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HARMALINE, A POTENT INHIBITOR OF SODIUM-DEPENDENT TRANSPORT

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Summary

Harmaline, a hallucinogenic alkaloid, inhibits sugar and amino acid transport by the guinea-pig intestinal mucosa in vitro during short and long incubations. It affects sodium-dependent transport systems in other tissues such as the dog colonic mucosa and renal cortex slices, but does not influence sodium-independent transport. It inhibits sodium entry into intestinal rings, both in the presence and absence of non-electrolytes. It is proposed that harmaline interacts with the sodium-binding site of the transport carriers in the membranes of intestinal and renal cells.

Harmaline is an alkaloid extracted from *Banisteriopsis caapi* [1], a Colombian liana used by indigenous tribes to prepare a hallucinogenic concoction [2]. It has been reported to compete with sodium for its binding site on the (Na^+-K^+)-ATPase of squid nerve axons and to block sodium transport in this tissue [3]. This finding prompted us to examine its action on the transport of sodium and non-electrolytes in intestinal and kidney slices in vitro, and we have found that its mode of action differs considerably from that of other known inhibitors. We conclude that this drug prevents sodium binding to the carrier mechanisms for non-electrolytes situated in the membranes of intestinal and renal epithelial cells.

The experiments were performed with guinea-pig intestinal rings, dog colonic mucosal strips or dog renal cortex slices, prepared as described previously [4—6]. The tissues were incubated for different periods at 37° C in oxygenated Krebs bicarbonate buffer (or equivalent buffer where all sodium ions had been replaced by choline) containing the substrate whose transport

was to be examined, namely ^{14}C-labelled amino acids, sugars or *p*-amino-hippuric acid, with or without harmaline (Sigma, St Louis, or Fluka, Buchs). In experiments concerning sodium influx, a tracer dose of ^{22}Na was added to the incubation medium. Following the incubation, the tissues were rinsed with ice-cold isotonic mannitol, weighed amd dissolved in 30% KOH, prior to counting in a liquid scintillation medium [4].

TABLE I

HARMALINE INHIBITION OF SUGAR AND AMINO ACID UPTAKE BY GUINEA-PIG INTESTINAL RINGS

Incubations carried out in 1 mM solutions of ^{14}C-labelled sugar or amino acid, with or without 4 mM harmaline. Results are means (± S.E.M.) of 5 animals. All inhibitions are significant at $P < 0.001$. Distribution ratios calculated on the basis of 83% tissue water, and 8% and 12% extracellular space respectively after 5 and 60 min [4,14].

Incubation medium	Incubation time	Uptake (μmol/g)	Distribution ratio	Inhibition (%)
Phenylalanine alone	5 min	1.27 ± 0.140	1.63	
Phenylalanine + harmaline	5 min	0.53 ± 0.024	0.63	58.7%
β-Methyl-glucoside alone	5 min	0.97 ± 0.102	1.22	
β-Methyl-glucoside + harmaline	5 min	0.55 ± 0.041	0.67	42.7%
Phenylalanine alone	1 h	7.71 ± 0.880	10.82	
Phenylalanine + harmaline	1 h	1.19 ± 0.066	1.55	84.6%
β-Methyl-glucoside alone	1 h	5.12 ± 0.499	7.14	
β-Methyl-glucoside + harmaline	1 h	0.65 ± 0.044	0.78	87.4%

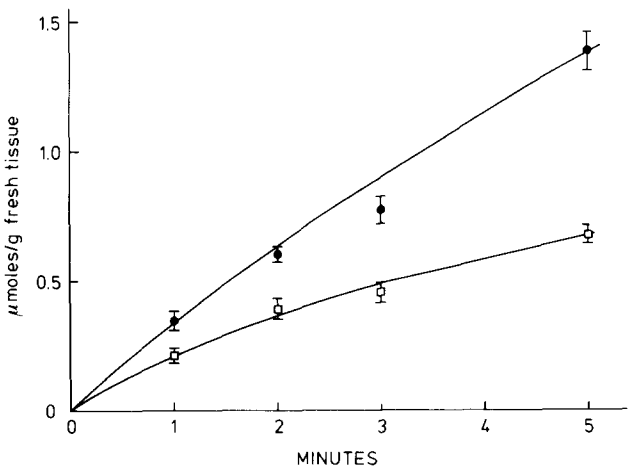

Fig.1. Time course of the inhibition of phenylalanine uptake in guinea-pig intestinal rings by harmaline. Phenylalanine concentration: 1 mM. Lower curve in presence of harmaline 4 mM. Results are means ± S.E.M. of six experiments.

The results given in Table I show that harmaline inhibits the uptake of L-phenylalanine or β-methyl-D-glucoside by intestinal rings during incubations of 5 or 60 min. Furthermore, the inhibition can be extrapolated to zero-time, as illustrated in Fig.1. This excludes the possibility that harmaline acts as a general metabolic poison, since such inhibitors do not influence the initial

rate of uptake of sugars and amino acids [7—9]. The inference is clear that the drug acts at the level of the entry mechanism in the brush-border membrane of the epithelial cell. Further support for this conclusion is gained from the finding (Table I) that the equilibrium uptake can be reduced below a tissue: medium distribution ratio of unity; it has been argued that only inhibitors acting on the entry mechanism are capable of producing such an effect [8].

