J. Pharm. Pharmacol. 1989, 41: 494–495
Communicated January 17, 1989

Effect of [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin and [D-Ala^2,D-Leu^5]enkephalin on ion and amino acid transport in rabbit ileum

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Abstract—The selective μ-opioid agonist [D-Ala^2,MePhe^4, Gly-ol^5]enkephalin (DAGO) (10 μM) reduced the short circuit (I_{sc}) and the L-valine induced increase of the transepithelial potential difference and I_{sc}(ΔV_{ms} and ΔI_{sc}) measured in-vitro in rabbit ileum, with a mechanism antagonized by naloxone (1 μM). [D-Ala^2, D-Leu^5]enkephalin (DADLE) (10 μM) had no significant effect on the transepithelial potential difference (V_{ms}), I_{sc}, ΔV_{ms} and ΔI_{sc}. In the ileum deprived of the serosa and muscolaris, DAGO reduced the ΔV_{ms} and ΔI_{sc}, but not the V_{ms} and I_{sc}, suggesting localization of the receptors responsible for this latter effect in the myenteric plexus and/or the muscularis mucosae. These preliminary results suggest that in the rabbit ileum opioids influence electrolyte and amino acid transport and these effects may be at least partly mediated by μ-receptors.

Opioids influence intestinal electrolyte and fluid transport, which might partly explain their antidiarrhoeal effects (McKay et al 1984). However, no data are available about effects on nutrient reabsorption, particularly amino acids. The δ-type of opioid receptor is mainly involved in the effect on ion and fluid transport in the guinea-pig ileum (Kachur et al 1980; Kachur & Miller 1982), but few data are available in the rabbit (Dobbins et al 1980; McKay et al 1981, 1982).

We tested the effects of [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (DAGO), a selective μ-ligand (Kosterlitz & Paterson 1981), and [D-Ala^2,D-Leu^5]enkephalin (DADLE), a δ-ligand with high cross reactivity at the μ-site (Gillan & Kosterlitz 1982), and their antagonism by naloxone on ion and L-valine transport, evaluated indirectly by their influence on the transepithelial electric potential difference (V_{ms}) and short circuit current (I_{sc}). To locate this effect in the rabbit ileum we tested the opioid effect in two different conditions: the intact ileum and the ileum without the serosa and muscularis mucosae.

Materials and methods

New Zealand male rabbits (Azienda Agricola Bernasconi, Valmorea, Como, Italy) were killed by a blow on the neck and 5 to 10 cm segments of distal ileum were rapidly excised and placed in Krebs-Henseleit saline (composition mM: 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 mucosae were removed by blunt dissection. The tissue was mounted between two lucite chambers (window 0·67 cm^2) and bathed on both sides by Krebs-Henseleit saline, kept at 30°C and bubbled with 95% O_2 and 5% CO_2 (pH 7·4).

The transepithelial potential difference (V_{ms}) and the short circuit current (I_{sc}) were measured as described by Field et al (1971) with a device made in our workshop that made automatic subtraction of the bathing fluid resistance for correct measurement of I_{sc}. To evaluate the maximal increase of V_{ms} and I_{sc} (ΔV_{ms} and ΔI_{sc}) induced by Na^+-dependent amino acid transport, the Krebs-Henseleit solution was replaced 5 min after the beginning of the experiment and then every 10 min, with a similar solution in which 10 mM NaCl was replaced by 20 mM L-valine. This solution was left for 2·5 min and then washed repeatedly with L-valine-free Krebs-Henseleit solution, restoring the initial conditions.

Naloxone was added to the Krebs-Henseleit solution before mounting the tissue (5 min before DAGO) and DADLE and DAGO were added to the serosal saline when the tissue was mounted. Molarity was calculated for the following salts: naloxone HCl (gift of Endo Laboratories, USA); [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin base (DAGO) and [D-Ala^2,D-Leu^5]enkephalin acetate (DADLE) (Bachem, Switzerland). Statistical evaluation of results was based on analysis of variance and Duncan's test or Student's t-test, as specified in the legends to the Tables.

Results

In intact tissue, 10 μM DAGO reduced I_{sc} and the L-valine-dependent increase of V_{ms} and I_{sc} (ΔV_{ms} and ΔI_{sc}), and the inhibition became significant for I_{sc}, ΔV_{ms} and ΔI_{sc} 25 min after treatment (Table 1). Naloxone (1μM), added to saline 5 min before saline or the agonist (see Methods), antagonized the agonist-induced effects, while alone it did not significantly modify these parameters compared with controls. Incubation with 10 μM DADLE induced a constant, but not significant, decrease of ΔV_{ms} and ΔI_{sc} for 25 min after mounting the tissue (Table 1), while V_{ms} and I_{sc} decreased from 45 min on (data not shown).

As DAGO has caused the greatest inhibition in intact tissue, only this μ-agonist was used to study the effects of opioids on the V_{ms} and I_{sc} and the relative L-valine-dependent changes (ΔV_{ms} and ΔI_{sc}) when the serosa and muscularis were removed (stripped tissue). Ten μM DAGO added to the serosal side significantly reduced only the L-valine-induced ΔV_{ms} and ΔI_{sc} (Table 2): the peak effect was 5 min after the drug addition and the reduction was still significant at 25 min (data not shown).

