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Regulation of L-valine absorption by opioids interacting with μ-receptors in rabbit ileum

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Abstract. In intact tissue, [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (10^{-5} M; μ-ligand), diminished short-circuit current (I_{sc}) and increased water, Na^+ and Cl^- net fluxes in vitro under open circuit conditions; it also inhibited L-valine absorption and L-valine-dependent variations of short-circuit current ($\Delta I_{sc,val}$). Naloxone (10^{-6} M) antagonized these effects. In the absence of the muscularis and myenteric plexus this enkephalin or morphine (μ-ligand) reduced I_{sc} and $\Delta I_{sc,val}$. These enkephalin effects occurred at different times. Different concentrations of enkephalin were tested for their effects on $\Delta I_{sc,val}$. [D-Ala^2,D-Leu^5]enkephalin (mainly a δ-ligand) significantly decreased I_{sc} but not $\Delta I_{sc,val}$. The reduction of L-valine absorption does not depend on the effects on basal ion transport. Interaction of opioids with μ-receptors located in the submucosal plexus and/or in the epithelial cell accounts for this reduction. This enkephalin effect seems to be at least partially under the control of the myenteric plexus.
Key words. Enkephalins; ion fluxes; amino acid transport; short-circuit current.

Absorption in the intestinal tract is subject to many regulatory processes[1-3]. Recently, there has been increasing interest in opioid-peptide, since the intestinal wall is rich in endogenous opioid-peptides, i.e. enkephalins, endorphins and dynorphins[4]. Early studies of these substances mainly focused on their analgesic and antidiarrheal effects[5,6], but recently their physiological role has been investigated[1,7,8]. Exogenous opioid-peptides with morphine-like activity, must also be considered. They can result from enzymatic cleavage of a variety of food proteins (e.g. milk, wheat, etc.) and react with specific receptors[9-12] present in the intestinal wall.

Opioid-receptors are widely distributed in the intestinal wall, with differences in type and localization that may be species-dependent. μ-Receptors have been identified in the myenteric and submucosal plexus in the guinea pig[13]; δ- and μ-receptors in the submucosal plexus and inside villi of the rat[13,14]; δ-, μ- and k-receptors in the myenteric plexus of the dog[15]. Contradictory results on specific binding of enkephalins to isolated enterocytes have been reported for the guinea pig[13,16,17], while rabbit and rat enterocytes seem to lack receptors[16,18].

The mechanisms underlying the opioid action are numerous and not yet exhaustively understood. The exogenous opioid-peptide-dependent increase in water absorption has been explained by an inhibition of peristalsis[19] and/or by action on ionic transport which involves δ-receptors in the guinea pig[20] and μ-receptors in the rat[21]. Enkephalin effects on fluid and ion transport seem to depend upon δ-receptors in the rabbit, though morphine, a typical agonist for μ-receptors, yields similar effects[22]. In this regard, it is interesting to note that naloxone, a specific μ-receptor antagonist, reduces water and electrolyte absorption in vivo[23]. Finally, very little is known about the effects of opioid-peptides on nutrient absorption. In everted sacs of rat jejunum, casomorphins or synthetic analogues determine variations in the accumulation of [^{3}H]leucine suggesting alterations in amino acid eptithelial transport[24]. Our preliminary data on rabbit ileum, based on transepithelial electrophysiological measurements, gave some indication of a possible interference of opioids on amino acid absorption[25,26].

To investigate this point, we have evaluated the effects of DAGO ([D-Ala^2,MePhe^4,Gly-ol^5]enkephalin), selective for μ-receptors, of morphine, with good μ-receptor reactivity[6,27], and of DADLE ([D-Ala^2,D-Leu^5]enkephalin), mainly a δ-ligand[28], on L-valine absorption. Drugs were used at the very beginning of the experiment (since we did not know their effects with time) or after a preset period which served as internal control (dose response curves and everted sac experiments). Moreover, DAGO effects on L-valine-dependent variations of electrical parameters were investigated in intact or in serosa- and muscularis-deprived intestinal wall as a first step towards the localization of the receptors involved. Experiments were also carried out in the absence of L-valine to elucidate the relationship between the effects on L-valine and basal ionic transports. Initial choice of DAGO and DADLE concentrations was made according to the doses reported to elicit maximal effects on basal ionic transport (measured through I_{sc}) for other opioids (morphine, [Met^5]enkephalin, [D-Ala^2,Met^5]enkephalin, β-casomorphine analogues, DADLE, etc.) in rabbit[10,22,29] or in guinea pig[20]. Dose response curve experiments were set up to verify the suitability of concentration choice for DAGO effects on L-transport.

