Dose-response effects of indomethacin and PGE$_2$ on electromechanical activity of in vivo rabbit ileum

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KOCH, K. L., A. DWYER, AND G. H. JEFFRIES. *Dose-response effects of indomethacin and PGE$_2$ on electromechanical activity of in vivo rabbit ileum.* Am. J. Physiol. 250 (Gastrointest. Liver Physiol. 13): G135–G139, 1986.—We determined dose-response characteristics of indomethacin and prostaglandin E$_2$ on the myoelectric activity in ileum of anesthetized New Zealand White rabbits. Monopolar electrodes and an intraluminal saline-filled catheter were used to simultaneously record electrical and mechanical activity. Thirty minutes after injection of 3.0, 5.0, and 10.0 mg/kg indomethacin, the percentage of slow waves with action potentials increased significantly from 10% to over 80%; at 60 min action potential activity decreased but remained dose dependent and significantly greater than controls. Action potential activity correlated with phasic increases in intraluminal pressure. Low-dose indomethacin (1.5 mg/kg) did not significantly alter action potential activity. Action potential activity induced by indomethacin (5 mg/kg) decreased dose dependently after infusion of prostaglandin E$_2$ (PGE$_2$, 1–28 μg/kg). In summary, dose-dependent action potential activity was induced by indomethacin and reversed by PGE$_2$. Endogenous inhibitory prostaglandins (PGE$_2$ or others) appear to modulate activity of specific excitatory neuromuscular circuits in in vivo ileum.

intestinal motility; anti-inflammatory drugs; prostaglandins

INDOMETHACIN is a potent inhibitor of prostaglandin synthesis (20). A variety of prostaglandins are produced in gut tissues, including the microsomes of smooth muscle cells (19). The effects of indomethacin on intestinal smooth muscles have been extensively studied in vitro: indomethacin inhibits longitudinal muscle contraction in a variety of conditions (2, 5, 8, 10, 16); conversely, indomethacin stimulates contraction of circular muscle (6, 18, 21). The effects of indomethacin on in vitro ileal circular muscle strips are due to inhibition of prostaglandin production, particularly prostaglandin E$_2$ (PGE$_2$) and prostacyclin (PGI$_2$) (18). On the basis of in vitro studies, it has been proposed that endogenous prostaglandin synthesis has a physiological role in propulsive and nonpropulsive movements of the intestine (24) and in the genesis of intestinal tone (9).

Few studies, however, have examined the effects of indomethacin on in vivo myoelectric activity of the small intestine. Although changes in intraluminal pressure and action potential activity have been individually described after administration of indomethacin (13, 15, 20), a systematic evaluation of the effects of indomethacin on in

vivo electromechanical activity of the intestine has not been reported. The present study was undertaken to investigate the effects of indomethacin on in vivo ileum by simultaneously recording myoelectric and mechanical activity from rabbit ileum. The dose-response effects of PGE$_2$ on the myoelectric activity induced by indomethacin were also examined.

MATERIALS AND METHODS

Surgical preparation. Male New Zealand White rabbits weighing 2.0–2.9 kg were used in all studies. Water but not food was allowed ad libitum for 18 h prior to the experiments. Pentobarbital sodium (30 mg/kg) was administered through an ear vein. A tracheostomy was performed and a catheter was placed into the internal jugular vein for administration of indomethacin or additional pentobarbital. Body temperature was maintained at 38°C throughout each experiment by a heating blanket. Through a midline abdominal incision, the ileum was identified. Three monopolar silver-silver chloride electrodes were placed perpendicular to the longitudinal axis of the ileum at 2.5-cm intervals and sewn to the serosa and outermost layers of the longitudinal muscle. The electrodes were connected to a rectilinear recorder (Beckman, R612, Fullerton, CA) through 8353A couplers. A reference electrode was placed in the hindlimb of the rabbit. All recordings were made with settings yielding a sensitivity of 0.05 mV/mm with a high-frequency cutoff of 30 Hz and low-frequency filter of 0.16 Hz. To record intraluminal pressure, a saline-filled, open-tipped polyurethane catheter (PEG-190) was inserted into the lumen of the ileum through a purse-string suture 5 cm orad to the proximal electrode, positioned beneath a serosal electrode, and connected to a Beckman pressure transducer (type 4-327-C).

