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Distribution of loperamide in the intestinal wall

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Loperamide [4-(4-chorophenyl)-4-hydroxy-*N*, *N*-dimethyl-α,α-diphenyl-1-piperidinebutanamide monohydrochloride], is a potent orally active antidiarrheal compound without central narcotic properties [1–3]. In the guinea pig ileum it selectively represses peristalsis by inhibiting the local reflex control of intestinal motility [4]. Loperamide binds to membrane sites in the longitudinal muscle–myenteric plexus preparation of the guinea pig ileum [5–7]. Studies of the distribution of labelled loperamide in rats indicate that a large part of the drug is found in the digestive tract [8, 9].

The aim of the present study was to determine, by radioimmunoassay, how exogenously added loperamide is distributed throughout the wall of the isolated guinea pig ileum.

Guinea pigs (350 to 400 g; $n = 19$) were killed by cervical translocation. The non-terminal ileum was isolated, and incubated in 100 ml Tyrode solution kept at 37° and aerated with a gas mixture of 95% O_2–5% CO_2 (pH 7.4). The incubation solution contained loperamide at concentrations of 1.25, 5 or 20 ng/ml; these concentrations produce an average of 7, 44 and 100 per cent inhibition of the peristaltic reflex activity, respectively [4]. At the end of the incubation period the ileum was washed repeatedly with drug-free Tyrode solutions. Six tissues were then prepared for radioimmunological determination of loperamide: an intact segment, a segment without mucosa, the longitudinal muscle with the myenteric plexus attached to it, the circular muscle with the mucosa, the circular muscle without the mucosa and the mucosa. Five to eight experiments were done per concentration of loperamide.

To determine the concentration of loperamide, different parts of the ileum, blotted with filter paper, were weighed and homogenized in methanol (1:5 w:v). After centrifugation, the supernatant was evaporated to dryness. The residue was dissolved in 0.05 ml of methanol and the concentration of loperamide was determined by radioimmunoassay as previously described [10]. Briefly, 0.2 ml of a 1/130 antiserum dilution and 0.5 ng of tritium-labelled loperamide (sp. act. 9 Ci/mM) was added to 0.05 ml of the reconstituted tissue extracts. The mixture was diluted with phosphate buffer (0.05 M, pH 7.4) to a final volume of 0.8 ml and incubated for 2 hr at room temperature. After the incubation period, free and specifically bound loperamide were separated by the addition of a suspension of dextran-coated charcoal (0.2 ml of a 0.5% suspension). The mixture was allowed to equilibrate for 1 hr at room temperature. The charcoal was then removed by centrifugation, and the radioactivity present in the supernatant was determined by liquid scintillation counting (Packard Tri-Carb, Model 3380, with an absolute activity analyzer model 544).

The uptake of loperamide by the 6 types of preparation is shown in Table 1. Loperamide accumulated in the longitudinal muscle–myenteric plexus preparation. The selective uptake of loperamide by the longitudinal muscle–myenteric plexus preparation was most pronounced at those concentrations inhibiting peristaltic reflex activity [4]. At these concentrations of 5 and 20 ng/ml the amounts of drug in the longitudinal muscle–myenteric plexus preparation, expressed per gram tissue, were respectively 9 and 9.5 times higher than in the whole ileal segment, and 8 to 12 times higher than in the circular muscle. The small amounts found in the mucosa were negligible, being near the limit of detection (±0.60 ng/g).

Both the selective uptake by the longitudinal muscle–myenteric plexus preparation and the inhibition of the peristaltic reflex activity increased with increasing loperamide concentrations in the incubation medium. This implies that the loperamide-induced inhibition of intestinal motility depends upon the amount of active drug reaching the longitudi-

Table 1. Distribution of loperamide in the wall of the guinea pig ileum

Tissue	Recovered loperamide, expressed in ng/g tissue (means ± S.E.M.; $n = 5$–8) I	II	III
Longitudinal muscle + myenteric plexus	10.09* ± 1.66	59.29† ± 12.35	196.84‡ ± 41.54
Total ileum segment	2.70 ± 0.80‖	6.59 ± 1.22‖	20.78 ± 2.80‖
Ileum segment without mucosa	4.62 ± 1.25§	21.70 ± 4.93‖	34.02 ± 5.46‖
Circular muscle + mucosa	2.73 ± 0.77‖	5.82 ± 0.72‖	16.55 ± 4.01‖
Circular muscle	6.50 ± 1.74	7.37 ± 1.01¶	16.57 ± 6.35‖
Mucosa	0.86 ± 0.24‖	0.79 ± 0.30‖	1.08 ± 0.39‖

The ileum was incubated with loperamide at 1.25 (I), 5 (II) and 20 (III) ng/ml. The significance of the differences of values *, † and ‡ from the other values of the corresponding concentrations, is calculated by the Mann–Whitney U-test two-tailed; §$P \leqslant 0.01$; ‖$P \leqslant 0.005$; ¶$P \leqslant 0.001$.

nal muscle–myenteric plexus of the ileum.

It has been suggested that the local inhibitory action of loperamide on intestinal motility may be due to its effect on nervous structures [4]. The present findings that the drug is found preferentially in the longitudinal muscle–myenteric plexus, rather than in either the circular muscle or the mucosa, indicate that loperamide acts on the intramural ganglia (myenteric plexus) and on nerve endings within the longitudinal muscle layer.

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Carbonic anhydrase activity of brush border and plasma membranes prepared from rat kidney cortex

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The distribution of carbonic anhydrase in the mammalian proximal tubule cell is of great physiological interest because of its role in bicarbonate reabsorption. The enzyme has been found in the microsomal membranes of rat, dog and human cortical cells as well as the cytoplasm of the cell [1, 2]. During the course of present work, carbonic anhydrase was found in the brush border and basal–lateral membranes of rat renal cortical cells [3]. Inhibition studies with these membranes indicated an enzyme 2–6 times less sensitive to the action of sulfanilamide, ethoxzolamide and CL 13475 than the cytoplasmically located enzyme [3]. If such differences were real, they point to a low activity form of the enzyme different than that found in the cytoplasm and perhaps an adjustment in dose or concentration of the drugs in physiological and clinical work. The present investigation was undertaken to reaffirm the localization of the enzyme within the plasma membranes of the rat renal cortical cell and to ascertain if the sensitivity of the membrane bound enzyme to the inhibitors methazolamide and acetazolamide is truly different from that of the cytoplasmic enzyme. The observation that isolated membranes contained carbonic anhydrase was confirmed but no difference in inhibitory sensitivity between the membrane-associated and cytoplasmic enzyme could be found.

Preparation of the kidney membranes. Male Sprague–Dawley rats, weighing 300–600 g, were killed by decapitation, the kidneys removed and perfused through the renal artery with cold sucrose–Tris buffer (0.25 M sucrose–0.01 M Tris–OH, pH 7.6 with HCl) to flush out contaminating red blood cells and thus their carbonic anhydrase. Cortical slices were obtained and a 10 per cent (w/v) renal homogenate was prepared in the sucrose–Tris buffer. Each experiment is from the kidneys of six rats. Plasma membranes were isolated by the method of Fitzpatrick *et al.* [4]. Brush border membranes were prepared by the method of Booth and Kenny [5] using 10 mM $CaCl_2$. The techniques are such that the preparation of the plasma membranes included both the brush border and basal–lateral membranes, but the brush border membrane preparation excludes basal–lateral membranes.

Enzyme assays. Prior to measuring the enzyme activities,