氏　　名	PUTRA SANTOSO
学位の種類	博士 (医学)
学位記番号	甲第 527 号
学位授与年月日	平成 29 年 3 月 21 日
学位授与の要件	自治医科大学学位規定第 4 条第 2 項該当
学位論文名	**光刺激による室傍核 oxytocin ニューロンの活性化と摂食抑制**
論文審査委員	(委員長) 教授 尾仲達史
	(委　員) 教授 西村　智　教授 須田史朗

論文内容の要旨

1　研究目的

Feeding behavior is regulated by complex processes involving both central and peripheral mechanisms. In rodents, feeding behavior exhibits a diurnal pattern, being highly promoted during dark phase and diminished during light phase. The feeding rhythm could be disrupted by irregular light exposure and thereby promotes metabolic diseases including obesity, diabetes and hypertension. Importantly, these metabolic disorders are also observed in humans who experience extended and/or irregular light exposure, due to the modern lifestyle. These findings suggest that light could entrain feeding behavior and thereby influence metabolism. However, how light could entrain feeding behavior, including feeding inhibition in rodents, remains unclear.

The master clock suprachiasmatic nucleus (SCN) in the hypothalamus functions as the endogenous circadian pacemaker of the body. SCN activity is implicated in maintaining of behavioral and physiological rhythms including feeding rhythm. Irregular and/or extended light exposure impairs the rhythm of SCN activity, and SCN lesion and blockade of SCN neurotransmission promote arrhythmic feeding in rodents. These findings suggest a role of SCN in light-entrained feeding behavior.

It is likely that the light-controlled SCN neurons regulate feeding behavior through the feeding centers located primarily in the hypothalamus. Arginine vasopressin (AVP) neurons in SCN are reported to project to feeding center paraventricular nucleus (PVN). Moreover, the expression of anorexigenic neuropeptide oxytocin (Oxt) in PVN tends to synchrony with light-dark cycle, suggesting its possible link to the light signals. It also has been suggested that activation of oxytocinergic signaling in the PVN profoundly suppresses feeding. Hence, it is intriguing whether the SCN-PVN pathway could be involved in the light-induced inhibition of feeding. The present study aimed to elucidate the neurocircuit in the hypothalamus that mediates light-induced feeding inhibition with a particular attention to the neurocircuit from SCN AVP to PVN Oxt.

2　研究方法

Male Wistar rats, Oxt-mRFP1 rats, and AVP-eGFP rats were reared in normal light-dark cycle and fed *ad libitum*. For the purpose of central injection experiments (AVP or Oxt-receptor antagonist injection), animals were stereotaxically cannulated in the lateral ventricle or intra-PVN. In the light exposure experiments, animals were exposed to the white light (200 lux) for 2 h (21:30-23:30) followed by feeding and locomotor activity measurements or for 90 min (21:30-23:00) followed by c-Fos examination. In another experiment, animals were intracerebroventricularly (icv) injected with an Oxt-receptor antagonist (OVT, right before dark phase, 19:30) and exposed to the light and 90 min later for 2 h (21:00-23:00) followed by feeding and locomotor activity measurements. Immunohistochemistry for c-Fos and Oxt was performed using a diaminobenzidine (DAB) immunostaining and immunofluorescent staining. A retrograde tracer study was carried out by injecting cholera toxin subunit B (CTB) into PVN followed by the confocal microscopic observation in SCN. The measurement of $[Ca^{2+}]_i$ in PVN Oxt neurons was performed using the ratiometric fura-2 fluorescent imaging, and the slice patch clamp recording on mRFP1 Oxt neurons in PVN was conducted using an Axopatch 200B patch-clamp amplifier.

3　研究成果

In the present study, it was observed that light exposure acutely suppressed food intake and elevated c-Fos expression in the SCN AVP and PVN Oxt neurons. The light-induced suppression of food intake was abolished by the administration of the Oxt receptor antagonist. Retrograde tracer analysis confirmed the projection of SCN AVP neurons to PVN. Furthermore, intracerebroventricular (icv) injection of AVP suppressed food intake and increased c-Fos in PVN Oxt neurons. Intra-PVN injection of AVP exerted an acute anorexigenic effect than icv injection. Moreover, AVP induced intracellular Ca^{2+} signaling and increased firing frequency of PVN Oxt neurons *in vitro*.

4　考察

Light exposure during dark phase rapidly suppressed food intake and increased c-Fos expressions in SCN AVP neurons and PVN Oxt neurons. Although it is known that light activates SCN neurons including those containing AVP, this is firstly suggested that light activates PVN Oxt neurons. Furthermore, icv injection of an Oxt-R antagonist abolished light-induced suppression of food intake, suggesting that Oxt is involved in this process. Icv injection of AVP increased c-Fos expression in PVN Oxt neurons and suppressed food intake, mimicking the effects of light exposure. Intra-PVN injection of AVP exerted feeding suppression. Moreover, AVP increased $[Ca^{2+}]_i$ and firing frequency in PVN Oxt neurons, indicative of a direct action of AVP on PVN Oxt neurons.

