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[COMMUNICATION]

Reduced Size of Preputial Glands and Absence of Aggressive Behavior in the Genetically Obese (ob/ob) Mouse

JUNKO YAMASHITA[1], SHIN-ICHI HAYASHI and YUKIO HIRATA[2]

Department of Nutrition, The Jikei University School of Medicine, Minato-ku, Tokyo 105, and [2]Department of Anatomy and Embryology, Tokyo Metropolitan Institute for Neurosciences, Musashidai, Tokyo 183, Japan

ABSTRACT—The preputial gland of freely fed genetically obese (ob/ob) male mice weighed less than that of the lean controls (21 mg vs. 40 mg for unilateral gland). The gland of the ob/ob mice pair-fed with their controls for 45 days was also small (22 mg). The gland of the ob/ob mice, either fed ad libitum or pair-fed, contained fewer lipid-secretory cells than that of the lean controls. Upon cohabitaion with an unfamiliar male mouse of either ob/ob or lean type, the ob/ob mouse did not exhibit any aggressive behavior which is commonly observed in an adult male mouse housed together with an unfamiliar one. These results indicate that the abnormal feature of the preputial glands in the ob/ob mice is not caused by hyperphagia and is probably related with the absence of aggressive behavior in the ob/ob mice.

INTRODUCTION

We have reported that the genetically obese (ob/ob) mouse has smaller submandibular glands with lower content of nerve growth factor than the lean control [1]. The physiological role of nerve growth factor in the submandibular glands of the mouse has not yet been clarified. Recent reports have shown that a massive amount of nerve growth factor is discharged from the submandibular glands into the bloodstream when adult male mice display aggressive behavior upon cohabitaion with unfamiliar male ones [2, 3]. Aggressive behavior is known to be related to the activity of the preputial glands [4–6]. We therefore expected that the ob/ob mice might have abnormal preputial glands and that their aggressive behavior might be different from the lean control's.

In the present study, we investigated the preputial glands of the ob/ob mice histologically, and also examined the effect of food restriction on the glands of these mice since hyperphagia is one of the causes for their obesity [7]. In addition, we observed whether aggressive behavior was provoked in the ob/ob mouse upon cohabitation with an unfamiliar ob/ob or lean mouse.

MATERIALS AND METHODS

The genetically obese (C57BL/6J-ob) and their lean control mice were bred in our laboratory from stock supplied kindly by Dr. M. Miyajima of the Animal Laboratory of Wakayama Medical College. Only male mice were used in this study.

At the age of 50 days, when the ob/ob mice were clearly distiguishable from lean mice, male mice were divided into the ob/ob and lean groups. The mice of each group were group-housed in a polypropylene box (30×20×13 cm) with sawdust, and fed laboratory chow (MF; Oriental Yeast Co. Ltd., Tokyo, Japan). In experiment 1, both the ob/ob and lean control mice were fed *ad libitum*. The experiments were done twice, firstly with four mice for each group and secondly with three mice

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[1] To whom reprint requests should be addressed.

[2] Present address: Department of Anatomy, School of Medicine, University of the Ryukyus, Okinawa 903-01, Japan.

for each group. In experiment 2, three lean mice were fed *ad libitum*, and three ob/ob mice were subjected to a pair-feeding experiment for 45 days. Thus, food intake of the lean group was measured daily, and the same quantity of food was given to the ob/ob group on the next day. All mice were kept in an air-conditioned room at a temperature of $25\pm1^{\circ}C$ and a relative humidity of $65\pm3\%$ with a 9:00 a.m. to 9:00 p.m. lighting schedule.

At the age of 95 days, each of the ob/ob mice was housed in a polypropylene box with a randomly selected unfamiliar lean or ob/ob mouse and their behaviors were observed for one hr. The mice were then deeply anesthetized with sodium pentobarbital, perfused through the heart with 4% paraformaldehyde in 0.05 M sodium phosphate buffer (pH 7.3) containing 5% sucrose, and then the preputial glands were dissected out. The left preputial gland was freed from adhering adipose and connective tissues, and weighed after the fluid contained in the gland was gently squeezed out according to Hayashi [6]. The right gland was subjected to histological study.

F-test and unpaired Student's t-test were used to compare the results.

