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SODIUM-DEPENDENT INHIBITION OF AMINO ACID AND DIPEPTIDE TRANSPORT BY HARMALINE IN MONKEY SMALL INTESTINE*

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Abstract—The effect of harmaline on amino acid and dipeptide uptake was studied in the monkey. Harmaline inhibited both the systems. Preincubation of the tissue with 4 mmole/l harmaline for 10 min was necessary for maximal inhibition. The uptake of glycyl-L-leucine- (^{14}C) and (^{14}C)-glycyl-L-leucine was inhibited to the same extent. The uptake of glycyl-L-proline, a dipeptide which was presumably taken up intact by the mucosal cells, was also inhibited. Total replacement of sodium in the medium reduced the uptake of both glycyl-L-leucine and glycyl-L-proline. But the uptake of these dipeptides was not inhibited by harmaline in the absence of sodium. However, the sodium-independent uptake of glycyl-L-leucine and glycyl-L-proline was also inhibited by other dipeptides.

Harmaline is an alkaloid extracted from *Banisteriopsis caapi*, a Colombian liana [1]. Apart from being a psychoactive drug, it has now become a chemical of much interest in the field of membrane transport because it is a competitive inhibitor of Na^+–K^+ adenosinetriphosphatase in neural tissue [2] and it appears that harmaline competes for the sodium site of the enzyme. More recently, a number of studies have shown that the alkaloid inhibits various transport systems in epithelial tissues. Harmaline reduces both sugar and amino acid uptake by slices of guinea pig small intestine *in vitro* [3]. It also inhibits other sodium-dependent transport mechanisms in other epithelia, such as sugar and amino acid uptake *in vitro* by renal cortex slices and dog colonic mucosa. However, the sodium-independent L-phenylalanine uptake by the guinea pig intestine is not at all inhibited by harmaline but is sensitive to competitive inhibition by other amino acids [4]. However, there are no reports available on the effect of harmaline on dipeptide transport. Since dipeptide transport, like amino acid transport, is also sodium-dependent, experiments in this line would be of some interest. The aim of the present work has therefore been to study the effect of harmaline on the uptake of dipeptides in the monkey small intestine.

MATERIALS AND METHODS

All the labelled dipeptides and amino acids were from The Radiochemical Center, Amersham, Bucks., U.K. Harmaline was kindly donated by Professor J. W. L. Robinson (University of Lausanne, Switzerland). All other chemicals were of analytical grade.

Adult monkeys (*Macaca radiata*) were used in all the uptake experiments. The region of the small intestine at 35–40 per cent distance of the total length from the pyloric end was used since this region of the intestine has been shown to be the most active site for amino acid and dipeptide uptake [5]. Small strips of this region weighing 20–30 mg were used. Uptake experiments were performed as described by Das and Radhakrishnan [6]. A short incubation time of 2 min was used throughout the present study to minimize surface hydrolysis. After incubation, the intestinal strip was gently blotted on a filter paper, weighed and then digested in a scintillation vial with 0.5 ml of NaOH (2 mole/l) at 75° for 2 hr. After digestion, 0.5 ml of water and 10 ml of scintillation mixture were added. The scintillation mixture was prepared by dissolving 10 g of naphthalene, 0.5 g of 3,5-diphenyloxazole and 3 g of Cab-O-Sil (Packard Instrument Co., U.S.A.) in 100 ml of dioxan. The radioactivity was measured in a liquid scintillation counter (Beckman model, LS-100).

All experiments were done in at least two animals to check the variation between animals. The variation were always below ± 15 per cent between animals and within ± 5 per cent in the same animal.

Paper chromatography was used to separate the free amino acids glycine and L-leucine and the intact dipeptide glycyl-L-leucine. The chromatograms were developed on Whatman No. 1 chromatography grade paper using a single phase solvent system (isopropanol: water, 4:1 v/v; 20 hr run). A 0.4% ninhydrin in 95% aqueous acetone was used as the staining reagent. The free amino acid spots (unstained) were cut out by comparing with the position of the standard amino acid spots (stained) run on the same paper and the radioactivity counted. Free glycine and leucine were estimated using (^{14}C)-glycyl-L-leucine and glycyl-L-leucine-(^{14}C), respectively. After 2 min incubation, free glycine concentration in the medium was less than 10 μmole/l and free L-leucine concentration was 4.5 μmoles/l.

