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# Effects of Ethanol on the Induction of Uncoupling Protein-1 (UCP1) mRNA in the Mouse Brown Adipose Tissue

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YOSHIMOTO, K., YASUHARA, M., KOMURA, S., MISUMI, Y., UCHIYAMA, Y., KOGURE, A., HIOKI, C., WAKABAYASHI, Y., SATOMI, Y., NISHIMURA, A., FUKUDA, F., HORI, M., YOKOYAMA, C. and YOSHIDA, T. *Effects of Ethanol on the Induction of Uncoupling Protein-1 (UCP1) mRNA in the Mouse Brown Adipose Tissue*. Tohoku J. Exp. Med., 2004, **204** (1), 45-51 —— Expression of uncoupling protein-1 (UCP1) is increased by cold acclimation and overfeeding, and reduced in fasting and genetic obesity. It is known that the mitochondrial UCP1 in the brown adipose tissue (BAT) is an important key molecule for non-shivering thermogenesis. On the other hand, ethanol (EtOH) alters thermoregulation in humans and laboratory animals. However, the relationship between EtOH intake and UCP1 expression is not yet clear. Accordingly, the present study employed the technique of real-time quantitative polymerase-chain reaction (PCR) to investigate the effects of EtOH (0.5 or 2.0 g/kg) on the expression of UCP1 mRNA in the mouse BAT. Control mice were injected with the same volume of physiological saline intraperitoneally (IP). IP injection of EtOH (0.5 g/kg) caused a decrease and an increase of the expression of BAT UCP1 mRNA at 1 and 4 hours, respectively. Treatment with EtOH (2.0 g/kg) caused an increases of the expression of BAT UCP1 mRNA at both 2 and 4 hours. BAT UCP1 mRNA levels in both groups increased at 4 hours after EtOH administration. The levels of UCP1 mRNA returned to the control levels by 8 hours after EtOH administration. The expression of BAT UCP1 mRNA was upregulated following EtOH administration, although a lower dose of EtOH initially reduced the expression of UCP1 mRNA in BAT. These findings suggest that EtOH-induced UCP1 mRNA expression in BAT reflects an alteration of the set point of thermogenesis. ——— uncoupling protein; ethanol; brown adipose tissue; mouse



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Thermogenesis depends on the action of uncoupling protein (UCP), a protein located in the inner membrane of mitochondria in the brown adipose tissue (BAT) (Horvath et al. 2002). UCP1 dissipates heat through the electrochemical gradients generated by respiratory activity (Cannon and Nedergaard 2004). Other uncoupling proteins, called UCP2 and UCP3, with high homology to the UCP1, have been discovered (Boss et al. 1997). UCP2 and UCP3 are also found in the BAT. UCP1 mainly controls thermoregulation in the BAT (Boss et al. 1997; Cinti et al. 2002).

Forensic studies have suggested a link between ethanol (EtOH) consumption and an impairment of thermoregulation (Hirvonen 1976). EtOH has been shown to produce a reduction in body temperature in humans and animals (Kalant and Le 1984; Alkana et al. 1996). EtOH generally lowers body temperature at normal ambient temperature. Also, it is well documented that neurons in the hypothalamic nuclei play a major role in integrating the various thermal inputs (Boulant et al. 1989). Changes in the releases of EtOH-induced serotonin (5-HT) and norepinephrine in this sympathetic nerve area have been implicated in possible mechanisms for the thermal regulation (Huttunen et al. 1988).

The effects of acute IP administration of EtOH on the extracellular concentrations of dopamine and 5-HT in the nucleus accumbens and the central amygdala of rats were studied using in vivo microdialysis coupled with the HPLC electrochemical detection procedure. EtOH increases the release of both dopamine and 5-HT in the CNS (Yoshimoto et al. 1991, 2003). Recently, a selective 5-$HT_{2c}$ receptor agonist, 1-(3-chrolophenyl) piperazine (mCPP), has been shown to lower food intake, reduce body weight, and accelerate the appearance of the behavioral satiety sequence (Vickers et al. 2003). Additionally, administration of mCPP reduced the time of EtOH-withdrawal (Rezazadeh et al. 1993). These findings suggest that the 5-$HT_{2c}$ receptor function is related to the development of alcohol dependence, alcohol drinking and eating disorders, so-called consummatory behavior. This behabior is influenced by the release of 5-HT, dopamine and other neurotransmitters in the CNS, especially the sympathetic nervous systems.

