Prostaglandin-like substances in *Propionibacterium acnes*. IV. Effect on isolated human vessels

E. Berlin, L. Hellgren, O. Thulesius and J. Vincent

Department of Clinical Physiology, Central Hospital, Växjö (Sweden), and Department of Dermatology, University of Trondheim, Trondheim (Norway), 2 May 1979

Summary. The present study demonstrates a powerful vasoconstrictor activity of prostaglandin-like substances (PLS), extracted from *P. acnes*, on human blood vessels. PLS is about equipotent to PGE_2 in its effect on human umbilical vessels, but the contractile response pattern is different. PLS therefore seems to have specific and different physiological characteristics.

The importance of various metabolites in *Propionibacterium acnes* as possible inflammatory mediators is unclear. Recently, prostaglandin-like substances (PLS) of the E-type have been isolated from the lipid fraction of *P. acnes*[1]. In a gerbil colon bioassay[2] as well as in vivo (hamster cheek pouch)[3], these compounds mimic E-prostaglandins. In a human utero-tubal junction bioassay where the muscle layers react differently to diverse prostaglandins, the effect of PLS was similar, although not identical with that of PGE_2[4]. Furthermore, PLS increases the cyclic AMP levels in rat ovaries approximately 2-fold[5] and possesses chemotactic properties[6].

The purpose of the present investigation was to test whether PLS evoked measurable physiological responses on human vessels, specifically umbilical arteries, which are especially sensitive to PGE_2-stimulation[7,9].

Vascular preparations were obtained during surgery of varicose veins on the lower extremities. Short vessel segments were taken from branches of the saphenous vein, which seemed to have retained the normal structure. Also, human umbilical arteries and veins were freshly obtained from the maternity ward.

The specimens were prepared by dissection, removing connective tissue. Small helical pieces were cut from each vessel and the preparation suspended in an organ bath with a capacity of 20 ml and filled with aerated Krebs-Ringer solution (95% O_2 + 5% CO_2). Isometric tension was recorded and drugs added with the aid of micropipets. Tension values were calculated per unit transsectional area of the preparation. Further details about the experimental procedure and calculations can be obtained from a recent publication[8]. The procedure of extraction and recovery of PLS from the lipid fraction of *P. acnes* was described earlier[1]. PGE_2 was used as a reference substance in order to compare the biological activity of PLS. As an indication of smooth muscle contractile response with different agonists we have calculated the kinetic parameter dT/dt (differential tension versus time) during the contraction with an ED_{50}-dose (half the dose eliciting maximal contraction).

In all preparations tested PLS administered to the organ bath induced contraction of the vascular preparation (increased tone). The results from 10 experiments are summarized in the table. As can be seen from these data as well as from figure 1, PLS is a potent smooth muscle stimulant. The dose response curve of PLS closely resembles that of PGE_2. The slightly higher maximal tension elicited after PGE_2 compared to PLS was not significant. PLS elicited much higher tension in umbilical arteries as compared to the saphenous veins; the opposite holds for noradrenaline. PLS after a short latency induces a forceful and stable increase in tension.

The kinetics of the response pattern of PLS, PGE_2 and noradrenaline are shown in the table and in figure 3.

The umbilical artery is a very useful preparation for bioassay of prostaglandins, since it has been postulated that these substances may be involved in the physiological closure of the umbilical vessels after birth[9]. The present investigation confirms previous results showing that PLS extracted from *P. acnes* has a biological activity closely resembling that of PGE_2 on human vessels. The most

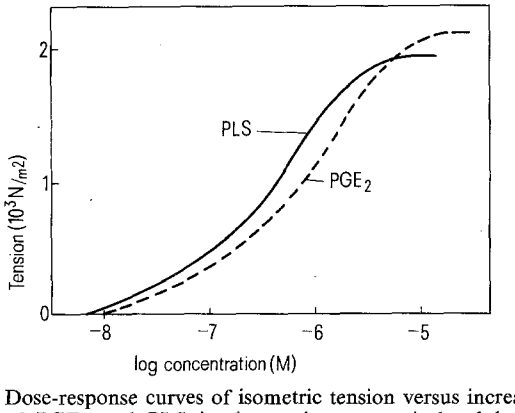

Fig. 1. Dose-response curves of isometric tension versus increasing doses of PGE_2 and PLS in 6 experiments on isolated human umbilical arteries (PLS-concentration based on bioassay studies using gerbil-colon).

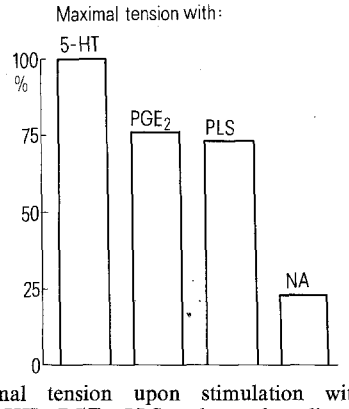

Fig. 2. Maximal tension upon stimulation with 5-hydroxytryptamine (5-HT), PGE_2, PLS and noradrenaline (NA), (human umbilical arteries).

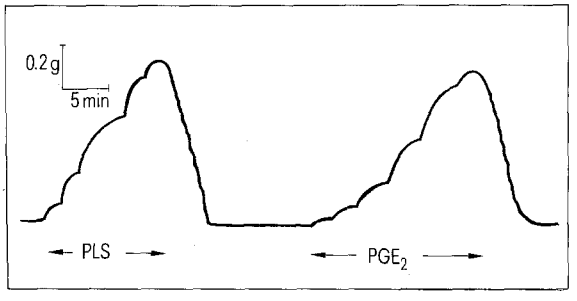

Fig. 3. Dose-response curves for PLS and PGE_2. Note rapid onset of tension in first part of the curve.

forceful increases in smooth muscle tone after administration of PLS in vitro were obtained with umbilical arteries. The responses were less pronounced in veins from the lower extremities. The contraction could not be blocked with pentholamine.

