COX inhibition excites enteric nerves that affect motility, alkaline secretion, and permeability in rat duodenum

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Nylander, Olof, Anneli Hälgren, and Manaf Sababi. COX inhibition excites enteric nerves that affect motility, alkaline secretion, and permeability in rat duodenum. Am J Physiol Gastrointest Liver Physiol 281: G1169–G1178, 2001—In anesthetized rats, the cyclooxygenase (COX) inhibitor indomethacin induces duodenal motility, increases duodenal mucosal alkaline secretion (DMAS), and evokes a transient increase in duodenal paracellular permeability (DPP). To examine whether enteric nerves influence these responses, the duodenum was perfused with lidocaine. Motility was assessed by measuring intraluminal pressure, and DPP was determined as blood-to-lumen clearance of $^{51}$Cr-EDTA. DMAS was assessed by titration. In control animals, few contractions occurred during saline perfusion and lidocaine did not alter this condition. Perfusion with 0.03–0.1% lidocaine did not affect DMAS or DPP whereas 0.3–1% lidocaine reduced DMAS and increased DPP. Indomethacin induced motility and doubled DMAS. Application of 0.03% lidocaine on the duodenal serosa reduced motility and DMAS whereas 0.03% lidocaine applied luminally inhibited DMAS only. Higher concentrations of lidocaine abolished the increase in DMAS and changed the motility pattern to numerous low-amplitude contractions, the latter effect being blocked by iloprost. The lidocaine-induced increases in DPP were markedly higher than in controls. We conclude that indomethacin activates enteric nerves that induce motility, increase DMAS, and decrease DPP.

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MATERIALS AND METHODS

Surgical Procedure

Male F$_1$ hybrids of Lewis-Dark Agouti rats (Animal Department, Biomedical Center, Uppsala, Sweden), weighing 200–300 g, were fasted overnight but given free access to drinking water. The animals, kept in groups of two or more,
were maintained under constant conditions (12:12-h light/dark cycle at 21°C). The operative procedures have been described before in detail (11), and a summary is provided here. Rats were anesthetized with thiobutabarbital sodium salt (Inactin; 125 mg/kg body wt ip), and a cannula was inserted into the trachea to facilitate respiration. Cannulas were inserted in the left external jugular and right femoral veins as well as in the right femoral artery. The veins were used for infusion of 51Cr-EDTA and drugs, respectively. The femoral artery was used for recordings of arterial blood pressure and blood sampling. Subsequently, the common bile duct was cannulated very close to its entrance into the duodenum (2–3 mm). A soft silicone tubing was introduced into the mouth and pushed gently along the esophagus into the stomach and through the pylorus and secured by ligatures. The proximal duodenal cannula was connected to a peristaltic pump, and the segment was continuously perfused with a 150 mM NaCl solution (saline). Closing the abdominal cavity with sutures completed the surgery. After surgery, at least 45 min were allowed to stabilize cardiovascular, respiratory, and gastrointestinal function before experiments commenced. All protocols were approved by the Uppsala University Ethics Committee for Animal Experiments.

Measurement of Duodenal Contractions

Measuring intraluminal pressure assessed duodenal contractions. The inlet perfusion cannula was connected to a pressure transducer positioned at the same level as the outlet cannula, and intraluminal pressure was recorded on a polyrecorder (polygraph 7D, Grass Instruments, Quincy, MA). Duodenal motility was quantified by measuring that fraction of time occupied by contractions (fractional contraction time, FCT) in 10-min periods. In some experiments, the amplitude of the duodenal contractions was determined. The amplitude of each duodenal contraction was determined before, during, and after lidocaine perfusion. Amplitudes were summated and divided by the total number of contractions. The mean amplitude was expressed as the percentage of control where 100% represented the mean amplitude of contractions before lidocaine exposure.