Discussion

Our preliminary results show that DAGO, a selective μ-receptor ligand (Kosterlitz & Paterson 1981), significantly reduced I_{sc} and the relative L-valine-dependent changes (ΔV_{ms} and ΔI_{sc}) with a mechanism reversed by naloxone, suggesting that opioids influence electrolyte and amino acid transport and that at least

Table 2. Effects of DAGO on V_{ms} and short circuit current I_{sc} and their changes (ΔV_{ms} and ΔI_{sc}) induced by L-valine in the stripped tissue.†

Drug		V_{ms} (mV)	I_{sc} (μA cm^{-2})	ΔV_{ms} (mV)	ΔI_{sc} (μA cm^{-2})
—	(11)	3·6±0·4	136·4±18·4	3·1±0·3	91·8±14·6
DAGO	(7)	3·8±0·4	100·5±10·7	2·0±0·4*	37·2±12·7*

Data are mean±s.e. of (n) experiments. For symbols see Table 1.
† The serosa and muscularis mucosae were removed from the small intestine. V_{ms}, I_{sc} ΔV_{ms} and ΔI_{sc} were recorded 5 min after addition of DAGO or DADLE (10 μM).
* $P<0·05$ from controls by Student's t-test.

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Table 1. Effects of DAGO and DADLE and effect of naloxone on DAGO-induced effects on the transepithelial electric potential difference (V_{ms}) and short circuit current (I_{sc}) and their changes (ΔV_{ms} and ΔI_{sc}) induced by L-valine.

Drug		V_{ms} (mV)	I_{sc} ($\mu A\,cm^{-2}$)	ΔV_{ms} (mV)	ΔI_{sc} ($\mu A\,cm^{-2}$)
—	(38)	2·60±0·14	50·71±3·70	1·88±0·13	35·53±2·75
Naloxone	(4)	2·14±0·19	45·52±8·95	1·49±0·30	29·85±9·05
DAGO	(7)	1·99±0·29	28·63±4·21*	1·17±0·22†	19·43±8·46††
Naloxone+DAGO	(5)	2·27±0·35	47·76±6·84	2·01±0·31	41·79±8·85
DADLE	(9)	3·07±0·35	54·31±7·55	1·52±0·33	24·99±5·58

Data are mean±s.e. of (n) experiments.

V_{ms}, I_{sc} ΔV_{ms} and ΔI_{sc} were recorded 25 min after addition of DAGO (10 μM) added 5 min after naloxone (1 μM) to the serosal medium.

* $P<0·05$ from controls and from the group treated with naloxone+DAGO and † $P<0·05$ and †† $P<0·01$ from the group treated with naloxone+DAGO by analysis of variance and Duncan's test.

the μ-receptors are involved in these processes. DAGO significantly reduced I_{sc} only when intact tissue was used. Conversely, McKay et al (1981) found that another slightly less selective μ-agonist, morphine, significantly lowered I_{sc} in rabbit stripped ileal tissue, indicating that the μ-receptor-mediated mechanism may not be the only one involved in this process. However, our data suggest that at least some of the μ-opioid receptors responsible for this action on ion transport are in the myenteric plexus and/or the muscularis mucosae. Conversely, the finding that the effect of the μ-opioid agonist DAGO on amino acid-induced changes (ΔV_{ms} and ΔI_{sc}) was still present in stripped tissue suggests that the receptors responsible for this effect are not in the myenteric plexus and muscularis mucosae. The rapid effect, as compared to intact tissue, possibly depends on the lack of muscularis mucosae, which might represent a barrier to drug diffusion.

DADLE, a δ-agonist with high cross-reactivity at the μ-sites (Gillan & Kosterlitz 1982), in our conditions produced no significant effect at 25 min after mounting the tissue. A non significant decrease on ΔV_{ms}, ΔI_{sc}, V_{ms} and I_{sc} was observed at 45 and 55 min. These experiments cannot explain the lack of activity of DADLE, which is usually an agonist at μ-receptors, and more experiments are needed to answer this question. However, as DADLE was used at a concentration which had maximal effects in similar experiments in guinea-pig tissue (Kachur et al 1980; Kachur & Miller 1982) a species specificity in the action of opioids is suggested, in agreement with the differences found between various species in the distribution of natural enkephalins and their presence in enterochromaffin cells (Alumets et al 1978).

Another synthetic enkephalin, [D-Ala^2,Met^5]enkephalin, reduced V_{ms} and I_{sc} (Dobbins et al 1980). However, the differences between these and our findings may be explained by the different experimental conditions used by Dobbins et al (different compounds, use of stripped tissue and the presence of glucose in the incubation medium). It is still not clear how—or even whether-the δ-receptor is involved in these processes and further experiments with more selective δ-agonists and antagonists are needed. As in rabbit ileum I_{sc} is mainly a lumped measurement of Na^+ and Cl^- transport (Field et al 1971; McKay et al 1981), our results do not distinguish which ion is responsible for the opioid-induced reductions of V_{ms} and I_{sc}. However, the reduction in L-valine-dependent ΔV_{ms} and ΔI_{sc} should indicate a decrease in Na^+-dependent amino acid transport.

In conclusion these preliminary results suggest that in the rabbit ileum opioids influence not only electrolyte, but also amino acid transport and that these effects may be at least partly mediated by the μ-receptor, while the involvement of δ- and κ-receptors remains to be elucidated.

This work was partly supported by Regione Lombardia Contract no. 334. The authors are indebted to Mrs Judy Baggott for style editing.

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