Effects of Pharmacological Agents on Nonelectrolyte Nutrients and Ion Transport in Secretory Diarrhea

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The present-day concepts on the pathophysiology of secretory diarrhea (1) have opened a new stage in the pharmacological treatment of diarrhea in children. Drugs used in the past as antidiarrheal agents and believed to act only on intestinal motility are now being viewed as antisecretory compounds, and their antidiarrheal action is being attributed, at least partially, to their action on the fluxes of ions and water in the intestine. Other compounds potentially useful as antidiarrheal agents because of their intestinal antisecretory effect are appearing on the scene. Powell and Field (2) have recently reviewed the pharmacological approaches to treatment of secretory diarrhea and they have discussed the possible mechanisms of action of currently used antidiarrheal agents as well as of newer, potentially useful drugs. The reader is referred to this excellent review for numbers, types, and possible mechanisms of action of antidiarrheal drugs.

Antidiarrheal agents can be grouped into two main classes. One class appears simply to enhance the normal absorptive processes, offsetting the secretion produced by diarrheagenic agents: the oral glucose-electrolyte solution and adrenal corticoids are among the best-known examples. Compounds in the other category predominantly seem to turn off intestinal secretory mechanisms. The long list of compounds in this group includes the following:

(a) **Heavy metals**, such as bismuth and aluminum. These compounds are well-known constipating agents, although their mechanism of action remains largely unknown.

(b) **Nonsteroidal antiinflammatory drugs**, including salicylates and indomethacin. These have been shown not only to stimulate Na and Cl absorption and inhibit HCO$_3$ intestinal secretion, but also to antagonize the diarrhea induced in experimental animals by a variety of bacterial agents (2).

(c) **Organic anions**, such as gallic acid (tannin), galacturonic acid (pectin), and nicotinic acid. These compounds, however, will probably not be of value as antidiarrheal agents since they have multiple effects and are able to stimulate either absorption or secretion in different experimental conditions (2).

(d) **Proteins** such as methylated casein, commonly used in France to treat
acute diarrhea, and recently shown to inhibit water and electrolyte secretion induced by cholera toxin in rat jejunum (3).

(e) **Alkaloids.** The most representative substance in this group is berberine, the potential usefulness of which as an antidiarrheal agent has been rediscovered recently after its use for thousands of years in oriental countries.

(f) **α-Adrenergic agonists.** These agents effectively stimulate Na and Cl absorption and reduce HCO$_3^-$ secretion, but their effects on the cardiovascular system have so far limited any possible application. The recent appearance of lidamidine, an α$_2$-adrenergic agent largely devoid of such systemic action, may represent an important advance (2,4).

(g) **Propranolol** and some local anesthetics with a similar structural formula. These have been shown to inhibit intestinal secretion induced in experimental models by a variety of secretagogues (2).

(h) **Gut hormones** such as somatostatin, which is capable of stimulating Na and Cl absorption and of partially inhibiting stimulated secretion (5,6).

(i) **Phenothiazines.** These have been shown to be potent antisecretory compounds, as discussed later in this chapter.

(j) **Opiates and opiate derivatives.** The well known antidiarrheal action of these drugs appears to be due, at least partially, to effects on the fluxes of ions and water across the intestine.

Obviously there is a large difference between identifying a compound as a candidate to be used in humans as an antidiarrheal drug and actually using it in therapy. As a matter of fact, the list of drugs of proven therapeutic value is much shorter than that of potential antidiarrheal drugs.

In this chapter we will focus on (a) some potential antidiarrheal agents, in terms of their effects on ion transport in the small intestine in vitro; (b) the effects of some potential antidiarrheal agents on nutrient transport in the intestine; and (c) the therapeutic use of some of these agents in infant diarrhea.

