Modulation of Spontaneous and Odorant-Evoked Activity of Rat Olfactory Sensory Neurons by Two Anorectic Peptides, Insulin and Leptin

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INTRODUCTION

The sense of smell is critically involved in eating behaviors from food detection to consumption. Addition of certain odorants to a diet has a profound influence on the amount of food rats consume (Le Magnen 1956, 2001), and loss of smell in bulbectomized rats leads to long-term compulsive overeating (Le Magnen 1956, 2001), and loss of smell in

The interplay between food intake and olfaction is mostly mediated by peptide hormones produced in various tissues according to an animal’s satiety status. Ghrelin, secreted from the stomach, signals hunger, while insulin from the pancreas and leptin from the adipose tissue mainly signal satiety. These peptides, carried by the blood flow, inform the food-intake regulatory centers in the brain including the hypothalamic nuclei, which in turn produce orexigenic (e.g., orexin) or anorexigenic peptides to initiate or to stop food intake (Arora and Anubhuti 2006; Plum et al. 2005; Schwartz et al. 2000; Stanley et al. 2005). In a behavioral study, intracerebroventricular injection of orexin or leptin increases or decreases the olfactory sensitivity, respectively (Julliard et al. 2007). Consistent with this finding, mutant mice lacking leptin or leptin receptors performed significantly better than wild-type animals in an olfactory-mediated, food-finding test (Getchell et al. 2006).

While the food intake peptides can exert their actions on the olfactory system centrally in the brain, e.g., the olfactory bulb (Fadool et al. 2004; Hardy et al. 2005; Palouzier-Paulignan et al. 2006), this study focuses on their effects on the peripheral sensory neurons in the olfactory epithelium. Several peptides (orexin, insulin, and leptin) are present in the olfactory mucosa, and their receptors and signaling machineries are identified in olfactory sensory neurons (OSNs) and other cell types (Baly et al. 2007; Getchell et al. 2006; Gorojankina et al. 2007; Lacroix et al. 2008). Insulin and leptin, two anorectic peptides, are of special interest because their levels as well as their receptor levels in the olfactory epithelium are regulated by the nutritional status (Baly et al. 2007; Getchell et al. 2006; Gorojankina et al. 2007; Lacroix et al. 2008). However, the effects of an elevated level of insulin or leptin (such as after a meal) on the functional properties of single OSNs are unknown.

Here we investigated the effects of insulin and leptin on individual OSNs in the absence or presence of odorants. We first studied the spontaneous activity, which is critical for the survival of OSNs by stabilizing their projections to the olfactory bulb (Yu et al. 2004). We characterized the firing patterns of rat OSNs in the intact epithelium preparation via patch-clamp recordings and examined the consequences of bath perfusion of insulin and leptin. Both peptides enhanced the excitability of OSNs by increasing the spontaneous firing frequency and reducing the interspike intervals in electrically evoked spike trains. This is surprising considering the recent report that insulin decreases the odorant responses of the olfactory epithelium in electroolfactogram (EOG) recordings (Lacroix et al. 2008). We then confirmed that insulin (as well as leptin) decreased the EOG signals induced by isoaamyl acetate. We further revealed that these two peptides reduced...
the odorant responses at the single-cell level using patch-clamp recordings. The results suggest that these circulating hormones, mostly known to regulate energy homeostasis, may modulate the smell function by altering the spontaneous and odorant-induced activity of OSNs.

METHODS


Patch-clamp recordings

Wistar rats were used at the age of P6-P15 because OSNs from younger preparations survive longer. Animals were deeply anesthetized using 0.02 ml ketamine (100 mg/ml) and decapitated. The whole head was quickly immersed in cold Ringer solution (2–4°C). A paramedial sagittal section of the nose allowed harvesting the medial septum that separates the two nasal cavities. Olfactory mucosa were peeled off from both sides of the septum and kept in cold, oxygenated Ringer solution. Under an upright microscope (Olympus BX61WI) equipped with a CCD camera (Dage-MTI) and a fourfold magnification changer, the dendritic knobs of OSNs were visualized through a ×40 water-immersion objective.