Part of this work has already appeared in preliminary form[25,26].

Materials and methods

New Zealand male rabbits were killed by cervical dislocation and a 5 to 10 cm segment of distal ileum was rapidly excised and placed in Krebs-Henseleit saline (mM concentrations: Na^+, 142.9; K^+, 5.9; Ca^{2+}, 2.5; Mg^{2+}, 1.2; Cl^-, 127.7; HCO_3^-, 24.9; $H_2PO_4^-$, 1.2; SO_4^{2-}, 1.2). In some experiments the serosa and muscularis were removed by blunt dissection: the effectiveness of the operation was checked, in the first experiments, by microscopic examinations.

Transepithelial electrical potential difference and short-circuit current. The intestine, opened as a flat sheet, was mounted between two Ussing chambers (exposed area: 0.67 cm^2) and bathed on both sides by Krebs-Henseleit saline (5 ml each side). Salines were kept at 30 °C in electrophysiological and everted sac experiments (see next section), on the basis of preliminary data showing more constant values of I_{sc} and ion and water transepithelial transport at 30 °C than at 37 °C in agreement with previous data[30]. Besides, I_{sc} values that we observed after 60 min were of the same order or even higher than those reported at the same time at 37 °C by other authors[22,29]. Solutions were bubbled with 95% O_2 and 5% CO_2 (pH 7.4). V_{ms} (transepithelial electrical potential difference) and I_{sc} were measured as reported by Field et al.[31] and utilizing an automatic device made in our workshop that allowed subtraction of the bathing fluid resistance for a correct measurement of I_{sc}. Every 10 min, starting from the 5th min, the initial luminal solution was replaced, for a 2.5 min period, by a similar solution in which 20 mM L-valine substituted for 10 mM NaCl so that osmolarity was maintained constant. L-Valine was not added to the serosal side to favor the electrogenic component (the Na^+-associated cotransport at the apical side) of the transepithelial transport. $\Delta V_{ms,val}$ and $\Delta I_{sc,val}$ represent the maximal increase due to L-valine presence, unless otherwise stated. Initial conditions (L-valine-free Krebs-Henseleit solution) were restored by three washings. If used, solutions containing DAGO or DADLE (10^{-5} M; Bachem, Switzerland) or morphine (10^{-4} M, a gift from M. Negri Institute, Milan, Italy) were present on the serosal side soon after mounting the tissue (that is, at the beginning of the experimental time). We chose addition at the serosal side since the effectiveness of natural enkephalins or synthetic analogues on transepithelial ion and water transport has been proved only at the serosal side[22,23] and in accordance with the in vivo situation; in preliminary experiments, application at the mucosal side had no effect. When naloxone (10^{-6} M; a gift from Endo Laboratories, USA) was used, tissue was kept in contact during the 5 min just after killing (that is, during the isolation, washing of contents and mounting). This was done to pretreat with the drug without altering the usual time-schedule.

To obtain the DAGO dose-response curve (5 min treatment with DAGO on the serosal side, tissue deprived of muscularis and serosa), we modified the previous procedure, starting from the 25th min. The solution containing L-valine was kept in contact with the luminal side for 7 min, followed by an equal period with the initial saline (L-valine free). The same operation was repeated starting from the 39th min. When used, enkephalin was added at the 41th min and naloxone at the 36th min (serosal side). Comparison was made between $\Delta I_{sc,val}$ observed at the end of each 7 min period.