**EFFECTS ON TRANSPORT OF IONS IN THE SMALL INTESTINE**

Traditionally, opiates and their analogues were thought to act as antidiarrheal agents via an effect on intestinal motility (7-9). In the last few years, however, it has become increasingly clear that loperamide (10-14), morphine (8,15,16), and enkephalins (17,18) also possess antisecretory properties.

Guandalini et al. (12) studied the effects of loperamide on intestinal ion transport in rabbit ileum. The drug induces a reduction in the short circuit current (Isc) (usually an antisecretory signal), and this effect is heightened when the concentration of loperamide is increased (12). As shown in Fig. 1, loperamide increases the net absorption of Cl, together with an increase in HCO$_3^-$ secretion. When added in vitro to a tissue stimulated by secretagogues, loperamide shows a distinct antisecretory type of action: secretion induced by theophylline, prostaglandin E$_2$ (PGE$_2$), and the calcium ionophore A23187 is reduced by the drug (Fig. 2). This effect is abolished by the opiate antagonist naloxone (12). Other
groups have shown that loperamide inhibits the secretion induced by the heat-labile *Escherichia coli* and cholera toxins *in vitro* (10,11,13), and that it also inhibits secretion induced in the rat *in vivo* by PGE₂, deoxycholic acid, and cholera toxin (19).

The data in Fig. 2 show that in the isolated rabbit ileum loperamide has no effect on the secretion induced by either the cGMP analogue 8-Br-cGMP or the cGMP agonist heat-stable *E. coli* toxin. On the other hand, loperamide has been shown to inhibit the secretion induced by heat-stable *E. coli* toxin in a different experimental system: the infant mouse *in vivo* (9).

Since naloxone abolishes the antisecretory activity of loperamide (11–13), this activity clearly appears to involve opiate receptors. Its precise mechanism, however, remains to be defined. We have failed to show any effect of loperamide on the intracellular concentrations of either cAMP or cGMP in the rabbit ileum (12), which suggests that the action of loperamide is either distal to or separate from the adenylate cyclase/cAMP and the guanylate cyclase/cGMP pathways.

On the other hand, studies with morphine have shown inhibition of adenylate cyclase activity and of tissue cAMP formation in brain tissue (7,20,21), whereas variable, conflicting results have been obtained in the intestine (15,22,23).

On the contrary, D-alanine methionine enkephalin amide (D-Ala₂-Met E)
appears to stimulate ion intestinal transport without affecting tissue cAMP levels (17).

While these discrepant results with various opiate analogues may be associated with differences in binding to specific opiate receptors, all opiates, including morphine, enkephalins, and loperamide, show a distinct stimulating effect on the net absorption of Cl, which is mainly due to a reduced serosa-to-mucosa flux of Cl (12, 23–25).

The role of intracellular Ca$^{2+}$, which is also known to be involved in the regulation of secretion, in the mechanism of action of the opiates remains to be established.

Berberine is an alkaloid derived from the plant Berberis aristata, extracts of which have been used as an antidiarrheal remedy in India and China since ancient times. Evidence accumulated when the drug was purified and tested in experimental settings suggests it has an antisecretory action. Dutta and Pause (26) found that it significantly reduced the severity of Vibrio cholerae infection in the infant rabbit model, and, more recently, it was shown to inhibit the effect of V. cholerae enterotoxin in animal models (27–29). Sack and Froehlich (30)
have shown that berberine inhibits the secretory response to heat-labile enterotoxins from *V. cholerae* and *E. coli* in the rabbit in vivo, even when administered after the toxins were bound to intestinal mucosa. Also, it inhibits the secretory response of the heat-stable enterotoxin of *E. coli* in the infant mouse model.

In the rat ileum, berberine had no sizable effect on the basal net absorption of ions, whereas it stimulated the absorption of Na and Cl in cholera toxin-treated tissues and reversed the ion secretion induced by dibutyryl cAMP and the heat-stable enterotoxin of *E. coli* (31).

