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Tetrodotoxin inhibits directly acting stimulants of intestinal fluid secretion

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Intravenous injection of tetrodotoxin (20 μg kg⁻¹) to anaesthetized rats blocks PGE_1- and inhibits VIP-induced fluid secretion from the mucosa of the jejunum without affecting the physiological rates of net water and glucose absorption. It is suggested that the toxin might release an endogenous inhibitor of secretion or that it might possess direct antisecretory activity on the epithelial cells of the jejunum. It is further suggested that results where tetrodotoxin has been used to investigate secretagogue action should be treated with caution.

The intestinal mucosa which normally absorbs fluid can secrete large amounts of fluid into the lumen resulting in diarrhoea, in a wide variety of pathological conditions. Such a complete reversal of physiological function must involve neurohumoral control. It is now well recognized that the intestinal mucosa is under not only the classical cholinergic and noradrenergic influence, but also receives direct peptidergic innervation (Turnberg 1983). The function and activities of these nerves remain mainly undetermined. Therefore, the selective nerve inhibitor tetrodotoxin (TTX) is used increasingly to investigate whether or not enteric nerves are involved in a response. Despite this, TTX has not been investigated for potential direct effects on the absorptive and secretory functions of the intestine in-vivo. This possibility is now investigated using the directly-acting secretagogues prostaglandin (PG) E_1 and vasoactive intestinal peptide (VIP).

Methods

Fluid absorption from, and PGE_1- and VIP-stimulated secretion into, the lumen of the rat jejunum was measured as described by Coupar (1985). In brief, male and female Hooded Wistar rats (230–290 g) were anaesthetized with pentobarbitone sodium (60 mg kg^{-1}). A cannula was introduced into the left jugular vein for drug administration and into the left common carotid artery for constant intra-arterial (i.a.) infusions of saline as control, PGE_1 (Upjohn) or VIP (Karolinska Institute) in saline at a rate of 40 μl min^{-1}. Mean systemic blood pressure was recorded from a side-arm off the carotid cannula by means of a Statham pressure transducer connected to a grass polygraph (Model 79C). A recirculation technique was used to measure the net amount of fluid transported by the jejunum. The perfusion solution (an isosmotic solution containing NaCl 8·57, KCl 0·37, dextrose 1 and phenol red 0·02 g $litre^{-1}$ as a non-absorbable marker) was recirculated through the intestinal loops from a reservoir maintained at 37 °C by gas-lift consisting of moistened 5% CO_2 in O_2. Animals treated with TTX (Sankyo) were respired artificially by an air pump at a rate of 53 strokes min^{-1} and 1·5 ml/100 g weight. Results are expressed as the net amount of water absorbed (+) or secreted (−) per gram wet weight of jejunum during the 20 min perfusion. Glucose absorption is measured by a commercially available hexokinase method (Glucoquant, Boehringer-Mannheim).

Segments of jejunum were also removed from anaesthetized animals 25 min after TTX and saline (1 ml kg^{-1}) as control, and suspended under an initial tension of 1 g in an organ bath at 37 °C containing Krebs-Henseleit solution with added glucose and gassed with 5% CO_2 in O_2. Tension changes were recorded by means of a Grass FTO3 force displacement transducer connected to a Grass Polygraph. After equilibration for 15 min the responses of the tissues were monitored to transmural electrical stimulation delivered by a Grass S48 stimulator and then to acetylcholine (Sigma).

Comparisons were made using the Student's unpaired *t*-test and differences considered statistically significant when $P < 0.05$. Values are means ± s.e.

Results

The mean rate of water absorption from the jejunum of animals injected i.v. with 20 μg kg^{-1} TTX 15 min before perfusing the lumen, was not significantly different from the saline-injected control preparations (Table 1). TTX did not alter the rate of glucose absorption either (control, 4·43 ± 0·11; TTX, 4·72 ± 0·11 mg g^{-1} in 20 min, P = 0·11). Intra-arterial infusion of PGE_1 (2 μg min^{-1}) or VIP (0·8 μg min^{-1}) starting 5 min before and continuing for the duration of the jejunal

Table 1. Effect of TTX on fluid absorption and secretion induced by PGE_1 and VIP. Animals received 20 μg kg^{-1} TTX i.v. 15 min before perfusion of the jejunum or saline as control. PGE_1, VIP or saline as control were infused intra-arterially 5 min before jejunal perfusion and continued for the duration of the 20 min perfusion period. TTX did not alter the rate of fluid absorption (P = 0·45) but caused highly significant inhibition of both PGE_1- and VIP-induced secretion rates (n = 5 for all means, ± s.e.m., units μl g^{-1} in 20 min).