Water, ion and L-valine transport with everted sac preparation. Direct measures of water, ion and L-valine transport in ileum were done on everted sac preparations. An intestine tract, about 10 cm long, was turned out quickly with the help of a glass rod, tied at one end and cannulated at the other. Water transport was evaluated following the dilution (spectrophotometric determination; $\lambda = 505$ nm) of an impermeant dye (0.07 mM phenol red) added to the serosal (i.e. internal) bathing medium[33]. Krebs-Henseleit solution was kept at 30 °C, with 95% O_2 and 5% CO_2 bubbled through on the mucosal side. When utilized, L-valine was present initially at the same concentration (20 mM) on both sides of the tissue (no substitution was done for other ions since no transepithelial osmotic gradient was present); initial volumes were 2.5 ml on the serosal side and 100 ml on the luminal (i.e. external) side. Ten min after mounting the tissue (an interval necessary to restore functional conditions after tissue isolation), 0.5 ml of solution were taken from the serosal side for transport measurement. The withdrawn solution was not replaced since it nearly equaled the volume of the transported fluid. The same amount (0.5 ml) of solution was withdrawn every 30 min between 10 and 100 min, to have three experimental periods. Enkephalin addition (with 20 µl of saline to a final concentration = 10^{-5} M, serosal side), took place, when needed, at the end of the first period of 30 min, preceded (5 min) in some experiments by naloxone addition (5 µl; final concentration = 10^{-6} M, serosal side). All subsequent calculations took into account all volume additions or removals. Samples were successively deproteinized with precipitations in 0.6 M trichloroacetic acid, and centrifuged. Serosal Na^+ and Cl^- concentrations were determined chemically with a flame photometer (model 943, Instrument Laboratory, Milan, Italy) and a chloridometer (Chlor-o-counter Mark II, Marius Instruments, Utrecht, Holland). L-Valine concentration was determined by HPLC (model 330-110, Beckman, San Ramon, CA, USA). In each experiment, derivatization by the dansyl-chloride method[34] was used for both samples

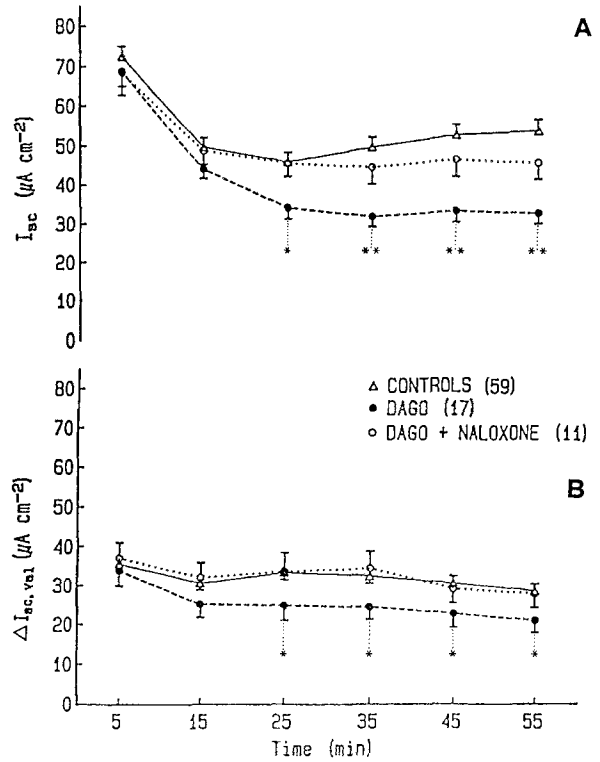

Figure 1. *A*) Effects on intact tissue of DAGO (10^{-5} M; serosal side) or DAGO (10^{-5} M) plus naloxone on I_{sc} or *B*) on L-valine-dependent variations of I_{sc} ($\Delta I_{sc,val}$). Data points represent mean values ± SE (bar). Number of experiments in parentheses. * = $p < 0.05$, ** = $p < 0.01$.

