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**Original article**

# Effect of D ala$_2$ metenkephalinamide on feline jejunal and ileal water and electrolyte transport

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**Summary** — The aim of this investigation was to compare the effect of an opioid, D ala$_2$ metenkephalinamide (DAMA), on net jejunal and ileal water and electrolyte fluxes using the gut perfusion technique in the anesthetized cat. Intestinal transport was measured during intravenous infusion of serial doses of 2, 6, and 18 $\mu g \cdot kg^{-1} \cdot h^{-1}$ of DAMA in 6 cats. Each cat was its own control during an intravenous infusion of 150 mmol/l NaCl preceding the first dose of peptide and following the last dose of DAMA. Both jejunal and ileal segments were isolated by inflated balloons and were studied at the same time. Fifteen ml of an iso-osmolar test solution with hypo-osmolar ion contents and complementary mannitol were administered in the uptstream tube and collected 1 h later in the downstream tube. In the jejunum, water secretion was dose-dependently reversed to an absorption from a control value of $+0.5 \pm 0.4$ to $-0.83 \pm 0.5$ $ml \cdot h^{-1} \cdot 10\ cm^{-1}$; in the ileum, water absorption was increased from $-0.5 \pm 0.3$ to $-1.5 \pm 0.2$ $ml \cdot h^{-1} \cdot 10\ cm^{-1}$. The net absorption of all electrolytes, *ie* sodium, chloride, bicarbonate, potassium and calcium also increased during peptide administration. However, a qualitative difference in the ion transport was observed between the jejunum and the ileum.

**absorption / cat / enkephalin / electrolyte flux / intestine / luminal perfusion**

**Résumé — Effet de la D Ala2 métenképhalinamide sur le transport d'eau et des électrolytes au niveau du jéjunum et de l'iléon chez le chat.** *Le but de ce travail a été de comparer l'effet d'un opiacé, la D Ala$_2$ métenképhalinamide (DAMA), sur les flux d'eau et d'électrolytes à 2 niveaux intestinaux, le jéjunum et l'iléon, en utilisant une technique modifiée de perfusion intestinale chez le chat anesthésié. Les transports intestinaux ont été mesurés pendant une perfusion intraveineuse continue de doses croissantes de DAMA de 2, 6 et 18 $\mu g \cdot kg^{-1} \cdot h^{-1}$, chez 6 chats. Chaque chat a été son propre témoin pendant une perfusion intraveineuse de chlorure de sodium 0,15 $mol \cdot l^{-1}$ précédant la première dose et suivant la dernière dose de DAMA. Chacun des 2 segments intestinaux a été délimité par 2 sondes à ballonet. Quinze ml d'une solution iso-osmolaire renfermant du mannitol pour compenser une hypo-osmolarité ionique ont été administrés dans la sonde en amont du segment. Le contenu du segment a été recueilli par la sonde située en aval, après une heure* in situ. *Dans le jéjunum, la sécrétion d'eau observée en période basale ($+0.5 \pm 0.4$ $ml \cdot h^{-1} \cdot 10\ cm^{-1}$) a été remplacée*

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*par une absorption (–0.83 ± 0.5 ml•h$^{-1}$•10 cm$^{-1}$). Dans l'iléon, l'absorption hydrique obtenue en période basale (–0.5 ± 0.3 ml•h$^{-1}$•10 cm$^{-1}$) a été augmentée (–1.5 ± 0.2 ml•h$^{-1}$•10 cm$^{-1}$). L'absorption nette de tous les électrolytes, sodium, chlorure, bicarbonate, potassium et calcium a été augmentée pendant l'administration de DAMA. Cependant, une différence qualitative du transport ionique a été observée entre le jéjunum et l'iléon.*