Experimental design. Control rabbits were prepared as described above, and after a 90-min equilibration period a 2-ml bolus of saline was administered intravenously. Myoelectric activity was recorded for the subsequent 5 h. After the equilibration period, a single experimental animal received a single dose of indomethacin (either 1.5, 3.0, 5.0, or 10.0 mg/kg).

Indomethacin (Merck Sharp & Dohme, West Point, PA) and sodium bicarbonate (3:1 ratio by weight) were dissolved in saline and injected intravenously in a 2-ml bolus. PGE$_2$ (Sigma Chemical, St. Louis, MO) was dissolved in ethanol, evaporated under nitrogen, and dis-

solved in physiological phosphate buffer. Thirty to sixty minutes after injection of indomethacin (5 mg/kg), PGE$_2$ was administered intravenously over 30 s at doses ranging from 1 to 28 μg/kg.

Myoelectric data. Myoelectric recordings were scored by hand for slow wave frequency per minute and the percentage of slow waves with action potentials. Myoelectric activity from electrodes yielding the clearest slow waves throughout the experiment were selected for analysis. Slow wave frequency and the percentage of slow waves with action potentials were assessed during the 10-min period immediately preceding the injection of indomethacin and the 20- to 30-min and 50- to 60-min periods after injection. The same myoelectric characteristics were determined during 10-min periods before and after injection of PGE$_2$. Data were recorded as means ± SE.

Statistical analysis. Slow wave frequencies and percentages of slow waves with action potentials from control and indomethacin experiments were compared by use of unpaired t tests. Paired t tests were used to determine the significance of myoelectric effects recorded after PGE$_2$. A value of $P < 0.05$ was considered significant.

RESULTS

Myoelectric activity. Action potentials occurred on 0–7% of the slow waves recorded from control rabbits, and simultaneously recorded intraluminal pressures were quiescent. This electromechanical pattern was recorded for at least 5 h in the control rabbits.

Typical electromechanical changes observed after injection of indomethacin (5 mg/kg) are shown in Fig. 1. Action potential activity commenced after a lag time of 13–18 min (mean 14.8 ± 1.0 min, $n = 5$) after indomethacin. The onset of action potential activity was progressive, and maximum action potential activity occurred between 30 and 60 min after injection of indomethacin. Action potential activity was recorded at each electrode site and appeared on discrete regions of the slow waves. Phasic increases in intraluminal pressure correlated in time with the onset of action potentials on individual slow waves. The amplitude of intraluminal pressures ranged from 10 to 15 mmHg. Visual inspection of the ileum revealed intermittent contractions during intense action potential activity induced by indomethacin.

The dose-response characteristics of indomethacin on action potential activity are shown in Fig. 2. Action potential activity for control and experimental rabbits was equivalent at *time 0.* Indomethacin, 3.0, 5.0, and 10.0 mg/kg, significantly increased the percentage of slow waves with action potentials when compared with controls at 30 min. In contrast, 1.5 mg/kg indomethacin had an insignificant effect on action potential activity at 30 min. The maximum increase in the percentage of slow waves with action potential activity observed in these experiments (81 ± 9%) occurred 30 min after 5 mg/kg indomethacin; 10 mg/kg indomethacin did not further increase action potential activity (62.2 ± 8 at 30 min). At 60 min, action potential activity decreased but re-
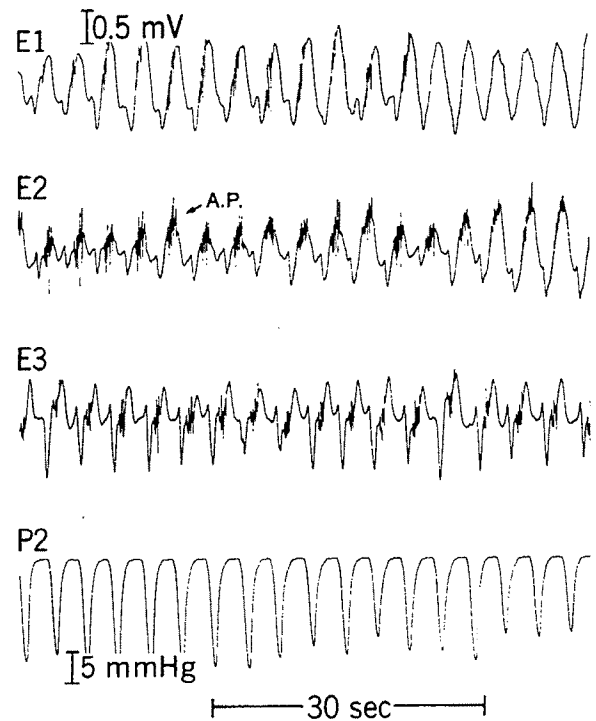

