mals per cage). Each animal was placed in the open-field apparatus and observed for 10 min using a video monitor system. Marking behavior was assessed by counting the number of ventral rubs to the pegs. The marking behavior was tested 1 day before and 30 days after isolation. 1 h after the last observation animals were sacrificed by decapitation. The brain was quickly removed and 8 regions - cortex, amygdala, striatum, hypothalamus, midbrain, hippocampus, olfactory bulbs, and pons plus medulla oblongata - were separated on an ice-cold glass plate. For the enzyme assays, tissues were homogenized in 5 mM Tris buffer containing 0.2% Triton X-100. Acetylcholine esterase (ACE) activities were determined at 37 °C by the spectrophotometric method of Ellman et al.[10] using 10^{-5} M of iso-OMPA. Choline acetyltransferase (CAT) activities were determined by the radiochemical micromethod of Fonnum[11] using [^{3}H]-acetylcoenzyme A as a substrate. Protein content was measured according to the method of Lowry et al.[12].

Results and discussion. After isolation housing, there was a significant difference ($p < 0.05$, Mann-Whitney U-test) in the frequency of marking between the isolated (22.5 ± 8.7) and aggregated groups (12.7 ± 6.7). ACE activity in the hippocampus was significantly higher in the isolated group than in the aggregated group ($t = 2.56$, $p < 0.05$, t-test), while there were no significant differences between the 2 groups in ACE activities of the other brain areas. The isolated group exhibited significantly high CAT activity in the hypothalamus as compared with the aggregated group ($t = 2.69$, $p < 0.02$, t-test). Essman[7] has reported that isolated mice exhibited a significant reduction in cortical bound acetylcholine (ACh) and a significant elevation in free cortical ACh as compared with aggregated mice. We also found previously that rats which manifested mouse-killing behavior following prolonged isolation showed higher ACh content in the diencephalon[8]. It would appear that determinations of the brain ACh dynamics can assist the understanding of scent marking behavior in gerbils. The results of the present study tend to support the notion that the brain cholinergic system may participate in the mediation of isolation-induced behavioral change.

1 The authors are indebted to Dr Masaru Sorimachi of the Department of Physiology, Ehime University, for pertinent advices and professional assistance in radiochemical assay.
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Prostaglandin-like substances in *Propionibacterium acnes*. V. Activity profiles using cascade superfusion bioassay and platelet aggregation

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Summary. The activity spectrum of prostaglandin-like substances (PLS) from *P. acnes* was investigated with cascade superfusion technique and by platelet aggregation assay. The biological activity of PLS resembles that of PGI_2: both relax bovine coronary artery, rabbit mesentric and coeliac arteries; both contract the rat stomach strip as well as both typically inhibit spontaneous movements of isolated guinea pig ileum. Also, similarly to PGI_2, PLS inhibits platelet aggregation regardless the inducer used. However, PLS possesses a specific antiaggregatory pattern on platelet, which indicates that these compounds are not indentical with primary prostaglandins or PGI_2.