Non-shivering thermogenesis associated with the UCP is a major component of energy expenditure to control body weight and lipid metabolism in rodents (Nicholls and Locke 1984). A better knowledge of UCP1 expression and its relationship to alcohol consumption could be very important for achieving the satiety of pharmacological sites for obesity treatment, and for the treatment of alcoholic disorders (Cannon and Nedergaad 2004). These findings suggest some linkage between EtOH-induced hypothermia and eating disorders. However, they did not show any causal relationship between the effect of EtOH on thermoregulation and feeding behavior. In the present study, we investigated whether EtOH acutely alters the expression of the UCP mRNA in the BAT of mice.

## Materials and Methods

A total of 90 male ICR mice were obtained from Clea Japan (Osaka) at 8 weeks of age and were housed in plastic cages at 22±2°C with a 12 hour light-dark cycle and free access to laboratory food (CE-2, Clea Japan) and tap water.

Animal care and experimental procedures were approved by the Animal Care Committee of Kyoto Prefectural University of Medicine. Animals were divided into two experimental groups and a control group. Mice group were injected intraperitoneally (IP) with 0.5 g/kg of EtOH (10%[v/v]), or 2.0 g/kg of EtOH (20% [v/v]). The control group was injected with physiological saline of the same volume.

After the administration of EtOH, the mice were killed by cervical dislocation at 0.5, 1.0, 2.0, 4.0, 8.0 and 16 hours. Interscapular BAT was removed rapidly and frozen in liquid nitrogen for real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis (Kogure et al. 2002a).

Total RNA was extracted from 0.1-0.3 g of

tissue using Sepazol-RNA I super (Nacalai Co., Kyoto). Total RNA (2 $\mu$g) was denatured at 80°C for 5 minutes, cooled immediately and reverse-transcribed using 100 units of Molony murine leukemia virus reverse transcriptase (Gibco BRL), 50 pmol of poly (dT) primer and 20 nmol of dNTPs in a total volume of 20 $\mu$l at 37°C for 1 hour.

Real-time quantitative PCR was performed in a fluorescence temperature cycler (LightCycler TM, Roche Diagnostics GmbH, Mannheim, Germany), with 6 $\mu$l of reaction mixture containing 3 mmol/liter MgCl, 50 mmol/liter Tris HCl (pH 8.3), 500 ng/$\mu$liter bovine serum albumin, 200 $\mu$mol/liter each dNTP, 1 : 30 000 dilution of SYBR Green I, 5 umol/liter each primer, 0.05 U/$\mu$l Taq DNA polymerase, 11 ng/$\mu$l TaqStart TM antibody (Clontech Laboratory, Palo Alto, CA, USA) and a template (Morrison et al. 1998).

Amplification was carried out using three-cycle procedure (denaturation, 95°C, 1 second, ramp rate 20°C/second; annealing 60°C, 10 seconds ramp rate 20°C/second; and extension 72°C, 26 seconds, ramp rate 2°C/second) for 40 cycles. The fluorescence signal was plotted against the cycle number for all samples and external standards.

Table 1 lists the primers used in the present study, and their predicted product length. Some amplification products performed in the LightCycler were checked by electrophoresis of 1.5% ethidium bromide-stained agarose gels. The estimated size of the amplified fragments matched the calculated size for UCP1 (197bp) and $\beta$-actin (584 bp), in all cases.

All data are expressed as the means±S.E.M. Statistical significance was evaluated using an analysis of variance (ANOVA) or Student's *t*-test, using Stat View 5.0 (SAS Institute, Cary, NC, USA).

## Results and Discussion

The time course of the induction of UCP1 mRNA showed significant changes following by the treatment of EtOH (0.5 g/kg i.p.) ($F[6,45]=6.228$, $p<0.001$). UCP1 mRNA in the 0.5 g/kg EtOH treatment group showed the maximum reduction 1 hour after EtOH administration, compared with the UCP1 mRNA levels of the control group ($p<0.01$). Thereafter, the levels of UCP1 mRNA increased rapidly and reached a maximum ($p<0.05$). It then returned to the levels of the control group after 8 hours (Fig. 1).

On the other hand, the UCP1 mRNA level in the 2.0 g/kg EtOH treatment groups was not different from that in the control group one hour after the administration. The level of UCP1 mRNA increased significantly at 2 hours after EtOH administration, and showed a maximum increase in the BAT at 4 hours ($p<0.01$) ($F[6,43]= 3.102$, $p<0.005$). The increased levels of UCP1 mRNA returned to the basal level at 8 hours (Fig. 2). There was no significant difference in body weight or BAT weight among the mice treated with either EtOH or physiological saline (data not shown).

