Effects of prostaglandin $F_{2\alpha}$ on the contractile responses of guinea-pig ileum to electrical stimulation

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Summary. Prostaglandin $F_{2\alpha}$ affects the contractile responses of the guinea-pig ileum probably through the increased acetylcholine release from the postganglionic cholinergic terminals.

The effect of prostaglandins of the E type (PGsE) on the intestinal smooth muscle is related to the cholinergic system[1-4] while the role of prostaglandins of the F type (PGsF) on the cholinergic transmission is not clear. Kadlec et al.[5] have not found any effect of $PGF_{2\alpha}$ on the release of the cholinergic transmitter, but we have data to suggest effects of $PGF_{2\alpha}$ both on smooth muscle and on acetylcholine release[6-8]. The present study was performed on guinea-pig ileum and was designed to evaluate the effects of $PGF_{2\alpha}$ on electrically induced contractions and electrically - evoked acetylcholine release.

Materials and methods. Segments from the distal ileum of male guinea-pigs were used. The preparations were suspended in modified Krebs solution bubbled with 95% O_2 and 5% CO_2 at 36.5 °C.

Contractile activity. The isotonic contractions of the longitudinal layer of both non-stimulated (used for bioassay) and electrically-stimulated preparations were recorded by means of a strain gauge, type M1000B (Microtechna, Czechoslovakia). The preparations were loaded with 1 g.

Electrical stimulation. Field stimulation[9] was applied to the preparations. Two types of stimulation were used, intermittent and continuous. The intermittent stimulation (5 Hz, 0.4 msec[5,10], 3 impulses, submaximal current) was applied in trains with a frequency of 0.1 Hz. The continuous stimulation had the same parameters and lasted for 3 min. In this case the acetylcholine output was determined.

Determination of acetylcholine output. The acetylcholine (ACh) output was determined in 1 ml of modified Krebs solution from the bath (3 ml) with the stimulated preparations by a bioassay on a segment from the aboral ileum[9,11]. In order to increase the sensitivity and decrease the spontaneous activity of the assay-organ physostigmin (5 ng/ml) and morphine (10 µg/ml) were added to the bath. The ACh output was determined before and after $PGF_{2\alpha}$ treatment. The standard dose-response curves for ACh were plotted on the basis of 4–6 concentrations of ACh.

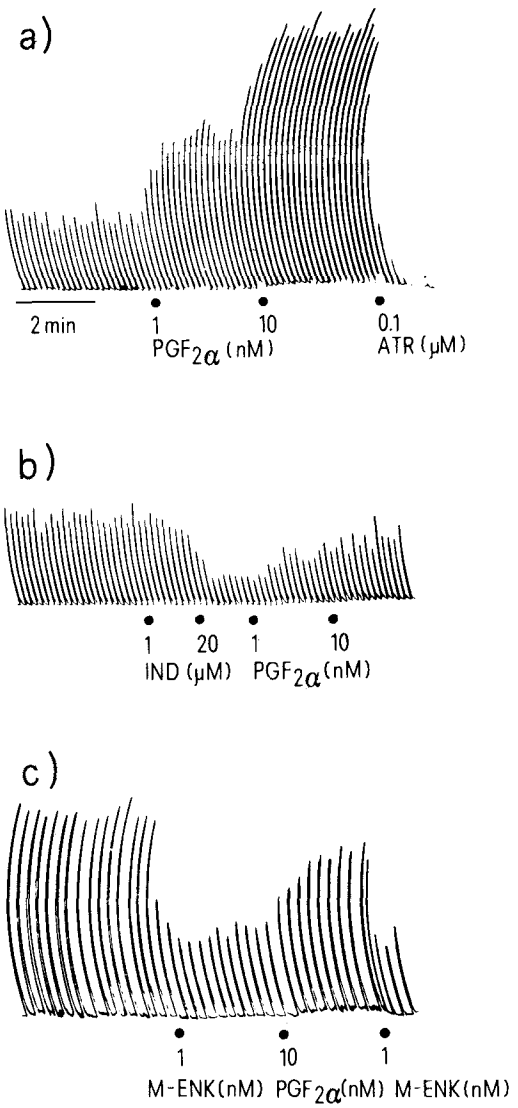
Drugs used. $PGF_{2\alpha}$-tromethamin salt, kindly supplied by Dr John Pike, Upjohn Co., Mich.; physostigminum salicylicum (Merck); morphine hydrochloride (Pharmachim); met-enkephalin (Calbiochem); indomethacin (Sigma); atropinum sulfuricum (Merck); acetylcholine chloride (Germed).

Results and discussion. The intermittent electrical stimulation elicited contractile responses of the longitudinal layer of the guinea-pig ileum. $PGF_{2\alpha}$ increased in a dose-dependent fashion the contractile amplitude, the maximum effect being observed at about the 2nd min. of the treatment. $PGF_{2\alpha}$ (1 nM) increased 1.95 ± 0.11 times the electrically-evoked response ($n=6$, $p < 0.01$). $PGF_{2\alpha}$ (10 nM) slightly increased the tone of the preparations and increased 2.63 ± 0.17 times the contraction amplitude ($n=6$, $p < 0.001$). Addition of atropine (0.1 µM) to the bath led to a complete inhibition of the contractile responses (fig. a).