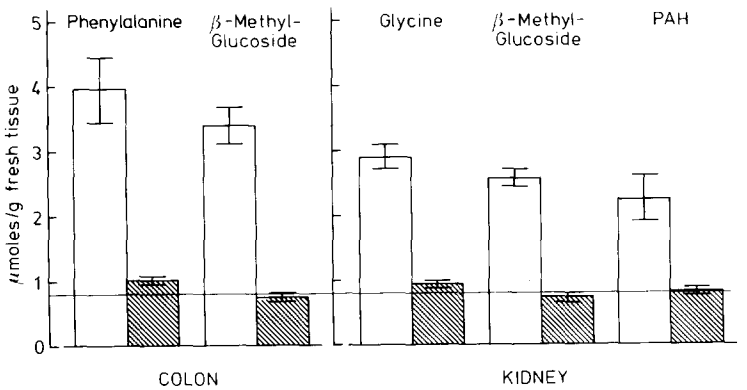

Fig.2. Inhibition of sodium-dependent transport systems in dog colon mucosa and renal cortex slices by harmaline. Substrate concentration always 0.1 mM, and harmaline concentration 4 mM. Shaded columns in presence of harmaline. Results are the means of four experiments ± S.E.M. The horizontal line represents a distribution ratio of unity. PAH represents *p*-aminohippuric acid.

The results in Fig.2 demonstrate that harmaline inhibits other Na^+-dependent transport mechanisms in various cells. Thus glycine, β-methyl-glucoside and *p*-aminohippuric acid transport by renal cortex slices is severely reduced, as is the sugar and amino-acid uptake by the colonic mucosa of the dog, a species that exceptionally transports these substrates actively [5]. These observations suggest that the drug interferes in some way with the sodium site on the membrane carrier which is the common factor in all these transport systems.

TABLE II

LACK OF HARMALINE INHIBITION IN THE ABSENCE OF SODIUM IONS IN GUINEA-PIG INTESTINAL RINGS

Incubations carried out in Na^+-free, choline-substituted bicarbonate buffer. The results are means of seven experiments. Statistical evaluation by random-block analysis of variance, considering each animal as a block [4,9]. *D* represents the least significant difference at given probability levels.

Incubation medium	Uptake after 5 min (μmol/g fresh tissue)	Uptake after 1 h
1 mM phenylalanine	0.225	0.775
1 mM phenylalanine + 4 mM harmaline	0.228	0.890
1 mM β-methyl-glucoside	0.061	0.348
1 mM β-methyl-glucoside + 4 mM harmaline	0.063	0.330
$D_{0.05}$	0.036	0.161
$D_{0.001}$	0.066	0.287

This inference is further strengthened by the results presented in Table II where it can be seen that the uptake of sugars and amino acids by intestinal rings incubated in the absence of sodium is not affected by harmaline. It would be unlikely that the drug affected sugar entry in Na^+-free buffer, since there is not even any interaction between homologous sugars under such conditions [10]. But there is a mediated component of amino acid transport in the absence of sodium which can be inhibited by homologues [10], and this is unaffected by harmaline. This result incidentally precludes a simple action of harmaline on membrane permeability.

TABLE III

EFFECT OF HARMALINE ON THE ENTRY OF SODIUM INTO GUINEA-PIG INTESTINAL RINGS IN THE PRESENCE AND ABSENCE OF SUGARS OR AMINO ACIDS

Incubations carried out 2 min in Krebs bicarbonate buffer containing a tracer dose of ^{22}Na. Results are means of six experiments; statistical evaluation by random-block analysis of variance.

Addition to incubation medium	Sodium influx (μequiv/g fresh tissue)
None	11.1
10 mM glucose	13.1
20 mM phenylalanine	13.0
4 mM harmaline	9.0
4 mM harmaline + 10 mM glucose	9.7
4 mM harmaline + 20 mM phenylalanine	10.4
$D_{0.05}$	0.98
$D_{0.001}$	1.77

Finally, the effect of harmaline on sodium entry into intestinal rings is shown in Table III. The method employed has been used and validated by other authors [11]. The entry of sodium is stimulated by sugars and amino acids, the extent of this enhancement corresponding to that reported by other authors who used methods that unequivocally limited sodium uptake to movement across the brush-border membrane [12]. Harmaline very significantly inhibits the entry of sodium, and furthermore reduces the entry in the presence of sugars or amino acids practically to the level observed in the absence of these substrates. In another series, not shown, harmaline also significantly inhibited sodium influx in one-minute incubations.

These results provide a strong indication that harmaline not only interferes with the sodium site of $(Na^+ + K^+)$-ATPase in neural tissue [3], but it also reacts with sodium-binding sites on membrane carriers. It is worth stressing that, if our hypothesis is confirmed, then harmaline may become a beneficial experimental tool, and in particular should be useful in distinguishing the merits of the two rival kinetic models that has been proposed to explain sodium-dependent amino acid transport in the intestinal epithelial cell [13,14].

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