We have studied the effect of berberine on fluxes of ions in the isolated rabbit ileum (32). In this animal model, the addition of berberine at a concentration of $2 \times 10^{-3}$ M on the serosal side results in a marked reduction of the Isc; the degree of this effect is significantly correlated with the initial Isc (correlation index of 0.8). These electrical changes are associated with a complete abolition of the net secretory flux of HCO$_3^-$, while the net absorptive fluxes of Na and Cl remain unaffected (32), which suggests that under basal conditions (i.e., in the absence of secretagogues) the effect of berberine is mainly, if not exclusively, an inhibition of HCO$_3^-$ secretion. On the other hand, berberine affects Na and Cl fluxes when added to secretagogue-stimulated tissues. The addition of berberine to PGE$_2$-stimulated ileum results in both a reduction in HCO$_3^-$ secretion and a stimulation of Na and Cl absorptive net fluxes, an effect which can also be thought of as a partial removal of the secretagogue-induced changes.

Figure 3 shows the effects of berberine on the secretion induced by a number of compounds including two cAMP-mediated secretagogues (PGE$_2$ and theophylline), the cyclic GMP analogue 8-Br-cGMP and the calcium ionophore A23187. The data show a striking antisecretory effect against agents acting by all three known messengers of secretion.

On the other hand, it should be emphasized that we were unable to show any effect of berberine at the intracellular level (32), which is in keeping with other previous observations in rat ileum that no change in adenylate cyclase activity after exposure to berberine was detectable (31).

Table 1 summarizes some of the results we have obtained in the isolated rabbit ileum model with loperamide and berberine.

The potential clinical usefulness of berberine is indicated by the fact that, in spite of the similarities between it and other agents in producing the antisecretory effects in the small intestine, berberine is effective on the mucosal side (31), whereas the other agents, such as aspirin (33), somatostatin (5,6), $\alpha$-adrenergic compounds (34,35), and neuroleptics (36), are only effective when added on the serosal side.

The observation in mice that chlorpromazine inhibited secretion by cholera toxin, PGE$_1$, and dibutyryl cAMP (37) prompted various studies, as a result of which it was established that several neuroleptic agents possess antisecretory activity. Smith and Field (36) tested four phenothiazine compounds in the isolated rabbit ileum and found that the order of antisecretory potencies was trifluoperazine > chlorpromazine > haloperidol > chlorprothixene. In this animal
FIG. 3. Berberine–secretagogues interaction. The previous addition of 2 mM berberine (BS; filled columns) significantly (p < 0.001) inhibited the Isc increments brought about by all intestinal secretagogues tested, as compared to controls (□). Concentrations of secretagogues as in the legend to Fig. 2.

model, trifluoperazine did not alter basal transport rates significantly but did partially inhibit the response to a variety of secretagogues, including theophylline, 8-Br-cAMP, vasoactive intestinal polypeptide (VIP), dimethyl PGE₂, and heat-stable E. coli enterotoxin and the calcium ionophore A23187 (36). Inhibition by trifluoperazine of the secretion induced by VIP and theophylline was demonstrated in the human small intestine, too (36). Like the other antisecretory compounds discussed above, trifluoperazine did not significantly alter cAMP or cGMP concentrations either under basal conditions or in the presence of secretagogues (36), which suggests that the mechanism of the antisecretory action of these drugs lies beyond the generation of cAMP and cGMP.

On the other hand, it is noteworthy that all four phenothiazines with demonstrated antisecretory activity in the intestine are potent inhibitors of the cal-

TABLE 1. Comparison between loperamide and berberine: in vitro effects on ion transport in rabbit ileum

<table>
<thead>
<tr>
<th>Drug</th>
<th>Isc</th>
<th>J^Na_\text{net}</th>
<th>J^O_\text{net}</th>
<th>J^R_\text{net}</th>
<th>cAMP</th>
<th>cGMP</th>
<th>Ca^{2+}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loperamide</td>
<td>†</td>
<td>=</td>
<td>†</td>
<td>†</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Berberine</td>
<td>††</td>
<td>=</td>
<td>††</td>
<td>††</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
</tbody>
</table>

