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# Influence of Aerobic Energy Deficit, Ouabain and Harmaline on the Phenylalanine and Galactose Active Transport by Snail Intestine*

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The effect of anaerobiosis ($N_2$ bubbling of the medium) or $10^{-4}$ M dinitrophenol on the penetration of 0.5 mM Phe in snail and rat everted intestine, in 2 min and 30 min incubation periods, has been studied. The aerobic energy deficit inhibits the amino acid net entry in both species, but whereas the active transport is annulled in rat, snail intestine is capable of continuing to accumulate Phe against a gradient. The prolonged action (30 min of preincubation) of 1 mM ouabain inhibits 0.1 mM Phe and 0.1 mM galactose entry in snail intestine. Amino acid uptake is far higher than the one obtained in the absence of $Na^+$, in which condition Phe keeps accumulating against a gradient in the tissue water. Galactose active transport, instead, becomes null in the presence of the glucoside or in the absence of $Na^+$. One mM harmaline is able to inhibit the initial entry of galactose into the tissue, while higher than 5 mM concentrations are required to inhibit that of Phe. Results confirm that snail intestine is capable of easily carrying out active transport processes with energy from anaerobic origin. On the other hand Phe transport is less sensitive to the absence of $Na^+$, presence of ouabain or harmaline than that of galactose, so that contrary to what has been observed for the sugar, the active transport of the amino acid is not annulled in any of the three conditions.

**Key words:** Intestinal active transport, Anaerobiosis, Harmaline, Ouabain.

Previous results with intestinal everted sacs of the snail *Helix aspersa* showed that the active transport of 5 mM galactose was independent of $O_2$ availability and scantly sensitive to DNP (2). In the present work, the effect of anaerobiosis (with $N_2$ bubbling of the medium) and DNP on phenylalanine uptake by snail everted intestine is studied and compared with that obtained

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in rat intestine under the same conditions. In addition, since the intestinal active transport of sugars and amino acids depends on $Na^+$ gradient, the effects of ouabain and harmaline on phenylalanine and galactose transport have also been investigated.

## Materials and Methods

Phenylalanine and galactose uptake by everted intestine of the snail *Helix aspersa* and the albino Wistar rats (7), has been measured.

As regards snail, everted whole intestine was used, incubated at 30°C in 25 ml erlenmeyers containing 10 ml of physiological solution (1, 12) with the substrate ($^{14}C$) at the required concentration. They were subjected to controlled mechanical stirring, the medium being bubbled with 95 % $O_2$, 5 % $CO_2$ or, where appropriate, with $N_2$. Four intestines (about 90 mg) corresponding to as many other animals were placed in each erlenmeyer. They were initially introduced in 5 ml substrate free physiological solution where the preincubation, when appropriate, was carried out in accordance with the experimental conditions. The addition of 5 ml of physiological solution at 30°C with the substrate ($^{14}C$) at twice the desired concentration marked zero time for the incubation periods, which lasted 2 min or 30 min.

Once the incubation was finished, the intestinal preparations were washed in cold physiological solution, dried on wet filtre paper and individually weighed being kept in 0.5 ml of 0.1 N $NO_3H$ during 24 h at 4°C. Aliquot samples of 100 µl and 150 µl from incubation solutions and tissue extracts were taken respectively, to determine their radioactivity with liquid scintillation counter.

The results are expressed as the net entry rate in µmoles per g of wet weight. To estimate the concentration that the substrate would reach in the total of tissue water, the dry weight of each preparation was also determined.

In the experiments carried out with rat everted jejunum, a maximum of 3 rings of about 25 mg and 0.5 cm in length, were incubated in each erlenmeyer. The rings were suspended in the physiological solution (11) at 37°C.

## Results

*Effect of anoxia and 2-4-dinitrophenol (DNP) on L-phenylalanine uptake by snail and rat everted intestine.* To eliminate the $O_2$ dissolved, the medium with the intestinal preparations was gassed with pure $N_2$ for 15 min before the incubation period and during it. In the experiments with DNP, they were also preincubated for 15 min in the presence of the inhibitor, which continued to be present during incubation. Controls were equally preincubated for 15 min with oxygenation. The net Phe uptake measured after 2 min of incubation is taken as indicative of the initial entry rate, whereas at 30 min, at least in control conditions, there was not any net flux.