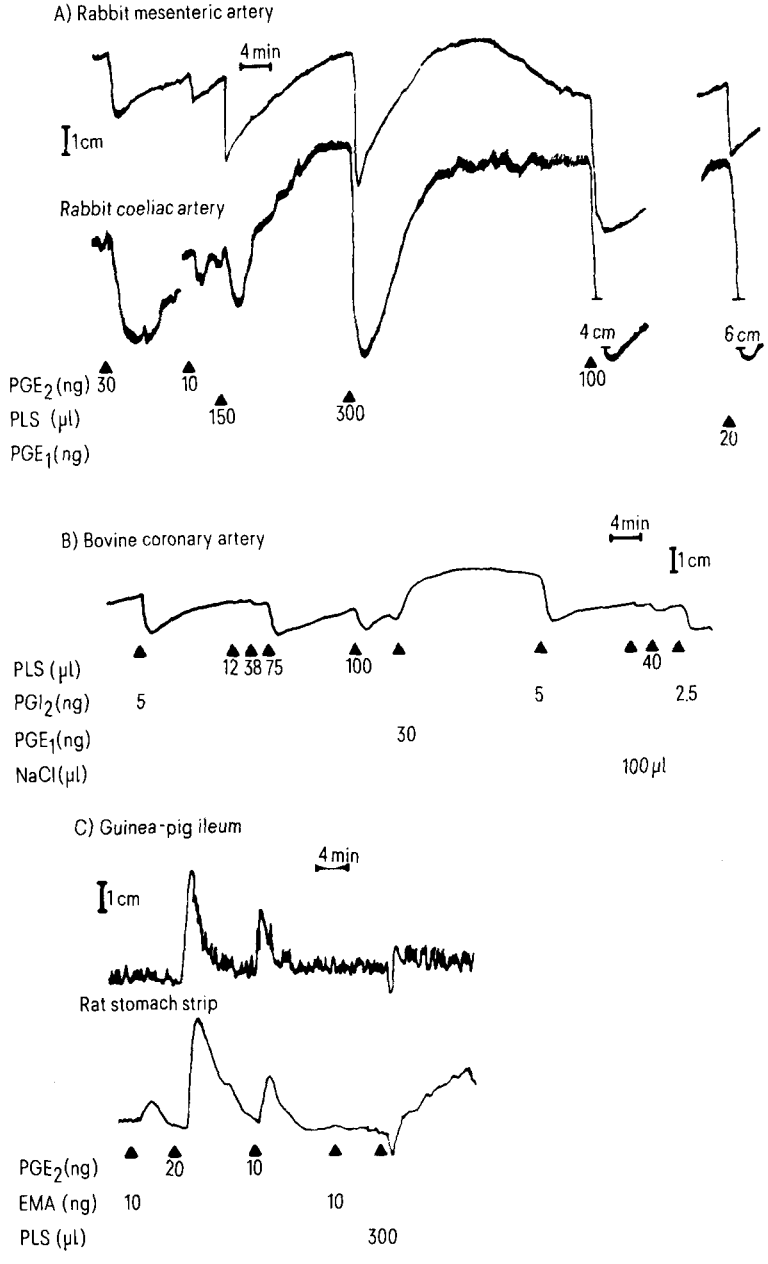
There is sufficient evidence supporting the fundamental role of *Propionibacterium acnes* in the development of inflammatory acne lesions. In a series of investigations we have paid particular attention to characterizing the prostaglandin-like substances (PLS) from the lipid fraction of this diphteroid. Our findings show a distinct biological activity associated with PLS. In various muscle preparations i.e. gerbil colon[1], strips of human Fallopian tube[2] or human vessels (umbilical arteries, vena saphena)[3] PLS mimic prostaglandins of the E-type. However, in spite of similarities, especially with PGE_2, PLS possess some specific properties. Thus PLS induce a significant increase in the cyclic AMP content in rat ovary[4] and act as potent chemoattractants[5]. Also the chemical analyses with reversed phase chromatography and gas chromatography-mass spectrometry demonstrated that PLS were not identical with PGE_2[6]. This paper describes new data concerning the biological activity of PLS studied by the cascade superfusion technique completed with a platelet aggregation assay.