It is not surprising to find a close resemblance when comparing the dose-response relationship of PLS and PGE_2 since the doses of PLS were determined with reference to PGE_2, the only difference being the target organ. We could, however, note specific characteristics regarding the kinetics of the response with shorter latency periods and faster contractions elicited by PLS which makes it reasonable to assume that PLS is not identical with PGE_2.

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Some factors affecting Malpighian tubule fluid secretion and transepithelial potential in *Locusta migratoria* L.

J.H. Anstee, D.M. Bell and D. Hyde

Department of Zoology, University of Durham, Durham DH13LE (England), 3 May 1979

Summary. Both fluid secretion and transepithelial potential were stimulated by cAMP. Fluid secretion was unaffected by 5-HT over the concentration range 10^{-8}–10^{-4} M. The presence of ouabain in the bathing medium effected a decrease in transepithelial potential.

Ramsay[1] demonstrated that the transepithelial potentials across insect Malpighian tubules, irrespective of polarity, did not obey the Nernst equation for K^+. On this basis he proposed that active K^+ transport was taking place across the Malpighian tubules. Subsequent studies suggest that cation transport is the 'prime mover' in fluid secretion by insect Malpighian tubules[2]. The rate at which *Carausius* and *Rhodnius* tubules secrete fluid is considerably stimulated by the presence of 5-hydroxytryptamine (5-HT) in the bathing medium[3]. Similarly, cyclic 3′, 5′-adenosine monophosphate (cAMP) was found to stimulate secretion by the tubules of both species. It was concluded that 5-HT and the diuretic hormones may interact with the Malpighian tubule cells of *Rhodnius* and *Carausius* at specific sites, probably on the cell membrane facing the haemolymph. As a result of this, secretion is induced, possibly through the action of intracellular cAMP produced as a response to 5-HT. In contrast, *Schistocerca* tubules, whilst stimulated by cAMP, are insensitive to 5-HT[4]. The present study has been carried out to determine the effect of cAMP and 5-HT on fluid secretion and the transepithelial potential in *Locusta*. In addition, the effect of ouabain on the transepithelial potential has been examined to ascertain the role of Na^+/K^+-activated ATPase in maintaining ion transport.

Materials and methods. Mature adult *Locusta migratoria* L. were used. In vitro measurements of fluid secretion by Malpighian tubules were carried out as described previously[5]. The secretion rate for each tubule was determined by measuring the diameter of the secreted droplet at 5-min intervals over a period of 30 min. At the end of this time, the 'normal' Ringer solution was replaced by a fresh solution which had either the same (control) or a different composition (experimental). The rate of secretion was redetermined over the next 60 min.

The transepithelial potential (PD) of actively secreting tubules was measured with KCl/agar bridges connected via 3 M KCl to 2 Calomel half cells. A high input impedance amplifier with a gain of 10× was used. The PD was measured by placing the recording electrode in contact with the lumen and the reference electrode in contact with the outside of the tubule. The amplifier output was adjusted to zero with the KCl/agar bridges in the same Ringer pool. The PD was displayed and recorded on a flat-bed recorder (Servoscribe). Initially, the PD was measured with the tubules bathed in normal Ringer solution. Continuous recordings were taken for 15 min to ensure that the PD was stable. The normal Ringer solution was then changed for a fresh solution of the same (control) or different (experimental) composition. The PD was then recorded continuously over the next 50 min. The temperature was maintained at 30 °C throughout. The 'normal' Ringer solution had the following composition (mM): NaCl 129; KCl 8.6; $MgCl_2$ 8.5; $CaCl_2$ 2; $NaHCO_3$ 10.2; NaH_2PO_4 4.3; glucose 34; pH 7. All solutions were made up in glass distilled, deionized water. All inorganic salts were AnalaR grade or the best commercially available. Ouabain, cAMP and 5-HT were obtained from the Sigma Chemical Co.

Results. The effects cAMP on fluid secretion are shown in table 1. Stimulation was most marked with 10^{-3} M cAMP in the bathing medium and the threshold was between 10^{-4} and 3×10^{-4} M. Some stimulation was noted almost immediately following the addition of cAMP although maximum stimulation was observed some 10–20 min later. Thereafter, the rate of secretion returned to approximately its pre-stimulated level. In contrast to this, no significant stimulation of fluid secretion was observed by the inclusion of 5-HT in normal Ringer solution over the concentration range 10^{-8}–10^{-4} M.

The presence of 10^{-3} M cAMP in the normal Ringer solution effected an increase in lumen positivity from $+8.8\pm1.6$ mV to $+14.1\pm2.0$ mV ($n=14$). Application of a paired t-test indicates that this difference is significant ($p<0.01$). In contrast, control tubules, maintained in normal Ringer solution throughout, showed a slight decrease in lumen positivity from $+9.5\pm1.0$ to $+8.2\pm3.0$ mV ($n=7$). As was observed with studies on fluid secretion, the effect of cAMP on PD was gradual; approximately 16 min elapsing before a new stable potential was established. On return to normal Ringer solution, the PD showed a substantial return to the value observed prior to cAMP addition.

When normal Ringer solution was replaced by normal Ringer solution containing 10^{-3} M ouabain, the PD changed