FIG. 1. Electromechanical recording from rabbit ileum 60 min after injection of indomethacin (5 mg/kg iv). Electrodes (E$_1$-E$_3$) are 2.5 cm apart, positioned perpendicular to longitudinal axis of ileum and located proximal (E$_1$) to distal (E$_3$) on ileal serosa. P$_2$ indicates intraluminal pressure recorded beneath E$_2$. Peak pressures point to bottom of tracing. Slow wave frequency is 16 cycles/min. Action potential activity (AP), particularly well seen at E$_2$, occurs on discrete portions of slow waves recorded at each electrode site. Onset of action potential activity on individual slow waves at E$_2$ corresponds with phasic increases in intraluminal pressure at P$_2$.
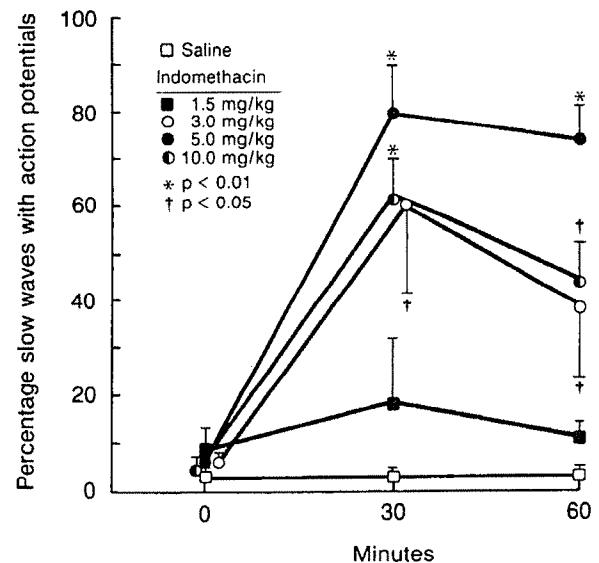

FIG. 2. Dose-response effect of indomethacin on percentage of slow waves (±SE) with action potentials before indomethacin (*time 0*) and 30 and 60 min after injection. Indomethacin significantly increases action potential activity. *†Significant differences between saline and indomethacin values. See text for description. Number of rabbits receiving each dose is shown on Table 1.

mained significantly greater than base-line activity after 3.0, 5.0, and 10 mg/kg indomethacin.

Base-line slow wave frequencies were not significantly altered 30 and 60 min after injection of either saline or

the various doses of indomethacin (Table 1). However, when slow wave frequencies from control and indomethacin-treated rabbits were compared (unpaired t tests), significantly lower frequencies were found 30 min after 1.5 and 3.0 mg/kg indomethacin and 60 min after 3.0 and 5.0 mg/kg indomethacin. In contrast, slow wave frequencies after 10 mg/kg indomethacin did not differ from control. The overall effect of indomethacin on slow wave frequency was small, and a dose-response effect was not apparent.

Effect of PGE$_2$ on indomethacin-induced electromechanical activity. In other experiments, PGE$_2$ (1–28 μg/kg) was injected intravenously 30–60 min after administration of 5 mg/kg indomethacin at a time when action potential activity occurred on at least 60% of the slow waves. The effect of each dose of PGE$_2$ was evaluated during the 10-min periods before and after injection in a minimum of four rabbits. PGE$_2$ decreased the indomethacin-induced action potential activity in a dose-dependent manner as shown in Fig. 3. PGE$_2$, 1 μg/kg, had no effect on action potential activity; 4 and 6 μg/kg PGE$_2$ reduced the percentage of slow waves with action potentials but not significantly. After 8 μg/kg PGE$_2$, the action

TABLE 1. *Effect of indomethacin on slow wave frequency in in vivo rabbit ileum*

	n	Slow Wave Frequency		
		0 min	30 min	60 min
Control	5	17.9±0.6	18.2±0.5	18.4±0.6
Indomethacin, mg/kg				
1.5	4	15.5±0.6	15.4±0.4*	15.6±0.5
3.0	4	15.9±0.7	15.4±0.6*	16.0±0.6†
5.0	5	17.3±0.5	16.5±0.6	15.9±0.3*
10.0	5	15.8±0.5	15.9±0.9	16.1±1.0

