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Effect of Indomethacin on Intestinal Water Transport in Salmonella-Infected Rhesus Monkeys

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Indomethacin, a nonsteroidal anti-inflammatory agent, will abolish salmonella-induced rabbit ileal secretion when given prior to the establishment of infection. In the present study, we assessed whether indomethacin can inhibit salmonella-induced intestinal secretion when administered after infection and net intestinal secretion are well established. A physiological model of salmonellosis, salmonella-infected rhesus monkeys, was used. This model also permitted an examination of the effects of indomethacin in both the small and large intestines. The effect of indomethacin in control monkeys was also studied. Indomethacin caused a striking enhancement of net intestinal water transport in the jejunum, ileum, and colon of salmonella-infected monkeys. These effects occurred promptly and were of sufficient magnitude in the ileum and colon to cause a reversal in the direction of net transport from net secretion to net absorption. Indomethacin also enhanced net water transport in the jejunum, ileum, and colon of normal animals. These data show that indomethacin markedly enhances net intestinal water transport in both the small and large intestines of salmonella-infected monkeys, even when administered after salmonella infection and intestinal secretion are well established. Similar enhancement also occurs in the normal intestine. The mechanism(s) by which indomethacin produces these effects is not known.

We have previously shown that indomethacin (a nonsteroidal anti-inflammatory agent) treatment of rabbits abolishes the intestinal fluid secretion evoked by infection with *Salmonella typhimurium* (7, 9). In these studies, however, indomethacin was given prior to the induction of salmonella infection, and the ability of indomethacin to modify salmonella-induced intestinal secretion after this secretion was well established was not studied. Furthermore, the rabbit ileal loop model (6), a somewhat artificial model of intestinal salmonellosis, was employed in these studies. This model of salmonellosis only permits assessment of the ileum, and it is not suitable for the study of intestinal transport rates since the infection is not uniform over the course of the test period (6).

It is the purpose of the present study to assess whether indomethacin can inhibit salmonella-induced intestinal secretion when the drug is administered after salmonella enterocolitis and intestinal secretion are well established. The rhesus monkey model (8, 19) was chosen because salmonellosis in monkeys more closely resembles the disease as it occurs in humans than does salmonellosis in various other animals (12, 19). In addition, the use of the monkey model permits an examination of the effects of indomethacin, simultaneously, on both the small and large intestines.

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MATERIALS AND METHODS

Bacterial strain. *S. typhimurium* (strain TML), isolated from an adult with severe cholera-like diarrhea (4, 5), is a virulent strain for humans and other animals and is described in previous publications (4-9, 19). The strain was maintained in the lyophilized state, and a new ampoule was used for each experiment. Organisms were grown overnight on Trypticase soy agar, harvested by centrifugation, and suspended in fresh Trypticase soy broth to the desired concentration.

Animal model. Male rhesus monkeys (*Macaca mulatta*), free from enteric pathogens and weighing 2 to 3 kg, were infected by orogastric inoculation as previously described (8, 19). Without anesthesia, monkeys were inoculated with 5×10^{10} *S. typhimurium* organisms in 20 ml of Trypticase soy broth. Control monkeys were given sterile broth. Animals developing diarrhea, defined as multiple, watery bowel movements on each of 2 successive days, were

studied (19). Diarrheal and control animals were studied at 48 to 72 h after inoculation when diarrhea was at its peak.

Measurement of intestinal water transport. Intestinal water transport was studied by the in vivo perfusion technique (8, 19). Segments of proximal jejunum, distal ileum, and transverse colon (15 cm long) were simultaneously perfused at a rate of 0.5 ml/min with an isotonic solution containing: Na^+, 150 mmol/liter; K^+, 5 mmol/liter; Cl^-, 125 mmol/liter; HCO_3^-, 30 mmol/liter; mannitol, 16 mmol/liter; and polyethylene glycol 4000 (PEG), 6 g/liter, with a pH of 7.5 and osmolality of 305 mosmol/liter. [^{14}C]PEG (3.6 mCi/mmol; New England Nuclear Corp., Boston, Mass.) was added as a nonabsorbable water marker. Intestinal segments were perfused for a 1.5-h equilibration period and three subsequent 1-h test periods.

[^{14}C]PEG was counted in duplicate in a toluene-based liquid scintillation cocktail in a Beckman LS-345 beta scintillation counter (Beckman Instruments Inc., Fullerton, Calif.). Water transport rates were calculated by standard formulas (1).