After being transferred to the recording chamber, mucosa explants were continuously perfused with oxygenated Ringer (2.5 ml/min at 25 ± 2°C). Recordings were performed on the knobs of OSNs in either cell-attached or perforated patch-clamp configuration. In cell-attached configuration, borosilicate pipettes (1.2 mm OD, 0.69 mm ID, Sutter Instruments) were filled with Ringer solution, and the seal resistance was 2–3 GΩ. In perforated patch-clamp configuration, the recording pipettes were filled with the internal solution (see following text), and the seal resistance was 1–2 GΩ. The membrane potential was hyperpolarized (V_mold = −80 mV) to prevent exhaustion of the cells from the frequent firing. The junction potential (10 mV) was corrected in all recordings a posteriori. Data were acquired with an EPC9 amplifier combined with the Pulse software (HEKA Electronic). The sampling frequency was 10 kHz in current-clamp and 5 kHz in voltage-clamp mode. Signals were filtered at a frequency equal to half of the sampling frequency.

Insulin (human recombinant insulin, 0.5 μg/ml, i.e., 86 nM, Sigma) or leptin (rat recombinant leptin, 50 nM, Sigma) was dissolved in Ringer solution and perfused the entire explants. We used the peptide concentration is efficient in the olfactory bulb without interacting with insulin like growth factor pathways (Fadool et al. 2000). Although the leptin concentration was well below the young rat leptinemia, it appeared to be efficient. Using Ringer solution, we first verified that switching perfusing solutions didn’t disrupt the spontaneous firing of OSNs by any mechanical stimulation.

Isoamyl acetate was used to elicit odorant responses because it is an effective ligand for a significant portion of OSNs during in vivo recordings (Duchamp-Viret et al. 2000). Isoamyl acetate was first dissolved in DMSO as a stock solution (0.5 M) and diluted in Ringer solution (final concentration: 100 μM). The odorant stimulation was delivered by pressure ejection via a picospritzer (General Valve) using a pipette placed downstream from the recorded cell. The distance between the puffing electrode and the recording site was adjusted to avoid mechanical stimulation (Grosmaire et al. 2007). For each cell, the parameters of stimulation were adjusted to elicit reliable middle strength responses (duration of the puff was between 0.1 and 0.5 s with a 10 psi ejection pressure). The procedure included three stimulations in the control condition, three under drug perfusion (insulin or leptin), and three during washout. The isoamyl acetate stimulations were interspaced by 2 min.

EOG recordings

Non-weaned P15–P20 or ad-libitum-fed adult Wistar rats were used in either in vivo or in vitro preparations. The in vivo recording procedure has been previously described (Duchamp-Viret et al. 2000). Briefly, animals were anesthetized by intraperitoneal (ip) injection of an initial dose of pentobarbital sodium and chloral hydrate (Equithesin, 3 ml/kg) and secured for surgery in a stereotaxic apparatus. Anesthetic was then supplemented to maintain a deep level of anesthesia. Access to the olfactory mucosa was gained by opening a dorsal slit in the nasal bones and the underlying dorsal recess. For in vitro recordings, animals were anesthetized by an injection of pentobarbital sodium (Nembutal 20 mg/kg ip) and then decapitated. The skin was quickly removed, and the skull was longitudinally cut along the medial suture to expose the nasal cavities. The preparation, surrounded by cottons saturated with Ringer, was fixed into a silicone chamber and was placed under the nozzle of the olfactometer (Vigouroux et al. 1988). For in vivo preparations, the dorsal turbinite was stimulated whereas all turbinates were tested with hemi-head in vitro preparations.