Studies on the uptake of dipeptides in the presence and absence of sodium were done using two different buffers. The composition of the sodium containing

* The work reported here was carried out at Wellcome Research Unit, Christian Medical College and Hospital, Vellore 632 004, India.

buffer, in mmoles/l, was: NaCl, 118.4; KCl, 4.73; $CaCl_2$ $2H_2O$, 0.84; KH_2PO_4, 1.17; $MgSO_4$, $7H_2O$, 1.17; $NaH_2PO_4.H_2O$, 5.44 and Na_2HPO_4, 14.15. This buffer is a modified Krebs–Ringer phosphate buffer [7]. For the preparation of sodium free buffer, choline chloride was used for the replacement of sodium and potassium phosphates for sodium phosphates.

RESULTS

Preliminary experiments with glycyl-L-leucine-(^{14}C) and L-leucine-(^{14}C) showed that the inhibition of the uptake of these solutes by harmaline was maximal if the tissue was preincubated with harmaline for 10 min. The inhibition was also dependent on the concentration of harmaline. The maximal inhibition was noticed when the harmaline concentration was 10 mmole/l. However, even at a concentration as low as 0.5 mmole/l, harmaline had a significant inhibitory effect on the uptake. Subsequent studies on the effect of harmaline on the uptake of amino acids and dipeptides were done employing the above optimal conditions to get the maximal inhibition.

Effect of harmaline on amino acid uptake. The effect of harmaline (4 mmole/l) on the uptake of L-leucine-(^{14}C) and glycine-(^{14}C) was studied. The concentration of amino acids in these studies was 1 mmole/l. Harmaline inhibited L-leucine uptake by 44 ± 3 per cent and glycine uptake by 58 ± 5 per cent. The results are shown in Fig. 1.

Effect of harmaline on dipeptide uptake. Using glycyl-L-leucine-(^{14}C) and (^{14}C)-glycyl-L-leucine, it has earlier been shown that there is a marked difference in the rates of accumulation inside the mucosal cell of glycine and L-leucine residues from glycyl-L-leucine [6]. It was suggested that one of the reasons for this phenomenon may be the finding that the rate of efflux of glycine from inside to outside the mucosal cell is at least twice that of L-leucine.

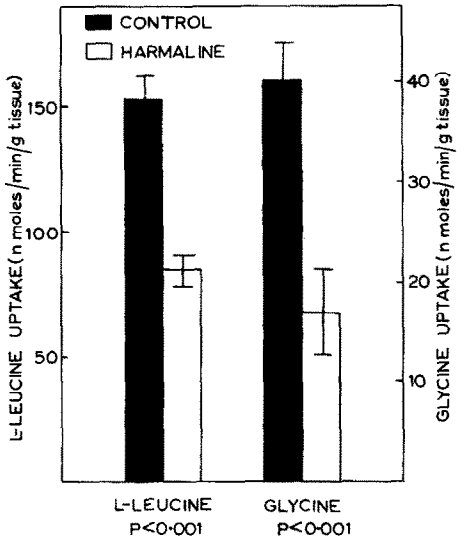

Fig. 1. Uptake of L-leucine (1 mmole/l) and glycine (1 mmole/l) by monkey intestinal strips in the presence and absence of harmaline (4 mmole/l). The mean and S.E. are shown (N = 6).

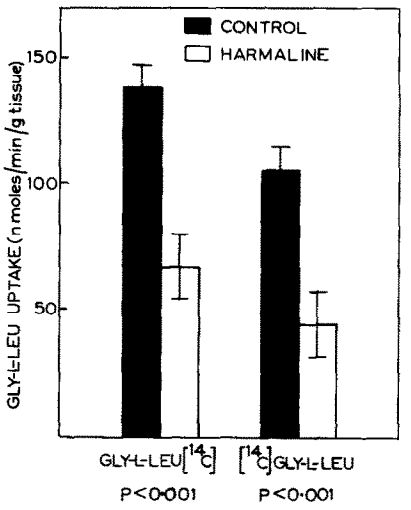

Fig. 2. Uptake of glycyl-L-leucine-(^{14}C) and (^{14}C)-glycyl-L-leucine by monkey intestinal strips in the presence and absence of harmaline (4 mmole/l). The dipeptide concentration was 1 mmole/l. The mean and S.E. are shown (N = 6).

