



Title	Effect of leptin on catecholamine release from rat chromaffin cells and pheochromocytoma cells
Author(s)	SEKIGUCHI, Michiko
Citation	Japanese Journal of Veterinary Research, 48(1), 58-59
Issue Date	2000-05-31
Doc URL	http://hdl.handle.net/2115/2812
Type	bulletin (article)
File Information	KJ00003408146.pdf



[Instructions for use](#)

tive than that obtained under conventional whole-cell voltage clamp condition with the low Cl^- intracellular solution. After measurement of V_{half} with nystatin-perforated configuration, the patch membrane rupture made the same cell to be under conventional whole-cell voltage clamp condition. This protocol shifted V_{half} by about 15 mV toward more negative potential.

12. These properties of the hyperpolarization-activated current (I_h) in rat DRG neurones in the present study almost agree with those in other neuronal cells. I_h in rat DRG neurones was found to be regulated by not only cytosolic cAMP but also cGMP and ATP. I_h channel may contribute towards setting the resting membrane potential in rat DRG neurones.

Effect of leptin on catecholamine release from rat chromaffin cells and pheochromocytoma cells.

Michiko Sekiguchi

*Laboratory of Physiology
Department of Biomedical Sciences
Faculty of Veterinary Medicine
Hokkaido University, Sapporo 060-0818, Japan*

To investigate the effects of leptin on catecholamine release from rat adrenal medullary chromaffin cells and rat pheochromocytoma cells (PC12), I performed RT-PCR to detect mRNA of leptin receptors (Ob-R) and Western blot to detect phosphorylation of MAP kinase which occurred when Ob-R was activated, and measured catecholamine release. The following results were obtained:

1. The mRNA of the long form leptin receptor (Ob-Rb) which had high ability of signal transduction was present in rat chromaffin cells and PC12.
2. Leptin caused phosphorylation of MAP kinase in both rat chromaffin cells and PC12.
3. Leptin had no acute effect on the spontaneous dopamine release in PC12. However, leptin added chronically to the culture medium for more than 4 days inhibited spontaneous dopamine release from PC12 differentiated by NGF, but not from undifferentiated cells.

4. Leptin had no effect on the dopamine release response to 100 μM ACh, 60 mM KCl and 100 μM ATP in NGF-differentiated PC12. Leptin also had no effect on the dose dependency of dopamine release response to ATP in NGF-differentiated PC12. Thus, the inhibition of calcium channels seems not to contribute to the inhibitory effect of leptin on spontaneous dopamine release from NGF-differentiated PC12.

5. Leptin increased the ratio of noradrenaline to dopamine in NGF-differentiated PC12.
6. Leptin had no acute effect on spontaneous and ACh-induced catecholamine release from rat chromaffin cells.

From results mentioned above, it was suggested that a functionally active Ob-R was present in rat chromaffin cells and PC12. Leptin had no effect on the catecholamine release response in rat chromaffin cells. Leptin chronically inhibited spontaneous dopamine release from PC12 differentiated by NGF. Fi-

nally, leptin modulated biosynthesis of noradrenaline from dopamine in PC12 differentiated by NGF.

Molecular Cloning of Mouse SSeCKS and Its Expression during Inflammation

Keisuke OKITA

*Department of Biomedical Sciences
Laboratory of Biochemistry
Graduate School of Veterinary Medicine
Hokkaido University, Sapporo 060-0818, Japan*

During inflammation, the brain mediates various physiological responses, such as fever and anorexia. To explore novel genes involved in these responses, brain mRNA of mice subjected to systemic inflammation by bacterial lipopolysaccharide (LPS) was examined by RNA arbitrarily primed-polymerase chain reaction. Apparent effects of LPS were found in the mRNA level of 34 out of 1500 genes, so far examined. I focused on one gene, 131.5, whose mRNA level was greatly increased by LPS administration. In order to isolate the full-length cDNA of 131.5, a mouse cDNA library was screened using a short-length cDNA fragment of 131.5 as a probe. Sequence analysis of an isolated full-length 131.5 cDNA had an open reading frame of 5.0 kbp encoding a polypeptide of 1684 amino acids, which was 84.8% identical to rat SSeCKS (rSSeCKS). Thus it was concluded that 131.5 was the gene of mouse SSeCKS (mSSeCKS). Predicted protein of mSSeCKS, like rSSeCKS, contained four protein kinase C phosphorylation sites, one protein kinase A binding site, and an N-terminal myristylation signal, but not the Zn finger domain. Expression of

mSSeCKS mRNA was examined in the brain and several peripheral organs by Northern blot analysis. In untreated control mice, mSSeCKS mRNA was expressed abundantly in the testis, but at undetectable levels in other organs. LPS administration induces mSSeCKS mRNA not only in the brain but also in the lung, heart, liver, spleen and kidney. In the lung and spleen, the mSSeCKS mRNA level was increased almost 10-fold after 1 hour, and was kept at high levels for several hours. *In situ* hybridization and immunohistochemical examinations showed strong signals of mSSeCKS mRNA and protein predominantly at microvascular endothelial cells of various tissues. Overexpression of rSSeCKS is known to cause cell flattening, formation of cellular projections, and the temporary loss of actin stress fibers and adhesion plaques, indicating rSSeCKS as a molecule to control the actin-based cytoskeletal architecture. Thus, my results suggest that mSSeCKS may play a role of the LPS-induced cellular responses, including the change of cytoskeleton in endothelial cells, and thereby an increase of vascular permeability.