FIG. 1. Rat olfactory sensory neurons (OSNs) show spontaneous bursting activity. A: the spontaneous activity of an OSN was recorded in cell-attached configuration. B: the period enlarged (right) where bursts were evident. B: the spontaneous activity of an OSN was recorded in perforated patch-clamp configuration. Bursts were still present, interspaced by more various interspike intervals (ISIs) than the cell-attached recordings. C: the probability density functions of ISIs are plotted for both cell-attached (CA) and perforated patch-clamp (PP) recordings. The table summarizes key values describing each distribution (in ms).
Recordings were done using borosilicate glass pipettes (1.5 mm OD, 1.17 mm ID, Clark Electromedical Instruments) filled with Ringer solution. The tip resistance was 2–4 MΩ. The EOG signal was amplified by a conventional amplifier (DC 30 Hz cutoff) and recorded on a CED-1401 data-acquisition system (Cambridge Electronic Design) connected to a computer for off-line analysis. The odorant stimulations were interspaced by 90 s. Depending on the recording site, the isomyl acetate concentration was adjusted to a fraction of the saturated vapor (SV) from SV/80 (80 times dilution) to SV/45 (45 times dilution) to obtain 10–15 mV EOG signals.

To apply insulin or leptin, small cubes of gelatin (Agarose VII-A, low gelling temperature, Sigma, 1% in Ringer) were extemporaneously soaked either with insulin or leptin solutions (86 and 50 nM, respectively, in Ringer). A test cube was applied close to the EOG recording site gently enough to avoid disrupting the electrode positioning. On one recording site, using the same isomyl acetate concentration, five EOG signals were elicited under each of the following conditions: control (with and without a gelatin cube soaked in Ringer), test (with a peptide soaked cube), and recovery (after cube removal). Only runs where the signals remained stable after the application of the Ringer-soaked cubes were included in the analysis.

**Recording solutions**

The same Ringer solution was used for both EOG and patch-clamp recordings (in mM): 125 NaCl, 4 KCl, 25 NaHCO₃, 2 CaCl₂, 1.25 NaH₂PO₄, 1 MgCl₂, and 5.5 glucose. The pH was kept at 7.6 by oxygenating with 95% O₂-5% CO₂, and the osmolarity was adjusted to 320 mOsm with sucrose. Tetrodotoxin (TTX, Sigma) at 1–2 μM was perfused in some of the experiments. As previously described by Ma and coworkers (2003), for perforated patch-clamp recordings, pipettes were filled with the internal solution containing (in mM) 70 Ma and coworkers (2003), for perforated patch-clamp recordings, was perfused in some of the experiments. As previously described by Ma and coworkers (2003), for perforated patch-clamp recordings, pipettes were filled with the internal solution containing (in mM) 70 KCl, 53 KOH, 30 methanesulfonic acid, 5 EGTA, 10 HEPES, and 70 sucrose; 310 mosM, pH 7.2 adjusted with KOH. Amphotericin B (stock solution: 3 mg amphotericin B/50 μl DMSO) was extemporaneously added at a final concentration of 130 μM.

**Data analysis**

The firing properties were analyzed using Open Electrophy (http://neuralensemble.org/trac/OpenElectrophy), SciPy, and MySql database software (Open Source licenses). The probability density functions of interspike intervals (ISIs) were plotted with an ISI distribution bin of 3 ms on the x axis, and the total integral of ISI distribution equals 1 (\( \int_{ISImin}^{ISImax} f(x)dx = 1 \)). Thus the probability for an ISI to fall within the interval \([a,b]\) is represented as the area under the graph within the interval \([a,b]\) (i.e., \( \int_{a}^{b} f(x)dx \)).

Statistics were carried out using R software (R Development Core Team 2007). Nonparametric Wilcoxon signed-rank tests were used to test the mean frequency distribution in the different cell populations recorded under cell-attached or perforated configurations. Nonparametric Wilcoxon matched-paired signed-rank tests were used to compare the firing frequencies, latencies, and ISIs with or without drug application.