Also, the present study shows that the rate of uptake of glycine by the mucosal cell is only about one fourth of that of L-leucine (Fig. 1). This means that glycine is recaptured at a slower rate that of L-leucine. It appears that this may also be an additional factor, though to a less significant extent because of the very low concentration of the amino acids in the medium, contributing to the higher rate of accumulation of L-leucine inside the cell. The present finding that the free glycine concentration in the medium at the end of the 2 min incubation is at least twice that of L-leucine (see Materials and Methods) is also in accordance with this idea. However, the accumulation of radioactivity from both the solutes in monkey small intestine was inhibited by a number of other dipeptides almost to the same extent. Hence, the effect of harmaline on the uptake of glycyl-L-leucine-(^{14}C) and (^{14}C)-glycyl-L-leucine was studied. Even though the accumulation of the dipeptide as measured by the radioactivity from (^{14}C)-glycyl-L-leucine was less than that from glycyl-L-leucine(^{14}C), harmaline inhibited both the systems to the same extent (50–60 per cent). The results are shown in Fig. 2.

Since harmaline inhibits amino acid transport, it might be argued that the inhibition of dipeptide uptake by harmaline might be due to the effect of the inhibitor on the uptake of free amino acids which might have resulted from the surface hydrolysis of the dipeptide. However, this possibility is ruled out by the finding that only negligible amount of free amino acids were found in the medium at the end of the incubation (see Materials and Methods). The bulk of the uptake as measured in 2 min period was due to the uptake of the intact dipeptide and hence the observed inhibition was due to the effect of harmaline on dipeptide uptake and not a result of its effect on free amino acid uptake.

To provide an additional and even stronger evidence that harmaline does inhibit the dipeptide

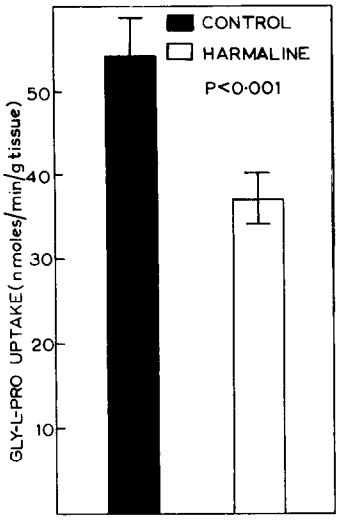

Fig. 3. Uptake of (^{14}C)-glycyl-L-proline (1 mmole/l) by monkey intestinal strips in the presence and absence of harmaline (4 mmole/l). The mean and S.E. are shown (N = 10).

uptake, the effect of this drug on the uptake of a dipeptide which is known to be taken up intact by the mucosal cells should be studied. Glycyl-L-proline has been shown to be transported intact across the brush border membrane of rabbit ileum because of the lack of glycyl-L-proline hydrolysing activity in the membrane [8]. The pellet fraction of the mucosal homogenate of monkey small intestine was checked for glycyl-L-proline hydrolysing activity. Even after the addition of a large amount of pellet protein to the incubation medium and with the incubation time increased to 1 hr, no hydrolysis of glycyl-L-proline was detectable, thus substantiating the findings of Rubino *et al.* [8] in the rabbit on the absence of this enzyme activity in the membrane fraction. Therefore, we studied the effect of harmaline on the uptake of (^{14}C)-glycyl-L-proline using slices of monkey small intestine. The results given in Fig. 3 show that harmaline inhibits the uptake of glycyl-L-proline by 35 ± 3 per cent.

