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# Influence of ricinoleic acid on the contractions elicited by $PGE_2$ on the guinea-pig ileum

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The effect of ricinoleic acid on prostaglandin $E_2$ ($PGE_2$)-evoked contractions was studied on guinea-pig isolated ileum. Addition of ricinoleic acid (10 μg $ml^{-1}$) to the organ bath increased the amplitude of the $PGE_2$-evoked responses. Ricinoleic acid (10 μg $ml^{-1}$) also sensitized the guinea-pig isolated ileum to acetylcholine and histamine. The effect of the ricinoleic acid was greatly reduced by indomethacin either in-vivo (10 mg $ml^{-1}$) or in-vitro (2 μg $ml^{-1}$).

Several independent studies have shown that prostaglandins are implicated as contributors to various gastrointestinal malfunctions including diarrhoea (for reviews see Collier 1974; Bennett 1978). Diarrhoea is also the most commonly associated side effect when prostaglandins are used for the termination of pregnancy (Karim & Amy 1975).

Release of prostaglandin-like material has been implicated as a mechanism involved in the cathartic action of ricinoleic acid and other laxatives and the effect may be reduced by the PG synthesis inhibitor indomethacin (Beubler & Juan 1979).

We have investigated whether ricinoleic acid potentiates responses evoked by prostaglandin $E_2$ ($PGE_2$) on guinea-pig isolated ileum.

## *Methods*

Male or female albino guinea-pigs, 250–280 g, were stunned by a blow to the head and from the terminal ileum a segment 4 cm long was taken (at least 10 cm from the caecum). The tissue was suspended in an organ bath containing 5 ml of Tyrode solution gassed with a mixture of 5% $CO_2$ in oxygen and maintained at 37 °C. The contractions of the longitudinal muscle were recorded on a kymograph by an isotonic lever (amplification 1:10; load 0·5 g). $PGE_2$ and other agonists, acetylcholine (ACh) and histamine, dissolved in 5 ml of the Tyrode solution, were added to the bath (muscle chamber) and allowed to remain in contact with the tissue until the maximal effect occurred (60 s) after addition and then washed out. The interval between additions was 12 min. After at least 3 control contractions, ricinoleic acid was added to the bath 10 min before the next addition of agonist. Then both agonist and ricinoleic acid were washed out and the ileum was again challenged at two consecutive intervals of 12 min with the agonist. Ricinoleic acid was also added to the Tyrode solution and allowed to act for 20 min after which time $PGE_2$ was injected into the bath.

In some experiments, indomethacin (2 μg $ml^{-1}$) was added separately to the Tyrode solution from the beginning of the experiment. Some animals were also treated intraperitoneally with indomethacin (10 mg $kg^{-1}$) 90 min before the removal of tissue.

Data from all experiments were statistically evaluated using Student's *t*-test.

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Table 1. Potentiating effect of ricinoleic acid (RA 10 μg $ml^{-1}$) on the responses of guinea-pig isolated ileum to various stimulant drugs.

| Drugs (n =) | Concn ng $ml^{-1}$ | Height of contraction (mm) Before R A | After R A | *P* value |
|---|---|---|---|---|
| | | mean ± s.e. | | |
| $PGE_2$ (9) | 10 | 37 ± 4·3 | 60 ± 4·0 (62)* | < 0·01 |
| ACh (4) | 20 | 50 ± 5·7 | 70 ± 6·1 (40) | < 0·05 |
| Histamine (5) | 10 | 45 ± 4·7 | 64 ± 6·0 (42) | < 0·05 |
| Indomethacin (4) | 2 μg | 38 ± 4·0 | 42 ± 3·7 (10) | < 0·01† |
| Indomethacin (5) | 10 mg | 37 ± 4·2 | 43 ± 5·1 (16) | < 0·01† |

% increase of response
† Controls without indomethacin: 40 ± 4·8 without indomethacin but + ricinoleic acid, 10 μg 63 ± 5·3.

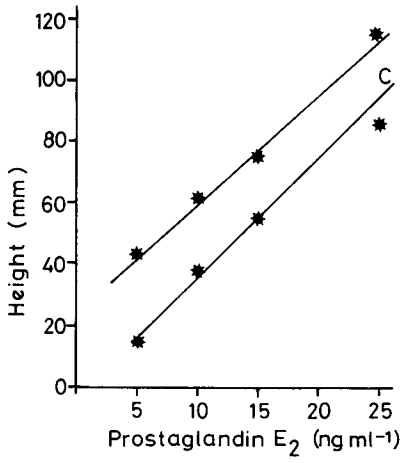


FIG. 1. Guinea-pig isolated ileum suspended in Tyrode solution. (c) represents the control response to $PGE_2$. Addition of ricinoleic acid (10 μg $ml^{-1}$) to the bath solution produced a marked potentiation of the $PGE_2$ responses.