RESULTS AND DISCUSSION

In the experiment 1, the preputial glands of the ob/ob mice were smaller and weighed less than those of the lean controls (t-test; $p<0.01$), while there was no significant difference between their variances (F-test; $p>0.1$). Since the results of two series of studies were similar for both the ob/ob and lean groups, they were combined in Table 1. The glands of the ob/ob mice had fewer folds with smaller number of lipid-secretory cells than those of lean controls, whereas the size of the lipid-secretroy cells in the glands was similar between the two groups.

The abnormalities of the preputial glands in the ob/ob mice were not corrected by food restriction carried out in the experiment 2 (Table 2, Fig. 1). The histological features of the glands of the ob/ob and lean control mice in the experiment 2 were very similar to those of the corresponding groups in the experiment 1. Accordingly, it was concluded that hyperphagia was not the cause of the abnormalities. It is possible, however, that obesity was responsible for that, since the ob/ob mice pair-fed with lean controls still weighed more than

TABLE 1. Body weight and weight of the left preputial gland of male ob/ob and lean mice fed *ad libitum*

Mice	(n)	Bosy weight(g)	Weight of left preputial gland(mg)
ob/ob	(7)	49.8 ± 1.9^{a}	21.1 ± 3.1^{a}
lean	(7)	26.8 ± 0.9	40.0 ± 1.5

The ob/ob and lean mice were each group-housed, and fed *ad libitum*. Results are mean±SEM at the age of 95 days.
[a] Significantly different from lean mice ($p<0.01$).

TABLE 2. Body weight and weight of the left preputial gland of male lean and pair-fed ob/ob mice

Mice	(n)	Body weight(g)		Weight of left preputial gland(mg)
		(50-day-old)	(95-day-old)	
ob/ob	(3)	26.9 ± 0.4^{a}	38.0 ± 0.7^{b}	22 ± 3^{b}
lean	(3)	21.8 ± 1.2	27.2 ± 1.1	40 ± 2

The ob/ob mice were group-housed, and pair-fed with group-housed lean mide. Results are mean±SEM for 3 mice of each group. Significantly different from lean mice (a: $p<0.05$, b: $p<0.01$).

