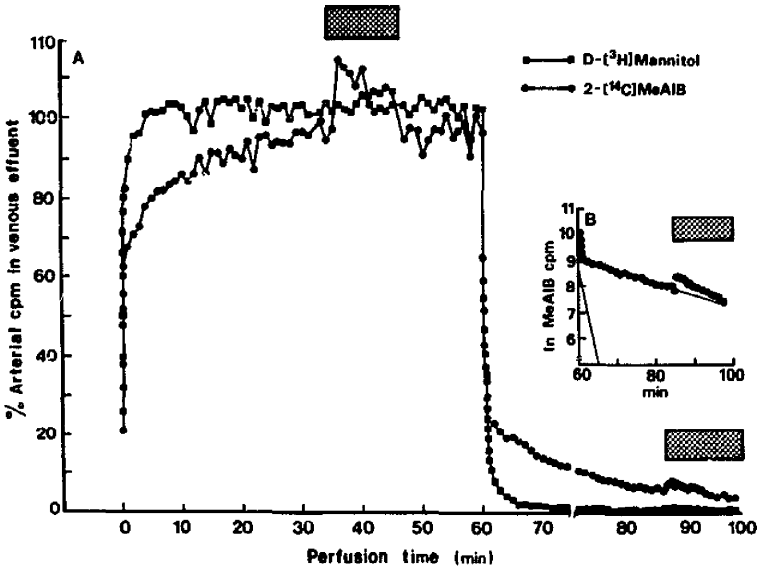

Fig. 1. Steady-state loading and washout experiment in the perfused rat pancreas. (A) pancreata were isolated from anesthetized rats (6 mg/100 g Sagatal, i.p.) and perfused at constant flow (1.75 ml/min) via the coeliac and superior mesenteric arteries with an oxygenated Krebs-Henseleit bicarbonate medium containing 2.5 mM D-glucose, 5% Dextran T70 and 0.25% bovine serum albumin (see Refs. 5, 6, 14). The pancreatic effluent was collected from the portal vein and pancreatic juice flow was sampled from a small cannula in the main exocrine duct. Following 20 min equilibration pancreata were preloaded for 60 min with D-[^{3}H]mannitol (0.2 μCi/ml) and the nonmetabolized amino acid analogue 2-[^{14}C]MeAIB (0.4 μCi/ml). Recoveries of both tracers in successive venous samples (100 μl) are expressed as a % of the radioactivity present in 100 μl of arterial reservoir perfusate. The abscissa denotes the time elapsed from commencing artificial perfusion of the isolated pancreas. At 60 min the perfusate was switched to an isotope-free solution and washout of both 2-[^{14}C]MeAIB and D-[^{3}H]mannitol was monitored for a further 40–45 min. Pancreata were challenged with $3 \cdot 10^{-7}$ M carbachol (hatched bars) during the loading (30–40 min) and washout (85–105 min) periods. (B) regression analysis of the semilog plot of the later part of 2-[^{14}C]MeAIB washout (perfusion time 65–100 min) yielded an exponential which could be distinguished from the initial rapid phase of washout. This line was extrapolated to $t = 0$ (end of 60 min loading) and linear values were subtracted from the early data points. Regression analysis of the remaining data yielded a second line for fast phase of washout. Reciprocals of the slopes of these two lines gave time constants for, respectively, the fast (τ_1) and slow (τ_2) phases of washout.

lustrated in Fig. 1A. Isolated pancreata were preperfused for 20 min with an amino acid-free and tracer-free solution and then loaded with 2-[^{14}C]MeAIB and D-[^{3}H]mannitiol for 60 min. Initially sequential samples of the pancreatic venous effluent were collected to monitor the rise in tracer concentrations, and thereafter the sampling rate was reduced to 1 min^{-1} throughout the 60 min loading period. The venous recovery of D-[^{3}H]mannitol rapidly approached 100%, suggesting that D-mannitol is a useful extracellular tracer in the perfused rat pancreas. The lower recovery of 2-[^{14}C]MeAIB in the venous effluent partly reflects cellular uptake and movement into the secretory duct system.

Tracer washout from extracellular and cellular compartments was monitored during perfusion of the preloaded pancreas with an isotope-free medium (Fig. 1A). D-[^{3}H]Mannitol was rapidly cleared from the pancreas and washout could be fitted by a double exponential (data not shown). The time constant for the prominent fast phase (τ_1) of washout was 1.5 ± 0.2 min (mean $\pm$ S.E., $n = 4$) compared to 35.1 ± 3.8 min ($n = 4$) for the slower phase of washout. The time constant for the fast phase (1.35 min) of 2-[^{14}C]MeAIB washout was similar to that for D-mannitol, whereas the slow phase of washout had a time constant of 22.1 min (Fig. 1B). In four experiments time constants for the fast (τ_1) and slow (τ_2) phases of 2-[^{14}C]MeAIB washout were, respectively, 1.4 ± 0.3 min and 20.7 ± 1.1 min.