Effect of indomethacin. After monkeys were perfused for 1 h to establish basal water transport rates, a single injection of indomethacin (10 mg/kg intravenously) was given and water transport was measured for 2 subsequent h. Water transport rates were calculated for each 1-h period (a 1-h control period and two post-indomethacin periods). Data are presented as mean ± standard error of the mean for each perfusion period and were evaluated statistically by the paired or unpaired *t* test, as appropriate (20).

RESULTS

Intestinal water transport in salmonella-infected monkeys. In control monkeys, net water absorption was observed in the jejunum, ileum, and colon (Fig. 1). In salmonella-infected animals, a marked decrease in net water absorption was noted in the jejunum and ileum. In the colon the defect in net water transport was even more marked. A reversal of net water transport, from absorption to secretion, was observed in this intestinal site. These results are comparable to the values observed in our previous studies (14, 19).

Effect of indomethacin on water transport in salmonella-infected monkeys. Indomethacin administration resulted in an increase in net water transport in the jejunum, ileum, and colon (Fig. 2). Although water transport rates improved in all three intestinal sites, only the changes observed in the ileum and colon were statistically significant. These effects were evident in h 1 after indomethacin administration and became more marked in h 2 after indomethacin administration. The most striking effects were seen in the ileum and colon, where the direction of net water movement was reversed from secretion to absorption. Although net water transport rates increased after indomethacin treatment, the water transport rates observed remained below those seen in normal monkeys (compare Fig. 1 and 2).

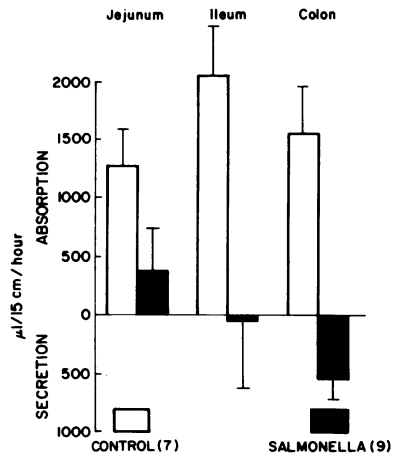

FIG. 1. *Net intestinal water transport in salmonella-infected and control monkeys. Bars represent mean ± standard error of the mean of a 1-h perfusion period. Numbers in parentheses represent the number of animals studied.*

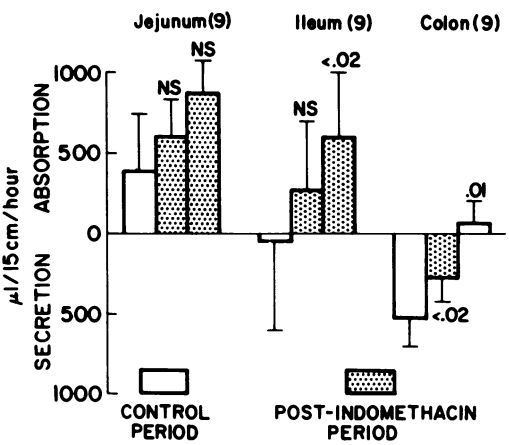

FIG. 2. *Effect of indomethacin on net intestinal water transport in salmonella-infected monkeys. Bars represent mean ± standard error of the mean of a 1-h perfusion period. The clear bars represent the control period (net transport prior to indomethacin administration). Each stippled bar represents a 1-h perfusion period after indomethacin administration. Numbers in parentheses represent the number of animals studied. P values were determined by a comparison with the control period. NS, Not significant.*

Effect of indomethacin on water transport in normal monkeys. As was seen in salmonella-infected monkeys, indomethacin administration resulted in increased net water trans-

port in the jejunum, ileum, and colon (Fig. 3). Only the changes observed in the ileum and colon achieved statistical significance and only in h 2 after indomethacin administration.

DISCUSSION

The results of the present study demonstrate that a nonsteroidal anti-inflammatory agent, indomethacin, given parenterally can greatly improve the intestinal water transport defect caused by salmonella infection. The effect is prompt, occurring within 1 to 2 h after intravenous administration, and occurs in both the small and large intestines. Although water transport rates in salmonella-infected animals did not return to control values after indomethacin administration, the effect was marked. Net water transport rates were more than doubled in the jejunum, ileum, and colon. In fact, in the ileum and colon, indomethacin caused a reversal in the direction of water transport from net water secretion to net water absorption. It is worthy of emphasis that our study shows that indomethacin has these effects on intestinal water transport in salmonellosis, even when administered after salmonella-induced intestinal secretion is well established.

