BBA Report

BBA 71076

Distinction between galactose and phenylalanine effects on alanine transport in rabbit ileum

RAYMOND A. FRIZZELL and STANLEY G. SCHULTZ

Department of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, Pa. 15213 (U.S.A.)

(Received March 12th, 1971)

SUMMARY

The ability of galactose to inhibit alanine transport by isolated rabbit ileum is abolished by metabolic inhibitors and/or ouabain. In contrast, inhibition of alanine transport by phenylalanine persists in the presence of metabolic inhibitors. These findings suggest that the effect of galactose on alanine transport does not reflect an interaction at the carrier level.

The inhibitory effects of actively transported sugars on the transport of a variety of neutral amino acids by *in vitro* small intestine are now well-documented phenomena[1,2]. For example, D-galactose inhibits the transmural transport and cellular accumulation of alanine[3] and stimulates efflux of previously accumulated phenylalanine[4] in segments of isolated rabbit ileum. Suggestions regarding the mechanism by which these interactions are produced include competitive interactions of the sugars and amino acids with a polyfunctional brush border carrier[1] and competition for a limited common energy supply[5]. In support of the latter hypothesis, Bingham *et al.*[6] have demonstrated that galactose, which inhibits amino acid transport *in vitro*, does not inhibit amino acid absorption by *in vivo* rat intestine where energy supplies are presumably non-limiting. Recently several authors[2,7] have argued that the mutual inhibitory interactions of sugars and amino acids in *in vitro* small intestine may be attributed to effects of Na^+-coupled solute transport on the intracellular Na^+ concentration and the Na^+ gradient across the mucosal membrane. To explore this possibility we have examined the effects of galactose on alanine transport under conditions in which the Na^+ gradient has already been abolished by either metabolic inhibition and/or exposure to ouabain.

All experiments were performed on segments of distal ileum from male white rabbits that were sacrificed by intravenous injection of pentobarbitol. The methods for determination of the unidirectional transmural flux of alanine across the intestine from mucosa to serosa have been described previously[8]. The mucosal surface of the tissue was

bathed by a buffered electrolyte solution[8] containing 5 mM alanine. L-[^{14}C] alanine was added to the mucosal solution at zero time and its appearance in the serosal bathing solution was determined. After a steady-state tracer flux was established, 30 mM D-galactose or 30 mM L-phenylalanine was added to the mucosal solution, and the mucosa-to-serosa alanine flux was monitored until a new steady state was obtained. In this manner, the effect of galactose or phenylalanine on transmural alanine transport could be evaluated with each tissue serving as its own control. In each instance, 30 mM mannitol was added to the serosal solution to maintain isotonicity. In several experiments, 10^{-3} M ouabain or 10^{-3} M ouabain *plus* 10^{-3} M KCN was added to both mucosal and serosal solutions and the tissue was incubated for 60 min prior to the addition of [^{14}C] alanine. All experiments were performed at 37° and the solutions were constantly gased with humidified 95% O_2–5% CO_2.

A typical experiment showing the effects of galactose and phenylalanine on the unidirectional alanine flux from mucosa-to-serosa is illustrated in Fig.1. In a total of four

