

optimized for processing both spatial position and spatial frequency information and that to regard them as spatial frequency analyzers is stress one side of their function at the expense of the other.

- 1 We thank Dr S. Marčelja for much helpful discussion.
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## Effects of later isolation housing on both scent marking behavior and brain cholinergic activities in Mongolian gerbils

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**Summary.** Significant difference in the frequency of marking was found between aggregated and isolated gerbils. As compared with the aggregated gerbils, isolated gerbils showed high acetylcholinesterase activity in the hippocampus and high choline acetyltransferase activity in the hypothalamus.

The Mongolian gerbil has recently been introduced into physiological and behavioral research. The gerbil marks low-lying objects in the environment by rubbing them with a midventral sebaceous scent gland. Thiessen and his colleagues<sup>2,3</sup> have indicated that the scent marking behavior is closely related to territoriality and dominance status. Brain and Benton<sup>4</sup> have proposed that in mice and rats individual housing alters their territorial dominance. It has also been reported in gerbils that marking behavior was affected by housing conditions<sup>5,6</sup>. In mice and rats, several investigators<sup>7-9</sup> have suggested that the central cholinergic system may play an important role in the mediation of isolation-induced behavioral changes. Little information, however, is available concerning the brain mechanisms of scent marking in the gerbil. This study was conducted to investigate changes in brain cholinergic activities and scent marking behavior in gerbils following later isolation housing.

**Materials and methods.** Male Mongolian gerbils (*Meriones unguiculatus*) weighing between 60 and 70 g were used. All the animals had free access to food and water. The temperature in the vivarium was maintained at  $23 \pm 1^\circ\text{C}$ , and the light-dark cycle (light on at 07.00 h, off at 19.00 h) was kept constant. The open-field apparatus was constructed of gray plexiglass (the size of the floor was  $60 \times 60$  cm, and enclosed by walls 46 cm high), except for a clear side panel which allowed observation from a lateral view. 6 pegs, made from clear plexiglass (2.5 cm long, 1.2 cm wide, and 0.6 cm high), were attached to the floor at regular intervals. Animals were weaned between 23 and 25 days after birth and reared with littermates. At 80 days of age male gerbils were randomly assigned to 2 groups: an isolated ( $n=8$ ) and an aggregated group ( $n=8$ , in 2 groups of 4 each). The isolated gerbils were housed in a isolation cage ( $30 \times 19 \times 13$  cm metal cage), while the aggregated gerbils were housed communally in a  $42 \times 25 \times 15$  cm polycarbonate cage (4 ani-

Table 1. Effect of later isolation housing on brain acetylcholinesterase activity in Mongolian gerbils (nmoles acetylthiocholine hydrolyzed/min/mg protein)

Brain areas	Aggregated group (n=8)	Isolated group (n=6)
Cortex	40.05 $\pm$ 3.18	41.39 $\pm$ 2.12
Striatum	534.62 $\pm$ 62.84	515.91 $\pm$ 61.67
Amygdala	104.51 $\pm$ 6.28	109.86 $\pm$ 7.72
Hypothalamus	120.26 $\pm$ 1.59	121.03 $\pm$ 2.88
Midbrain	151.50 $\pm$ 5.60	153.66 $\pm$ 6.25
Hippocampus	64.77 $\pm$ 3.65	69.45 $\pm$ 2.99*
Olfactory bulbs	40.75 $\pm$ 2.48	41.58 $\pm$ 1.54
Pons+ medulla oblongata	122.92 $\pm$ 4.81	126.19 $\pm$ 5.13

Each value is shown as mean  $\pm$  SD. \* Significance was evaluated by means of the 2-tailed Student's t-test comparing the isolated group with the aggregated group ( $p < 0.05$ ).

Table 2. Effect of later isolation housing on brain choline acetyltransferase activity in Mongolian gerbils (nmoles acetylcholine synthesized/h/mg protein)

Brain areas	Aggregated group (n=8)	Isolated group (n=6)
Cortex	36.56 $\pm$ 2.84	37.20 $\pm$ 2.47
Striatum	223.49 $\pm$ 21.69	214.60 $\pm$ 25.89
Amygdala	99.47 $\pm$ 5.59	102.58 $\pm$ 8.84
Hypothalamus	53.95 $\pm$ 4.02	59.77 $\pm$ 4.00*
Midbrain	100.59 $\pm$ 4.92	95.91 $\pm$ 9.18
Hippocampus	42.13 $\pm$ 4.72	43.41 $\pm$ 3.68
Olfactory bulbs	24.32 $\pm$ 2.21	23.06 $\pm$ 1.24
Pons+ medulla oblongata	119.20 $\pm$ 11.45	123.79 $\pm$ 10.51

Each value is shown mean  $\pm$  SD. \* Significance was evaluated by means of the 2-tailed Student's t-test comparing the isolated group with the aggregated group ( $p < 0.02$ ).

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.<sup>10</sup> using  $10^{-5}$  M of iso-OMPA. Choline acetyltransferase (CAT) activities were determined by the radiochemical micromethod of Fonnum<sup>11</sup> using [<sup>3</sup>H]-acetylcoenzyme A as a substrate. Protein content was measured according to the method of Lowry et al.<sup>12</sup>

**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 ( $22.5 \pm 8.7$ ) and aggregated groups ( $12.7 \pm 6.7$ ). 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<sup>7</sup> 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<sup>8</sup>. 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<sub>2</sub>: both relax bovine coronary artery, rabbit mesenteric 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<sub>2</sub>, PLS inhibits platelet aggregation regardless the inducer used. However, PLS possesses a specific antiaggregatory pattern on platelet, which indicates that these compounds are not identical with primary prostaglandins or PGI<sub>2</sub>.

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 diptheroid. Our findings show a distinct biological activity associated with PLS. In various muscle preparations i.e. gerbil colon<sup>1</sup>, strips of human Fallopian tube<sup>2</sup> or human vessels (umbilical arteries, vena saphena)<sup>3</sup> PLS mimic prostaglandins of the E-type. However, in spite of similarities, especially with PGE<sub>2</sub>, PLS possess some specific properties. Thus PLS induce a significant increase in the cyclic AMP content in rat ovary<sup>4</sup> and act as potent chemoattractants<sup>5</sup>. Also the chemical analyses with reversed phase chromatography and gas chromatography-mass spectrometry demonstrated that PLS were not identical with PGE<sub>2</sub><sup>6</sup>. 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<sup>7</sup>. The final sample (biological activity ~ 200 ng PGE<sub>2</sub> 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<sup>8</sup> superfused in cascade<sup>9,10</sup>. 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<sub>2</sub>, PGF<sub>2α</sub>, PGI<sub>2</sub> and (15S)-hydroxy-11,9-(epoxymethano)prosta-5Z,13-dienoic acid