***absorption / chat / enképhaline / électrolytes / flux / intestin / perfusion luminale***

## INTRODUCTION

The intestinal mucosa is innervated by cholinergic, adrenergic, peptidergic and intrinsic amine-handling nerve fibers (Furness and Costa, 1980; Thomas and Templeton, 1981). Both parasympathetic (Morris and Turnberg, 1980) and sympathetic (Morris and Turnberg, 1981) autonomic pathways have been implicated in the physiological control of intestinal transport. Stimulation of sympathetic nerves has resulted in a 2-fold increase in water absorption in the cat small intestine (Brunsson *et al,* 1979), while parasympathetic pathways are involved in intestinal secretion (Morris and Turnberg, 1980). Enkephalins are naturally occurring peptides (Polak *et al,* 1977) which are present in the enteric nervous system, located in extrinsic nerves, in cells and fibers of the myenteric plexus. They have an affinity for delta and mu opiate receptors (Bradbury *et al,* 1976; Lord *et al,* 1977), which are present in myenteric and submucosal plexus in gastro-intestinal smooth muscle (Furness and Costa, 1980) but not on enterocytes (Binder *et al,* 1984) in rats. There is evidence that opiates and enkephalins exert a large spectrum of effects in the digestive tract (Olson *et al,* 1987); in particular, they inhibit intestinal secretion and increase intestinal absorption. The antisecretory effect has been demonstrated *in vivo* either on basal jejunal secretion in the dog (Barbezat and Reasbeck, 1983) or in the rat (Fogel and Kaplan, 1984), or on cholera toxin, prostaglandin E1 and VIP-induced secretion in the rat (Beubler and Lembeck, 1979; Coupar, 1983). The absorptive effect has been showed principally *in vitro* at the ileal level in the rabbit (Dobbins *et al,* 1980; McKay *et al,* 1981) and the guinea-pig (Kachur *et al,* 1980), as a decrease of short-circuit current and an increase in net NaCl absorption.

The aim of this experiment was to study the effect of enkephalin on water and electrolyte transport in the cat in which the action of opioid peptides has not been studied. The hydroelectrolytic transport was studied simultaneously in the jejunum and the ileum of the same animal. The cats were kept anesthetized for several days, to repeat such a comparison, using a technique (Vagne *et al,* 1986) derived from the gut perfusion technique (Barbezat and Reasbeck, 1983). The well-known inhibitory effect of enkephalins on motility in the cat as in other species (Edin *et al,* 1980) was overcome by the complete recovery of the intestinal segment contents, at the end of each hour, based on marker recovery

## MATERIALS AND METHODS

### *Animals*

Studies were performed in 6 cats (purchased from Iffa-Credo, BP 109, 69210 l'Arbresle, France) which were anesthetized iv with pentobarbital (25 mg•kg$^{-1}$). After a median incision of the abdomen, a balloon-equipped tube (armou-

red tracheal tube, ch 18, Rüsch, FRG) was inserted into the jejunum just beneath Treitz'ligament, through a small incision and attached with a purse-shaped surgical ligation; a second identical tube was placed ≈ 30 cm below in the opposite position so that when the balloons were inflated a loop of about 30 cm was delimited. The exact length was determined at the completion of the study. In the same way, a tube was placed at the end of the ileum and a second tube was placed upstream in the opposite position. Between the 2 limited segments a catheter was inserted and connected to a syringe filled with ethchlorvinol (Placidyl, Abbott). The luminal instillation of 0.2–0.3 ml/d permitted a long-lasting hypnosis (Vagne *et al,* 1986). A gastric cannula was inserted in the gastric fundus and brought to the outside through an incision in the abdominal wall (fig 1). The animals were kept in a ventilated incubator at 30°C with 50% humidity and ventilation. Each day, the animals were weight-controled and received penicillin, glucose and saline by subcutaneous route as described previously (Vagne *et al,* 1986).