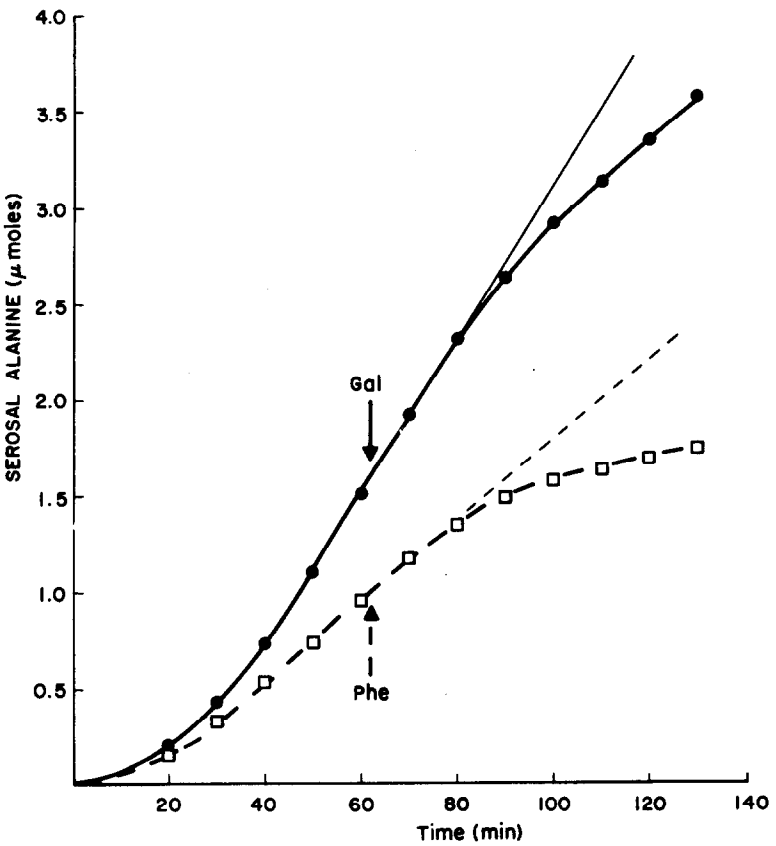

Fig.1. The effect of galactose and phenylalanine on the unidirectional alanine flux from mucosa to serosa. The figure shows the appearance of alanine in the serosal solution before and after the addition of galactose or phenylalanine (at the times indicated by the arrows) to the solution bathing the mucosal surface of adjacent segments of ileum from the same animal. See text for other details.

such experiments, alanine transport was inhibited 34 ± 4% by 30 mM galactose and 68 ± 6% by 30 mM phenylalanine. These results are in good agreement with the effect of galactose on transmural alanine transport reported previously[3]. If, however, the tissue was preincubated in 10^{-3} M ouabain or 10^{-3} M ouabain *plus* 10^{-3} M cyanide for 60 min prior to the flux determination, the inhibition by galactose is abolished as shown in Fig.2. In five experiments on poisoned tissue the inhibition by phenylalanine averaged 39 ± 3% whereas no discernable effect was produced by galactose.

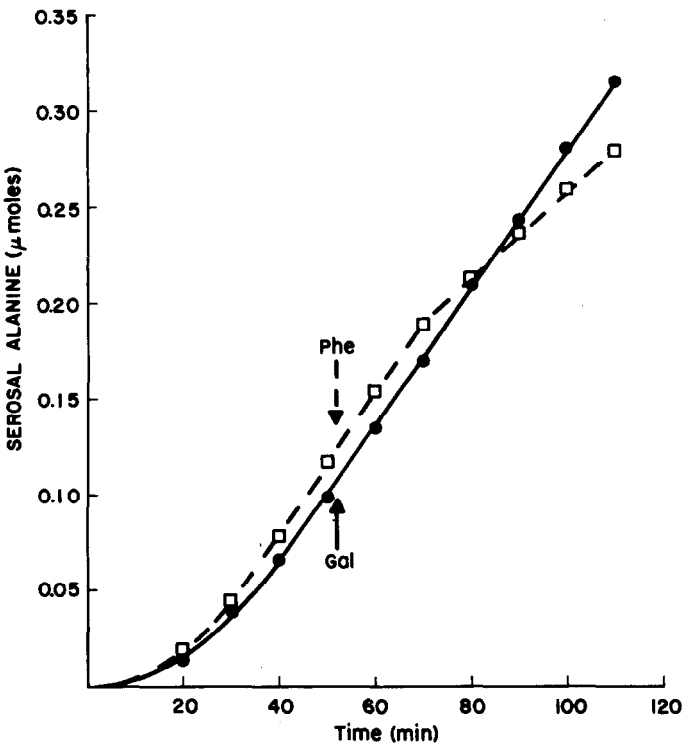

Fig.2. The effect of galactose and phenylalanine on mucosa-to-serosa alanine flux in the presence of 10^{-3} M ouabain *plus* 10^{-3} M KCN. See text and legend of Fig.1 for details.