**RESULTS**

**OSNs fire spontaneously in a bursting mode**

We recorded the spontaneous activity of OSNs in the intact olfactory epithelium under either cell-attached or perforated patch-clamp configuration. The extracellular cell-attached recording permits observation of the cells in a less disturbed condition, whereas the intracellular perforated patch-clamp allows subthreshold events recording and manipulation of the membrane potential by current injection. In either configuration, we could record the cells for as long as 30 min.

Under cell-attached configuration, all neurons (100%, n = 27) showed a spontaneous activity with a mean firing frequency of 3.0 ± 1.7 (SE) Hz. Most of the OSNs displayed a phasic firing pattern, consisting of bursts of action potentials (Fig. 1A). Under perforated patch clamp, 85.7% (n = 21) of the...
neurons showed a spontaneous activity with a mean firing frequency of $1.7 \pm 1.3$ Hz. Bursts of action potentials were also present but isolated spikes occurred more often (Fig. 1B). The lower firing frequency obtained under this configuration was presumably due to the hyperpolarizing current injected into the cells (see METHODS). Despite the difference in the mean firing frequency, the ISI probability density functions obtained from cell-attached (12,458 ISIs from all recorded cells) or perforated patch clamp (4,712 ISIs) were very similar (Fig. 1C). Both curves were centered at 41.9 ms (bin = 3 ms) with comparable median values (62.5 ms for cell-attached and 62.8 ms for perforated patch clamp) corresponding to the intraburst firing. These results indicate that OSNs in the intact epithelium fire spontaneously in a bursting mode.

**Insulin and leptin enhance the excitability of OSNs**

We next tested the effects of an elevated level of insulin or leptin on the spontaneous activity of OSNs. Bath perfusion of insulin (86 nM) or leptin (50 nM) had a strong stimulatory effect on the OSNs, regardless of their basal spontaneous firing frequency (Fig. 2A). Neurons were considered as sensitive to these peptides when the mean firing frequency changed by $\geq 10\%$ of the control value. Insulin significantly ($P < 0.0001$) increased the firing frequency in 22 of 24 cells (91.7%) within 1 min and had no effect on the remaining cells (Fig. 2B, left). During the washout period (2–6 min), nine neurons (40.9%) recovered and the remaining neurons kept firing at a higher frequency. Leptin was tested in another set of 24 neurons. The peptide significantly ($P < 0.002$) increased the mean firing frequency in 18 cells (75.0%) within 1 min (Fig. 2B, right). Among the remaining neurons, three did not respond to leptin and three showed a decrease in the spontaneous firing. During the washout period (2–6 min), 11 (61.1%) of the excited neurons showed a recovery back to the control frequency, and the remaining 7 (38.9%) kept firing at a higher frequency.

We also tested the effects of insulin and leptin on the evoked activity in rat OSNs. In perforated patch-clamp configuration, during silent periods, a 0.4 nA depolarizing step was repeatedly injected into the neurons under the control condition and during insulin (Fig. 3A) or leptin perfusion (B). If a spontaneous burst occurred during current injection, the trial was excluded from further analysis. Regardless of the basal excitability level, insulin shortened the latency to the first action potential by 27.5% (from 66.6 ± 24.7 to 48.3 ± 19.1 ms, $n = 6$, $P < 0.05$) and leptin by 24.9% (from 43.4 ± 15.6 to 32.6 ± 12.6 ms, $n = 6$, $P < 0.005$; Fig. 3C). The ISIs were analyzed to evaluate the effects of both peptides on the firing frequency (Fig. 3D). Both insulin and leptin decreased the mean ISIs by $\sim 13\%$: from 49.2 ± 7.7 to 42.0 ± 7.0 ms by insulin ($n = 6$, $P < 0.02$) and from 44.2 ± 11.9 to 38.4 ± 8.3 ms by leptin ($n = 7$, $P < 0.005$). These data reveal that insulin and leptin increase the neuronal excitability of OSNs.