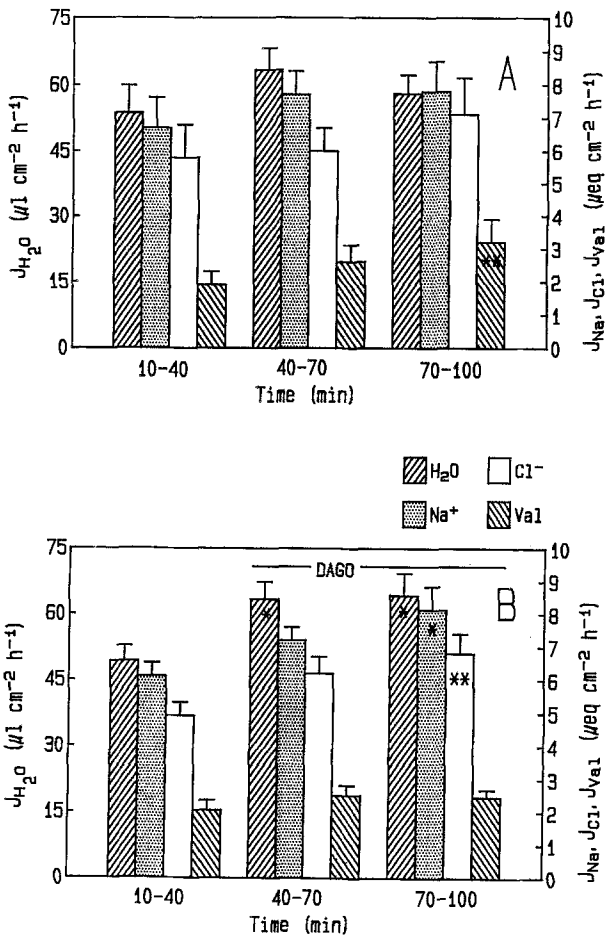

Figure 2. Water, Na^+, Cl^- and L-valine absorption measured in intact tissue, in the absence (*A*) or presence (*B*) of DAGO (10^{-5} M; addition at 40 min on the serosal side). L-Valine was present at the same concentration (20 mM) on both sides, from the beginning of the experiment; number of experiments = 7. Histograms represent mean values ± SE (bar).
Significance from the control period (10–40 min) of the same experiments: * = $p < 0.05$; ** = $p < 0.01$.

and standards for calibration. Analysis did not reveal significant traces of L-valine dependent on a possible degradation of subepithelial layers.

Statistical analysis. Statistical analysis was performed for electrophysiological experiments by the t-test for unpaired data and for experiments with the intestinal everted sacs by a sequentially rejective multiple test[35]. Data are reported as mean values ± SE with number of experiments (n).

Results

Variations of transepithelial electrical parameters in intact tissue in the presence of DAGO or DADLE. I_{sc}, in controls, decreases spontaneously for the first 15 min, after which the tendency was towards stabilization (fig. 1A). DAGO (10^{-5} M) significantly reduced I_{sc} starting from the 25th min of treatment. Quite similar effects on V_{ms} were observed. Naloxone (10^{-6} M) prevented these effects (fig. 1A).

DADLE (10^{-5} M, n = 17), like DAGO, reduced I_{sc} and V_{ms}, but significance was observed only after the 45th min and, in addition, the percentage of inhibition was lower (maximal I_{sc} inhibition was about 21% against 41%). These effects were not prevented by naloxone (10^{-6} M).

In the absence of DAGO, substitution of the luminal solution with one containing L-valine caused an increase in I_{sc} and V_{ms} (reported as $\Delta I_{sc,val}$ and a $\Delta V_{ms,val}$) in agreement with the coupled entrance of Na^+ and L-valine through the luminal membrane. All L-valine additions caused, within the same experiment, $\Delta I_{sc,val}$ of about the same entity. Injection per se (placebo) did not cause any significant effect. Washing out of L-valine caused a recovery of I_{sc} to its basal value.

DAGO decreased $\Delta I_{sc,val}$ (inhibition of about 30%, fig. 1B) and $\Delta V_{ms,val}$ (from 1.7 ± 0.1 mV, n = 59, to 1.3 ± 0.1 mV, n = 17; $p < 0.01$) significantly after 25 min. Naloxone (10^{-6} M) prevented these effects (fig. 1B). Preliminary data show that the presence of tetrodotoxin (TTX; 3×10^{-7} M) also prevented DAGO effects ($\Delta I_{sc,val}$ at 25 min: treatment = 33.7 ± 4.8 μA × cm^{-2}; n = 11; control = 33.3 ± 1.8 μA × cm^{-2}; n = 59). As for $\Delta I_{sc,val}$ (and $\Delta V_{ms,val}$), DADLE addition did not produce any significant difference from control values.