## *Results and discussion*

$PGE_2$ in concentrations ranging from 5 to 25 ng $ml^{-1}$ caused dose-dependent contractions of guinea-pig isolated ileum. The contractions were slow with the latent

period varying from 10 to 25 s. However, ricinoleic acid 10 μg $ml^{-1}$ introduced to the bath elicited increased contractions by the $PGE_2$ (Fig. 1), an effect totally reversible by washing out the test compound. Addition of ricinoleic acid, 10 μg $ml^{-1}$, to the Tyrode solution also produced a pronounced increase in the amplitude of the prostaglandin-evoked responses. But ricinoleic acid itself did not induce contractions in the concentration used.

The effect of ricinoleic acid on agonists other than $PGE_2$ was also examined. Ricinoleic acid, 10 μg $ml^{-1}$ increased contractions induced by ACh, 20 ng $ml^{-1}$, and by histamine, 10 ng $ml^{-1}$ (see Table 1).

Small amounts of indomethacin (2 μg $ml^{-1}$) added to the Tyrode solution prevented the potentiating effect of ricinoleic acid (Table 1). The intraperitoneal administration of indomethacin (10 mg $kg^{-1}$) also desensitized the guinea-pig ileum to the action of $PGE_2$ in presence of ricinoleic acid. However, contractions induced by $PGE_2$ alone were unaffected by indomethacin.

Our results indicate that ricinoleic acid is able to sensitize the ileal longitudinal muscle to the contractile effect induced by $PGE_2$, ACh and histamine but ricinoleic acid has a more pronounced effect on contractions to $PGE_2$ than on those of the other agonists. It has been suggested that ricinoleic acid stimulates the release of PG-like substances (Beubler & Juan 1979). That PG modulate the responses of the ileal smooth muscle to various agonists including ACh and histamine has been shown by Bennett et al (1975). An effect on intestinal concentration of PG/or a stimulation of PG-like release, or both, could explain those observations.

Furthermore, the observation that indomethacin, a PG synthesis inhibitor, in-vitro or in-vivo inhibits the potentiation of $PGE_2$, supports the concept that ricinoleate stimulates the synthesis of endogenous ileal prostaglandins, an action probably important for the cathartic properties of ricinoleic acid, the active constituent of castor oil.

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# Possible role of prostaglandins in post-tetanic potentiation at the nerve-muscle junction in the longitudinal muscle strip of guinea-pig ileum

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The effect of tetanic stimulation on the twitch responses of the longitudinal muscle-myenteric plexus preparation of the guinea-pig ileum to electrical stimulation was investigated in the presence of naloxone. Under this condition, or after the addition of $PGE_2$, twitch contractions were maximal and no potentiation of twitches following tetanus was observed. In the presence of indomethacin (1 μmol $litre^{-1}$) twitches were diminished and post-tetanic potentiation (PTP) was manifested. PTP was seen with indomethacin concentrations of 1 to 20 μmol $litre^{-1}$ or after simultaneous addition of diphloretin phosphate (16 μmol $litre^{-1}$). Thus it seems unlikely that the effect of prostaglandins released during tetanic stimulation would be of key importance for the manifestation of PTP. Rather it is thought that a decrease in the release of acetylcholine from motor nerve terminals, and consequently smaller twitches in the presence of indomethacin, offer favourable conditions for PTP.

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The phenomenon of presynaptic post-tetanic potentiation (PTP), a transient increase in evoked transmitter release after tetanic stimulation, has been described at both nicotinic (for refs see MacIntosh & Collier 1976; Ginsborg & Jenkinson 1976) and muscarinic (Kadlec et al 1979) synapses. At the muscarinic synapse of the longitudinal muscle-myenteric plexus in the guinea-pig ileum either kind of post-tetanic inhibition (Puig et al 1978) or PTP (Kadlec et al 1982) is observed depending on the stimulus parameters during tetanus. PTP is more pronounced in the presence of naloxone and of low concentrations of indomethacin; naloxone prevents those effects of endogenous opiate ligands released during tetanus that are responsible for the major part of post-tetanic twitch inhibition (Puig et al 1978). The output of acetylcholine (ACh) and resulting twitch contractions might be modified by prostaglandin E