The results showed (fig. 1) that, in the absence of $O_2$, Phe entry in snail intestine is smaller for 10 min and 30 min long experiments than with oxygenation. Such effect does not appear in 2 min long experiments. The inhibitory effect due to DNP was apparent as early as 2 min after the beginning of incubation, and at 30 min it proved to be analogous to that in the absence of $O_2$. The amino acid entry in both conditions of inhibition and in all the incubation times, takes place against a concentration gradient, by active transport, as Phe accumulates in the total of tissue water at a greater concentration than in the medium. Such an entry was, furthermore, much higher than that obtained with oxygenation in the absence of $Na^+$, an experimental situation in which Phe entry included accumulative transport and clearly exceeded the one that takes

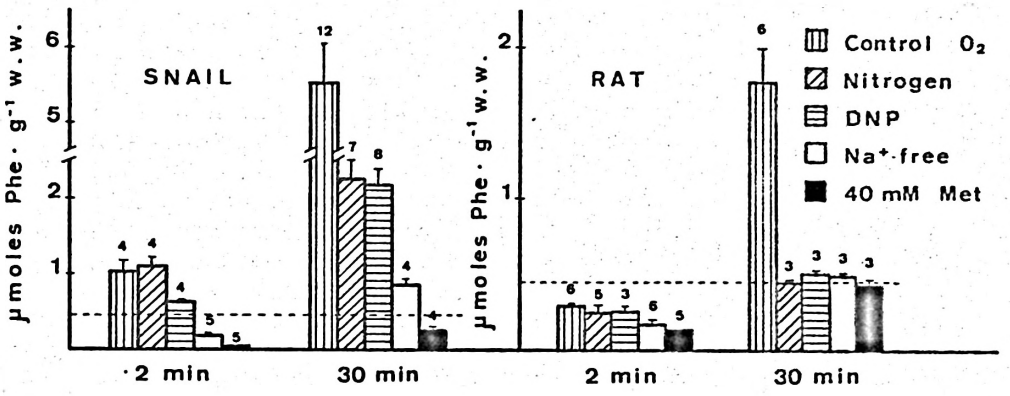


Fig. 1. *Influence of anaerobiosis ($N_2$ bubbling) and $10^{-4}$ M 2-4-dinitrophenol on the uptake of 0.5 mM Phenylalanine by snail and rat everted intestine.*
Bars represent S.E.M. for the number of experiments indicated at the top. The dashed line represents the uptake value where tissue Phe concentration is equal to medium Phe concentration.

place passively in the presence of 40 mM methionine.

The results from equivalent experiments carried out with rat intestine are qualitatively comparable, but they show important quantitative differences. The absence of $O_2$ or the presence of DNP make the Phe active transport null. Thus, the amino acid penetration after 30 min incubation in such conditions does not differ significantly from the one obtained in the absence of $Na^+$, a situation in which Phe entry is not concentrative and is similar to the one obtained in the presence of 40 mM methionine.

