Leptin 和 Neurotensin 在大鼠迷走神經運動神經核之作用 Effects of Leptin and Neurotensin in Dorsal Motor Nucleus of the Vagus in Rats

中文摘要

根據流行病學的調查，在許多國家包括台灣，近幾年之肥胖人口明顯成長，肥胖除了影響外觀外也會導致一連串的健康問題，如心血管疾病、高血壓、第 2 型糖尿病，因此，肥胖屬於全球性的健康問題。體重的控制是維持能量衡定的重要方式，下視丘爲調節能量衡定之重要中樞，其能透過感測週邊的訊息，以瞭解目前身體之能量狀態，並藉由週邊與中樞之神經網路來調節食慾與能量消耗，進而維持體內能量衡定。證據顯示腦幹也是參與能量衡定之重要區域。

Leptin 是由脂肪細胞以一定比例分泌而來，其能作用於下視丘進而抑制食慾;neurotensin 是神經傳導物，從中樞給予 neurotensin 能抑制食慾，因此，leptin 與 neurotensin 被認爲參與能量衡定之月生月太。研究顯示下視丘與腦幹具有 leptin 與 neurotensin receptor存在，其他研究顯示在腦幹中之迷走神經運動神經核(dorsal motor nucleus of vagus; DMNV)也是 leptin 與 neurotensin 作用之腦區。本研究是以腦幹組織切片爲材料，以電生理之 whole-cell patch-clamp 之技術觀察 leptin 與 neurotensin 對迷走神經運動神經核之影響。

本研究結果顯示 85 個成功紀錄的神經元中，leptin 的投予造成 DMNV 神經細胞去極化(19%)或引發內流電流，而且隨著 leptin 濃度增加(濃度範圍 0.25-300nM)或 leptin 也能造成 DMNV 神經細胞過極化(15%)。河豚毒素存在下，leptin 仍然可以引發 DMNV 神經細胞產生去極化或內流電流反應，膜電位變化爲 8±1mV (n=9)，也能誘發過極化或外流電流，膜電位變化爲 11±3mV (n=10)，因此證明 leptin 能直接作用於 DMNV 神經細胞造成膜電位變化而改變其興奮性。另外，河豚毒素存在下，投予 neurotensin 造成 DMNV 神經元去極化(52%)，平均膜電位變化爲 11　2 mV，證明 neurotensin 能直接作用 DMNV 神經細胞造成膜電位變化而改變其興奮性。

在電位嵌制下，觀察 leptin 或 neurotensin 誘發之 I-V relationships，結果就 leptin 引起之去極化而言，leptin 誘發之電流斜率爲負值，得到反轉電位平均爲-88±4 mV (n=12)；當神經細胞胞外鉀離子濃度增高時，此時 leptin 引發之內流電流變小，反轉電位爲由-87 mV 轉移至-60 mV (n=1)，因此可以確認 leptin 關閉鉀離子通道造成膜電位去極化反應。就 leptin 引起之過極化而言，leptin 誘發之電流斜率爲正値，得到反轉電位平均爲-85±3mV (n=4)，由實驗條件推算出鉀離子通道平衡電位爲-94mV，因此推測 leptin 開啓鉀離子通道造成膜電位過極化反應。就 neurotensin 引起之去極化而言，neurotensin 誘發之電流斜率爲負値，得到反轉電位平均爲-92±3mV (n=9)，由實驗條件推算出鉀離子通道平衡電位爲-94mV，因此推測關閉鉀離子通道造成膜電位去極化反

應。
電生理紀錄前，從腹腔注射 fluoro-gold，以反向運輸標定副交感節前神經細胞，電生理紀錄中，在電極溶液中加入 lucifer yellow (0.2%)，以標定被記錄到神經細胞之位置，藉此方式確認被記錄到神經細胞之位置是否位於 DMNV 核區之副交感節前神經細胞。本研究顯示 leptin 或 neurotensin 作用而產生細胞膜去極化或過極化之 DMNV 神經細胞中，也包括副交感節前神經細胞。因此，本研究證實 leptin 或 neurotensin 可直接作用於腦幹之 DMNV 神經細胞，調節副交感神經活性。

英文摘要

Obesity is one of the major public health problems in many countries in the world including Taiwan and is also a serious threat to health. Regulation of energy homeostasis is critical in body weight control. The hypothalamus, capable of detecting peripheral signals, and thus regulating energy intake (feeding) and expenditure by forming links between central and peripheral systems, appears to be the major integrating site of the adiposity signaling in the central nervous system (CNS). Evidences also suggest brain stem as an important structure involving in the regulation of energy balance.
It has been shown that leptin is secreted in proportion to adiposity to serve as negative feedback signaling molecules on the regulation of body adiposity by acting in the hypothalamus. Neurotensin is a neurotransmitter known to be involved in energy homeostasis by reducing food intake. The receptors of leptin and neurotensin were found not only in the hypothalamus but also in the brain stem. Other anatomical evidences imply the dorsal motor nucleus of the vagus (DMNV) as a potential site of action for both leptin and neurotensin. By using whole-cell patch-clamp recording in brainstem slices, the effects of leptin and neurotensin were investigated in the present study.
Leptin was found to either depolarize or hyperpolarize 19% and 15% of DMNV neurons, respectively, in a dose-dependent manner in the range of 0.5 nM to 300 nM. Neurotensin depolarize 52% of DMNV neurons. The effects of leptin and neurotensin persisted in the presence of tetrodotoxin, indicating direct effects of leptin and neurotensin on DMNV neurons.
With the use of voltage-clamp recording techniques, the current-voltage (I-V) relationship of leptin- or neurotensin-induced currents were examined. Leptin-induced inward current exhibited a negative slope with a reversal potential of -87mV and the reversal potential was shift to -60mV in a high potassium Krebs solution. These results indicate that a decrease of potassium conductance may underlie leptin-induced inward current (or depolarization). Leptin-induced outward current exhibited a

positive slope with a reversal potential of -85±3(n=4)mV implying that an increase of potassium conductance may be the major ionic mechanism of leptin-induced outward current (or hyperpolarization). Similar to the results of leptin-induced inward current a negative slope of the I-V curve and a reversal potential of -92±3mV(n=9) were obtained for neurotensin-induced inward current suggesting a decrease of potassium conductance as the underlying mechanism.

By using retrograde labeling and intracellular staining techniques, the parasympathetic preganglionic neurons in the DMNV were identified. Our results show that leptin- and neurotensin-depolarized or hyperpolarized DMNV neurons include identified parasympathetic preganglionic neurons. The results suggest that DMNV is one of the target sites of leptin and neurotensin in the central nervous system and imply a role of leptin and neurotensin in the regulation of parasympathetic activity.