The effect of $PGF_{2\alpha}$ was evaluated on the background of indomethacin and met-enkephalin. Indomethacin (1 µM, 20 µM) decreased in a dose-dependent way the contractions produced by intermittent electrical stimulation. Addition of $PGF_{2\alpha}$ to the bath decreased the inhibitory effect of indomethacin (fig. b). $PGF_{2\alpha}$ also restored the met-enkephalin (1 nM)-reduced contractions (fig. c).

The ACh output upon continuous stimulation varied with the different preparations. In all cases, however, the ACh output increased in the presence of $PGF_{2\alpha}$ (table).

It is known that the contractile responses of the guinea-pig ileum to electrical stimulation are due to ACh release from the postganglionic cholinergic nerve terminals[9]. In the



Longitudinal muscle layer of guinea-pig ileum. Contractile responses to intermittent electrical stimulation (5 Hz, 0.4 msec, 3 impulses, submaximal current, trains every 10 sec). Effects of $PGF_{2\alpha}$ and atropine (ATR) (a); indomethacin (IND) and $PGF_{2\alpha}$ (b); and met-enkephalin (M-ENK) and $PGF_{2\alpha}$ (c). The second drug was administered without washing out the first one. Designations: ●, drugs application.

present experiments $PGF_{2\alpha}$ produced a dose-dependent increase of the contractions upon intermittent stimulation as well as an increase of the ACh output in response to continuous stimulation. The fact that atropine abolished the response to electrical stimulation shows that the contractile response was mediated by acetylcholine, and the demonstration that $PGF_{2\alpha}$ increased the acetylcholine liberation indicates that its effect was central to the neuromuscular junction, most probably on the postganglionic neurone.

Table. Guinea-pig ileum. ACh output in pg/g/min in response to continuous electrical stimulation (5 Hz, 0.4 msec, submaximal current, 3 min) before and after $PGF_{2\alpha}$ 1 nM

Segments	1	2	3	4	5
Before $PGF_{2\alpha}$	3.35	3.00	4.92	3.26	7.4
After $PGF_{2\alpha}$	5.74	4.90	6.80	3.89	13.0

Modulation of the ACh release[1-3,5] nonspecific sensitization of the smooth-muscle membrane to ACh[4] has been proposed for explanation of the mechanism of action of PGsE in the guinea-pig ileum. The present data show an effect of $PGF_{2\alpha}$ on the cholinergic system too and suggest an action of $PGF_{2\alpha}$ central to the neuromuscular junction. The latter suggestion was supported by the finding that $PGF_{2\alpha}$ counteracted the effects of drugs which inhibited the postganglionic ACh release - indomethacin[1, 2] and metenkephalin[12]. Thus similar to the effects of PGsE[10], the $PGF_{2\alpha}$ action on the contractile responses of the guinea-pig ileum might be attributed to an increased ACh release.

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Dantrolene: evidence for effects on Na permeability properties of the nodal membrane[1]

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Summary. The effects of dantrolene on myelinated frog nerve fibers were studied in voltage clamp experiments. Dantrolene shifted the potential-dependent parameters describing Na^+ permeability towards more negative membrane potentials. The findings are interpreted as a change in the negative surface charge of the membrane.

Dantrolene-Na (DaNa) is a muscle relaxant and has been used for the treatment of spasticity[2]. Furthermore an exacerbation of paramyotonic muscle weakness by DaNa has been observed[3]. It has been assumed that DaNa reduces contraction of skeletal muscle either by decreasing the amount of Ca^{++} released from the sarcoplasmic reticulum[4-6] and/or from some presynaptic Ca^{++} stores[7]. No direct effects on neuromuscular transmission[8] or on the electrical properties of the skeletal muscle membrane[9] have been detected. On the other hand it has recently been reported that DaNa affected frog myelinated nerve fibers and that it increased their excitability[3]. We confirmed these results by performing voltage clamp experiments. We describe effects of DaNa on the nodal membrane which we interprete as an influence of the drug on fixed negative surface charges of the nerve membrane.

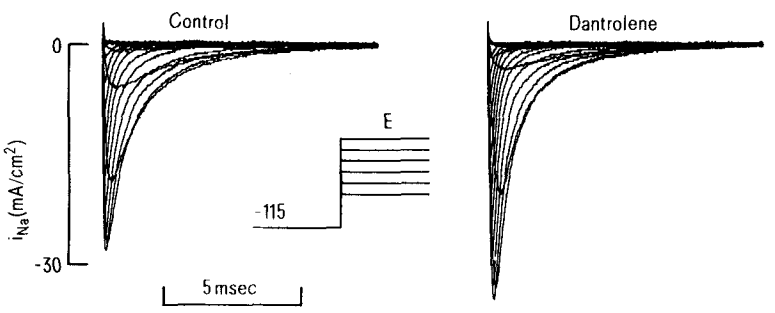

Figure 1. Membrane currents in Ringer (control) and in the presence of dantrolene-Na (saturated solution), recorded during depolarizing potential steps of increasing amplitude. Each pulse preceded by a 50-msec pulse of −115 mV. Corrected for leakage and capacity currents. K^+ currents were blocked by external TEA and internal CsCl.