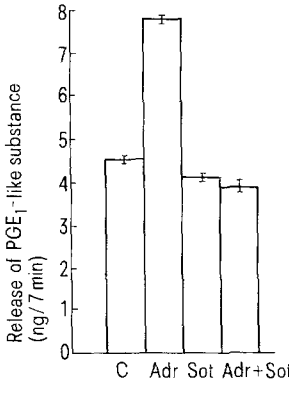
($p < 0.001$). Sotalol decreased ($p < 0.001$) the PGE_1-like activity slightly but significantly to 4.1 ± 0.36 ng ($n = 5$). Administration of adrenaline and sotalol in combination did not produce any significant alteration in the PGE_1 content in the effluent as compared to the control value ($p > 0.05$).

These results thus suggest that there was a basal efflux of PGE_1-like material which increased after the administration of adrenaline. There was a decrease in the efflux of PGE_1 after sotalol, a β-adrenoceptor blocking agent. In the presence of sotalol, adrenaline was unable to augment the PGE_1 content.

The present results extend the previous studies of Botting[6] who suggested that the PGE_1-like activity released from the isolated guinea-pig ileum during field stimulation was caused by the release of noradrenaline from intramural sympathetic nerves which acted on α- and β-adrenoceptors. Our finding that adrenaline increased the release of PGs suggests that β-adrenoceptor stimulation participates in PG release. Shaw and Ramwell[7] also postulated an interrelationship between sympathetic neurohumoral transmitter and PGs on the basis of findings that noradrenaline-stimulated lipolysis is associated with increased release of PGE_1-like substances. The increased amount of PGs released from rabbit heart by adrenaline can be regarded as an overflow resulting from an increase in PG production or from a decreased inactivation of PG. PG release in the heart may have an inhibitory regulatory function on sympathetic influence on the heart[3,8,9]. It is known that tissue damage of various kinds leads to PG formation[10]; this suggests that at least part of the continuous basal release of PGs is due to tissue damage. Sotalol decreased slightly the spontaneous efflux of PGE_1-like activity suggesting that endogenous noradrenaline accounted for only a small fraction of PG release in the non-stimulated heart.



The effect of β-adrenoceptor agonist and antagonist drugs on the output of prostaglandin (assayed against standard PGE_1) from perfusate of the isolated heart of rabbit. C, Control; Adr, adrenaline; Sot, sotalol; Adr, + Sot, adrenaline + sotalol.

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Effects of prostaglandin E_1 on $^{45}Ca^{++}$-incorporation and spike activity in longitudinal smooth muscle of cat jejunum

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Summary. Prostaglandin E_1 (0.3 µM) decreased both the $^{45}Ca^{++}$-incorporation and the spike activity in isolated longitudinal smooth muscle preparations of the cat jejunum probably by an inhibition on the Ca^{++} influx.

Several studies have linked the effects of prostaglandins of the E group with transmembrane calcium transport and intracellular calcium mobilization, leading to an increased or decreased Ca^{++} availability[1-3]. Our earlier investigations[4,5] have shown that the inhibitory effect of prostaglandin E_1 on smooth muscles of the cat jejunum depends on the Na^+/Ca^{++} ratio and the Ca^{++} concentration.

In this study we compared the effects of prostaglandin E_1 (PGE_1) on the $^{45}Ca^{++}$ incorporation with the PGE_1 effects on the calcium-dependent[6] generation of spike potentials in longitudinal smooth muscle of the cat jejunum.

Materials and methods. Strips from the proximal jejunum of male cats anaesthetized with chloralose (80 mg/kg) were used. The preparations were suspended in Krebs solution bubbled with O_2 at 36 °C. A radioautographic method was used for semiquantitative evaluation of the $^{45}Ca^{++}$ incorporation by the smooth muscle cells[7]. The electrical activity was recorded by pressure electrodes[8] and the mechanical activity by a strain gauge at initial tension of 1 g.

The effects of PGE_1 (0.3 µM) were observed under the following experimental conditions: A) in normal Krebs solution containing 2.5 mM calcium; B) in Krebs solution with increased calcium, 3.5 mM; C) in Krebs solution with decreased sodium (normal content of $Na^+ = 135.4$ mM), as 35% of sodium was substituted by sucrose; and D) on preparations pretreated with caffeine (0.5 mM) in normal Krebs solution. $^{45}Ca^{++}$ was added as a label (2 µCi/ml; 10 nM/ml) and after 5 min exposure the smooth muscle tissue was prefixed in glutaraldehyde (2.25% in 0.1 M cacodylate buffer, pH 7.2, 4 °C, for 4 h) and postfixed in osmium tetroxide (2% in 0.1 M cacodylate buffer, for 2 h). The silver grains (s.g.) in the radioautograms were counted in an area of 1000 µm² for assessment of the $^{45}Ca^{++}$ incorporation. The changes in the number of silver grains as well as slow waves with spike potentials (s.w.) per min were followed before and at the 5th min of the PGE_1 treatment. The data from groups B, C and D were compared statistically with the data from group A using Student's t-test.