Values are means ± SE in cycles/min; n, no. of measurements. *$P < 0.01$ Indo vs. control. †$P < 0.02$ Indo vs. control.
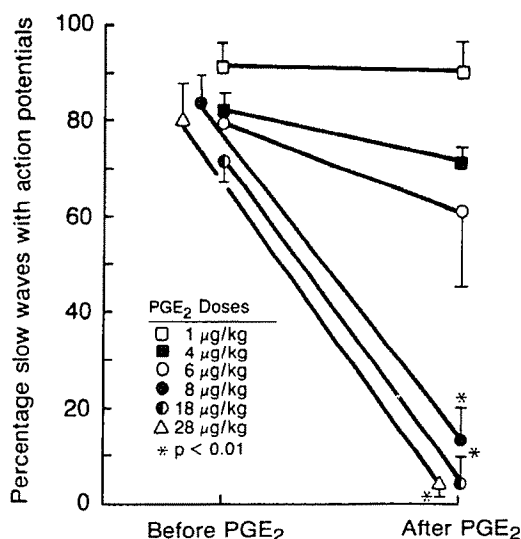

FIG. 3. Dose-response effects of PGE$_2$ on percentage of slow waves with action potentials (±SE) induced by indomethacin. PGE$_2$ was administered 30–60 min after injection of indomethacin (5 mg/kg) when action potentials were occurring on 60% or more of slow waves (Before PGE$_2$). Increasing doses of PGE$_2$ abolish indomethacin-induced action potentials (After PGE$_2$). Number of rabbits receiving each dose is shown on Table 2. *Significant differences between effects of 1 μg/kg PGE$_2$ and other PGE$_2$ doses.

TABLE 2. *Effect of PGE$_2$ on slow wave frequency in rabbits pretreated with 5 mg/kg indomethacin*

PGE$_2$, μg/kg	n	Slow Wave Frequencies		
		Before PGE$_2$	After PGE$_2$	P
1	4	16.1±0.2	15.9±0.2	NS
4	5	16.3±0.2	16.1±0.4	NS
6	5	17.7±0.4	18.8±0.6	NS
8	9	16.7±0.4	19.1±0.6	<0.01
18	8	17.0±0.3	19.7±0.5	<0.01
28	7	17.5±0.7	19.3±0.3	<0.01

Values are means ± SE in cycles/min.

potential activity was significantly reduced to 13 ± 7%; after 18 and 28 μg/kg action potential activity was virtually abolished (5 ± 5 and 4 ± 2%, respectively). Action potential activity and phasic increases in intraluminal pressure were temporarily abolished by 18 and 28 μg/kg PGE$_2$; the duration of the inhibitory PGE$_2$ effect was 10–12 min.

Slow wave frequencies 10 min before and after the various doses of PGE$_2$ are listed in Table 2. PGE$_2$ at 1, 4, and 6 μg/kg did not affect slow wave frequency, whereas 8, 18, and 28 μg/kg significantly increased the slow wave frequency in the indomethacin-treated rabbits. In other experiments ($n = 6$), a dose 18 or 28 μg/kg PGE$_2$ was administered after the equilibration period but before indomethacin. Neither slow wave frequency nor action potential activity was altered by the two PGE$_2$ doses. Combined data from these experiments are listed below: slow wave frequency before and after PGE$_2$ was 19.0 ± 0.4 and 19.1 ± 0.3, respectively, and the percentage of slow waves with action potential activity was 5.0 ± 2.0 and 4.4 ± 1.0, respectively.

Histological examination. Histological examination of ileum from control and indomethacin-treated rabbits revealed no mucosal or muscle wall abnormalities by routine light microscopy.

DISCUSSION

Endogenous prostaglandins may have a physiological role in modulating intestinal motility (5, 9, 24). By altering biosynthesis of prostaglandins with indomethacin and measuring subsequent electromechanical activity, the effect of ongoing endogenous production of prostaglandins on intestinal motility may be assessed (18, 20). Although indomethacin is a well-known and potent inhibitor of prostaglandin synthesis (22), a detailed examination of the effects of indomethacin on in vivo electromechanical activity of the intestine has not been reported. The present in vivo studies show that indomethacin induces dose-dependent action potential activity that is associated with phasic increases in intraluminal pressure. The lag time to onset of action potential activity after indomethacin is consistent with lag times reported for inhibition of cyclooxygenase and depletion of prostaglandins in ileal and antral smooth muscle (18, 20, 21). Although tissue prostaglandin levels were not measured in these experiments, Wilson et al. (25) previously showed that PGE was significantly reduced in rabbit intestine after 5 mg/kg indomethacin, the most active dose used in the present studies.