	Saline	PGE_1	VIP
Saline	216 ± 27	−82 ± 35	−396 ± 24
TTX	258 ± 46	+267 ± 26	−3 ± 25

perfusion both produced large net secretions. The values attained were near maximal responses achievable with each of these secretagogues (Coupar 1985). Intravenous injection with 20 μg kg^{-1} TTX 15 min before perfusion of the jejunum resulted in a total block of PGE_1-induced secretion and a large inhibition in the VIP-induced response (Table 1).

The dose of TTX used reduced mean systemic blood pressure from 127 ± 3 to 38 ± 0·9 mmHg. Also, contractions induced by transmural stimulation of jejunal segments taken from anaesthetized animals pretreated with TTX were significantly reduced compared with controls (Fig. 1). However, responses to acetylcholine were unaffected, the ED50 values being 0·33 (0·38–0·29, 95% confidence interval) in control, and 0·29 (0·38–0·19) μmol $litre^{-1}$ in segments taken from TTX-pretreated animals.

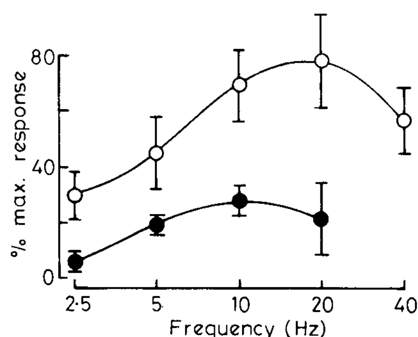

FIG. 1. Frequency-response curves to transmural electrical stimulation (supramaximal voltage, 1 ms pulse width, 10 s duration) in jejunal segments ex-vivo. TTX pretreatment (●) significantly decreased contraction compared with control (○) ($P < 0·05$) except at 5 Hz ($P = 0·097$, n = 4 for all means). The contractions are presumed to be cholinergic since they were blocked by atropine (0·1 μg ml^{-1}, n = 3) and are expressed as percent response compared with the maximum response obtained by acetylcholine (100%).

Discussion

It has been shown recently that TTX enhances Na^+ and Cl^- absorption from the rat isolated colon. This has been taken as evidence that nerves of the submucosal plexus keep the activity of the mucosa at a set point below its maximum absorptive capacity (Andres et al 1985). The results of the present study do not show that fluid absorption is any higher in the jejunum of anaesthetized rats pretreated with TTX. However, the major finding is that TTX blocks PGE_1- and inhibits VIP-induced intestinal fluid secretion. Although the dose of TTX used caused a large fall in mean systemic blood pressure, it is highly unlikely that this effect is causally related to the large reduction in fluid secretion for the following reasons. The toxin did not adversely affect the normal function of the jejunum since the absorption rates for both water and glucose were normal in TTX-treated animals. The level of the reduced blood pressure following injection of TTX is similar to that resulting from destruction of the CNS, yet fluid secretion, not absorption, occurs in the jejunum of pithed rats. This has been shown to be a result of PGE_2-like material release from the intestine, the amount of secretion being similar to that induced by PGE_1 in this study (Lee & Coupar 1982). Also, the vasodilator effect of TTX could result in the delivery of more of the secretagogues to the intestinal mucosa which would be expected to enhance, rather than reduce the secretory rates. The dose of TTX used in this study is not much above the LD50 value (8 μg kg^{-1} i.v. in mice, manufacturers information). It was also not totally effective in blocking cholinergic nerve transmission ex-vivo. However, it is conceded that some of the toxin could have washed from the tissue during the equilibration period.