### *Experimental design*

Each day, the 4 balloons were inflated with 10 ml of air and the delimited jejunal and ileal loops were rinsed with warmed distilled water which was drained into the distal tubes. Some air was pushed into the loop to get rid of the water. The loops were then washed with 20 ml of water containing 30 mg/l of phenol red (PR). PR acted as a marker for the water left in the loop after pushing some air through and before administering 15 ml of the test solution. The solution contained 70 mM of NaCl, 10 mM of $NaHCO_3$, 5.2 mmol•l$^{-1}$ of KCl, 1.2 mmol•l$^{-1}$ of $CaCl_2$, 136 mmol•l$^{-1}$ of mannitol. $^{14}$C Polyethylene glycol 4 000 (PEG) (NEN, France) mixed with 5 g•l$^{-1}$ of cold PEG 4 000 (PEG) (NEN, France) mixed with 5 g•l$^{-1}$ of cold PEG 4 000 was used as a non-absorbable marker (3.7 kBq/ml). The test solution was drained after 1 h and completely recovered by washing with 20 ml of water containing phenol red as a marker (30 mg/l). The same test was repeated 6 times per day, with a lag time of 10 min between 2 tests. The first 2 tests were given during saline intravenous administration, the next 3 tests during intravenous administration of serial doses of DAMA of 2, 6 and 18 µg/kg per h, and the last test during saline iv again. The tests for the jejunum and for the ileum were separated by 5-min intervals.

### *Drugs*

D $Ala_2$ metenkephalinamide was purchased from Sigma Chemicals, France.

### *Laboratory analysis*

$^{14}$C PEG activity was determined by liquid scitillimetry with an external standard to correct for quenching, in the test solution, in the recovered solution and in the phenol red washing solution. Radioactivity was corrected for interference with the amount of phenol red present in the solution according to a determined linear relationship. Only the experiments where the recovery of labeled PEG was 80% or more were analyzed.

Phenol red was measured at pH 10.5 using an automated technique (Auto-analyzer, Technicon) and a spectrophotometric measurement at 560 nm (Beckman DB). Osmolality was measured by depression of freezing point using a Fiske osmometer.

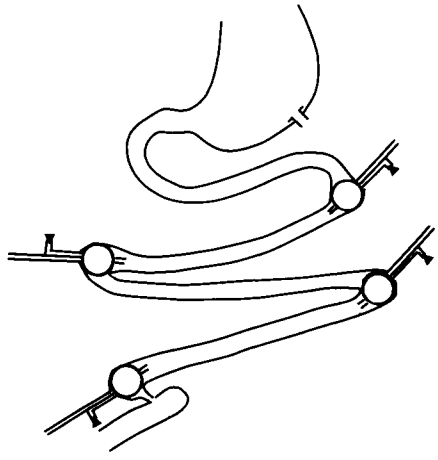

**Fig 1.** Position of the 4 tubes with their balloons inflated showing the delimitation of the jejunal and ileal segments. A gastric cannula was inserted to remove gastric juice and reflux biliary secretion.

Na, Ca and K contents were determined by flame photometry. Cl was measured by potentiometric titration. Bicarbonate concentration was determined by acid back-titration using an automatic titrator (Radiometer) (Vagne *et al*, 1986).

## *Calculations*

*Vt* = volume of test solution injected into the intestine (15 ml)

*CtA* = concentration of $^{14}C$ PEG in the test solution.

*CtE* = concentration in each electrolyte in the test solution.

*Vr* = volume of washing phenol red containing water (20 ml)

*Cr* = concentration of phenol red in the washing solution

*Vs* = volume recovered after 1 h from the intestine

*CsA* = concentration of $^{14}C$ PEG

*CsR* = concentration of phenol red

*CsE* = concentration of each electrolyte

*Vw* = volume of washing water recovered

*CwA* = concentration of $^{14}C$ PEG

*CwR* = concentration of phenol red

Net flux was calculated as the difference between the recovered and the administered amounts for volume and ions.