**Odorant stimulation induces various response patterns in OSNs**

We characterized the activity patterns of OSNs induced by isoamyl acetate, an odorant that elicited responses in 74.7% of the neurons ($n = 95$) with 54.7% showing excitatory and 20.0% showing inhibitory responses. Among the excitatory responses, we observed two predominant patterns. First, isoamyl acetate stimulation induced tonic bursts in some neurons, recorded in cell-attached (Fig. 4A, top) or perforated patch-clamp configurations (Fig. 4A, bottom). In the intracellular recordings, the action potentials appeared to be grafted on the depolarizing receptor potential. Second, isoamyl acetate stimulation induced an initial high-frequency burst with decrementing spikes (b), followed by a silence (s), and a rebound (r) pattern (Fig. 4B). In perforated patch-clamp recordings, the initial burst (b; 52.4 ± 9.2 Hz, $n = 8$) was grafted on the rising phase of a long-lasting receptor potential (Fig. 4B, bottom). The sustained depolarization (mean peak amplitude = 38.6 ± 12.3 mV, $n = 13$) corresponded to a period (s) during which no action potentials were elicited. The b/s/r pattern was observed in 61.3% of the OSNs excited by isoamyl acetate versus the simple burst pattern in 38.7% ($n = 31$). We then measured the inward transduction currents underlying the excitatory responses elicited by isoamyl acetate puffs under voltage-clamp mode. The peak current amplitude ranged between 4 and 478 pA ($n = 22$, Fig. 4C), with 45% < 25 pA, 32% between 25 and 100 pA, and 23% > 100 pA. These results demonstrate that rat OSNs display a variety of activity patterns (dominated by the b/s/r pattern) in response to the same odorant.

Notably, addition of 1–2 μM TTX in the bath failed to completely block the spontaneous firing ($n = 6$, data not shown) or the odorant-induced spikes (Fig. 4C, →). The TTX...
sensitivity of Na\(^+\) channels in rat OSNs is somewhat controversial in the literature. Acutely dissociated OSNs are highly sensitive (Trombley and Westbrook 1991) in contrast to cultured OSNs (Rajendra et al. 1992). Here we showed that the OSNs in the intact epithelium were not as sensitive as the dissociated neurons.

Insulin and leptin mainly reduce odorant responses of OSNs

We examined the effects of these two peptides on the odorant-induced activity of OSNs. We initially used EOG recordings to measure the odorant responses from a large population of sensory neurons. The peptide delivery procedure is shown in Fig. 5A, and the high percentage (87\%) of stable control runs verified the undisturbed recording conditions (see METHODS). Results obtained from in vivo and in vitro preparations in young and adult animals were similar and thus pooled together. Insulin decreased the peak amplitude of isoamyl. Acetate-induced EOG signals to 46\% of the control (\(n = 13, P < 0.0002\)), and a recovery to 84\% was observed in 10 recording sessions (Fig. 5B). Leptin decreased the peak amplitude to 38\% of the control (\(n = 14, P < 0.0001\)), and a recovery to 70\% was observed in nine recording sessions (Fig. 5C).

Because the EOG recording does not provide the information at the single-cell level, we next tested the effects of these two peptides on individual OSNs using patch-clamp recordings. Fourteen of 17 neurons (82\%) with stable excitatory isoamyl acetate responses showed odorant responses modulated by peptide perfusion. While insulin and leptin increased the spontaneous firing (Fig. 6, A and B), both peptides mainly reduced the odorant responses by slowing down the initial burst (Fig. 6, C and D) or decreasing the transduction currents (66.7\%, \(n = 12\) for insulin; 60.0\%, \(n = 5\) for leptin; Fig. 6E), consistent with the decreased EOG signals. However, three neurons (2 under insulin and 1 under leptin perfusion) showed an increase of the firing frequency during the initial burst (Fig. 7, A and B). We suspected that these three cells had such a profound increase in the spontaneous firing frequency, which might have masked the peptide effects on the odorant responses. To test this possibility, we plotted the normalized firing frequencies (to