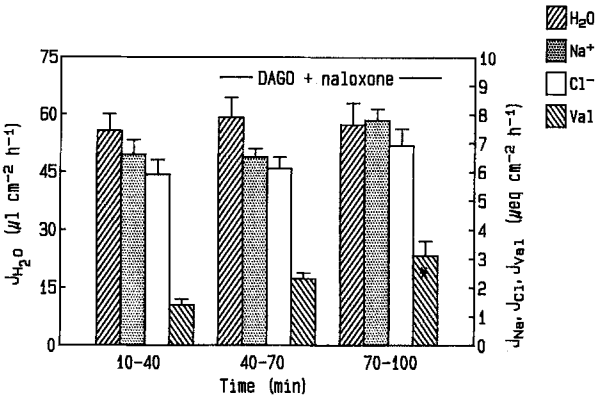

Figure 3. Water, Na^+, Cl^- and L-valine absorption in intact tissue, in the presence of DAGO (10^{-5} M; added at 40 min) and naloxone (10^{-6} M, added at 35 M) at the serosal side; number of experiments = 9. Histograms represent mean values ± SE (bar). Significance from the control period (10–40 min) of the same experiments: * = $p < 0.05$.

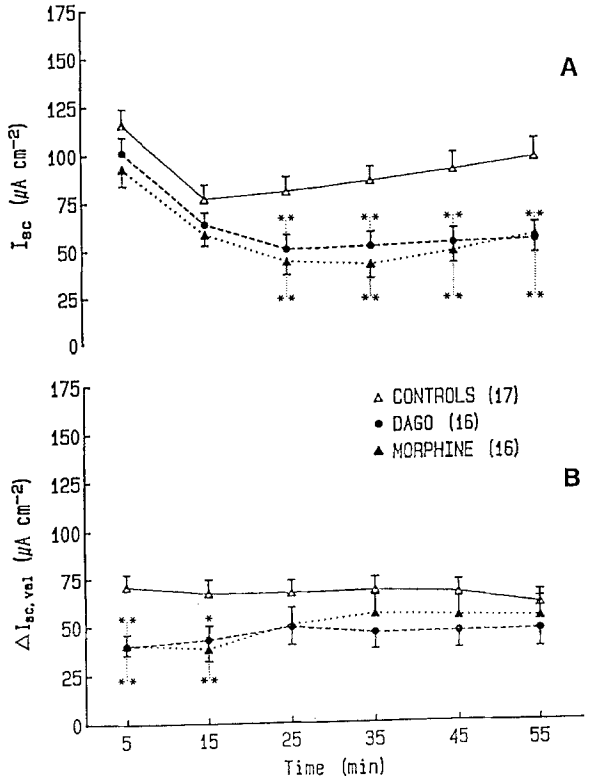

Figure 4. Effects of 10^{-5} M DAGO or 10^{-4} M morphine on I_{sc} (*A*) or on $\Delta I_{sc,val}$ (*B*) in stripped tissue. Data points represent mean values ± SE (bar). Number of experiments in parentheses. * = $p < 0.05$; ** = $p < 0.01$.

Effects of DAGO on water, ion and L-valine transport in everted sac preparation. In control conditions without L-valine, water and ion transport remained constant from 10 to 100 min after setting the tissue; addition of DAGO (10^{-5} M) at 40 min caused a significant increase in water, Na^+ and Cl^- transport in the two 30 min periods that followed enkephalin addition.