The main action of TTX is to block sodium conductance in excitable tissues (Catterall 1980). However, it has been emphasized that TTX should not be used indiscriminately, since some nerves are resistant to its action while some non-neuronal tissues are blocked by it, such as cardiac and skeletal muscle and vascular smooth muscle (Narahashi 1974). Therefore, the results suggest either that TTX blocks nerve pathways resulting in the release of a substance that inhibits intestinal secretion or that it acts directly on the epithelial cells to prevent them secreting. A large number of VIPergic nerve fibres innervate the small intestinal epithelium of the rat (Schultzberg et al 1980) and prostaglandins are synthesized by the intestinal mucosa (Peskar et al 1981). Both secretagogues bind to epithelial cell membranes of the small intestine (Binder et al 1980; Beubler 1981; Tepperman & Soper 1981; Sarrieau et al 1983). The mechanism of intestinal fluid secretion is complex and not clearly understood. There are many secretagogues and several possible intracellular mediators of their action, but ultimately the process results in active Cl^- secretion (for further details, refer to Frizzell et al 1980). Further experiments are therefore required to elucidate the mechanism of the TTX antisecretory effect. Until such results are available interpretations of secretagogue action using TTX should be made with caution.

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Clonidine reduces plasma melatonin levels

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Clonidine, an α-adrenoceptor agonist, reduces human plasma melatonin levels when administered intravenously at 2–3 μg kg^{-1} to sleeping volunteers. Measurement of plasma melatonin levels after administration of clonidine could be the basis for a clinical in-vivo test of α-adrenoceptors.

In mammals, synthesis and secretion of melatonin by the pineal gland is initiated and maintained through stimulation of the β_1-adrenoceptors of the pinealocytes by noradrenaline released from peripheral postganglionic sympathetic neurons; this occurs mainly at night (Ariens Kappers 1960; Wurtman et al 1971; Deguchi & Axelrod 1972; Klein & Weller 1973; Parfitt et al 1976; Zatz et al 1976). Although the neurotransmitters and receptors remain to be identified, melatonin production also seems to be under peripheral sympathetic regulation in man, because: (i) bedtime administration of propranolol (120–140 mg p.o.) can block the night-time rise of plasma melatonin levels (Vaughan et al 1976; Hanssen et al 1977; Moore et al 1979; Lewy 1983), (ii) cervical cordotomy disrupts the nocturnal increase in human urinary melatonin levels (Kneisley et al 1978), and (iii) patients with sympatholytic diseases, such as Shy-Drager syndrome and idiopathic orthostatic hypotension, have markedly reduced plasma melatonin (Vaughan et al 1979) and urinary 6-hydroxymelatonin levels (Tetsuo et al 1981). Martin et al (1984) have reported decreased 6-hydroxymelatonin excretion in Korsakoff's psychosis. We now report reduction of human plasma melatonin levels after administration of clonidine, an α-adrenoceptor agonist.

Methods

Four healthy volunteer subjects (three males, ages 21–24 and one female, age 57) were studied after having given written consent and with the approval of the appropriate ethical committee. Clonidine (2·0, 2·5 or 3·0 μg kg^{-1}) was administered intravenously shortly after sleep onset (between midnight and 0200h). Blood was sampled through an indwelling venous cannula every 30 min between midnight and 0500h. Samples were also taken at these intervals on a baseline (placebo control) night after administration of saline. Subjects did not know which night they received clonidine or which night they received saline. Per cent reduction of plasma melatonin was determined by dividing the value obtained 1 h after infusion of clonidine by the value obtained at the same clock time on the control (baseline) night. In addition, the 24-year-old male subject received clonidine 2·7 μg kg^{-1} p.o. at 2350h on another occasion. Plasma melatonin was measured using the gas chromatographic-negative chemical ionization mass spectrometric assay of Lewy & Markey (1978). This assay uses a deuterated internal standard and has a high degree of specificity, accuracy and sensitivity.

Results and discussion

There was a highly linear dose-response relationship between the amount of clonidine administered and the reduction in the concentration of plasma melatonin 1 h later. One h after infusion of clonidine or saline, respectively (which was at the beginning of or within the nadir), plasma levels of melatonin were 65·5/82·5 pg ml^{-1} (79%) and 27·0/37·0 pg ml^{-1} (73%) after 2·0 μg kg^{-1}; 7·5/13·0 pg ml^{-1} (58%) after 2·5 μg kg^{-1}, and 13·4/35·5 pg ml^{-1} (38%) and 27·5/82·5 pg ml^{-1} (33·3%) after 3·0 μg kg^{-1}. Although the data points were few, the correlation coefficient (r = −0·99) was highly significant ($P < 0·01$).

Oral administration of clonidine to one volunteer at midnight also reduced melatonin secretion by between 20–50%, with a lag time of 1–2 h, a maximum difference at 4 h (50%) and a longer duration of action (5 h).

Peripherally, clonidine is a relatively selective agonist

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