Measurement of Luminal Alkalization

The rate of DMAS was determined by back titration of the effluent to pH 5.00 with 50 mM HCl under continuous gassing (100% N₂) using pH stat equipment. The pH electrode was routinely calibrated with a standard buffer before the start of the titration. The lidocaine solutions contained different amounts of NaOH depending on the concentrations used (the pH of the solution containing 1% lidocaine was 6.7). To avoid “false” (too high) DMAS values, blanks (solutions that had not passed through the duodenum) were titrated back to pH 5.0 to determine the amount of alkali added to saline in each lidocaine solution. In individual experiments, the perfusion rate was determined by measuring effluent saline that had not passed through the duodenum. An excellent recovery was obtained for all solutions (98–104%). DMAS was expressed as the amount (micromoles) of base secreted per centimeter of intestine per hour.

Measurement of Duodenal Paracellular Permeability

After completion of surgery 51Cr-EDTA was administered intravenously as a bolus of 75 µCi followed by a continuous infusion at a rate of 50 µCi per hour. The radioactive isotope was diluted in a Ringer-HCO₃ solution and infused at a rate of 1 ml/h. One hour was permitted for tissue equilibration of the 51Cr-EDTA. Three blood samples (0.2 ml each) were collected at regular time intervals during the experiment, and the blood volume loss compensated for by injection of a 7% bovine albumin solution. After centrifugation, 50 µl of the plasma were removed for measurement of radioactivity. The duodenal segment was perfused with saline at a rate of ~0.4 ml/min, and the effluent was collected in 10-min samples. The luminal perfusate and the blood plasma were analyzed for 51Cr activity in a gamma counter (1282 Compugamma CS, Pharmacia, Uppsala, Sweden). A linear regression analysis of the plasma samples was made to obtain a corresponding plasma value for each effluent sample. The clearance of 51Cr-EDTA from blood to lumen was calculated as previously described (11). 51Cr-EDTA clearance was expressed as milliliters per minute per gram wet tissue weight.

Measurement of Duodenal Mucosal Blood Flow

Laser-Doppler flowmetry was used for blood flow measurements. The instrument used (Periflux PF 3, Perimed, Stockholm, Sweden) permits continuous linear measurements of the red blood cell perfusion (number of red blood cells × velocity) in a tissue hemisphere with a radius of ~1 mm. The laser light is guided to the tissue by an optical fiber (diameter, 0.5 mm), and a pair of similar-sized fibers picks up the backscattered light. To measure duodenal blood flow, the duodenum was covered with plastic foil, and a chamber with a circular opening (diameter, 4.5 mm) in the center was placed on top of the duodenal segment. The bottom of the chamber was sealed to the plastic foil by silicone grease (Dow Corning). The chamber opening was filled with saline at 37°C, and the temperature of the preparation was maintained by perfusion of the chamber with water at 37°C. The laser-Doppler probe, which was fixed to a micromanipulator, was inserted in the chamber opening and placed immediately above the surface of the serosal side of the duodenum. Care was taken to keep the probe in position at the same distance from the duodenal surface throughout the experiments.

Experimental Protocol

Controls. The experiment started with a 30-min control period with saline perfusion. This was followed by perfusion of the duodenum with lidocaine at a concentration of 0.03 or 0.1% for 30 min and then by perfusion with saline for 30 min. Subsequently, the duodenum was perfused with lidocaine at a concentration of 0.3% or 1% for 30 min followed by saline for 30 min (n = 6/group).

Indomethacin experiments. After a 20-min control period, indomethacin was injected intravenously as a bolus at a dose of 5 mg/kg. Fifty minutes after indomethacin administration, the duodenum was perfused luminally for 30 min with lidocaine at a concentration of 0.03% (n = 6), 0.1% (n = 6), 0.3% (n = 8), or 1% (n = 5). The experiments ended with a 30-min saline perfusion period.
**Indomethacin plus iloprost.** After a 20-min control period, indomethacin was injected intravenously as a bolus at a dose of 5 mg/kg. Twenty minutes after indomethacin injection, iloprost was administered as a constant intravenous infusion at a dose of 15 μg·kg⁻¹·h⁻¹ during the remaining part of the experiment. Thirty minutes after the start of the iloprost infusion, the duodenum was perfused for 30 min with lidocaine at a concentration of 1% followed by a 30-min saline perfusion period (n = 5).