### For volume

The 15 ml of test solution *(Vt)* administered were corrected to give the exact initial volume *(Vi)* as the volume corresponding to the amount of $^{14}C$ PEG recovered in the test solution and in the washing solutions:

$Vi = (Vs \times CsA) / CtA + (VW \times CwA) / CtA$

The volume of test solution recovered *(Vs)* was corrected to give the exact final volume *(Vf)* by adding the amount of the test solution left in the washing solution ($^{14}C$ PEG) and by substracting the volume of washing solution remaining from the preceding washing (phenol red).

$Vf = Vs + [(Vw \times CwA) / CsA] - [(Vs \times CsR) / CtR]$

The water net flux was equal to : $Vf - Vi$, expressed in $ml \cdot h^{-1}$ and given per 10 cm of intestine.

Net water absorption from the lumen was then expressed as a negative value and net secretion into the lumen as a positive value.

### For electrolytes

The initial amount administered was:

$Vi \times CtE$

– the recovered amount was:

$Vfe \times CsE$

$Vfe = Vs + [(Vw \times CwA) / CsA]$

The electrolyte net flux was equal to = $(Vfe \times CsE) - (Vi \times CtE)$, expressed in $\mu Eq \cdot h^{-1}$ and is given per 10 cm of intestine. Positive value indicates a secretion, negative value an absorption.

## *Statistical analysis*

The comparison of the data obtained from the same animal during basal state and during DAMA administration was calculated by Student's *t*-test for paired small samples values. As too small a sample size might fail to detect that the distribution differed from the approximately normal distribution, the Mann–Whitney U-test was also systematically calculated. The variation was considered as statistically significant when both statistical tests were significant ($P < 0.05$).

## *Histological control*

The animals were sacrificed after 4 d. The lengths of jejunum and ileum delimited by the 2 balloons were measured. Fragments were submitted to histological control. Jejunal and ileal mucosae were not significantly altered after 4 d of intestinal perfusion.

Two cats were surgically relieved of their tubes and gastric cannula and were allowed to recover with appropriate medical care. They were kept in the laboratory for several months

after the test period in excellent condition. The recovery indicates that no irreversible change was induced during the experiments. They are now living peacefully in a friendly veterinarian family.

## RESULTS

*In basal conditions,* the jejunum secreted water, $Na^+$ ($P < 0.01$), $Cl^-$ ($P < 0.01$), $K^+$ and $Ca^{2+}$, and slightly absorbed bicarbonate. In contrast, the ileum absorbed water and $Cl^-$ and secreted $Na^+$ and bicarbonate ($P < 0.001$) with $K^+$ and $Ca^{2+}$ ($P < 0.02$) (fig 2). The next flux of bicarbonate and $Cl^-$ were different in the jejunum and the ileum (*t*-test for paired values respectively 4.51, $P < 0.01$ and 3.09, $P < 0.02$).

The electrolyte concentration of the jejunal contents did not differ statistically from that of the test solution, except for bicarbonate concentration which was lower. Bicarbonate concentration was increased in the ileal contents while $Cl^-$ concentration was lowered by the same amount without any change in $Na^+$ concentration (table I).

**Table I.** Electrolyte concentrations after 1 h.

| | *Jejunum* | | | *Ileum* | | |
|---|---|---|---|---|---|---|
| *mmol/l* | *Test solution* | *Basal* | *DAMA (18 µg/kg•h)* | *Test solution* | *Basal* | *DAMA (18 µg/kg•h)* |
| $[Na^+]$ | | | | | | |
| Mean | 80 | 86 | 74* | 80 | 80 | 60* |
| SEM | | 4 | 4 | | 6 | 6 |
| $[CL^-]$ | | | | | | |
| Mean | 78 | 83 | 73* | 78 | 62* | 44* |
| SEM | | 4 | 3 | | 6 | 6 |
| $[K^+]$ | | | | | | |
| Mean | 5.2 | 5.1 | 4.9 | 5.2 | 5.1 | 4.4 |
| SEM | | 0.2 | 0.4 | | 0.4 | 0.3 |
| $[Ca^{2+}]$ | | | | | | |
| Mean | 1.2 | 1.03 | 0.99 | 1.2 | 1.34 | 1.27 |
| SEM | | 0.08 | 0.08 | | 0.05 | 0.06 |
| $[HCO_3^-]$ | | | | | | |
| Mean | 10 | 5.9* | 3.7 | 10 | 21.5** | 15.3 |
| SEM | | 1.4 | 1 | | 2.8 | 2.4 |
| mOsm | | | | | | |
| Osmolarity | | | | | | |
| Mean | 329.2 | 322 | 317** | 330.2 | 317 | 306.6* |
| SEM | 3.1 | 5.7 | 6 | 3.2 | 7.9 | 6.8 |