Net tracer 2-MeAIB uptake was determined from the difference in the venous recoveries of the labelled extracellular tracer and test amino acid (see Fig. 1A). The uptake measured for 2-[^{14}C]MeAIB ($37 \pm 4\%$, mean $\pm$ S.E., $n = 4$) during the early loading phase was identical to the unidirectional uptake ($37 \pm 2\%$, $n = 8$) measured previously under zero-trans conditions in the isolated perfused rat pancreas [15]. Increasing efflux of 2-[^{14}C]MeAIB from the epithelium decreased net tracer uptake, and after 25–30 min a steady-state uptake of $13 \pm 1\%$ was measured (Fig. 2A). When the pancreas was challenged with $3 \cdot 10^{-7}$ M carbachol during the isotope loading period net uptake of 2-[^{14}C]MeAIB was immediately inhibited (Fig. 2A). Reference to Fig. 1A shows that venous recoveries of 2-[^{14}C]MeAIB actually exceeded recoveries for D-[^{3}H]mannitol during the first 4 min of carbachol infusion, suggesting that cellular efflux may also have been stimulated. At

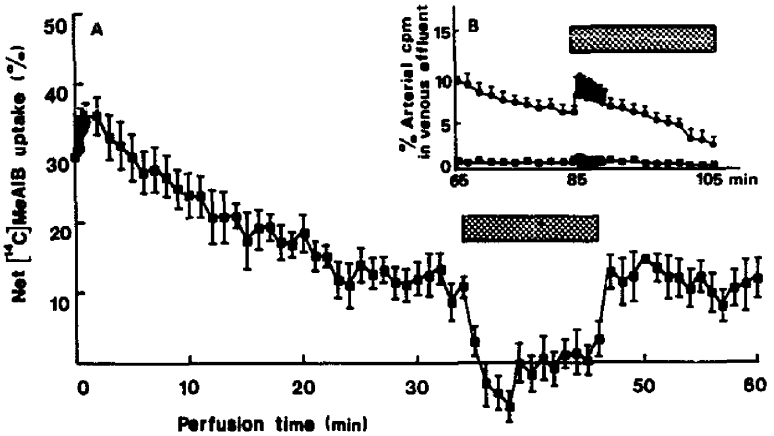

Fig. 2. Effects of carbachol on 2-MeAIB uptake and efflux in the pancreatic exocrine epithelium. (A) 2-[^{14}C]MeAIB uptake relative to D-[^{3}H]mannitol was measured during the 60 min tracer loading period: % uptake = $(1-(2\text{-}[^{14}C]MeAIB/D\text{-}[^{3}H]mannitol)) \times 100$. After 35 min of loading the perfusate was switched for 10 min to a solution containing the same specific isotope activities and $3 \cdot 10^{-7}$ M carbachol. Tracer loading was then continued for another 15 min in the absence of carbachol. At 60 min the perfusate was switched to an isotope-free solution and tracer washout was monitored for a further 40–45 min (see Fig. 1A). (B) Effects of carbachol on washout profile of 2-[^{14}C]MeAIB (●——●) and D-[^{3}H]mannitol (■——■). Carbachol was infused after 25 min of washout and the venous sampling rate was initially increased from 1 min^{-1} to 25 samples per min. Values denote the mean $\pm$ S.E. or +S.E. ($n = 4$), and if not shown the error falls within the symbol.

an arterial flow rate of 1.75 ml/min it seems unlikely that the carbachol evoked juice flow of 6 μl/2 min would have led to a concentration of counts in the venous outflow. Upon removal of carbachol net 2-[^{14}C]MeAIB uptake returned to prestimulus levels.