The in vivo electromechanical activity observed after indomethacin is characterized by rhythmic bursts of action potentials on slow waves and phasic increases in intraluminal pressure, all of which indicate contractile activity of the circular muscle layer (1). Similar action potential activity after indomethacin has been noted in the jejunum of fasted conscious dogs (13). Thus, anesthesia and laparotomy per se do not appear relevant to the electromechanical activity noted after indomethacin. Indomethacin also stimulates contractions of in vitro circular muscle from human and canine ileum (6, 18, 19). The increase in in vitro muscle contractility is associated with decreased tissue levels of PGE$_2$ and PGI$_2$, changes which occurred after a lag phase of 20 min duration (18). Similar lag periods preceded the onset of indomethacin-induced electromechanical activity in the present in vivo studies. Taken together, these data suggest that the dose-dependent in vivo action potential activity observed after indomethacin was due to depletion of inhibitory endogenous prostaglandins that modulate circular muscle contractility.

Administration of PGE$_2$ resulted in dose-dependent reversal of the in vivo action potential activity induced by indomethacin. It is well documented that PGE$_2$ inhibits contraction of circular muscle in vitro (3, 4, 21). Other studies have shown that PGE$_2$ decreases intraluminal pressure, an effect interpreted as inhibition of circular muscle contraction (3, 7). The inhibition of in vivo action potential activity (i.e., circular muscle contraction) by PGE$_2$ is consistent with these previous studies. Furthermore, in vitro circular muscle contractions induced by indomethacin are also inhibited by PGE$_2$ and PGI$_2$ (18). It has been previously proposed that PGE$_2$ and PGI$_2$ inhibit potential excitatory neuromuscular activity in the circular muscle (13, 18, 20). Although PGI$_2$ was not studied, the effects of indomethacin and PGE$_2$ reported here strongly support the concept that inhibitory endogenous prostaglandins such as PGE$_2$ modulate intestinal motility. Specifically, the present dose-response experiments suggest that inhibitory prostaglandins regulate the activity of excitatory neuromuscular circuits, which mediate phasic contractions of in vivo ileal circular muscle.

Aspirin (100 mg/kg iv) produced electromechanical activity in rabbit ileum which was similar to indomethacin-induced responses (12). These findings indicate that indomethacin-induced action potential activity is the result of inhibition of prostaglandin synthesis rather than a nonspecific effect of the drug itself. Thus, by inhibiting endogenous prostaglandins indomethacin may unmask excitatory neuromuscular pathway(s) that are ordinarily suppressed by the ongoing synthesis of prostaglandin products such as PGE$_2$ or PGI$_2$ (13, 18, 20). The dose-dependent reversal of indomethacin-induced action potential activity by PGE$_2$ supports this notion. Furthermore, previous studies showed that indomethacin-induced action potentials were significantly decreased by scopalamine but were unaffected by hexamethonium, phentolamine, propranolol, and bilateral cervical vagotomy (11). Thus, indomethacin appears to unmask an enteric cholinergic circuit in rabbit ileum by

inhibiting local synthesis of prostaglandins. The dose-dependent effects of indomethacin on in vivo action potential activity support the concept that a distinct enteric neuromuscular circuit(s) or receptor(s) mediates the electromechanical response observed after indomethacin.

The effect of indomethacin and PGE$_2$ on in vivo slow wave frequency deserves comment. Slow waves probably originate in the longitudinal muscle layer of the intestine, generated by metabolic processes within the muscle that affect membrane depolarization and repolarization (23). In the present studies some doses of indomethacin caused a slight decrease in slow wave frequency, but a dose-response effect was not seen. Thus, indomethacin would appear to have little effect on the longitudinal muscle. However, if indomethacin induced a deficiency of prostaglandins and altered cellular processes that affected slow waves, then administration of prostaglandin should reverse the effect. As shown in Table 2, infusions of PGE$_2$ resulted in significant dose-dependent increases in slow wave frequency, an effect observed only in indomethacin-treated rabbits. Increased slow wave frequency was also noted in ex vivo canine stomach after flash or continuous infusions of PGE$_2$, a finding attributed to selective action on the longitudinal muscle (14). Thus, prostaglandins may also have a role in the generation of in vivo slow waves through as-yet-undefined metabolic effects in the longitudinal muscle.

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