Comparison with the preceding column; *t*-test for paired values and small samples; * = $P < 0.05$, ** = $P < 0.01$; $N$ = 9 experiments in 7 cats.

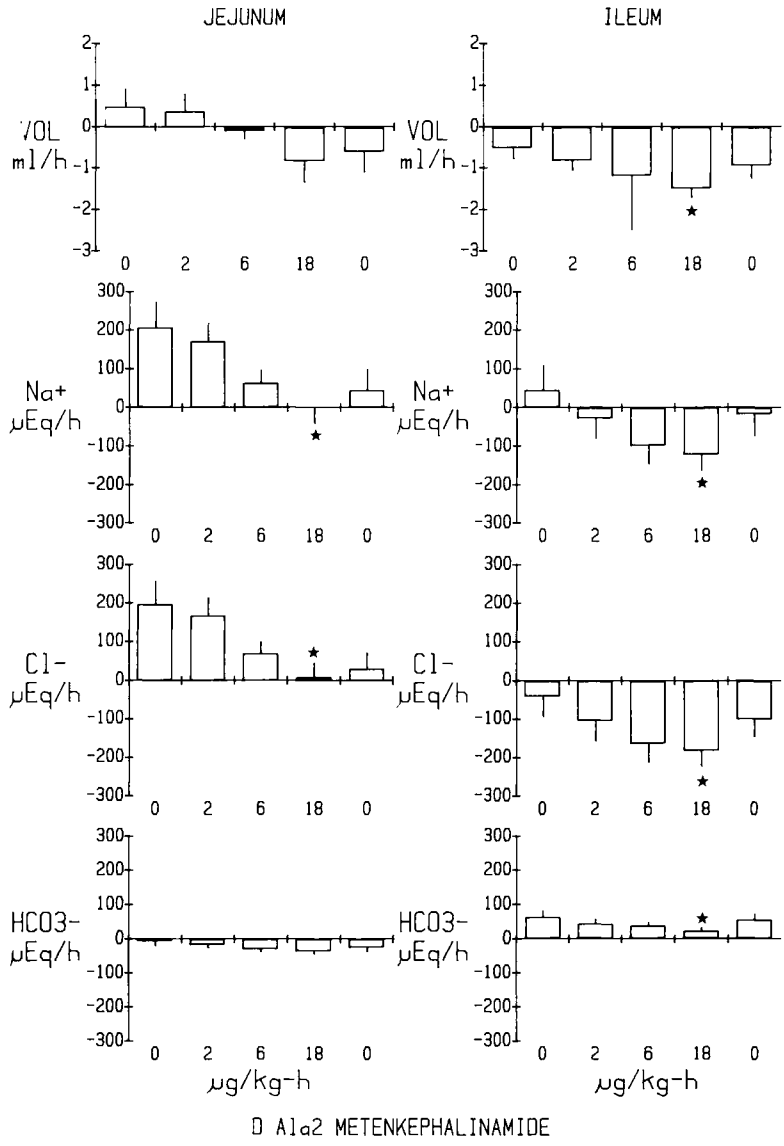


**Fig 2.** Net fluxes (per h and per 10 cm of intestine) of water, $Na^+$, $Cl^-$, $HCO3^-$ from the lumen of jejunum (left) and ileum (right), during intravenous saline infusion (0), D ala2 metenkephalinamide (2, 6 and 18 $\mu g \cdot kg^{-1} \cdot h^{-1}$), and saline infusion again (0). Net secretion has positive value and net absorption negative value. The histograms represent the mean of 9 experiments in 6 cats and the vertical bars are the SEM. The star indicates a statistical difference ($P < 0.05$) between the value and the saline value taken as control.