Material and methods. Cascade superfusion bioassay. PLS were isolated from *P. acnes* and purified according to previously reported procedures[7]. The final sample (biological activity ~ 200 ng PGE_2 equivalent, gerbil colon bioassay) was dissolved in saline and tested (~ 20 µl) on rabbit mesenteric and coeliac arteries and aorta; on bovine coronary arteries, rat stomach strip, guinea-pig ileum and lung parenchymal strips[8] superfused in cascade[9,10]. Superfusion was performed using Krebs bicarbonate buffer (5 ml/min, 37 °C) containing the following antagonists: mepyramine (0.1 µg/ml), atropine (0.1 µg/ml), phenoxybenzamine (0.1 µg/ml), methysergide (0.2 µg/ml), indomethacin (1 µg/ml), and propranolol (2 µg/ml). Biological activity of PLS was compared with that of PGE_2, $PGF_{2\alpha}$, PGI_2 and (15S)-hydroxy-11,9-(epoxymethano)prosta-5Z,13-dienoic acid

(EMA). The stock solution of PGI_2 was prepared in 0.1 M Tris buffer, pH 9.0. Experimental solutions of PGI_2 were prepared in ice cold Krebs buffer or 0.05 M Tris buffer, pH 8.2 and used immediately.

Platelet aggregation assay. Rabbit arterial blood (cardiac puncture) was withdrawn into tubes containing trisodium citrate (final concentration: 0.38%). Following centrifugation (250× g for 8 min) platelet-rich plasma (PRP) was collected and used for platelet aggregation. Platelet aggregation was performed in 1-ml cuvettes using an Upchurch aggregometer (37 °C; magnetic stirring: 1100 rpm). The light transmittance (%) was set at 0 for PRP and 100 for the platelet-poor plasma which was obtained after centrifugation of citrated blood at 1200× g for 15 min. Following incubation of PRP for 3 min (37 °C) the substances to be tested, or their respective controls (i.e., 0.05 M Tris, PGI_2 dissolved in Tris, physiological saline, PLS dissolved in physiological saline, were added to PRP for 1 min in volumes not exceeding 20 µl. Aggregation was then induced with ADP (Sigma) or collagen (Horm). For controls an extract from the nutrient substrate without the bacteria, prepared identically, was used. These control samples were found to be devoid of smooth muscle or anti-aggregatory activities.

Results and discussion. The present investigations have revealed that PLS relaxes rabbit mesenteric and coeliac arteries. This effect is qualitatively similar to the relaxation produced by either PGE_1 or PGE_2 (figure, A). However, the figure, B, shows that PLS similarly to PGI_2 but unlike PGE_1 or PGE_2 (not shown) relaxes bovine coronary artery strip. The response of isolated guinea-pig ileum to PLS, if any, is very weak, which contrasts with the strong contraction induced by PGE_2 (figure, C). Qualitatively the response of rat stomach strip to PLS differs from that to EMA or PGE_2 (figure, C); PLS induces relaxation followed by sustained contraction. Up to 300 µl of PLS did not affect



Poligraph record of 3 bioassay experiments with prostaglandin-like substances (PLS) from *P. acnes*. Detector organs (as stated) were superfused in cascade with Krebs bicarbonate buffer (5 ml/min).

the tonus of isolated guinea-pig lung parenchymal strip. Among the smooth muscle preparations tested, the most sensitive to PLS was found to be isolated, helically cut strips of rabbit arteries.

Based on the present findings the biological activity of PLS differs from that of PGE_2 or PGE_1 which eliminates the possibility that PLS could be one of the E-prostaglandins or a mixture of them, which confirms our previous findings. The observed spectrum of activity of PLS resembles that of PGI_2[10]; both relax bovine coronary artery and rabbit mesenteric and coeliac arteries; both contract the rat stomach strip and both typically inhibit spontaneous movements of the isolated guinea-pig ileum. Therefore, to check the possibility that PLS could be identical with PGI_2, their effects on platelet aggregation induced either by ADP or collagen have been determined.