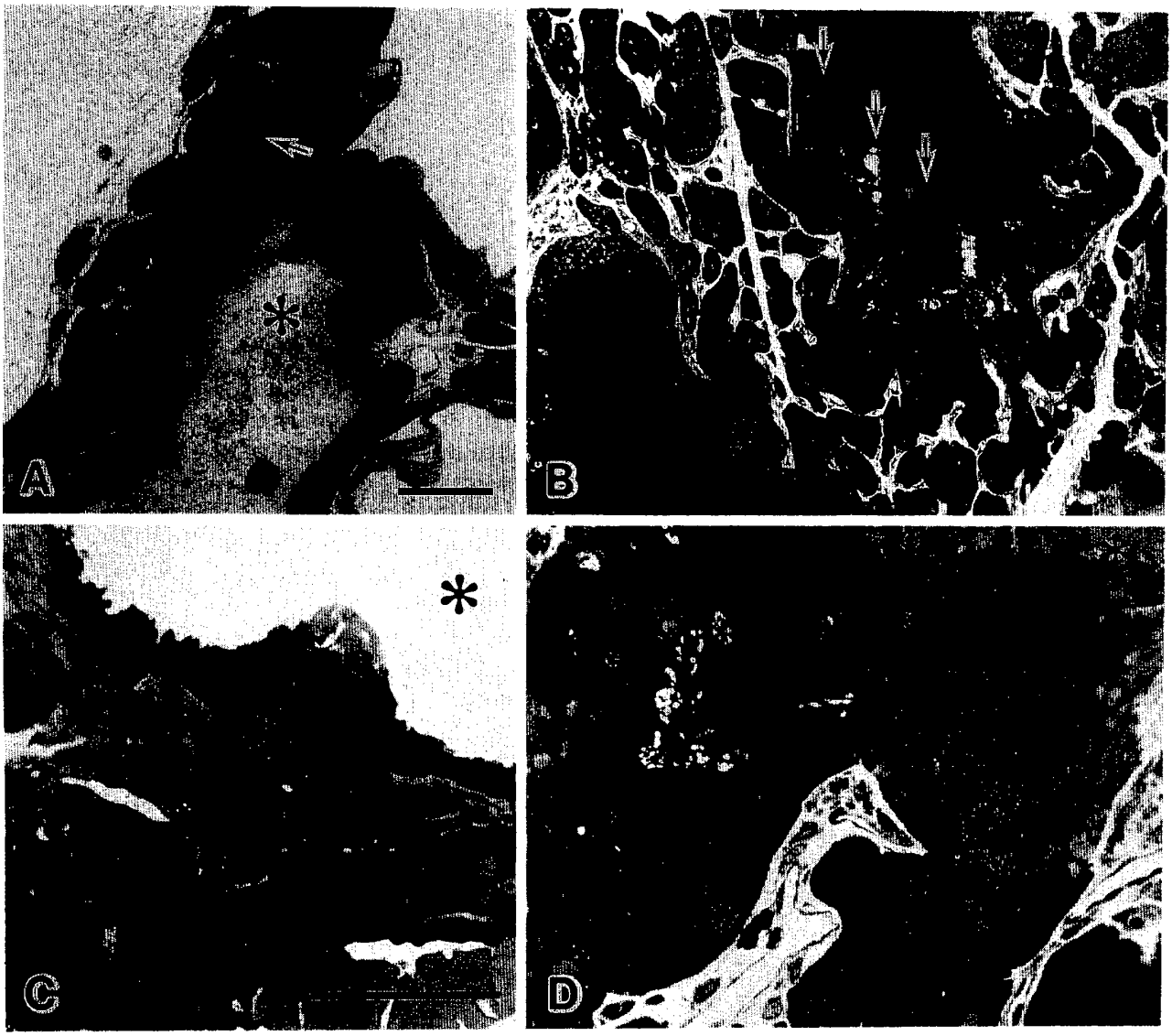

FIG. 1. Photomicrographs of the preputial glands from the ob/ob (A, C) and the lean control (B, D) mice. In the ob/ob mouse of the experiment 2 (body weight 37.4 g, preputial gland 15 mg), the glandular lumen (asterisks) is much wider than that of the lean control (body weight 29.5 g, preputial gland 37 mg), and the secretory acini are less numerous and less complicated than those of the lean mouse. Arrow heads: Opening of the secretory acini into the lumen. 1.5 μm resin sections stained with toluidine blue/safranin-O. Scale bars: 100 μm in A and 50 μm in C and D.

the lean controls. This overweight was probably due to low metaboric rate of the ob/ob mouse compared with the lean control [8].

Meanwhile, intermale spontaneous aggression is a common phenomenon in this species [9]. It is known that the preputial gland of a male mouse plays an important role maintaining the aggressivity not only of a cohabitant male but also of its owner [10]. Hence we supposed that the ob/ob mouse might not elicit aggressive behavior of a cohabitant, and/or not display aggressive behavior against a cohabitant when it was housed together with an unfamiliar male mouse. We therefore examined their behavior during one hr cohabitation.

Upon cohabitation with a lean mouse, four of ten lean mice in the experiments 1 and 2 attacked a cohabitant 1~5 times with a latency of 2~21 min and sometimes mounted on the cohabitant after fighting. Upon cohabitation with the ob/ob mouse three of them also chased a cohabitant ob/ob mouse and tried to mount 1~3 times with the

latency of 33～36 min. The mounting behavior against the ob/ob mouse was always displayed without mutual aggressive behavior. On the other hand, none of the ob/ob mice displayed any aggressive behavior against a cohabitant, irrespective as to whether it was an ob/ob or lean mouse. In addition, we have often observed that lean mice of the present strain sometimes display intermale aggressive behavior even against familiar lean mice, whereas we have never observed mutual aggressive behavior between ob/ob mice.

The ob/ob mouse shows depressed serum concentration of testosterone [11], and it is also suggested that it has an impaired thyroid function [12]. These hormones are known to be related to the preputial gland weight as well as the aggressiveness in the mouse [13, 14]. Abnormal status of these hormones may directly affect the preputial gland as well as the aggressive behavior. Alternatively, nerve growth factor released from the submandibular glands may play an essential role in the development of preputial glands in the normal mouse and this process may be impaired in the ob/ob mouse, since it is reported that these hormones increase the tissue weight and nerve growth factor content in the submandibular glands of the ob/ob mouse [15]. Further studies are needed to examine these possibilities.

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