### *The continuous IV infusion of DAMA*

It resulted in a change from jejunal water secretion to water absorption (from 0.5 ± 0.4 to –0.8 ± 0.5 ml/h) and in an increase of ileal water absorption ($P < 0.05$) (from –0.5 ± 0.3 to –1.5 ± 0.2 ml/h). The changes were related to the dose of DAMA and in our experimental design the dose of 18 $\mu g \cdot kg^{-1} \cdot h^{-1}$ was the most efficient dose for all the effects which were determined. Jejunal $Na^+$ and $Cl^+$ secretions were decreased (respectively from 208 ± 5 to –3 ± 38 and 197 ± 59 to 9 ± 35 µEq/h) while ileal Na secretion was replaced by an absorption (from 46 ± 62 to –122 ± 39 µEq/h) and Cl– absorption was increased (from –42 ± 53 to –184 ± 39 µEq/h), ($P < 0.05$), (fig 2).

For bicarbonate, the increase in jejunal absorption was not statistically significant but the decrease in ileal bicarbonate secretion was significant ($P < 0.05$) (from 64 ± 15 to 23 ± 8 µEq/h). Both $K^+$ and $Ca^{2+}$ secretions from jejunum and ileum were decreased with a significant change, leading to absorption in the ileum only for $K^+$ (from 1.6 ± 4 to –6.1 ± 1.7 µEq/h) and in the jejunum for $Ca^{2+}$ (from 0.48 ± 0.77 to –0.87 ±0.66 µmol/h).

During peptide administration, $Na^+$ and $Cl^-$ concentrations of the jejunal contents collected after 1 h were decreased ($P < 0.05$). Bicarbonate concentration was decreased (table I).

$Na^+$ and $Cl^-$ concentrations of the ileal contents were decreased ($P < 0.05$, 0.02). Bicarbonate concentration decrease was at the limit of significance. A significant decrease in osmolarity was observed in both jejunal and ileal contents ($P < 0.05$) (table I).

### *The return to basal conditions*

During the replacement of DAMA perfusion by saline resulted in a more or less complete return to basal water and ion transport (fig 2). Electrolyte concentrations in the jejunum as well as in the ileum returned to near basal levels.

## DISCUSSION

We have studied simultaneously in the same cat water and electrolyte transport in the jejunum and in the ileum in basal conditions and in response to DAMA intravenous infusion.

We determined in preliminary experiments the low concentration of sodium and chloride solution which would be suitable for approaching the zero flux ion concentration in both segments (Fromm and Hegel, 1987) in order to amplify an effect on absorption. Using an iso-osmolar test solution containing 80 mM Na and 78 $mmol \cdot l^{-1}$ Cl, we induced, in basal conditions, in the jejunum, a slight water and ion secretion including Na, Cl, K and Ca. Only bicarbonate was slightly absorbed. In the ileum, we induced a slight absorption of water and Cl and a secretion of bicarbonate, Na, K and Ca. The jejunal net fluxes of bicarbonate and Cl statistically differed from the ileal ones. The difference in the mechanism of ion exchanges in both segments was objectified by the difference in final concentrations. In the jejunum, a significant decrease of bicarbonate concentration was observed without any change in Cl concentration, while in the ileum the increase of bicarbonate concentration was associated with a decrease in Cl concentration of the same magnitude. The variation obtained in the ileum was compatible with $HCO_3$/Cl exchange, inducing the chloride absorption against the chemical gradient.