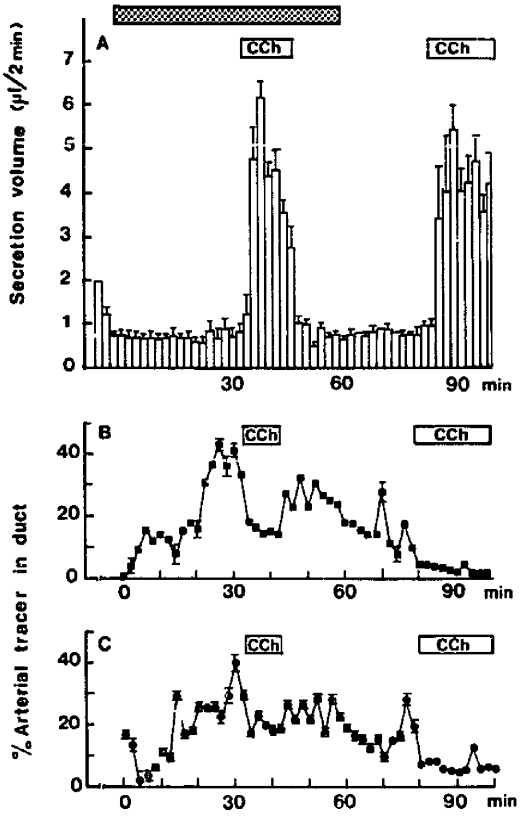

Fig. 3. Effects of carbachol on transepithelial permeability and secretion in the pancreas. (A) Basal and carbachol-induced (CCh, $3 \cdot 10^{-7}$ M) pancreatic secretion was collected from the pancreatic duct at two minute intervals during the isotope loading (0–60 min perfusion) and tracer washout (60–105) periods. The hatched rectangle denotes the 60 min tracer loading period. (B) Concentration of D-[^{3}H]mannitol in the pancreatic secretion collected under basal conditions and during infusion of carbachol in the loading and washout phases of the experiment. Tracer concentrations in pancreatic juice are expressed as the % of activity in an identical volume of arterial perfusate. (C) Concentration of 2-[^{14}C]MeAIB in pancreatic juice in the absence and presence of $3 \cdot 10^{-7}$ M carbachol. Values denote the mean ± S.E. or + S.E. ($n = 4$), and if not shown error bars fall within the symbols.

In these experiments we simultaneously measured transfer of labelled D-mannitol (Fig. 3B) and 2-MeAIB (Fig. 3C) into the basal and carbachol-evoked pancreatic secretion (Fig. 3A). Pancreatic juice flow increased from a basal value of 0.75 ± 0.04 μl/2 min ($n = 69$ observations) to 3.63 ± 0.38 μl/2 min (mean ± S.E., $n = 28$) during a vascular challenge with $3 \cdot 10^{-7}$ M carbachol. Tracer recoveries per μl of pancreatic juice were expressed as a % of the radioactivity present in an equal volume of arterial perfusate. Both tracers appeared in the basal secretion within 5 min, and after 25–30 min loading the collected pancreatic juice contained between 25–41% of the arterial D-[^{3}H]mannitol and [^{14}C]MeAIB activity. Although carbachol increased the total number of counts in pancreatic juice, the concentration of both tracers was decreased by the enhanced fluid secretion.

To further characterize the mechanism underlying the carbachol-induced inhibition of 2-MeAIB uptake, we monitored amino acid efflux into the circulation under zero-trans conditions following 60 min tracer loading. After 25 min washout with an isotope-free perfusate the pancreas was challenged with $3 \cdot 10^{-7}$ M carbachol. Fig. 2B shows the venous recoveries for both tracers before and during stimulation with carbachol. The venous recovery of D-[^{3}H]mannitol was now very low compared with that for 2-[^{14}C]MeAIB. Carbachol transiently accelerated efflux of 2-[^{14}C]MeAIB but had no effect on the residual washout of D-[^{3}H]mannitol. During the initial period of carbachol infusion the time constant for the slow phase of 2-[^{14}C]MeAIB washout decreased from 20.7 min to 15.1 ± 3.5 min ($n = 4$). Pancreatic juice flow increased from a basal value of 0.81 ± 0.03 μl/2 min to 4.27 ± 0.31 μl/2 min (Fig. 3A) and concentrations of 2-[^{14}C]MeAIB and D-[^{3}H]mannitol were again decreased (Figs. 3B and 3C).

Previous studies in vitro using an isolated rabbit pancreas immersed in a saline-filled bath [18] reported that extracellular sucrose [19] or D-mannitol [20] rapidly appeared in the secreted fluid. Ductal activities of D-mannitol in rabbit pancreas (60% of bath activity, see Table 1 in Ref. 20) are somewhat higher than values measured in the perfused rat pancreas (Fig. 3B). In the isolated

rabbit pancreas cholinergic agonists appear to decrease juice flow [20] but to increase protein output and the concentration of sucrose [19] and D-mannitol [20] in the secretion. Spontaneous juice secretion from the isolated [18] or vascularly perfused [21] rabbit pancreas occurs at near maximal rates and appears to be insensitive to high doses of a cholinergic agonist [20,21]. The marked increase in pancreatic juice flow evoked by carbachol in the rat pancreas would tend to dilute isotope activities (Figs. 3B and 3C), whereas in the rabbit pancreas ductal concentrations may increase as a result of a decreased juice flow and, as Jansen et al. [20] suggested, a secretagogue evoked increase in paracellular permeability. By contrast, the perfused cat pancreas has been shown to be impermeable to D-mannitol [22].