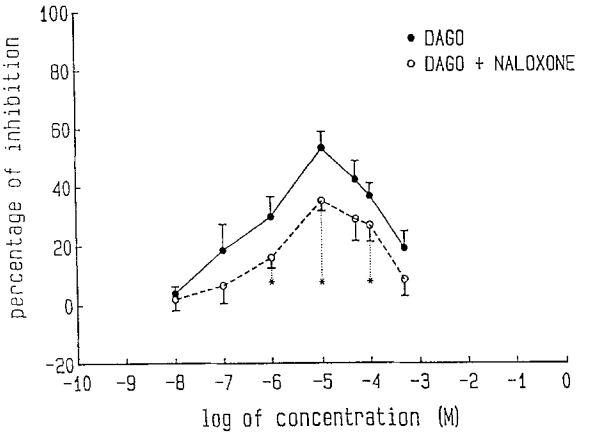

Figure 5. Dose response curves relating the percentage of inhibition of L-valine-dependent I_{sc} variations in stripped tissue to different doses of DAGO (significantly different from zero starting from 10^{-7} M) or DAGO plus 10^{-6} M naloxone (significantly different from zero between 10^{-6} and 10^{-4} M DAGO). Each point represents the mean ± SE of four to ten experiments. Significance between the two curves: * = $p < 0.05$.

Presence of L-valine (fig. 2A) from the beginning of the experiment caused no significant variations with time of net fluxes of water, Na^+ and Cl^-. L-Valine transport significantly increased in the third experimental period. Addition of DAGO (10^{-5} M) at 40 min eliminated the previously observed increase in L-valine transport in the third period and significantly increased water, Na^+ and Cl^- transport (fig. 2B). Naloxone (10^{-6} M) addition at 40 min prevented DAGO effects (fig. 3).

Effects of DAGO or morphine on transepithelial electrical parameters in tissue deprived of muscularis. In the absence of L-valine and in control conditions, values of I_{sc} were almost twofold (average ratio obtained at all experimental points = 1.7, fig. 4A) greater than those in the intact tissue, while V_{ms} was not significantly different; resistance was proportionally diminished (to 55%). Treatment with DAGO (10^{-5} M) or morphine (10^{-4} M) caused decreases of I_{sc} (fig. 4A) and V_{ms} (with similar time courses to those of I_{sc}) from control values. Significance was reached at the 25th min, as with the intact tissue.

DAGO (10^{-5} M) or morphine (10^{-4} M) caused a significant decrease in $\Delta I_{sc,val}$ (about 50%) that occurred earlier (at 5 min) than in intact tissue; a marginal significance was observed from the 25th min ($p < 0.10$): measured values were always lower throughout the experiment than for the controls (fig. 4B). DAGO or morphine induced significant reductions of $\Delta V_{ms,val}$ from the 5th min (time courses in control and treatment conditions were similar to those of $\Delta I_{sc,val}$).

Dose response curve for DAGO on $\Delta I_{sc,val}$ in tissue deprived of muscularis. To verify the suitability of concentrations of DAGO and DADLE chosen on the basis of literature data[10, 20, 22, 29], we set up experiments to obtain dose response curves of DAGO concentration vs $\Delta I_{sc,val}$.

Significant inhibition was observed at 10^{-7} M; maximum inhibition (5 min treatment, see 'Materials and

methods') was reached when the enkephalin was 10^{-5} M; at higher DAGO concentrations, the inhibition decreased (fig. 5). Addition of a placebo with the same procedure had no effect on $\Delta I_{sc,val}$. If DAGO was added in the absence of the amino acid, there was no significant effect on I_{sc} in the subsequent 5-min period. When naloxone (10^{-6} M) was added to L-valine and DAGO, inhibition was less than that observed with the sole enkephalin, at all concentrations of DAGO from 10^{-8} to 5×10^{-4} M (fig. 5).

Discussion

Effects of DAGO on amino acid transport. In everted sac experiments, if L-valine was present, all transports increased with time; variations in Na^+ and amino acid transfer were of the same magnitude. However, only the L-valine transport increase is statistically significant (since, as a percentage, it is the greatest change from initial values). Conversely, DAGO addition significantly enhanced water, Na^+ and Cl^- transports, but abolished the increase in L-valine transport. Electrophysiological experiments also indicate an inhibitory effect of DAGO on the amino acid transport, as shown by the significant decrease of $\Delta I_{sc,val}$ in intact tissue after the 25th min of treatment.

