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# Failure of tetrodotoxin to inhibit the prostaglandin-induced secretory response of rat small intestine in-vitro

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**Abstract**—Tetrodotoxin (TTX, 10 μM) did not inhibit prostaglandin $E_2$ ($PGE_2$)-stimulated increases in electrical activity in intact or stripped sheets of rat small intestine, although it reduced basal electrical activity in the intact preparation. Basal and $PGE_2$-stimulated cAMP production by enterocytes isolated from the small intestine was unaffected by TTX. Thus its reported ability to inhibit prostaglandin-induced fluid secretion in-vivo does not appear to represent a direct interaction of the neurotoxin with the mechanism of prostaglandin action at the enterocyte.

There is evidence that the secretory response of the intestinal mucosa involves not only a direct action of secretagogues on the transporting cells, but also the participation of the enteric nervous system (ENS, Lundgren et al 1989). Prostaglandins are potent stimulants of intestinal secretion in-vivo (Matuchansky & Coutrot 1978; Hardcastle et al 1981) and the observation that in such preparations the neurotoxin, tetrodotoxin (TTX), abolishes the secretory response to prostaglandin $E_1$ ($PGE_1$) (Coupar 1986) suggests that the prostaglandin does not directly affect the enterocyte but acts via a neural pathway. The fact that prostaglandins can stimulate secretion in in-vitro preparations of intestine does not exclude this possibility as such preparations retain a large proportion of their intramural nerve networks. It is, however, less easy to reconcile with reports that prostaglandins can stimulate transporting cells in the absence of neural networks; they can enhance cAMP production in isolated enterocytes (Hardcastle et al 1981) and increase the short-circuit current in a colonic cell line (Dharmsathaphorn et al 1984). To account for the abolition of prostaglandin-induced intestinal secretion by TTX in-vivo, despite the direct action of prostanoids on the transporting cells, it has been proposed that the toxin might release an endogenous inhibitor or that it might exert a direct antisecretory action on the transporting cells (Coupar 1986). This latter possibility was examined by testing the ability of TTX to influence the secretory actions of $PGE_2$ in in-vitro preparations of rat small intestine.

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## Materials and methods

Experiments were carried out on male albino rats (Sheffield strain, 230–250 g) allowed free access to food and water.

*Measurement of transintestinal electrical activity.* Transintestinal electrical activity was measured in-vitro in paired sheets of mid-intestine, removed from anaesthetized rats (60 mg $kg^{-1}$ sodium pentobarbitone, i.p.). In some experiments the muscle layers were removed (stripped sheets), while in others they were not (intact sheets). The sheets were clamped between two Perspex chambers (exposed tissue area = 1·925 $cm^2$) and incubated at 37°C in Krebs bicarbonate saline (5 mL in each chamber) gassed with 95%$O_2$–5%$CO_2$. The potential difference (PD) was measured with salt bridge electrodes, connected via calomel half-cells to a differential input electrometer. Current was applied through Ag/AgCl electrodes in contact with mucosal and serosal solutions via wide-bore salt bridges. When determining the short-circuit current (SCC) a correction for fluid resistance was

applied as described by Field et al (1971). Resistance (R) was calculated from PD and SCC values using Ohm's law and for each tissue pair, R values did not differ by more than 25%.

After setting up, tissues were allowed to stabilize for 10 min by which time steady values had been achieved. Basal readings were taken every min for 5 min and then $PGE_2$ was added to the serosal solution and readings continued for a further 10 min. TTX (10 $\mu$M) was added to the serosal solution of the test sheet on setting up the preparation, while control sheets received an equivalent volume of the saline vehicle (25 $\mu$L).

*Measurement of cAMP production by isolated enterocytes.* The small intestine was removed from rats killed by a blow to the head and cervical dislocation. Epithelial cells were isolated using the method described by Hardcastle et al (1980) which yields a mixed population of villous and crypt cells. cAMP was assayed using the competitive binding protein method of Brown et al (1971) and the levels related to the protein content, determined by the method of Lowry et al (1951).

*Expression of results.* Results are expressed as mean values ± s.e. of the mean of the number of observations indicated. The significance of TTX action was assessed using a paired *t*-test.

*Chemicals.* $PGE_2$ was obtained from Upjohn Co, Kalamazoo, MI, USA; TTX and adenosine 3′, 5′-cyclic phosphoric acid from Sigma Chemical Co, St Louis, MO, USA. Radioactive tracers were supplied by Amersham International plc, Amersham, UK.

## Results

$PGE_2$ caused concentration-dependent increases in both the SCC generated by stripped intestinal sheets and cAMP production by isolated enterocytes (Fig. 1). The electrical response was, however, more sensitive to $PGE_2$, with an EC50 value of 0·15 $\mu$M compared with 0·95 $\mu$M for the stimulation of cAMP production.