*Effect of ouabain and harmaline on phenylalanine and D-galactose entry in*

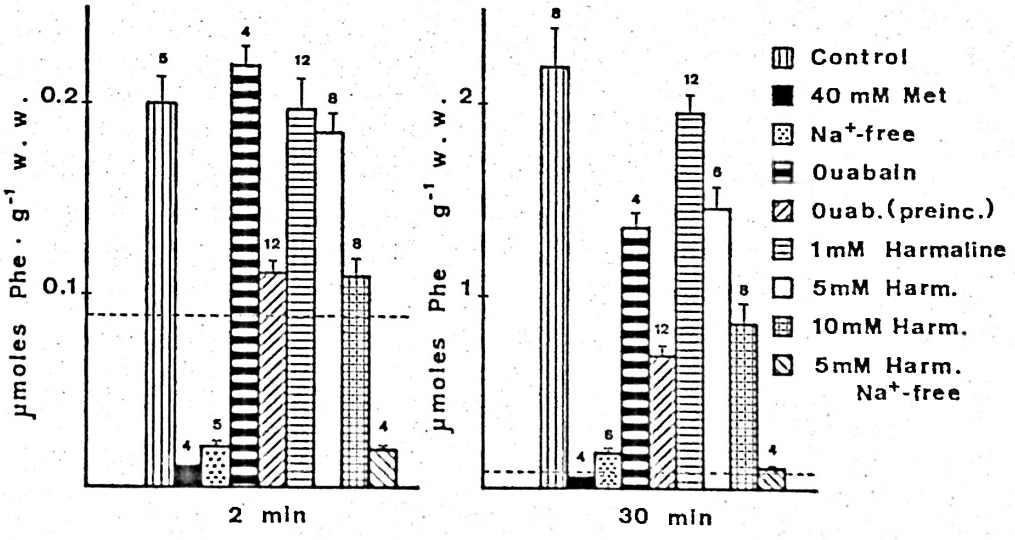


Fig. 2. *Inhibition by 1 mM ouabain and different concentrations of harmaline on the uptake of 0.1 mM Phenylalanine by snail everted intestine.*
Legend as in figure 1.

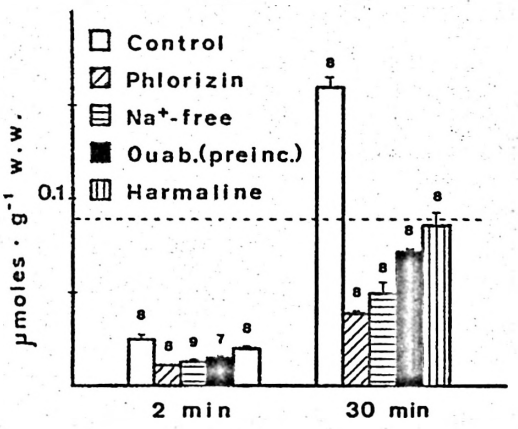


Fig. 3. *Effects of 1 mM ouabain and harmaline on the entry of 0.1 mM galactose in snail everted intestine.*
Legend as in figure 1.

*snail everted intestine.* The effect of ouabain and harmaline on the entry of 0.1 mM Phe and 0.1 mM galactose in snail everted intestine has been investigated. The results (figs. 2 and 3) are compared with the entry in the absence of $Na^+$ and passive entry of Phe (with 40 mM methionine) or galactose ($10^{-4}$ M phlorizin).

The presence of 1 mM ouabain exclusively during incubation, inhibited the entry of 0.1 mM Phe after 30 min, without producing any differences in the 2 min long experiments. However, if the preparations were preincubated for 30 min in the presence of the glycoside, the inhibition already appeared after 2 min incubation (44 %), becoming more noticeable after 30 min (68 %). Such inhibition does not entail the abolition of transport since even after 2 min of incubation, the amino acid concentration in the total tissue water is higher than in the medium. On the other hand, the entry amply exceeds the corresponding one in the absence of $Na^+$.

One mM harmaline does not modify Phe penetration in snail intestine. At 5 mM concentration, inhibition is observed after 30 min (33 %), and at 10 mM, the effect is more pronounced and appears by 2 min time. Transport inhibition is only partial, as the amino acid entry, even with 10 mM harmaline, is much higher than when $Na^+$ is suppressed and it is conducted against a concentration gradient. When the intestine preparations were incubated in a $Na^+$ lacking medium, 5 mM harmaline did not modify Phe penetration by 2 min time, but reduced the one corresponding to 30 min.

As regards sugar transport (fig. 3), 1 mM ouabain during the 30 min preincubation and subsequent incubation with 0.1 mM galactose, strongly inhibits sugar entry, in such a way that not even in 30 min long incubations accumulation in the tissue is produced. In this latter situation, inhibition by ouabain is significantly inferior to the one produced by $Na^+$ suppression in the medium.