Sodium dependent System A amino acid uptake is driven by the electrochemical gradient for Na^+, and an increase in intracellular sodium (Na_i^+) may decrease unidirectional transport (see Ref. 23). However, experiments in Ehrlich cells, using voltage sensitive dyes, have shown that an elevation in intracellular Na^+ in a metabolically active cell can also increase the membrane potential thereby compensating for increased intracellular Na^+ [24]. The membrane potential in these cells appears to be influenced by coupled Na^+-amino acid efflux and the activity of the Na^+/K^+ pump [24]. Recent studies in Ehrlich cells have shown that System A amino acid transport is stimulated 2–3-fold by a 60 mV membrane hyperpolarization [25]. Studies in pancreatic acinar cells suggested that cholinergic stimulation of basolateral Na^+/K^+ pumps may be mediated indirectly via Na^+ influx [26]. Although there is no direct evidence that pancreatic secretagogues maintain an elevated steady-state intracellular Na^+ concentration (see Refs. 27, 28), microelectrode studies in mouse pancreatic fragments have confirmed that acetylcholine induces a rapid membrane depolarization and an increase in both cytoplasmic Na^+ and Ca^{2+} [29,30]. In this study activation of pancreatic secretion by acetylcholine appeared to be linked to an increase in membrane permeability to Na^+ which resulted in an increased intracellular Na^+ concentration. Furthermore, in the rat pancreas uptake of ^{22}Na has been reported to be stimulated transiently by acetylcholine [31]. In the perfused rat pancreas acetylcholine causes only a small transient depolarization of the acinar cell [32], and it seems unlikely that this change in potential could fully account for the observed inhibition of 2-MeAIB uptake. Without direct measurements of cellular Na^+ or μ_{Na} in our study it is difficult to establish whether the Na^+ gradient or other factors, unrelated to this ion gradient, are involved.

Our findings in the perfused rat pancreas confirm earlier studies in pancreatic acini (see Table 3 in Ref. 4) and parotid gland fragments (see Table 1 in Ref. 33) in which carbachol inhibited accumulation of the less specific transport analogue aminoisobutyric acid (2-AIB) by, respectively, 71% and 53%. Although A23187 mimicked the inhibitory effects of caerulein (and presumably carbachol) in pancreatic acini [4], the calcium ionophore had no effect on 2-AIB accumulation in parotid gland fragments [33]. Iwamoto and Williams [4] concluded that the inhibition of 2-AIB accumulation in mouse pancreatic acini may have been caused directly by changes in cytoplasmic Ca^{2+} or indirectly by changes in cell metabolism or the Na^+ gradient. In pancreatic fragments A23187 has previously been shown to evoke a membrane depolarization and parallel increases in cytosolic Ca^{2+} and Na^+ [30]. Moreover, the magnitude of changes in intracellular Ca^{2+} and Na^+ paralleled those observed in cells exposed to acetylcholine [30]. Keryer and Rossignol [33] have argued that the decreased accumulation of 2-AIB in the parotid gland was not directly related to variations in the membrane potential but mainly due to alterations in the Na^+ concentration gradient.

Our observation that carbachol evoked efflux of 2-MeAIB from the preloaded rat pancreas corroborates earlier studies with 2-AIB in mouse pancreatic acini [4], however, the cellular mechanism(s) mediating these effects remain unclear. Efflux of 2-MeAIB has been reported to be accelerated from HTC hepatoma cells containing high intracellular Na^+, and exit via System A was attributed to ouabain-induced changes in the Na^+ gradient [34]. Furthermore, in Ehrlich cells efflux of amino acids is stimulated by cellular Na^+ suggesting coupled Na^+-amino acid efflux [35]. The present findings demonstrate that cholinergic secretagogues rapidly inhibit influx and accelerate

efflux of Na^+-dependent System A amino acids at the basolateral membrane of the exocrine pancreatic epithelium.

This investigation was supported by a Medical Research Council Project Grant (G8420750SB) and a Wellcome Trust Summer Studentship awarded to Dr. G.E. Mann. We thank Dr. I.C.H. Smith for helpful discussions and Mr. K. Doolabh for his assistance with the illustrations.

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