**Duodenal mucosal blood flow.** The same protocol used for indomethacin experiments was used in this series of experiments. The duodenum was perfused with 0.3% lidocaine for a 30-min period (n = 6). DMAS, ⁵¹Cr-EDTA clearance, and duodenal motility were not determined in these experiments.

**Serosal application of lidocaine.** The abdominal cavity was left opened but covered with plastic foil. Twenty to thirty minutes before the start of effluent collection, indomethacin was administered as a bolus at a dose of 5 mg/kg. The duodenum was perfused with saline throughout the experiment. Gauze was prepared to match the size of the duodenum and placed on top of the segment and around its lateral sides, thereby minimizing contact with the mesentery and/or surrounding structures. During a 30-min control period, gauze soaked in warm saline was placed around the duodenum. Thereafter the saline-soaked gauze was replaced by one soaked in 0.03% (n = 4) or 1% lidocaine (n = 5) and left there for 30 min. Subsequently, the lidocaine-soaked gauze was replaced by a saline-soaked one, and the experiments ended after 30–40 min.

**Chemicals**

⁵¹Cr-EDTA and Inactin were obtained from NEN (Boston, MA) and RBI (Natick, MA), respectively. Bovine albumin and NaCl were purchased from Sigma Chemical (St. Louis, MO). Heparin was from Pharmacia (Stockholm, Sweden) and indomethacin (Confortid for injection) from Dumex Denmark. Lidocaine hydrochloride (Xylocaine for injection; 10 mg/ml) was purchased from AstraZeneca (Södertälje, Sweden).

**Statistics**

Values are expressed as means ± SE. The statistical significance of data was tested by ANOVA with contrast (Fisher’s protected least-significant difference test) with comparison of results obtained before, during, and after lidocaine exposure (repeated measures) or of differences between groups of animals (nonrepeated measures). All statistical analyses were performed on a Macintosh computer using Statview software (Abacus Concepts, Berkeley, CA). P < 0.05 was considered significant (2-tailed test).

**RESULTS**

**Control Animals**

In control rats, no or very few duodenal contractions (mean FCT, 0.02 ± 0.01) occurred during saline perfusion or in response to lidocaine perfusion (mean FCT, 0.02 ± 0.01). Perfusion of the duodenum with 0.03% or 0.1% lidocaine did not affect DMAS (the changes in secretion were +0.7 ± 1.1 μmol·cm⁻¹·h⁻¹ and −1.4 ± 0.2 μmol·cm⁻¹·h⁻¹, respectively). Lидocaine at luminal concentrations of 0.3% or 1% decreased basal DMAS from 6.5 ± 1.7 to 3.7 ± 0.9 μmol·cm⁻¹·h⁻¹ (P < 0.05) and from 5.3 ± 1.5 to 1.4 ± 0.7 μmol·cm⁻¹·h⁻¹ (P < 0.05), respectively. Neither 0.03% nor 0.1% of lidocaine affected basal paracellular permeability, whereas 0.3% induced a small and transient increase (P < 0.05) in ⁵¹Cr-EDTA clearance (Fig. 1). Perfusion of the duodenum with 1% lidocaine increased (P < 0.05) ⁵¹Cr-EDTA clearance from 0.31 ± 0.04 to 0.90 ± 0.12 ml·min⁻¹·100 g⁻¹, the latter value representing the mean for the whole 30-min period of lidocaine perfusion. During the first and last 20 min of the experiment, the mean arterial blood pressure (MABP) was 102 ± 3 and 94 ± 5 mmHg, respectively.