PLS, similarly to PGI_2, inhibits platelet aggregation regardless of the inducer used. However, a few distinctions between the anti-aggregatory activity of PLS and that of PGI_2 have been noted. One is that the anti-aggregatory activity of PLS is heat resistant, i.e., it is not destroyed by immersion in a 100 °C water bath for 15 sec. Secondly, the anti-aggregatory activity of PLS is preferentially directed against collagen. When 10 µM of ADP is used to induce platelet aggregation, the IC_{50} for PLS is between 70–80 µl/ml; and when 5 µg of collagen per ml of PRP is used to induce platelet aggregation the IC_{50} for PLS is between 0.5–2.0 µl/ml. It therefore seems that the anti-aggregatory activity of PLS against collagen is about 70 times higher than that against ADP. Third, there is a quantitative difference between the de-aggregatory activities of PLS and PGI_2 (de-aggregation is defined as the light transmittance decrease, correlated to the amount of PGI_2 added at the height of collagen-induced aggregation). In that respect PLS is about 10 times weaker than PGI_2.

The prostaglandins and related compounds constitute a complex system both regarding their effects as smooth muscle stimulants and their ability to enhance or else inhibit platelet aggregation. This study indicates that PLS is not identical with primary prostaglandins or PGI_2. The present data confirm the powerful and specific biological activity of these compounds. Moreover, PLS possess a unique pattern in their anti-aggregatory effect on platelets. It seems that PLS constitute a new type of bacterial metabolite. However, how these substances contribute on the inflammatory sequelae of acne vulgaris remains to be determined.

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The control of amygdaloid seizures by the globus pallidus[1]

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Summary. Both in acute and chronic cats entopeduncular stimulation inhibits, to a greater extent than caudate activation, focal paroxysmal activity in the ventro-basal complex of the amygdala. Lesion of entopeduncular neurons, by means of kainic acid injection, induces a decrease of the caudate inhibitory effect. It is suggested that neostriatal control of the amygdaloid seizures occurs partly through the globus pallidus.

It has been shown previously that caudate conditioning stimulation controls localized seizures in the amygdala, hippocampus and temporal cortex[2–5]. As regards the anatomical pathways through which the caudate nucleus may influence the activity of the amygdaloid neurons, no direct anatomical connection between neostriatum and amygdaloid complex has been demonstrated, whereas it is known that caudate efferent fibres project both to the substantia nigra and the globus pallidus[6]. In order to study the role of the globus pallidus in the control of focal paroxysmal activity of the amygdala, an experiment has been performed in which the effects of both stimulation and lesion of this structure have been observed. Preliminary accounts of these results have been presented[7].

Material and methods. The experiments were performed on 8 'encéphale isolé' cats with local anaesthesia of painful points and on 5 cats with chronically implanted electrodes. Focal paroxysmal activity in the ventro-basal complex of the amygdala has been obtained by repetitive stimulation of the contralateral nucleus (A 10–12, L 8–10, H 3–5)[8] or by activation of the ipsilateral pyriform cortex. Stimulation parameters changed between 20–60 c/sec; 3–5 sec; 0.2–3.5 mA; 0.1–1 msec (duration of single shock). Conditioning stimulation of the caudate (A 15–17, L 4–6, H 15–17) and entopeduncular nucleus (A 10–12, L 5–7, H 7–9), which in the cat corresponds to the internal pallidal segment of the primates, was performed by a bipolar method with coaxial electrodes (external diameter 0.5 mm, tip 25–50 µm), obliquely oriented in order to avoid lesions of the internal capsule. This stimulation immediately preceded the test stimulus apt to evoke the amygdaloid AD. Striatal nuclei, ipsilateral to the amygdaloid recording electrodes, have been stimulated with trains varying between 2 and 5 sec; 30–80 c/sec; 0.2–3 mA; 0.1–1 msec. Injection of kainic acid (0.5–1.5 µg in 0.5–1.5 µl, phosphate buffer, pH 7.4) into the entopeduncular nucleus was made by means of a Hamilton microsyringe. The position of the electrode tips, and the lesion of the entopeduncular nucleus induced by kainic acid injection, were controlled on serial Nissl sections.

Results and discussion. Stimulation of the ventro-basal complex of the amygdala evoked in the contralateral nucleus a focal paroxysmal activity (after-discharge, AD),