One mM harmaline, on the other hand, inhibits galactose entry into the tissue when it is added to the incubation medium. The effect is appreciable not only after 30 min incubations, but also after 2 min ones. The inhibition after 30 min is somewhat lower than the one observed in the experiments with ouabain and in those conducted in the absence of $Na^+$.

## Discussion

The phenylalanine uptake in control conditions is much higher in snail intestine than in rat. After 30 min incubation with 0.5 mM Phe, the amino acid concentration reached in the total tissue water is higher than 6 mM in snail intestine (twelvefold that of the medium), as opposed to a little more than 2 mM for rat. The passive penetration of Phe, instead, obtained in the presence of high methionine concentrations, is greater in rat intestine that in snail. The relative contribution of the transport component in the total entry is far higher in snail intestine than in rat. Such differences may be related to specific differences in the efficiency of the transport systems, but also to the absorbing surface/weight distinct ratio and other characteristics of the preparations of either species.

The results obtained in the absence of $O_2$ and in the presence of $10^{-4}$ M DNP, show that in both rat and snail intestine, the Phe active transport is affected by energy deficit from aerobic origin. Nevertheless, whereas rat intestine is unable to actively transport Phe when the aerobic metabolism is blocked, snail intestine is able in such circumstances to keep accumulating amino acid uphill although to a lesser extent than controls.

In accordance with the sodium gradient hypothesis, such active transport inhibition must be related to the inefficiency of the $Na^+$ pump by aerobic energy deficit, and to the consequent weakening of the cationic gradient. The absence of $Na^+$ in the medium, however, inhibits much more Phe entry into snail intestine than the deficit of aerobic energy does, which suggests that $Na^+$ pumping may persist to a certain extent due to ATP from anaerobic origin.

As previously reported (4, 10), the net Phe entry decreases markedly in the absence of $Na^+$, but keeps including mediated transport and even accumulation against a gradient.

In rat intestine, on the contrary, the amino acid penetration in 30 min, in the absence of aerobic energy does not differ from the one obtained in the absence of $Na^+$, which reveals a strict dependence of the $Na^+$ pumping as regards oxidative metabolism. The 2 min incubation time proves to be insufficient in either species for effects from energetic deficit to be observed.

Phe uptake in the absence of $Na^+$ by rat intestine, as opposed to what occurs in snail, is scarcely different from passive entry, which indicates that the amino acid transport across the mucosal membrane in rat is far more strictly dependent on $Na^+$. The lesser $Na^+$ dependence of neutral amino acid transport by snail intestine in relation to that of sugars, had already been reported (5, 8), and it likewise occurs in other species (13, 17).

The results obtained with ouabain and harmaline on 0.1 mM Phe and 0.1 mM galactose transport by snail intestine show those very differences. From such low concentrations, the amino acid penetrates snail intestine much faster than sugar does, so that the amino acid concentration in the total tissue water after 30 min incubation exceeds twentyfold that of the medium, whereas that of galactose does not quite reach a twofold concentration.

Phe passive entry also exceeds that of galactose, but to a much lesser degree. This entry is slow with either substrate and the distribution equilibrium between medium and tissues is not reached even after 30 min. If the substrate concentration in the extracellular liquid is assumed to be in such a time equal that of the medium, and the extracellular water to be 38 % of the total water (3), the Phe intracellular concentration would prove to be half that of the incubation medium, while that of galactose would be negligible, as if the passive membrane permeability to sugar were markedly inferior to that of the amino acid.

Contrary to what was observed with Phe, the absence of $Na^+$ completely blocks the galactose active transport by snail intestine, the entry being similar to that obtained in the presence of $10^{-4}$ M Phlorizin.