The effect of harmaline on the uptake of glycyl-L-leucine and glycyl-L-proline was studied in the presence and absence of sodium ions. The results are shown in Table 1. Absence of sodium reduced glycyl-L-leucine uptake by 57 ± 6 per cent and glycyl-L-proline uptake by 35 ± 4 per cent. Harmaline inhibited glycyl-L-leucine uptake by 60 ± 3 per cent and glycyl-L-proline uptake by 36 ± 4 per cent when sodium containing buffer was used. But the uptake of these two dipeptides was not affected by harmaline when sodium free buffer was used. Thus, harmaline was inhibitory only when sodium ions were present in the medium. However, the sodium-independent uptake of glycyl-L-leucine and glycyl-L-proline was inhibited by other dipeptides. L-Alanyl-L-phenylalanine inhibited glycyl-L-leucine uptake by 62 ± 5 per cent and glycyl-L-leucine inhibited glycyl-L-proline uptake by 49 ± 4 per cent when sodium free buffer was used.

DISCUSSION

Sodium ions play an important role in the transport of amino acids and monosaccharides [9, 10]. But the transport of glucose from disaccharides does not need sodium ions [11, 12]. In contrast to this, di- and tripeptides require sodium for their optimal transport [13–16]. However, an appreciable amount of peptide is transported even after the total replacement of sodium in the medium [17]. The present study also confirms this partial dependence of peptide transport on sodium ions.

The influence of harmaline on sodium dependent transport and sodium transport mechanisms has been discussed in detail by Sepulveda and Robinson [18]. Sepulveda and Robinson have found that 4 mmole/l harmaline inhibited L-phenylalanine (1 mmole/l) influx by about 90 per cent in guinea pig intestine [19]. In the present study, the inhibition of L-leucine and glycine uptake by harmaline was less than the

Table 1. Effect of harmaline on dipeptide uptake in the presence and absence of sodium ions*

Solutions	Uptake (nmole/min/g tissue)	
	Sodium containing buffer	Sodium free buffer
Glycyl-L-leucine (1 mmole/l)	148 ± 12.3	63 ± 6.8
Glycyl-L-leucine (1 mmole/l) + Harmaline (4 mmole/l)	58 ± 3.2	65 ± 7.5
Glycyl-L-leucine (1 mmole/l) + L-Alanyl-L-phenylalanine (25 mmole/l)	60 ± 3.9	24 ± 3.2
Glycyl-L-proline (1 mmole/l)	106 ± 15.7	69 ± 5.6
Glycyl-L-proline (1 mmole/l)+ Harmaline (4 mmole/l)	68 ± 7.1	65 ± 4.8
Glycyl-L-proline (1 mmole/l) + Glycly-L-leucine (25 mmole/l)	60 ± 4.3	35 ± 5.4

* The results indicate the mean of three different experiments each done in duplicate.

inhibition noted in this earlier study. The most likely reason for this may be the difference in the incubation time. In our study, the tissue was incubated only for 2 min. Sepulveda and Robinson [19] have found that the longer the time of incubation, the higher was the inhibition for any given concentration of harmaline. They have shown that 1 mmole/l harmaline inhibited L-phenylalanine influx by 50 per cent when the incubation time was only 5 min but the same concentration of harmaline inhibited L-phenylalanine influx by 90 per cent when the incubation time was 60 min.

Since harmaline inhibited the transport of glycyl-L-leucine as well as glycyl-L-proline, which shared a common transport system in the monkey [6], it appears that harmaline is a general inhibitor of dipeptide transport system.

Harmaline did not inhibit dipeptide transport when sodium was absent from the medium. Thus, harmaline seems to interfere only with sodium-dependent transport systems. The sodium-independent transport of dipeptides was not due to simple diffusion but it was also a carrier mediated process, since the transport of glycyl-L-leucine and glycyl-L-proline was inhibited by other dipeptides even when sodium free buffer was used. These findings are very similar to the observations made by Robinson [4] in the case of amino acids.

The actual mechanism of inhibition of sodium-dependent transport systems by harmaline is not yet clear. Harmaline may interact either with Na^+–K^+ adenosinetriphosphatase [2] or with a common binding site for sodium in the brush border membrane shared by the different transport systems [20]. This would account for the lack of inhibition of the alkaloid on the sodium-independent amino acid and dipeptide transport systems.

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