**Indomethacin-Treated Animals**

Indomethacin induced duodenal contractions in all animals. Thirty minutes after indomethacin administration, FCT stabilized and the motility pattern was characterized by bursts of contractions followed by a period of quiescence (Fig. 2A). Indomethacin also increased DMAS, a response that paralleled the indomethacin-induced increase in motility (Figs. 3, 4, 5, and 6). In accordance with previous experiments (23), indomethacin induced a brief increase followed by a decrease in ⁵¹Cr-EDTA clearance.

Perfusion of the duodenum with 0.03% lidocaine did not affect FCT or the amplitude of the contractions (Figs. 2A and 3) but did reduce indomethacin-stimulated DMAS by 54 ± 10% (P < 0.05). After cessation of the lidocaine perfusion, DMAS returned to prelidocaine levels. Perfusion with 0.03% of lidocaine did not affect ⁵¹Cr-EDTA clearance. During the first and last 20 min of the experiment, the MABP was 128 ± 5 and 110 ± 6 mmHg, respectively.

Luminal perfusion with 0.1% lidocaine did not affect FCT or the motility pattern except that the amplitude of the contractions decreased (P < 0.05) by 64 ± 5% (Figs. 2B and 4). The indomethacin-stimulated DMAS was strongly reduced (P < 0.05) by 0.1% lidocaine. This inhibition was significantly greater (P < 0.05) than that obtained by 0.03% lidocaine and completely re-

![Fig. 1. Effects of luminal perfusion of the duodenum with lidocaine (Lido) on basal ⁵¹Cr-EDTA clearance. The duodenum was perfused with saline for 30 min and then with lidocaine at a concentration of 0.03% (●) or 0.1% (○). Subsequently, the duodenum was perfused with saline for 30 min and then with lidocaine at a concentration of 0.3% (●) or 1% (○). The open bar indicates the periods of lidocaine perfusion. Values are means ± SE; n = 6/group. *P < 0.05.](image-url)
versible after cessation of the lidocaine perfusion. A small and transient increase in $\text{Cr}^{51}$-EDTA clearance (the mean increase was $0.18 \pm 0.04 \text{ ml/min} \cdot \text{g}$) was obtained in response to perfusion with 0.1% lidocaine ($P < 0.05$). During the first and last 20 min of the experiment, the MABP was $124 \pm 8$ and $107 \pm 5 \text{ mmHg}$, respectively.

The typical indomethacin-induced duodenal motility pattern was abolished by perfusion with 0.3% or 1% lidocaine and was replaced by irregular low-amplitude contractions (Fig. 2C). During the first 10-min perfusion with 0.3% lidocaine the FCT tended to decrease, but this was followed by an increase in FCT to a level not different from the two FCT values preceding the lidocaine perfusion (Fig. 5). Lidocaine at 1% increased FCT to a level that was significantly higher ($P < 0.05$) than that seen before the lidocaine perfusion (Fig. 6). The amplitude of the contractions and FCT were significantly higher ($P < 0.05$) in response to 1% than to 0.3% lidocaine. Ten minutes after cessation of the perfusion with 0.3% or 1% lidocaine, the typical indomethacin motility pattern recurred.

Perfusion with 0.3% or 1% lidocaine abolished indomethacin-stimulated DMAS, an effect completely reversible 20 min after cessation of the lidocaine perfusion (Figs. 5 and 6). The increase in $\text{Cr}^{51}$-EDTA clearance was significantly higher in response to 0.3% lidocaine (the mean increase was $0.80 \pm 0.08 \text{ ml/min} \cdot \text{g}$) than that obtained with 0.1% or in control animals exposed to 0.3% lidocaine only. Perfusion of the duodenum with 1% lidocaine induced a substantial increase in $\text{Cr}^{51}$-EDTA clearance (the mean increase was $1.81 \pm 0.20 \text{ ml/min} \cdot \text{g}$), which was significantly higher ($P < 0.05$) than in control rats perfused with the same lidocaine solution. The effect of lidocaine on permeability was completely reversible 20 min after cessation of lidocaine perfusion. During the first and last 20 min of the experiment, in rats treated with indomethacin plus 0.3% lidocaine, the MABP was $112 \pm 4$ and $92 \pm 6 \text{ mmHg}$, respec-