![FIG. 4](https://example.com/fig4.png)

*FIG. 4.* Odorant stimulation induces various activity patterns in individual OSNs. Two types of responses were frequently observed following stimulation with isoamyl acetate (ISO, 100 \(\mu\)M, duration is indicated on each picture). A: in some neurons, brief puffs of ISO elicited a nondecrementing burst, observed either in cell-attached (top) or perforated patch-clamp (current-clamp mode) configuration (bottom). B: in other neurons, brief puffs of ISO elicited an initial burst (b) followed by a silence (s) and a firing rebound (r) in cell-attached configuration (top). A similar pattern was observed in perforated patch-clamp configuration (bottom): the initial burst (b) was grafted on a large receptor potential, which kept the membrane potential in a depolarized state (s). During repolarization, the rebound (r) firing appeared before a complete return to the spontaneous activity. C: 2 neurons were stimulated for 250 ms by ISO puffs that elicited a small (top) or a large (bottom) inward transduction current recorded under voltage-clamp mode. Despite the presence of 1 \(\mu\)M TTX in the bath perfusion, some action potentials (→) occurred in response to ISO. The holding potential for both cells was −80 mV.

![FIG. 5](https://example.com/fig5.png)

*FIG. 5.* Insulin and leptin decrease odorant-induced electroolfactogram (EOG) signals. EOG signals were recorded from the surface of olfactory turbinates in response to isoamyl acetate puffs (ISO, SV/80, 0.5 s). A: during each experiment, 5 ISO stimulations were delivered in each condition: without any cube, with Ringer-soaked cube, with peptide (insulin or leptin)-soaked cube, and after cube removal. B: in 1 preparation, insulin (red lines, 4 traces being shown) reduced the EOG signals (control recordings in black solid lines, 2 traces from each control condition being shown) with partial recovery (black dotted lines, 3 traces being shown). The graph on the right summarizes the normalized EOG signals from 13 preparations. C: in 1 preparation, leptin (green lines) reduced the EOG signals (control recordings in black solid lines) with partial recovery (black dotted lines). Right: graph summarizes the normalized EOG signals from 14 preparations.
the control) under peptide (insulin or leptin) perfusion for the ten cells with a nonzero baseline firing (Fig. 7C). The three cells with an increased isoamyl acetate-induced firing (orange) tend to show a more profound increase in the spontaneous firing frequency than those displaying a decreased isoamyl acetate-induced firing (blue). Overall, the EOG and single-cell recordings indicate that insulin and leptin mainly reduce odorant-induced responses in OSNs.

DISCUSSION

Using electrophysiological approaches, we have investigated the influence of two anorectic peptides, insulin and leptin, on the functional properties of OSNs in the absence or presence of odorants. While insulin and leptin dramatically enhance the excitability of OSNs assessed by spontaneous or electrically evoked events, they significantly reduced the odorant-induced activity measured by either EOG or single-cell recordings. The results suggest that an elevated blood level of insulin and leptin after meals may result in a decreased signal-to-noise ratio of the odor inputs and thus modulate the smell function to match the satiety status. The potential role of these food intake peptides in stabilizing the epithelio-bulbar connections by upregulating the spontaneous activity of OSNs is also discussed.