These results strongly suggest that the mechanisms underlying the inhibitory actions of phenylalanine and galactose on transmural alanine transport differ. Previous studies have shown that metabolic inhibitors and/or ouabain do not affect carrier-mediated unidirectional influx of alanine across brush border of rabbit ileum[9]. The inhibitory effect of phenylalanine on mucosa-to-serosa transmural alanine transport across poisoned tissue can therefore be attributed to a competitive interaction with the brush border mechanism responsible for alanine influx. If, as suggested by Alvarado[1], the inhibitory effect of galactose is also the result of a competitive interaction at the carrier level it is difficult to see why this interaction, and not that of phenylalanine, should be abolished by metabolic inhibition and/or ouabain.

Although these results are difficult to reconcile with the notion of a polyfunctional carrier they are readily interpretable in terms of the Na^+-gradient hypothesis and the more general concept of energy limitation. Accordingly, the inhibitory effect of galactose on transmural transport and accumulation of alanine can be attributed to the increase in intracellular Na^+ concentration that is observed in the presence of galactose[10]. An increased Na^+ influx across the brush border coupled to the influx of galactose as well as energy limitation of the active Na^+ extrusion mechanism are responsible for this increased intracellular Na^+ concentration and the decrease in the Na^+ gradient across the brush border[2]. If, however, the Na^+ gradient has already been abolished by means of metabolic inhibitors or ouabain, inhibition of alanine transport by galactose is no longer observed.

This argument may be generalized to the mutual inhibitory interactions observed with sugars and amino acids in *in vitro* small intestine. If influx of a sugar or amino acid is associated with a concomitant increase in Na^+ influx and if energy supplies to the active Na^+ extrusion mechanisms are limiting, the local or general increase in cell Na^+ concentration will result in a secondary inhibition of all other transport processes that are coupled to the Na^+ gradient. The inhibition will be due primarily to an increased efflux of the organic solute from the cell across the mucosal border; however, in some instances a decreased influx can be observed due to trans-inhibitory effects of intracellular Na^+ that have been discussed in detail previously[2]. The former argument provides an explanation for the observation that sugars stimulate efflux of previously accumulated amino acids[4]. Alvarado *et al.*[4] have considered these observations as examples of countertransport and as strong evidence that sugars and amino acids share a common carrier. Clearly, the enhancement of amino acid efflux can be attributed to a local or general increase in cell Na^+ concentration[2,10] Thus these observations need not imply that amino acids and sugars share a common carrier but rather that two distinct carrier systems share a common co-substrate, Na^+. If this argument is correct, these phenomena should not be considered examples of countertransport.

This work was supported by research grants from the U.S. Public Health Service National Institute of Arthritis and Metabolic Diseases (AM-13744) and the American Heart Association (70-633). R.A.F. is a U.S. Public Health Service Postdoctoral Research Fellow (AM 49585) and S.G.S. is supported by a Research Career Development Award from the National Institute of Arthritis and Metabolic Diseases (AM 9013).

REFERENCES

1 F. Alvarado, *Am. J. Clin. Nutr.*, 23 (1970) 824.
2 S.G. Schultz and P.F. Curran, *Physiol. Rev.*, 50 (1970) 637.
3 R.A. Chez, S.G. Schultz and P.F. Curran, *Science*, 153 (1966) 1012.
4 F. Alvarado, R. Torres-Pinedo, L. Mateu and J.W.L. Robinson, *FEBS Letters*, 8 (1970) 153.
5 H. Newey and D.H. Smyth, *Nature*, 202 (1964) 400.
6 J.K. Bingham, H. Newey and D.H. Smyth, *Biochim. Biophys. Acta*, 130 (1966) 281.
7 C.P. Read, *Biol. Bull.*, 133 (1967) 630.
8 S.G. Schultz and R. Zalusky, *J. Gen. Physiol.*, 47 (1964) 567.
9 R.A. Chez, R.R. Palmer, S.G. Schultz and P.F. Curran, *J. Gen. Physiol.*, 50 (1967) 2357.
10 W. Koopman and S.G. Schultz, *Biochim. Biophys. Acta*, 173 (1969) 338.