Light exposure during dark phase acutely inhibited food intake is consistent with the previous reports. Regarding the possible mechanism underlying the light-associated feeding

inhibition, the present study showed that light-induced feeding inhibition paralleled with the activation of PVN Oxt neuron. Moreover, icv injection of Oxt receptor antagonist abolished the light action to suppress feeding, but partially attenuated the light action to suppress locomotor activity. These results suggest that light-induced feeding inhibition is not simply secondary to suppression of locomotor activity, but may be associated with the activation of Oxt neurons. However, it should be considered that an Oxt-R antagonist (OVT) used in this study is not a selective antagonist to Oxt-R but also could possibly bind to the AVP receptors. Therefore, the effect of OVT to counteract light exposure on feeding and locomotor activity suppression might be also due to the blockade of AVP action.

The current study confirms the projection of SCN AVP neurons to PVN, in consistent with the previous studies in hamsters, rats, and humans. Thus, SCN neurons could directly act on PVN neurons. Furthermore, it is found that AVP induced Ca^{2+} signaling and increased firing frequency in PVN Oxt neurons *in vitro*. These results extended the previous report that AVP excited PVN neurons *in vitro* and identified the Oxt neuron as a target.

The present study suggests a plausible role of the SCN to PVN neurocircuit in feeding regulation. However, previous studies have mainly suggested the implication of this circuit in glucose metabolism. Hence this recent finding, together with these previous reports, may gain an evidence that the SCN to PVN neurocircuit is involved in the regulation of energy homeostasis with a wider spectrum covering feeding and glucose metabolism.

5　結論

The current study suggests a neurocircuit from SCN AVP to PVN Oxt that relays light reception to inhibition of feeding behavior.

論文審査の結果の要旨

申請者の論文は、暗期の光照射による摂食抑制作用がオキシトシン受容体アンタゴニストで阻害されること、暗期の光刺激で視交叉上核のバゾプレシン細胞と視床下部室傍核のオキシトシン細胞の Fos 蛋白質発現が増加すること、視床下部室傍核に入れた逆行性トレーサーCTB に陽性となる視交叉上核のバゾプレシン細胞が存在すること、バゾプレシンの脳室内あるいは視床下部室傍核内投与で摂食が抑制されること、in vitro 実験でオキシトシン細胞の発火頻度あるいは細胞内 Ca 濃度がバゾプレシンで増加することを示した。これらのデータから、視交叉上核のバゾプレシン細胞が視床下部室傍核に投射すること、この経路が光照射で活性化されることで摂食抑制が誘発される可能性を申請者は主張している。本論文は、夜間の光照射による摂食抑制にオキシトシン受容体が関与する可能性を示したもので新しい発見と考えられる。

タイトルの適切性、一つのグラフの縦軸の記載漏れ、単数字をグラフに表示する意義、用いたオキシトシン受容体アンタゴニストの特異性に関する議論がされていないこと、一部表現が強すぎるという指摘がなされた。これらは適切に修正された。論文は全体として明確に論理立てて記述されており、新規な発見で学位論文として十分にふさわしいと満場一致で判断された。

最終試験の結果の要旨

発表は、日内変動と光による日内変動へ修飾の概説から始まり、光照射による視交叉上核バゾプレシンン細胞と視床下部オキシトシン細胞の活性化、視床下部室傍核領域に投射する視索上核バゾプレシンニューロンの存在、バゾプレシンのオキシトシンニューロン興奮作用、バゾプレシン脳室内或は視床下部室傍核内投与の摂食抑制作用、光照射後の摂食抑制作用のオキシトシン受容体アンタゴニストによる阻害のデータが発表された。その発表は分かり易く明快であった。引き続き質疑応答がなされた。質疑は、ヒトにおける日内変動障害と代謝異常との関連、夜行動物における光照射の機構の昼行性のヒトにおける意義、昼行性動物と夜行性動物のオキシトシン細胞の活動の日内変動の異同、日内変動による摂食変動と光照射による短期的摂食抑制との関係、この両者におけるオキシトシンの働き、用いたオキシトシン受容体アンタゴニストの選択性、薬物の用量依存性、ノックアウトマウスの既報データとの整合性に関してなされた。候補者はこれらの質問に真摯な姿勢で適切に回答した。

候補者は関連領域にわたり幅広い知識と教養を持ち合わせており、博士（医学）の学位にふさわしいと満場一致で判断された。