![Fig. 2. A: a representative experiment showing the duodenal motility pattern in response to indomethacin (5 mg/kg iv) before and during perfusion with 0.03% lidocaine. B: recordings from 1 of 6 rats. Perfusion of the duodenum with 0.1% lidocaine did not affect the indomethacin-induced duodenal motility pattern, characterized by bursts of contractions followed by a period of quiescence, but reduced the amplitude of the contractions. C: perfusion of the duodenum with 1% lidocaine changed the indomethacin duodenal motility pattern from intermittent bursts of contractions to more or less continuous low-amplitude contractions. A recording from 1 of 5 rats is shown.](http://ajpgi.physiology.org/)
tively. In rats treated with indomethacin plus 1% lidocaine and at the same time points as above, the MABP was 118 ± 10 and 101 ± 14 mmHg, respectively. The mean increase in \( \frac{51\text{Cr-EDTA clearance}}{100 \text{g}} \) in response to 1% lidocaine in the presence of iloprost was 1.16 ± 0.06 ml-min\(^{-1}\cdot100 \text{g}^{-1}\), i.e., significantly lower (\( P < 0.05 \)) than in rats treated with indomethacin plus lidocaine but significantly higher (\( P < 0.05 \)) than the increase in rats treated with lidocaine (1%) alone. Dur-

Effects of Iloprost in Indomethacin-Treated Rats

Experiments were performed to investigate whether iloprost, a stable prostacyclin analog, could prevent the irregular, low-amplitude contractions obtained in response to luminal perfusion with 1% lidocaine or diminish the lidocaine-induced increase in mucosal permeability. In accordance with previous findings (26), iloprost abolished indomethacin-induced motility and the stimulation of DMAS (Fig. 7). Iloprost also prevented the occurrence of the numerous low-amplitude duodenal contractions obtained in response to lidocaine perfusion. In iloprost-treated rats, lidocaine significantly (\( P < 0.05 \)) decreased DMAS, a response reversible 20 min after cessation of the lidocaine perfusion. The mean increase in \( \frac{51\text{Cr-EDTA clearance}}{100 \text{g}} \) in response to 1% lidocaine in the presence of iloprost was 1.16 ± 0.06 ml-min\(^{-1}\cdot100 \text{g}^{-1}\), i.e., significantly lower (\( P < 0.05 \)) than in rats treated with indomethacin plus lidocaine but significantly higher (\( P < 0.05 \)) than the increase in rats treated with lidocaine (1%) alone. Dur-

Fig. 4. Effects of luminal perfusion of the duodenum with lidocaine at a concentration of 0.1% on \( 51\text{Cr-EDTA clearance} \) (A), DMAS (B), FCT (C), and the amplitude of the contractions (D) in rats treated with indomethacin. Indomethacin was administered intravenously as a bolus dose of 5 mg/kg starting at the time point indicated by the arrow. The open bar indicates the period of lidocaine perfusion. Values are means ± SE; \( n = 6 \). * \( P < 0.05 \) compared with values at 60 and 70 min.

Fig. 5. Effects of luminal perfusion of the duodenum with lidocaine at a concentration of 0.3% on \( 51\text{Cr-EDTA clearance} \) (A), DMAS (B), FCT (C), and the amplitude of the contractions (D) in rats treated with indomethacin. Indomethacin was administered intravenously as a bolus dose of 5 mg/kg starting at the time point indicated by the arrow. The open bar indicates the period of lidocaine perfusion. Values are means ± SE; \( n = 8 \). * \( P < 0.05 \) compared with values at 60 and 70 min.
ing the first and last 20 min of the experiment, the MABP was 110 ± 6 and 90 ± 2 mmHg, respectively.