The ability of indomethacin to enhance intestinal water transport is not confined to the salmonella-infected intestine. Our present results also show that indomethacin enhances intestinal water transport in the normal jejunum, ileum, and colon. The ability of nonsteroidal anti-inflammatory agents to enhance intestinal water transport in the normal small intestine has also been observed by Wald et al. (21) and Farris et al. (2). In addition to improving water transport in salmonella-infected and normal intestines, nonsteroidal anti-inflammatory agents can also enhance intestinal water transport in a variety of other secretory disorders. We have previously reported that indomethacin pretreatment of rabbits can inhibit intestinal secretion induced by infection with *Shigella flexneri* or *Vibrio cholerae* or secretion induced by cholera toxin (7, 9). The ability of indomethacin or aspirin to reduce cholera-mediated secretion in various animal species has also been reported by several other groups (2, 3, 11, 15, 21). Thus, it seems that nonsteroidal anti-inflammatory agents have the ability to enhance intestinal water transport in the normal intestine as well as the intestine secreting in response to a variety of stimuli.

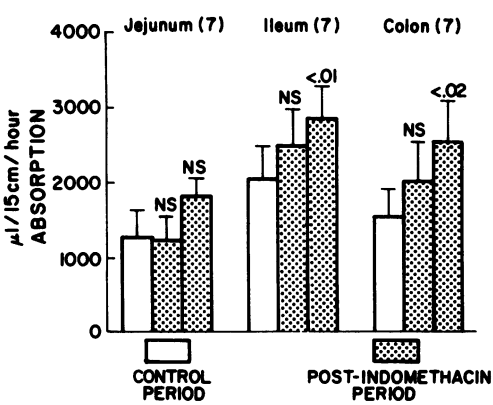

FIG. 3. *Effect of indomethacin on net intestinal water transport in normal monkeys. Bars represent mean ± 1 standard error of the mean of a 1-h perfusion period. The clear bars represent the control period (net transport prior to indomethacin administration). Each stippled bar represents a 1-h perfusion period after indomethacin administration. Numbers in parentheses represent the number of animals studied. P values were determined by a comparison with the control period. NS, Not significant.*

The mechanism by which nonsteroidal anti-inflammatory agents increase net intestinal water transport is unclear. Net transport, as measured in the in vivo perfusion model, is the sum of two oppositely directed fluxes—an inwardly directed flux from lumen to blood, i.e., absorption, and an outwardly directed flux from blood to lumen, i.e., secretion. Thus, indomethacin could enhance net transport by stimulating absorption or by diminishing secretion. The directionality of flux alterations cannot be defined in our present studies. However, the recent study of Farris et al. (2) may help elucidate the mechanism by which nonsteroidal anti-inflammatory agents increase net water transport. These investigators studied the effects of aspirin on the in vitro absorptive and secretory fluxes in the normal rabbit ileum. Their findings showed that aspirin increases net water absorption in the normal small intestine by promoting the unidirectional flux of sodium and chloride from intestinal lumen to blood, i.e., absorption. This effect was not mediated by intestinal adrenergic mechanisms. They also showed that aspirin enhanced net transport in the cholera toxin-exposed intestine by stimulating sodium and chloride absorption and concluded that these effects occur at a step beyond cyclic adenosine 5'-monophosphate production and degradation. This conclusion is consistent with the observations of Giannella et al. (7) and Wald et al. (21), who reported that indomethacin improves net water transport in the cholera toxin-exposed rabbit intestine while not altering intestinal concentrations of either adenyl cyclase or cyclic adenosine 5'-monophosphate.

In spite of these data indicating a nonspecific enhancement of absorptive flux by nonsteroidal anti-inflammatory agents, it is possible that indomethacin may enhance intestinal water transport in the salmonella-infected intestine by another mechanism (7). Although most available evidence suggests that prostaglandins are not involved in the intestinal water transport defect caused by cholera toxin (7, 13), intestinal infection with invasive salmonellae may result in local prostaglandin synthesis. This seems likely since intestinal infections with virulent salmonellae regularly result in a mucosal inflammatory reaction (6, 7), and acute inflammatory reactions elsewhere result in the synthesis and release of prostaglandins (10, 22). Many investigators have shown that prostaglandins cause intestinal secretion (13, 16–18, 22). Thus, it is possible that prostaglandins may be involved in the salmonella-induced water transport defect (7). That indomethacin enhances net transport in the salmonella-infected intestine by virtue of its ability to inhibit prostaglandin synthesis and thereby interferes with a prostaglandin-dependent secretory system is consistent with our findings, but must remain speculative at present. Further studies are underway to help answer this question.

Our data suggest that indomethacin, or other similar agents, might provide useful therapy for the intestinal fluid loss seen in various diarrheal disorders.

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