OSNs fire spontaneously in a bursting mode

We analyzed the spontaneous activity of OSNs in either cell-attached or perforated patch-clamp configuration. The cell-attached recording is less invasive and thus keeps the neurons closer to their physiological conditions, whereas the perforated patch-clamp enables direct recording and manipulation of the membrane potential. The spontaneous activity of OSNs is clearly bursting even though the mean firing frequency is lower in perforated patch-clamp recordings (Fig. 1). This is due to a small hyperpolarizing current injected into the neurons to avoid “overfiring,” so that the cells can be recorded long enough to study the peptide effects. The spontaneous firing frequency of rat OSNs has also been obtained with an in vivo approach using the extracellular, single-unit recording (Duchamp-Viret et al. 1999, 2005). In this latter report, 40% of in vivo OSNs fire at >1.7 Hz compared with 77% of in situ cells in the intact epithelium recorded in cell-attached configuration in the current study. This is probably due to the differences between the two preparations and...
OSNs display various response patterns to odorant stimulation

We observed a large proportion of OSNs (54.7%) showing an excitatory response to isoamyl acetate, consistent with a previous in vivo study (60%) (Duchamp-Viret et al. 2000). However, we detected more inhibitory responses (20 vs. 5%) in perforated patch-clamp recordings, in which inhibition is readily identified by membrane hyperpolarization. In contrast, in vivo single-unit recordings, inhibition appears as a decrease in the firing frequency, which may be too subtle to be noticed or simply hidden under a low-frequency spontaneous firing.

When the rat OSNs were stimulated by brief puffs of isoamyl acetate, we frequently encountered two response patterns: the simple nondecrementing burst and the “burst/silence/rebound (b/s/r) pattern” (Fig. 4). During the initial burst of the b/s/r pattern, the amplitude of action potentials decreases. This is likely attributed to a current shunt (a decreased membrane resistance) caused by the transduction current and progressive inactivation of voltage-gated Na\(^+\) channels caused by depolarization (Lynch and Barry 1991; Narusuye et al. 2003; Trotier and MacLeod 1983). The same mechanisms may also lead to the silence period. Initiation of the rebound spikes relies on decrease of this shunt and on re-activation of voltage-gated Na\(^+\) currents when the membrane potential repolarizes.

Similar response patterns have been reported in mammalian and amphibian OSNs (Duchamp-Viret et al. 2000; Reisert and Matthews 2001; Rospars et al. 2003). In single-unit recordings from in vivo rat OSNs (Duchamp-Viret et al. 2000), a low concentration odorant stimulation evokes a simple nondecrementing burst, and a medium concentration elicits a “b/s/r” pattern. For a high concentration, the duration of the initial burst decreases and its firing frequency increases, while the silence becomes longer. The concentration-dependent changes do not rely on the odorant quality but depend on the odorant sensitivity of the OSNs. In our in vitro recordings, the same isoamyl acetate stimulation elicited patterns corresponding to low-, medium-, and high-strength responses, indicating a broad sensitivity among OSNs. Taken together, the in vitro OSNs in the intact epithelium closely resemble those under in vivo conditions in their odorant-induced activity.

Insulin and leptin may play several roles in the olfactory system

The most striking finding in our study is that bath perfusion of insulin or leptin significantly increases the spontaneous firing but reduces odorant-induced activity in OSNs. The underlying mechanisms are likely attributed to two factors: the ionic channels responsible for the spontaneous activity are different from those involved in odorant signal transduction (Kleene 2008; Narusuye et al. 2003) and insulin and leptin interact with different channels (Fadool et al. 2004; Shanley et al. 2002; Spanwick et al. 1997, 2000) and signaling pathways (Bjorbaek and Kahn 2004; Capeau 2005). It is also possible that these two hormones act on the same targets via different signaling pathways as reported in the hypothalamus (Plum et al. 2005). Based on our findings, we propose that insulin and leptin may modulate the smell function at the peripheral level by exerting several actions.

First, insulin and leptin may help stabilizing the epithelio-bulbar connections by enhancing the spontaneous activity of OSNs, which undergo continuous cell death and neurogenesis. In the competitive environment of the olfactory epithelium, less-active OSNs have a disadvantage in maintaining their synaptic connections in the olfactory bulb and likely disappear (Yu et al. 2004; Zou et al. 2004). A recent study reveals that genetic deletion of the shaker Kv1.3 channel in the OSNs disturbs the refinement of the primary olfactory projections (Biju et al. 2008; Fadool et al. 2004). More interestingly, insulin inhibits this potassium channel (Fadool et al. 2000), which might lead to the increased firing frequency we observed in the OSNs. Although it requires further investigation to identify the molecular targets for insulin and leptin in the OSNs, the strong effects of these two peptides on the spontaneous activity support a role of these blood-circulating hormones in the olfactory epithelium in the absence of odorant stimulation.