Based on the data in Figs. 1 and 2, the relative changes of the mRNA levels of UCP1 in the BAT are shown in Fig. 3. The lower dose of EtOH (0.5 g/kg IP) caused a reduction in the level

Table 1 *Primer sets used for real time PCR*

| | GenBank | | Sequence | Position | Expected size (bp) |
|---|---|---|---|---|---|
| $\beta$-actin | X63672 | Forward | ATG AAG ATC CTG ACC GAG GGT | 568-588 | 584 |
| | | Reverse | AAC GCA GCT CAG TAA CAG TCC G | 1128-1151 | |
| UCP1 | U63418 | Forward | GTG AAG GTC AGA ATG CAA GC | 409-428 | 197 |
| | | Reverse | AGG GCC CCC TTC ATG AGG TC | 586-605 | |

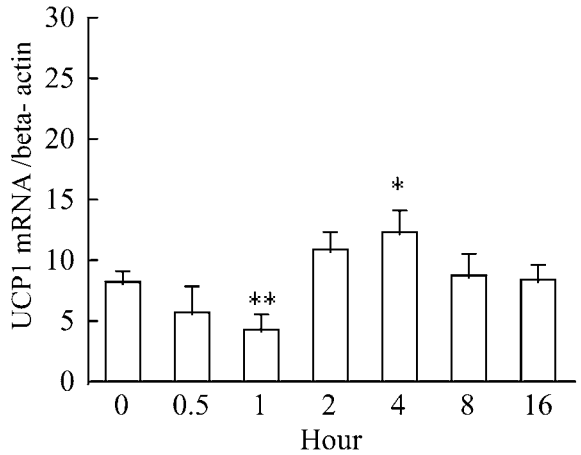


Fig. 1. Real-time quantitative RT-PCR analysis of uncoupling protein 1(UCP1) mRNA of interscapular brown adipose tissue (BAT). Mice were treated with EtOH (0.5 g/kg i.p.) or physiological saline (Control), respectively. Values are means±S.E.M. for 6-10 ICR mice at each time. $^{*}p<0.05$ and $^{**}p<0.01$ are compared with the control group (0 hour).

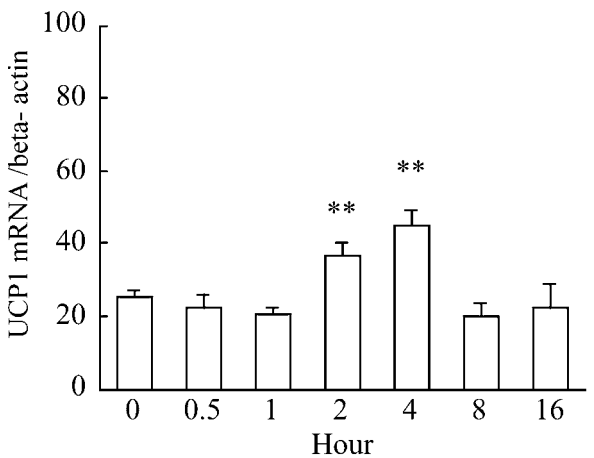


Fig. 2. Real-time quantitative RT-PCR analysis of uncoupling protein 1(UCP1) mRNA of interscapular brown adipose tissue (BAT). Mice were treated with EtOH (2.0 g/kg i.p.) or physiological saline, respectively. Values are means ±S.E.M. for 6-8 ICR mice at each time. $^{**}p<0.01$ is compared with the control group (0 hour).

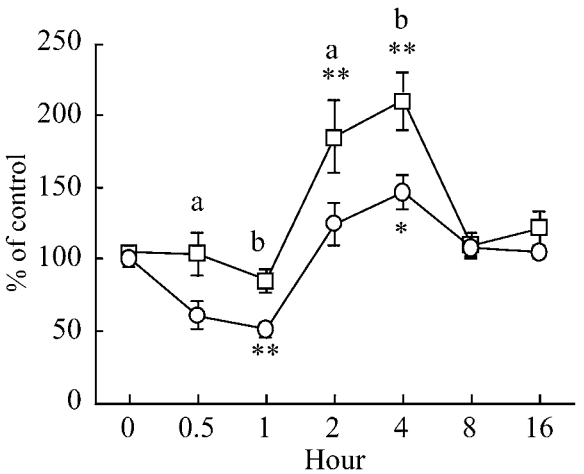


Fig. 3. Time course of relative changes in the induction of uncoupling protein 1 (UCP1) mRNA with the real-time quantitative RT-PCR analysis. $^{*}p<0.05$ and $^{**}p<0.01$ are compared with the control group (0 hour), $^{a}p<0.05$ and $^{b}p<0.01$ are compared with the EtOH (0.5 g/kg)-treated group (open circles). Open squares indicate the changes in mice treated with EtOH (2.0 g/kg).

of UCP1 mRNA at one hour, and the level thereafter returned to the control basal level. The high dose of EtOH (2.0 g/kg i.p.) caused an increase in the level of UCP1 mRNA at two hours. Two hours after administration, the levels of UCP1 mRNA in both treatment groups showed the same remarkable pattern of the change of the UCP1 mRNA level.