**Effects of Lidocaine on Duodenal Mucosal Blood Flow**

Lidocaine may induce dilatation of duodenal arterioles, thus increasing capillary pressure. The rise in capillary pressure may evoke net filtration of fluid across duodenal capillaries, thereby elevating interstitial fluid pressure, which in turn may increase paracellular permeability to $^{51}$Cr-EDTA. Experiments were therefore performed to investigate whether the lidocaine-induced increase in $^{51}$Cr-EDTA clearance is associated with an increase in duodenal blood flow or a decrease in vascular resistance. Lidocaine, at a luminal concentration of 0.3%, did not, however, affect duodenal blood flow or vascular resistance (Fig. 8).

**Serosal Application of Lidocaine**

Is duodenal motility more susceptible to inhibition by lidocaine if applied on the serosal instead of the mucosal surface and if so, what happens with DMAS? Application of 0.03% lidocaine on the serosal surface of the duodenum for 30 min reduced FCT from 0.20 ± 0.04 to 0.13 ± 0.04 ($P < 0.05, n = 4$) and the mean amplitude of the contractions by 52 ± 8.5% ($P < 0.05, n = 4$). These changes in motility were associated with a reduction of DMAS from 12.2 ± 2.2 to 7.9 ± 1.2 μmol·cm$^{-1}$·h$^{-1}$ ($P < 0.05, n = 4$).

Luminal lidocaine may increase paracellular permeability by a nonspecific action on duodenocytes, especially at high concentrations. We therefore tested the effect of serosal application of lidocaine on paracellular permeability. Application of 1% lidocaine on the serosal surface of the duodenum for 30 min in indomethacin-treated rats significantly increased $^{51}$Cr-EDTA clearance.

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**Fig. 6.** Effects of luminal perfusion of the duodenum with lidocaine at a concentration of 1% on $^{51}$Cr-EDTA clearance (A), DMAS (B), FCT (C), and the amplitude of the contractions (D) in rats treated with indomethacin. Indomethacin was administered intravenously as a bolus dose of 5 mg/kg starting at the time point indicated by the arrow. The open bar indicates the period of lidocaine perfusion. Values are means ± SE; $n = 5$. *$P < 0.05$ compared with values at 60 and 70 min.

**Fig. 7.** Effects of luminal perfusion of the duodenum with lidocaine at a concentration of 1% on $^{51}$Cr-EDTA clearance (A), DMAS (B), and FCT (C) in rats treated with indomethacin and iloprost. Indomethacin was administered intravenously as a bolus dose of 5 mg/kg. Iloprost was administered as a constant intravenous infusion at a dose of 15 μg·kg$^{-1}$·h$^{-1}$. The open bar indicates the period of lidocaine perfusion. Values are means ± SE; $n = 5$. *$P < 0.05$ compared with values at 60 and 70 min.
clearance (Fig. 9). However, the increase in $^{51}$Cr-EDTA clearance was lower ($P < 0.05$), more transient, and not as consistent as that in response to luminal perfusion with lidocaine. Three out of five animals responded with an increase, whereas no effect on $^{51}$Cr-EDTA clearance was obtained in two animals.

**DISCUSSION**

The enteric nervous system is an independent integrative system placed in close proximity to the effector cells it controls. It has been suggested (32) that a limited number of critical neurons in the myenteric plexus control the activity of the intrinsic microcircuits of the enteric nervous system that regulate motility, secretion, and blood flow. It thus seems reasonable to assume that inhibition of these neurons or those innervating the target cells should induce effects on secretion, motility, and blood flow and perhaps also on paracellular permeability. In the present study, we perfused rat duodenum with various concentrations of lidocaine, a widely used local anesthetic (5). Lidocaine exerts its inhibitory action by blockage of voltage-gated Na$^+$ channels, thereby preventing the generation of action potentials responsible for nerve conduction (25). Hence by increasing the concentration of lidocaine in the luminal solution, it might be possible to gradually “paralyze” the enteric nervous system.