J^\text{net}_i refers to the effect of either drug on the net transepithelial flux of the ion species. (†) indicates increased, (†) decreased, (=) unchanged. The antisecretory capacity of the drug is here intended as a measure of its effectiveness in inhibiting the short circuit current (Isc) increments evoked by cAMP, cGMP, or Ca^{2+}-related agonists. (−) indicates no effect; (+) to (+++) moderate to potent effect.
cium-dependent regulatory protein calmodulin in bovine brain (38–40). This observation may give some insight into the mechanism of the antisecretory action, which might well apply to antisecretory compounds other than the phenothiazines. Increases in cytosolic Ca^{2+} stimulate the activity of calmodulin, an ubiquitous cytosolic protein. The activated calmodulin, among its several other effects, stimulates protein kinases. An inhibition of the Na Cl influx process and an increase of Cl secretion then ensue. By this pathway, any decrease in cytosolic Ca^{2+} can theoretically produce changes of the antisecretory type. In other words, any agent—phenothiazines among them—capable of reducing Ca^{2+} gating and/or intracellular release of Ca^{2+} can exert an antisecretory effect.

To summarize the current information on the mechanism of action of the most studied antisecretory compounds, taking into consideration the three established regulatory pathways (cAMP, cGMP, Ca^{2+}–calmodulin), it would seem that while the cyclic nucleotides are mostly uninvolved, there are at least theoretically sound reasons to support the hypothesis that the Ca^{2+}–calmodulin pathway is involved. It goes without saying that further studies are necessary to test this hypothesis for neuroleptics as well as for other compounds with demonstrated antisecretory activity in the small intestine.

EFFECTS ON TRANSPORT OF NUTRIENTS

Before any single antisecretory substance can be suggested for therapeutic use as an antidiarrheal drug, one should first know whether the drug has any effect on the absorption of nutrients. Since diarrheal diseases are commonly treated with oral solutions containing glucose as the nonelectrolyte component, it becomes of particular importance to ascertain whether the candidate drug has any effect on the absorption of glucose. We have studied the effects of loperamide, chlorpromazine, berberine, and somatostatin on the lumen-to-cell influx processes for glucose and phenylalanine in rabbit ileum. The results are shown in Table 2.

None of the drugs tested significantly alters the influx of glucose, thus suggesting that these drugs should not interfere with the oral use of glucose–electrolyte solutions. On the contrary, the influx of phenylalanine, which is unaffected by loperamide and somatostatin and is slightly but not significantly reduced by berberine, is markedly inhibited by chlorpromazine. Therefore, chlorpromazine should be used therapeutically with caution, and any widespread use of the compounds mentioned above should await further studies.

Some available in vivo absorption studies focus attention on possible discrepancies between results obtained in vitro and in vivo. For instance, while in our in vitro study somatostatin did not affect the influx of glucose, it did reduce the absorption of glucose, and that of glycine and lysine as well, in human subjects treated intravenously (41). If we refer to another drug proposed as a therapeutic tool because of its intestinal antisecretory activity, i.e., aspirin, studies on its effect on the absorption of monosaccharides and amino acids have yielded
conflicting results (42,43), again suggesting that caution be exercised while awaiting more extensive studies.

**USE OF ANTISECRETORY COMPOUNDS AS ANTDIARRHEAL AGENTS IN INFANT DIARRHEA**

**Glucocorticoid Steroids**

Glucocorticoid steroids have been used for at least 20 years in the treatment of acute celiac disease in both adults and children. While their beneficial effect was attributed to their anti-immune activity, recent evidence suggests that, at least in part, it must be attributed to an antisecretory action on the small intestine (44).