Second, insulin and leptin may reduce the signal-to-noise ratio of the odor inputs to match the smell function with the satiety status. While these two peptides increase the spontaneous activity of OSNs (Fig. 2), they mainly reduce the odorant-induced activity in the olfactory epithelium (Figs. 5 and 6). We first explored the peptide effects using EOG recordings, which summate the receptor potentials from a population of OSNs (Getchell 1974). Consistent with the results from a recent study.
examining the role of insulin in adult rats (Lacroix et al. 2008), here we report similar inhibition induced by insulin (as well as by leptin) in either young or adult rats.

The physiological concentrations of insulin and leptin in the olfactory mucosa have not been experimentally measured. The peptide dose we used is within the physiological range of insulinemia and leptinemia (blood levels of insulin and leptin) and mimics an elevation after a meal for adult rats. Compared with adults, young rats have different insulinemia and leptinemia (Aalinkeel et al. 1999; Bouret and Simerly 2007; Bruder et al. 2004; Li et al. 2004; Mistry et al. 1999; Smith and Waddell 2003) and display different secretion rhythms (Bouret and Simerly 2007; Nagatani et al. 2000). Nevertheless, the similar effects we observed on the EOG signals at both ages indicate that insulin or leptin has comparable functional effects on odorant-induced responses in young and adult tissues.

Although the olfactory epithelium has tight junctions, peptides like insulin and leptin have been shown to enter the circulating systems and the CNS after intranasal instillation (el Khafagy et al. 2007; Foster-Schubert and Cummings 2006). This is likely due to the mucociliary clearance of the peptides and the vascularization of lamina propria in which the capillaries have a porous endothelial basement membrane. Therefore in the EOG recordings, these two peptides should reach their receptors on the OSNs and cause the observed effects. In each experiment, by performing controls using Ringer solution and observing at least a partial recovery after peptide washout, we ruled out nonspecific effects caused by possible disturbance of the recording configurations during drug application. However, these experiments do not completely rule out a nonspecific effect of these peptides unrelated to food intake. Future studies using some circulant orexigenic peptides to show opposite effects on the activity of OSNs would help to tease out the consequences of these peptides.

We then used patch-clamp recordings to analyze the peptide effects on odorant-induced responses of individual OSNs. Both peptides mainly reduce the burst firing and the transduction currents (Fig. 6). Because of the strong stimulatory effects of these two peptides on the spontaneous firing of OSNs, their effects on odorant-induced firing could be concealed (Fig. 7). Therefore the peptide effects are better assessed in changes in the transduction currents or EOG signals, which all show a reduction under peptide application. What would be the consequence of high insulinemia and leptinemia on the olfactory coding in the postprandial context? At the epithelium level, the signal-to-noise ratio of the sensory input transmitted to the olfactory bulb will be reduced. The weaker odorant response will tend to be concealed in the reinforced spontaneous activity.

While our study focuses on the effects of food intake peptides on the olfactory epithelium, they influence the smell function by targeting the olfactory system centrally as well. The olfactory bulb is a target for insulin, leptin, and orexin (Fadool et al. 2004; Getchell et al. 2006; Julliard et al. 2007; Palouzier-Palugnan et al. 2006; Shioda et al. 1998), and the anterior piriform cortex acts as a critical sensor of amino-acids deficiency (Gietzen et al. 2007). Overall, the olfactory system plays an essential role in modulating eating behaviors to match the metabolic status. Conversely, the peptide hormones, which regulate food intake, modulate the sense of smell to match the satiety status.

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