For ouabain to manifest inhibition of galactose intestinal transport in snail a prolonged action is required (5). The same is observed with its effect on Phe transport, but the amino acid entry in the presence of ouabain and following 30 min preincubation with the glycoside is clearly superior to that obtained in the absence of $Na^+$, which suggests that the $Na^+$ pump has not been blocked completely, or that a residual sodium gradient trans-luminal membrane persists. As regards sugar,the prolonged action of 1 mM ouabain totally annuls the active transport. The fact that the entry values measured exceed those corresponding to the absence of $Na^+$, at least in 30 min incubations, could be explained if the persistence of a certain $Na^+$ gradient, as in the case of Phe, were accepted. Similar experi-

ments in snail intestine showed that 0.5 mM galactose, with 1 mM ouabain, penetrated the tissue only passively (5).

It can, therefore, be deduced that galactose active transport in snail intestine is more sensitive to ouabain than that of Phe. For an equal concentration of inhibitor, and the same degree of dissipation of the $Na^+$ gradient, Phe accumulates in the tissue, whereas the sugar only penetrates down its concentration gradient.

Something similar is observed with harmaline. At least two effects on the intestinal transport in various mammal species (15, 16) have been described for this alkaloid: one rapid and direct, related to the competition with $Na^+$ for the same site of the cotransport system, and another indirect, which requires prolonged action and high concentrations, on $Na^+$, $K^+$-ATPase.

The results obtained show that the possible direct inhibitory effect on the luminal membrane in Phe transport would require higher than 5 mM concentrations, whereas the indirect one would be apparent at that concentration. The presence of 5 mM harmaline in a $Na^+$ lacking medium does not exert any additional effects on the initial entry of Phe, whereas in 30 min experiments, it accentuates the inhibition by the absence of $Na^+$. Perhaps the inhibitory effect of 5 mM harmaline on the $Na^+$ pump contributes to minimizing the possible apical reservoir of the cation to which some authors have referred (6).

The transport of 0.1 mM galactose is sensitive to harmaline even at the lowest assayed concentration, the observed inhibition being likely attributable, in accordance with what was obtained for Phe, to a direct effect of harmaline on the sugar transport system. The values obtained are somewhat higher than those in the absence of $Na^+$, as reported in mammal species (14). In snail intestine, 1 mM harmaline was capable of annulling the specific entry of 0.5 mM galactose in similar experiments of 2 min duration (5).

It seems, therefore, that Phe intestinal transport is much less sensitive to harmaline than that of galactose, as regards both the direct inhibitory action and the indirect one.

This bears on the fact that Phe transport is much less strict than that of galactose in its $Na^+$ dependence. Equivalent results have also been reported in other species, basically mammals (9). What is peculiar to snail intestine is its great capacity for Phe accumulation in the absence of $Na^+$ and its marked tolerance against a metabolic energy deficit from aerobic origin.

## Resumen

Se ha estudiado la influencia de la anaerobiosis (gaseado del medio con $N_2$) o del 2-4 dinitrofenol $10^{-4}$ M sobre la penetración de Phe 0,5 mM en intestino evertido de caracol y de rata, en incubaciones de 2 y de 30 min. El déficit de energía aerobia inhibe la entrada neta del aminoácido en ambas especies, pero mientras en rata se anula el transporte activo, el intestino de caracol es capaz de seguir acumulando Phe contra gradiente. La acción prolongada (30 min de preincubación) de ouabaína 1 mM inhibe la entrada de Phe y galactosa 0,1 mM en intestino de caracol. En el caso de aminoácido, la penetración es muy superior a la obtenida en ausencia de $Na^+$ en el medio, condición en la que la Phe sigue acumulándose contra gradiente en el agua del tejido. En cambio, el transporte activo de galactosa se anula en presencia del glucósido o en ausencia de $Na^+$. La harmalina 1 mM es capaz de inhibir la entrada inicial de galactosa al tejido, mientras que se requieren concentraciones superiores a 5 mM para que se inhiba la de Phe. Los resultados confirman que el intestino de caracol es capaz de llevar a cabo con facilidad procesos de transporte activo con energía de origen anaerobio. De otra parte, el transporte de Phe es menos sensible a la ausencia de $Na^+$, presencia de ouabaína o de harmalina que el transporte de galactosa, de modo que, a diferencia de lo observado para el azúcar, en ninguna de las tres condiciones se anula el transporte activo del aminoácido.

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