**Chlorpromazine**

In 1979, Rabbani and colleagues (45) treated adult patients having severe cholera by administering chlorpromazine either orally or intravenously at the dosage of 1 or 4 mg/kg body weight. The stool output during the 32-hr period after the administration of the drug was dramatically reduced by an average of 66%, with no significant difference between the two routes of drug administration.

We have used chlorpromazine to treat acute diarrhea in patients ranging in age from 1 to 16 months (average 8 months) and weighing (body weight) from 2.4 to 10.2 kg (average 6.3). The acute diarrhea was of a secretory type in all cases, and was of various etiologies including *Salmonellae*, heat-labile *E. coli* enterotoxin, and celiac crisis. Chlorpromazine was given orally at the dosage of 1 mg/kg body weight per day; the treated group was compared with a placebo group matched for age, body weight, and severity of diarrhea. Daily stool outputs before and after either chlorpromazine or placebo are given in Fig. 4, which shows a marked reduction of stool output in the treated group.

**TABLE 2. Effect of drugs on the influxes of glucose and phenylalanine in the rabbit ileum**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Glucose influx (μmol/cm²·hr)</th>
<th>Phenylalanine influx (μmol/cm²·hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.53 ± 0.08</td>
<td>4.52 ± 0.78</td>
</tr>
<tr>
<td>50 μM Loperamide</td>
<td>0.87 ± 0.27</td>
<td>4.30 ± 0.89</td>
</tr>
<tr>
<td>0.2 mM Chlorpromazine</td>
<td>0.33 ± 0.08</td>
<td>0.77 ± 0.16*</td>
</tr>
<tr>
<td>2 mM Berberine</td>
<td>0.76 ± 0.23</td>
<td>2.81 ± 0.88</td>
</tr>
<tr>
<td>1 μM Somatostatin</td>
<td>0.57 ± 0.15</td>
<td>5.0 ± 0.77</td>
</tr>
</tbody>
</table>

Data are means ± 1 SE. Drugs were added to both sides of the tissues 20–30 min before the incubation and were present on the mucosal side only during incubation. Initial rates of unidirectional flux ("influxes") of the nutrients from the incubation solution into the epithelium across the brush border were measured utilizing radiolabeled substrates as described by Guandalini et al. (56).

* p < 0.001. All other sets of influx values in the presence of drugs are not significantly different from the corresponding control values.
Aspirin

Aspirin has been given by mouth at the dosage of 25 mg/kg body weight per day to malnourished infants and young children having gastroenteritis and dehydration. In comparison with the control group, the treated group displayed reduced fecal fluid loss and enhanced weight gain (46).

Berberine

Berberine has been used at the dosage of 5–10 mg/kg/day orally, with reported success for those patients having acute diarrhea due to giardiasis or cholera (47,48). Berberine has a long history of use in traditional Oriental medicine.

Loperamide

Loperamide has been known for several years to be an effective antidiarrheal agent in adults (49–54). It is also widely used in diarrheic children, although well-controlled studies are few. Sandhu and colleagues (55) used loperamide (approximately 1.3 mg/kg body weight per day) in severe protracted diarrhea,
and provided convincing evidence that the drug can be lifesaving in some infants in severe diarrheal states. However, due to the effect of loperamide on smooth muscle, the risk of complicating ileus and/or bacterial overgrowth should be kept in mind (55).

As drugs of demonstrated usefulness in the treatment of children having secretory diarrhea, glucocorticoids, aspirin, berberine, chlorpromazine, and loperamide are probably only the first on a list which is expected to grow rapidly in the near future. There is no doubt that a new dimension has been discovered in the treatment of diarrhea and that newly recognized antisecretory drugs can be very useful in selected cases of both acute and chronic secretory diarrhea in which dietary manipulation and other treatments have failed. It should be emphasized, however, that rehydration, appropriate dietary treatment, and chemotherapy when indicated remain the major tools in the treatment of diarrhea, and that antidiarrheal drugs of the antisecretory type should await further studies before their widespread use can be recommended.

REFERENCES