We showed that DAMA induced an increase in water and electrolyte absorption in both jejunum and ileum. This effect was

secondary to a decrease in secretion of water, Na and Cl in the jejunum. In the ileum, DAMA induced an increase in the absorption of water Na and Cl and a decrease in bicarbonate secretion. For both intestinal segments, the effect of DAMA was dose-dependent and the dose of 18 $\mu g \cdot kg^{-1} \cdot h^{-1}$ was the most efficient. The decrease in Na and Cl concentrations and in osmolarity in the solution after 1 hour of DAMA infusion showed that more ions than water were absorbed in the jejunum as well as in the ileum. In both intestinal segments the induction of absorption was realized against the chemical gradient. The lack of change of K and Ca concentration suggests that their transport was only related to water movement.

An increase in water and ion absorption has been demonstrated following serosal administration of enkephalin *in vitro* in the rabbit ileum (Dobbins *et al,* 1980) and in the guinea pig (Kachur *et al,* 1980), *in vivo* in the dog (Barbezat and Reasbeck, 1983) and the rat (Fogel and Kaplan, 1984) jejunum. Doses used in the dog (20 $\mu g \cdot kg^{-1} \cdot h^{-1}$) were similar to those used in the cat, and the decrease of ionic concentration of the fluid after absorption was also observed (Barbezat and Reasbeck, 1983).

The *in vitro* effect of DAMA, studied on ileal tissue, has been shown to be associated with a decrease in the short-circuit current (Dobbins *et al,* 1980; McKay *et al,* 1981; Binder *et al,* 1984), suggesting an increase in electrogenic chloride absorption (Dobbins *et al,* 1980; McKay *et al,* 1981). Sodium changes were a little at variance with some studies reporting no change (McKay *et al,* 1981) and others showing an increased absorption (Dobbins *et al,* 1980) during exposure to enkephalins. *In vivo* studies (Barbezat and Reasbeck, 1983) revealed a more pronounced increase in net absorption of sodium and bicarbonate during enkephalin infusion in dog than in cat or rat (Coupar, 1978). Our protocol provides information on the attained equilibrium, as opposed to experiments exploring the difference of potential induced by the electrogenic transport. However, we were unable to demonstrate any change in the cation–anion ratio. In basal conditions, the luminal solution collected after 1 h contained more cations than anions (delta in the jejunum and ileum, respectively 26 ± 10 and 28 ± 8 μmol/h). During DAMA stimulation a difference of the same magnitude was observed (delta in the jejunum and the ileum respectively 35 ± 13 and 31 ± 12 μmol/h). This cation–anion difference could be consecutive to the secretion of unidentified anion(s) that we did not determine. The same cation–anion difference was observed for the jejunum and the ileum.

Our results indicate that DAMA is effective in the cat, as in other species (Cooke, 1986), at 2 different levels of the intestine, jejunum and ileum, even though it is clearly evident that the mechanisms of ion transport differ. A different motility response to metenkephalin was also observed at the 3 levels of the small intestine in the cat (Radomirov *et al,* 1990). This confirms that intestinal transport is controlled by several neurotransmitters and that opioids intervene in cat as in other species in increasing absorption. Our protocol excludes the possibility that this effect could be due to motility inhibition, which is a well-known property of the opioid peptides (Rozé and Dubrasquet, 1983) and which has traditionally been thought to underlie their antidiarrheal or constipating activity (Manara and Bianchetti, 1985). Previous work has shown that mucosal blood flow can influence absorption (Mailman, 1984), a factor which has not been studied in our experiments. Since no enkephallin receptors have been discovered on dog mucosal cells but in membranes of myenteric and

deep muscular plexus (Allescher *et al*, 1989), it has been proposed that enkaphalin (which reduces acetylcholine release at muscle level: Paton, 1957), induces absorption by adrenergic preponderance (Taper, 1983) of the balance of adrenergic and cholinergic control of intestinal transport. But local non-cholinergic, non adrenergic pathways, like peptidergic release, might be involved too. Our data show that our model is convenient for studying the direct effect of drugs on intestinal absorption independent of intestinal motility.

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