L-Valine transport inhibition could depend on general variations in basal ionic transport due to DAGO; in fact DAGO presence resulted in altered ionic basal trasports both in everted sac and I_{sc} experiments. Evidence that the mechanisms through which DAGO interacts with basal ionic transports or L-valine transport are independent of each other comes from experiments on stripped tissue: DAGO effect on $\Delta I_{sc,val}$ is significant in 5 min, 20 min before the effects on basal I_{sc}. DADLE also affects I_{sc} but not $\Delta I_{sc,val}$, thus confirming that the two effects are not obligatorily linked. This conclusion is also suggested by the fact that L-valine absorption is inhibited though apical membrane should result hyperpolarized (so favoring electrogenic Na^+-L-valine apical entry) as a consequence of DAGO effects on ionic basal transport (as dealt with in the rest of the paragraph) and in agreement with the observed decrease of V_{ms}. Actually DAGO, in the absence of L-valine, increases ionic absorption (everted sac experiments) and reduces I_{sc}. In the rabbit ileum, I_{sc} is mainly the result of active, electrogenic Na^+ absorption and Cl^- secretion[31] though one cannot exclude a minor participation of secretion of bicarbonate and to a lesser extent of potassium. Thus, I_{sc} inhibition and the increase of ionic transepithelial absorption due to DAGO can be accounted for mainly by a reduction of Cl^- (anion) secretion. This effect has been reported for other opioids[22,30]. DAGO inhibition of elctrogenic Cl^- secretion, in accordance with the proposed models for intestinal epithelium[36], would result in a hyperpolarization of the apical membrane which should not prevent Na^+-L-valine coupled uptake.

The reduction of L-valine transport might also reflect increased intracellular metabolism of the amino acid. However, the subsequent decrease in its intracellular concentration should increase its entry across the apical membrane, and this is not in accordance with the observed reduction of $\Delta V_{ms,val}$.

All these considerations suggest that there is direct involvement of opioids in amino acid transport, and this agrees with results obtained by other researchers[24] who studied the effects of exorphins (β-casomorphins and synthetic analogues) on L-leucine transport. Moreover, inhibition of amino acid uptake by enkephalins has also been found in rat brain synaptosomes[37,38].

We observed a significant inhibition of $\Delta I_{sc,val}$ using DAGO starting from 10^{-7} M; this concentration is in the range as those found in rabbit myenteric plexus and gastrointestinal tract[39]. The concentration of DAGO at which we observed 50% of the maximal effect was about 10^{-6} M; similar doses are reported for inhibition of synaptosomal proline uptake by some enkephalins[37]. No comparison is possible with the results obtained by Ermisch et al.[24] in rat intestine, who observed alterations in accumulation of L-leucine following β-casomorphine administration, since these authors do not show the dose response curve. These doses are very high if one considers DAGO affinity[40] to opioid receptors in guinea pig brain (K_i 1.9 nM), and in comparison to DADLE potency assay (IC_{50} about 10^{-8} M) in muscular tissue of guinea pig ileum[41] or mouse vas deferens[42] or DADLE effects[20] on I_{sc} (ionic basal transport) in guinea pig ileum (IC_{50} of about 10^{-8} M). Yet, doses reported to elicit half maximal effects on I_{sc} in rabbit intestine by different opioids[10,22,29] are in the range from 10^{-5} to 10^{-7} M. Moreover, when evaluating a dose response relationship, differences in tissue and species must be considered; besides, the steps involved before the ultimate effect is observed can be complex and numerous[19,43] (see also introduction and next sections) as suggested also by the bell-shaped dose response curve of DAGO on $\Delta I_{sc,val}$. About this point, we can only put forward some possible explanations for the observed diminution of inhibition when DAGO was added at concentrations greater than 10^{-5} M: 1) a mixed agonist-antagonist effect similar to the analgesic effects of some opioids[6], and 2) a biphasic response resulting from induced release of different neurotransmitters[44]. A bell-shaped dose response curve was also found for rabbit ileum[22,45] in the reduction of I_{sc} caused by morphine or an enkephaline-like pentapeptide.

We did not set up experiments to obtain a dose response curve (DAGO concentrations vs I_{sc} as a measure of basal ionic transport) since we principally wanted to evaluate the effects of the drug on L-valine transport. Furthermore, in the conditions under which we obtained the dose response curve for the inhibition of L-valine transport (tissue deprived of muscularis, 5 min

of treatment), we did not observe any significant effect on I_{sc} (basal ionic transport) in agreement with our previous observation that these effects occur later, that is from 25 min.