The presence of 10 $\mu$M TTX did not alter basal electrical activity in stripped sheets, nor did it influence the rises in PD (control: 1·6 ± 0·1 (9) mV, + TTX: 1·5 ± 0·2 (9) mV; $P > 0·05$) and SCC (control: 62·1 ± 6·2 (9) $\mu$A cm$^{-2}$, + TTX: 66·4 ± 10·2 (9) $\mu$A cm$^{-2}$; $P > 0·05$) induced by 1·4 $\mu$M $PGE_2$ (Table 1). In intact intestinal sheets, the basal PD and SCC were significantly lower in the presence of 10 $\mu$M TTX, both under basal conditions and with $PGE_2$ (Table 1). However, the increases in PD (control: 2·0 ± 0·3 (5) mV, + TTX: 2·1 ± 0·2 (5) mV; $P > 0·05$) and SCC (control: 58·2 ± 3·0 (5) $\mu$A cm$^{-2}$, + TTX: 57·6 ± 3·3 (5) $\mu$A cm$^{-2}$; $P > 0·05$) induced by 2·8 $\mu$M $PGE_2$ were unaffected by TTX.

In isolated enterocytes, the presence of 10 $\mu$M TTX in the incubation medium did not alter either basal or $PGE_2$-stimulated cAMP levels (Table 2).

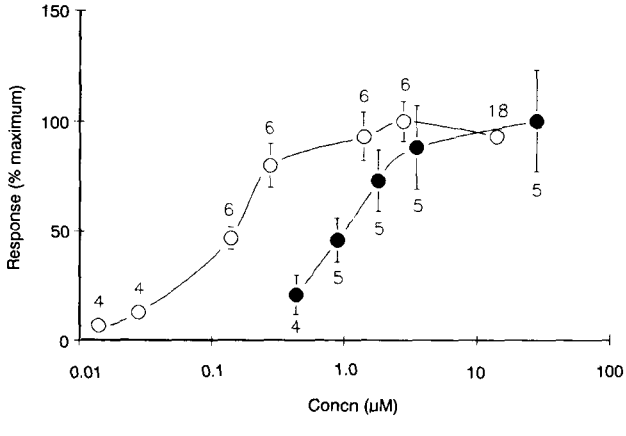


FIG. 1. Increases in SCC (○) and cAMP production (●) induced by varying concentrations of $PGE_2$ in rat small intestine. The SCC was measured in stripped intestinal sheets while cAMP production was determined in triplicate in isolated enterocytes. Each point represents the mean ± s.e. of the mean of the number of observations indicated. Basal SCC was 75·2 ± 3·5 (50) $\mu$A cm$^{-2}$ and 100% response to $PGE_2$ was 116·9 ± 11·1 (6) $\mu$A cm$^{-2}$. Basal cAMP production was 1·85 ± 0·21 (10) pmol (mg protein)$^{-1}$/10 min and 100% response to $PGE_2$ was 2·99 ± 0·70 (5) pmol (mg protein)$^{-1}$/10 min.

Table 1. Effect of tetrodotoxin (TTX, 10 $\mu$M in serosal solution) on the electrical activity of intact and stripped sheets of rat mid-intestine under basal conditions and in the presence of serosal $PGE_2$. Each value represents the mean ± s.e.m. of the number of tissues indicated in parentheses and a paired *t*-test was used to assess the significance of the effects of TTX ($P_{TTX}$) and $PGE_2$.* $P < 0·01$,** $P < 0·001$ for the effect of $PGE_2$.

| | PD (mV) | SCC ($\mu$A cm$^{-2}$) | R (ohm cm$^2$) |
|---|---|---|---|
| Intact sheets (5) | | | |
| Basal | | | |
| Control | 3·8 ± 0·7 | 96·6 ± 15·6 | 43·2 ± 9·4 |
| + TTX | 1·9 ± 0·2 | 52·5 ± 5·8 | 36·5 ± 3·4 |
| $P_{TTX}$ | < 0·05 | < 0·05 | > 0·05 |
| + 2·8 $\mu$M $PGE_2$ | | | |
| Control | 5·8 ± 0·6* | 154·8 ± 16·0** | 34·8 ± 4·2 |
| + TTX | 4·0 ± 0·4** | 110·1 ± 6·2** | 36·4 ± 3·1 |
| $P_{TTX}$ | < 0·05 | < 0·05 | > 0·05 |
| Stripped sheets (9) | | | |
| Basal | | | |
| Control | 2·5 ± 0·2 | 84·8 ± 5·7 | 29·4 ± 1·1 |
| + TTX | 2·2 ± 0·2 | 78·8 ± 8·3 | 28·0 ± 1·4 |
| $P_{TTX}$ | > 0·05 | > 0·05 | > 0·05 |
| + 1·4 $\mu$M $PGE_2$ | | | |
| Control | 4·1 ± 0·2** | 146·9 ± 8·5* | 27·7 ± 1·4* |
| + TTX | 3·7 ± 0·2** | 145·2 ± 13·5** | 25·9 ± 1·5** |
| $P_{TTX}$ | > 0·05 | > 0·05 | > 0·05 |