**Effects of Lidocaine on Basal and Indomethacin-Stimulated DMAS**

In control rats, perfusion with lidocaine at 0.03% or 0.1% had no effect on basal DMAS. Higher luminal concentrations of lidocaine did, however, reduce basal DMAS. These results confirm previous findings in the rat (9, 14) and suggest that DMAS may be influenced by neural mechanisms. Interestingly, in indomethacin-treated animals, a reduction of DMAS was obtained already at a luminal lidocaine concentration of 0.03% and 0.1% lidocaine almost abolished indomethacin-stimulated DMAS. It is known that the effectiveness of lidocaine in blocking neural activity is “frequency and voltage” dependent, i.e., lidocaine is more effective at blocking neurons that are firing at a high frequency (5, 13). It thus seems likely that inhibition of prostaglandin synthesis increases the firing of action potentials in enteric neurons, possibly mucosal nerves, that innervate the secretory epithelium, thereby facilitating the binding of lidocaine to the receptor site in the Na$^+$ channel. This could explain why lidocaine is more effective in inhibiting DMAS in indomethacin-treated rats than in control rats.

**Effect of Lidocaine on Duodenal Contractions**

In rats during luminal perfusion with saline before indomethacin administration, no or very few duodenal contractions occurred; this is a condition commonly referred to as postoperative ileus (4). According to Wood (32), the circular intestinal smooth muscle is under tonic influence of inhibitory motoneurons. Contractions are evoked when the inhibitory motoneurons are suppressed by inhibitory synaptic input from enteric interneurons. Hence one possible explanation for the postoperative inhibition of duodenal motility might be that the inhibitory motoneurons remain tonically active. If this is the case, and assuming that duodenal circular smooth muscle activity is controlled entirely by neural mechanisms, then inhibition of motoneurons by lidocaine should reveal intrinsic myogenic activity,
i.e., contractions at the same frequency of electrical slow waves (2, 31). However, none of the lidocaine solutions tested affected duodenal motility in control rats, suggesting that 1) lidocaine did not reach the muscularis externa in a concentration high enough to block the motoneurons or 2) humoral factors also contribute to the postoperative inhibition of duodenal motility in anesthetized rats.

In accord with previous findings (26), indomethacin induced a cyclical stereotyped motility pattern, characterized by bursts of contractions followed by a period of quiescence. All luminally applied lidocaine solutions, except 0.03%, affected indomethacin-induced duodenal motility. Perfusion with 0.1% of lidocaine reduced the amplitude of the contractions, suggesting attenuation in force of contraction. Increasing the luminal lidocaine concentration 10-fold (1%) completely changed the indomethacin-induced motility pattern to numerous irregular low-amplitude contractions that persisted during the whole lidocaine perfusion period. One explanation for this motility pattern might be that lidocaine reached the circular muscle layer in a sufficient concentration to block the inhibitory motoneurons, thereby inducing intrinsic myogenic activity. The reason why the same lidocaine concentration did not induce a similar motility pattern in control rats is probably due to a tonic inhibitory effect of prostacyclin on smooth muscle activity. Indirect support for this is the finding that intravenous infusion of iloprost, a stable prostacyclin analog, prevented the occurrence of these low-amplitude contractions in response to lidocaine in indomethacin-treated animals.

**Relationship Between Motility and Secretion**

Our working hypothesis is that indomethacin increases DMAS via a reflex activated by induction of duodenal contractions (Fig. 10). In the present investigation, further evidence is provided supporting this hypothesis. Luminal perfusion of the duodenum with 0.03% lidocaine reduced DMAS but had no effect on motility, whereas serosal application of lidocaine, at the same concentration, reduced both motility and DMAS. It should be noted though that serosal application of lidocaine might inhibit extrinsic autonomic neurons as well. Whether or not inhibition of these nerves contributes to the reduction of DMAS in response to serosal application of lidocaine is difficult to elucidate. However, it has previously been shown that indomethacin stimulates DMAS in vagotomized animals (28) and that inhibition of $\alpha$-adrenoceptors increases rather than decreases DMAS (8).