Localization and identification of the receptors. DAGO is known to be a selective μ-receptor ligand[27]. In intact tissue its effects on $\Delta I_{sc,val}$ are completely eliminated by naloxone; this indicates the presence of a specific interaction with opioid receptors. Though naloxone preferentially elicits its action when μ-receptors are involved[6], the concentration used in our experiments (10^{-6} M) implies a possible interaction with other receptor types[40]. The high degree of inhibition elicited by naloxone in intact tissue on DAGO effects, even though its K_i for μ-receptors is of the same order of magnitude of that of DAGO[40], could depend on the presence of different μ-receptor subpopulations[46] and/or on effects exerted in intact tissue not related to DAGO inhibition. We found that naloxone, at doses greater than 10^{-6} M, stimulated I_{sc} in a significant manner (data not shown) in accordance with the results reported by Dobbins et al.[29]. In stripped tissue, the concentration of DAGO that caused the same percentage of $\Delta I_{sc,val}$ inhibition is roughly ten times greater in the presence than in the absence of naloxone (in the range of DAGO concentrations from 10^{-7} to 10^{-5} M). This is possibly due to a competition mechanism.

Involvement of μ-receptors is also in agreement with the action of morphine, that mainly acts on μ-receptors. Interactions of DAGO with δ-receptors could not be ruled out at the 10^{-5} M concentration but it seems unlikely since DADLE (a δ-ligand with cross reactivity with the μ site) does not elicit appreciable effects on $\Delta I_{sc,val}$ at the same concentration of DAGO. However, the lack of significant effects of DADLE on $\Delta I_{sc,val}$ and the fact that its action on I_{sc} is not antagonized by naloxone at the same concentration effective on DAGO, suggest a mechanism which differs from that involved with DAGO. δ-receptors (besides μ) could act on basic ion transport[32]. Alternatively, DAGO and DADLE might act on two different subpopulations of μ-receptors[46], sensitive in different ways to naloxone. Experiments with a more selective δ-ligand such as [D-Pen^2,p-chloro-Phe^4,D-Pen^5]enkephalin (pCl-Phe^4-DPDPE) will definitively exclude an involvement of δ-receptors.

As for the localization of the receptors, we must note that in the absence of the myenteric plexus we still observe the effects of DAGO on $\Delta I_{sc,val}$. This suggests that the receptors involved should be present on the submucosal plexus and/or on enterocytes. Preliminary experiments with TTX are in agreement with the involvement of a release of endogenous neurotransmitters. We tried to localize DAGO binding sites on rabbit ileal stripped tissue by means of autoradiography. However, the evaluation of specific binding of [^{3}H] DAGO, calculated as the difference of values obtained in the presence or absence of 10 μM naloxone, did not provide evidence for binding sites, since the values we obtained were too close to the lower limits of sensitivity of the method because of the high nonspecific binding.

Possible role of the myenteric plexus. The percentage of $\Delta I_{sc,val}$ inhibition by DAGO is lower in the intact tissue (30%), than in the stripped tissue (53%) and the effect is more delayed. The reduction and delay of inhibition might be due to: 1) presence of unstirred subepithelial layers, or 2) the intervention of the myenteric plexus that controls, to some extent, the effects of DAGO on L-valine absorption. The latter hypothesis seems to be the sounder, since the inhibition of basal I_{sc} occurs at the 25th min in both cases, independent of tissue stripping, thereby showing that the unstirred layer effects are not the limiting factor. In the light of the previous considerations, we can hypothesize that 1) DAGO operates as a neuromodulator at the submucosal plexus, modifying the release of one or more neurotransmitters which regulate enterocyte transport, and 2) the DAGO-dependent release of the neurotransmitters, responsible for the effect of L-valine absorption, is partially under the control of the myenteric plexus. In agreement with such control, naloxone in intact tissue completely antagonizes DAGO effects, whereas in stripped tissue, its antagonism is only partial (about 40%).

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