Table 2. Effect of tetrodotoxin (TTX, 10 $\mu$M) on basal and $PGE_2$-stimulated cAMP production by enterocytes isolated from rat small intestine. cAMP production is expressed as pmol (mg protein)$^{-1}$/10 min and each point represents the mean ± s.e. of the mean of 5 individual experiments each carried out in triplicate. A paired *t*-test was used to assess the significance of the effects of TTX ($P_{TTX}$) and $PGE_2$. *$P < 0·05$, **$P < 0·01$ for the effect of $PGE_2$.

| | Control | + TTX | $P_{TTX}$ |
|---|---|---|---|
| Basal | 1·82 ± 0·23 | 1·97 ± 0·40 | > 0·05 |
| + 3·5 $\mu$M $PGE_2$ | 4·45 ± 0·80** | 3·99 ± 0·79* | > 0·05 |
| + 28·0 $\mu$M $PGE_2$ | 4·81 ± 0·89** | 4·95 ± 0·91** | > 0·05 |

## Discussion

The intestinal response to $PGE_2$ was measured both as an increase in electrical activity in stripped and intact intestinal sheets and as enhanced cAMP production by isolated enterocytes. It has been suggested that there is a causal relationship between the prostaglandin-induced increase in cAMP levels in the enterocyte and the stimulation of $Cl^-$ secretion (Kimberg et al 1971). However, the fact that the electrical response was more sensitive to $PGE_2$ (Fig. 1) suggests that this is not the case and supports the view that the secretory response to prostaglandins is mediated primarily by calcium, rather than by cAMP (Beubler et al 1986).

In stripped intestinal sheets, TTX did not affect basal electrical activity, although it reduced basal PD and SCC in intact sheets (Table 1). This confirms that the concentration of

TTX used was effective and suggests that neurones in the myenteric plexus, which are removed by stripping, can influence basal ion transport. This is in agreement with observations in rabbit ileal preparations with an intact myenteric plexus where TTX has also been shown to reduce the basal SCC (Hubel 1978). The neural elements that remain after stripping do not appear to alter basal ion transport in rat small intestine. Similarly in stripped sheets of rat colon it has been reported that TTX does not affect the basal SCC or fluxes of $Na^+$ and $Cl^-$ (Zimmerman & Binder 1983). There may, however, be some species differences, as in guinea-pig ileum (Carey & Cooke 1986) TTX was found to cause a significant reduction in the SCC in stripped preparations.

The ability of TTX to inhibit the secretory response of the intestine to prostaglandins in-vivo (Coupar 1986) is not observed under in-vitro conditions. The rise in SCC induced by $PGE_2$ is a reflection of its stimulation of electrogenic $Cl^-$ secretion (Hardcastle et al 1981). This aspect of the response was not inhibited by TTX in either intact or stripped sheets (Table 1). Similarly it has been reported that in rabbit ileum TTX does not reduce the rises in SCC induced by the secretagogues carbachol and theophylline (Hubel 1978). It has been suggested that the secretory effects of $PGE_2$ in stripped sheets of rat colon involve an intramural cholinergic neural pathway in addition to a direct action on the transporting cells (Diener et al 1988) as atropine causes a 50% reduction in the response. The concentration of atropine used, however, was rather high so that non-specific actions cannot be excluded.

In addition to its effects on $Cl^-$ secretion, $PGE_2$ also inhibits the coupled absorption of NaCl and this contributes to its secretory action (Hardcastle et al 1981). Since this process is electrically silent, a change in its activity cannot be detected using electrical techniques. It is therefore possible that the ENS may be involved in the inhibition of NaCl absorption caused by $PGE_2$, but not in its stimulation of $Cl^-$ secretion. A selective action of antagonists on these two transport mechanisms is not unknown. 5-Hydroxytryptamine also stimulates $Cl^-$ secretion and inhibits NaCl absorption and recent observations indicate that the electrical response is antagonized by cisapride but not by ketanserin, while the fluid secretory response is reduced by ketanserin, but not by cisapride (Beubler et al 1990). It therefore seems that in the secretory response, there is not an obligatory linkage between the inhibition of NaCl co-transport and the stimulation of $Cl^-$ secretion.

$PGE_2$ increased cAMP production by isolated enterocytes and this response was unaffected by TTX. Since cAMP also inhibits NaCl co-transport (Field 1971), the lack of effect of TTX on prostaglandin-induced cAMP production argues against its possessing the ability to reduce NaCl absorption under in-vitro conditions.

The failure of TTX to influence the intestinal secretory response to $PGE_2$ in-vitro suggests a lack of involvement of neural mechanisms. It is possible that in-vivo, the net movement of fluid into the intestinal lumen in response to prostaglandin stimulation results in distension of the intestinal loop which could activate a local enteric reflex leading to a further stimulation of the secretory process (Caren et al 1974). This secondary effect would be blocked by TTX and may explain why secretagogue action in-vivo is reduced by agents that inhibit nervous activity (Lundgren et al 1989).

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