Effects of PGE_1 (0.3 μM) on the $^{45}Ca^{++}$ incorporation and spike activity in isolated longitudinal smooth muscle of cat jejunum: A) normal Krebs solution with 2.5 mM Ca^{++}; B) Krebs solution with 3.5 mM Ca^{++}; C) Krebs solution with 35% of sodium substituted by sucrose; and D) caffeine pretreated preparations in normal Krebs solution. Designations: s.g./1000 μm^2 - silver grains in radioautograms; s.w./min - slow waves

Solutions	No. of experiments		Control $^{45}Ca^{++}$ incorporation s.g./1000 μm^2	Control spike activity		PGE_1 (0.3 μM)-treatment		
	$^{45}Ca^{++}$ incorporation	Spike activity		s.w./min total	s.w./min with spike potentials	$^{45}Ca^{++}$ incorporation s.g./1000 μm^2	s.w./min total	s.w./min with spike potentials
A	8	9	0.31±0.02	11.3±0.6	9.1±0.5	0.11±0.01*	11.5±0.7	2.2±0.5*
B	8	8	0.38±0.01	10.8±0.8	9.3±0.7	0.32±0.04*	10.8±0.8	4.6±0.3*
C	8	8	0.42±0.01	8.6±0.4	7.6±0.3	0.24±0.02*	7.3±0.2	5.3±0.3*
D	8	12	0.34±0.02	11.2±0.7	9.1±0.7	0.18±0.01*	11.1±0.7	5.4±0.4*

* Statistically significant difference ($p < 0.05$). Means ± SEM are presented.

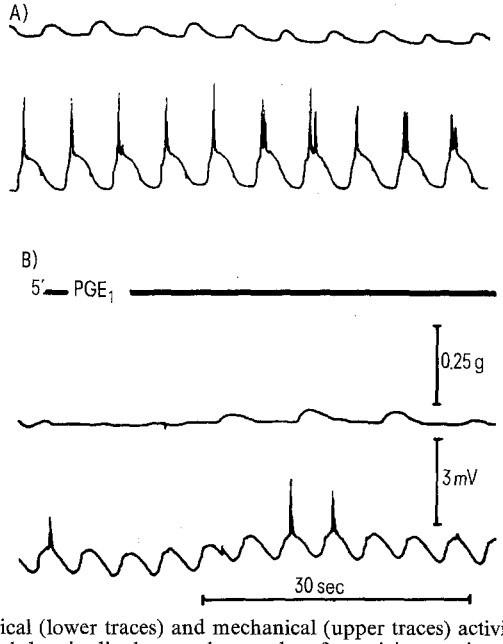
Drugs used. PGE_1 was kindly provided by Dr J. Pike, Upjohn Co., Kalamazoo, Mich.; $^{45}Ca^{++}$, Radiochemical Centre, Amersham, U.K., and Caffeinum purum from Merck.

Results and discussion. The $^{45}Ca^{++}$ incorporation in the longitudinal smooth muscle of the cat jejunum in normal Krebs solution was 0.31 ± 0.02 s.g./1000 μm^2 smooth muscle tissue. Spike potentials accompanied 9.1 ± 0.5 s.w./min of the total number of 11.3 ± 0.6 s.w./min.

After PGE_1 treatment, the $^{45}Ca^{++}$ incorporation decreased nearly 3 times and the number of slow waves with spike potentials was markedly reduced (figure).

The increase of calcium to 3.5 mM keeping specific radioactivity constant or the substitution of 35% of sodium by sucrose enhanced the $^{45}Ca^{++}$ incorporation and decreased the inhibitory effect of PGE_1. The enhancement of the $^{45}Ca^{++}$ incorporation in the smooth muscle corresponded to an increase of the number of the slow waves accompanied by spike potentials.

The effect of PGE_1 on the $^{45}Ca^{++}$ incorporation was decreased to a smaller degree in caffeine-pretreated preparations. Less pronounced was also the inhibitory effect on the spike potential generation (table).



Electrical (lower traces) and mechanical (upper traces) activities of isolated longitudinal smooth muscle of cat jejunum in normal Krebs solution: *A* Spontaneous activity; *B* the 5th min of PGE_1 (0.3 μM)-treatment.

It is well known that the calcium influx plays an important role in the generation of spike potentials in the intestinal smooth muscle. In response to PGE_1 there occurred an inhibition of the spontaneous spike generation in the longitudinal smooth muscle of the cat jejunum. This phenomenon was linked with the considerable decrease of the $^{45}Ca^{++}$ incorporation.

The effects of PGE_1 were shown to depend on Ca^{++} concentration[9] and as shown by Michibayashi[10] PGE_1 exerted an effect on the transmembrane transport of calcium. In the present experiments, the inhibitory effects of PGE_1 on the $^{45}Ca^{++}$ incorporation and on the generation of spike potentials were much decreased with the increase of calcium or the decrease of sodium in the nutrient medium, i.e. when similar to other observations[11,12] the Ca^{++} influx into the smooth muscle cells was increased. There are some differences in PGE_1 effects in these 2 groups. They might be due to changes in membrane saturable system for calcium[13] produced by the absolute or relative increase of calcium concentration in the extracellular medium. The effects of PGE_1 were also decreased after caffeine which activated the release of membrane-bound calcium and enhanced the membrane permeability to sodium[14]. However, our experiments with $^{45}Ca^{++}$ incorporation suggest that there also occurs an increase of Ca^{++} influx. Thus, as in other smooth muscles, the PGE_1 effects on the longitudinal muscle of the cat jejunum probably depend on intracellular calcium availability, but the effects of PGE_1 on $^{45}Ca^{++}$ incorporation and spike activity suggest an inhibitory action of PGE_1 on calcium influx also.

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