**Effect of Lidocaine on Duodenal Paracellular Permeability**

The physical-chemical properties of $^{51}$Cr-EDTA (3, 15, 19) imply that the water-filled paracellular shunts act as the predominant route of epithelial passage for this molecule. An increased paracellular transport of $^{51}$Cr-EDTA could reflect a change of the convective forces across the mucosa or an increase in the surface area available for diffusion, i.e., widening of paracellular shunts (24). In the present study, it is shown that lidocaine increases the blood-to-lumen clearance of $^{51}$Cr-EDTA. The complexity of the in vivo system makes it difficult to define exactly the mechanism of action of lidocaine in increasing $^{51}$Cr-EDTA clearance. One alternative is that lidocaine increases $^{51}$Cr-EDTA clearance via dilation of arterioles in the submucosa. However, this alternative seems less likely because 0.3% lidocaine had no effect on duodenal blood flow or vascular resistance.

The tight junction is the main structure restricting solutes and ions from moving freely across the epithelium by the paracellular route. Most of the classic second messengers and intracellular signaling pathways have been shown to influence the permeability of tight junctions (1, 7, 16). Accordingly, neurotransmitters, released from mucosal nerves may affect tight junction permeability via receptor-mediated increases in second messengers. Lidocaine might thus increase duodenal paracellular permeability by blocking impulse conduction in a population of mucosal nerves that exerts a tonic inhibitory influence on permeability. Furthermore, it is possible that inhibition of cyclooxy-
genase excites these mucosal nerves because both the sensitivity to lidocaine and the maximal response to lidocaine-induced increases in paracellular permeability were augmented after indomethacin treatment.

In human red blood cells, lidocaine has been shown to induce conformational changes in cytoskeletal protein network (22). The possibility thus exists that lidocaine, independent of neural blockade, induces contraction of the duodenocyte cytoskeleton, which is anatomically and functionally tied to the junctional structure (20), thereby promoting an increase in paracellular permeability. Using an autoradiographic technique, Cassuto et al. (6) examined the distribution of [14C]lidocaine in the rat jejunum after serosal application and found a high density of silver grains in the muscle layer but only weak blackening of the submucosa. In the present study, it was shown that serosal application of 1% lidocaine increased the blood-to-lumen clearance of 51Cr-EDTA, suggesting a neural mechanism of action rather than a direct effect of lidocaine on duodenocytes. It is important to note, though, that the increase in 51Cr-EDTA clearance was smaller and more transient in response to serosal than mucosal application of lidocaine. We do not know the reason for this difference, but one explanation might be that serosal application of lidocaine blocks neurons located in the muscularis externa, including extrinsic nerves, whereas luminal perfusion of lidocaine blocks impulse conduction in nerves situated in the mucosa and submucosal plexus. Furthermore, we cannot rule out the possibility that part of the increase in permeability, in response to luminal lidocaine, is due to a direct effect of lidocaine on duodenocytes.

We conclude that lidocaine is more effective in inhibiting indomethacin-stimulated than basal alkaline secretion, suggesting that inhibition of prostaglandin synthesis increases impulse conduction in a population of secretomotor neurons that stimulates DMAS. Interestingly, lidocaine induced almost continuous phasic secretion, suggesting that inhibition of prostaglandin synthesis excites or reveals enteric nerves because both the increase in paracellular permeability and the maximal response to lidocaine-induced increases in paracellular permeability were augmented after indomethacin treatment. Further, we cannot rule out the possibility that the increase in permeability, in response to luminal lidocaine, is due to a direct effect of lidocaine on duodenocytes.

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