It is commonly believed that EtOH impairs thermoregulatory mechanisms and also lowers the hypothermic set point for body temperature control. This effect of EtOH in the CNS results in an alteration of the induction of UCP1 mRNA in the peripheral area, BAT. It is hypothesized that levels of UCP1 mRNA may reflect the response to the sympathetic nervous system in the hypothalamus.

BAT is essential for non-shivering thermogenesis, and appears to be a site for an adaptive response to cold acclimation-recruited norepinephrine-induced thermogenesis. It transfers energy from food into heat and plays a crucial role in the energy balance by dissipating excess energy

intake as heat (Himms-Hagen 1989). Information on body temperature, feeding state, and body energy reserves is coordinated in an area of the brain, the so-called ventromedial hypothalamic nucleus (Ricquier et al. 1986). Increases in the rate of food combustion that decrease metabolic efficiency, or increases in the rate of heat production, are stimulated by the released transmitter norepinephrine via $\beta$-adrenergic receptors, especially $\beta_3$ subtype (Yoshida et al. 1991; Cannon and Nedergaard 2004).

In the present study, UCP1 m RNA levels decreased significantly at 1 hour after the administration of the lower dose of EtOH (Fig. 1). A gradual decrease in the level of UCP1 mRNA was also observed at one hour in the higher-dose EtOH group (Fig. 2). Next, a rapid and significant increase in the level of UCP1 mRNA was observed at 4 hours (Figs. 1, 2 and 3). Similar rapid changes of UCP were reported in rats exposed to the cold (Ricquier et al. 1986). These findings may indicate that the set point of thermoregulation occurs at an early stage in the EtOH-exposed animals. Le et al. (1981) reported that the maximum reduction in rectal temperature usually occurs at about 30-60 minutes after EtOH administration. The reduction of the UCP1 mRNA levels in the present study is consistent with the changes in body temperature due to hypothermia, as shown in animal models.

Furthermore, administration of 0.5 g/kg EtOH caused more marked changes in the levels of UCP1 mRNA than administration of 2.0 g/kg EtOH (Fig. 3). This finding is also consistent with the fact that a dose of 0.5 g/kg EtOH lowers rectal temperatures (Lomax et al. 1980). Small doses of EtOH affect the function of UCP, which regulates cellular energy homeostasis by promoting energy dissipation as heat, and may alter the hypothalamic set point for thermoregulation. Such a remarkable change of the induction of BAT UCP1 mRNA suggests an effect at the transcriptional level of *UCP* genes, but it is still not clearly understood.

In this study, 0.5 g/kg EtOH-treated mice showed a more marked decrease in the level of UCP1 mRNA than 2.0 g/kg EtOH-treated treated mice (Figs. 1, 2 and 3). BAT thermogenetic capacity was reduced in mice offered only 10% (v/v) EtOH for 10 days as voluntary drinking fluid (Muralidhara and Desauntels 1996). Light to moderate EtOH intake influences thermoregulation through UCP1 mRNA expression (Huttunen et al. 1998). Following i.p. injection of subhypnotic or hypnotic EtOH doses, the maximum hypothermia occurs around 30-60 minutes post injection and can last for more than 2 hours (Malcolm and Alkana 1981). The effect of EtOH on core temperature depends on the time of the measurement with respect to EtOH adminstration (O'Conner et al. 1989).

After a single EtOH administration, the changes of the expression of BAT UCP1 mRNA depend on the EtOH absorption. On the other hand, increases of UCP1 mRNA levels may reflect the effects of EtOH metabolites, acetaldehyde and acetate, and EtOH elimination at late times. Furthermore, alcohol ingestion induces the mitochondrial cytochrome oxidative capactiy of the BAT in the rat (Huttunen and Kortelainen 1988). These findings raise a question about whether the mechanism of EtOH-induced changes in UCP function is directly or indirectly related to the BAT with a U-shaped pattern; however, we are unable to clearly explain this discrepancy based on the present study.

Recently, a 5-$HT_1$ receptor agonist, 8-OH-DPAT, was shown to cause a reversal of the leptin-evoked stimulation of the BAT sympathetic nerve activity and thermogenesis (Morrison 2004). We previously reported that ethanol-induced 5-HT release in the nucleus accumbens could be suppressed with the administration of 8-OH-DPAT (Yoshimoto et al. 1991). EtOH-induced UCP1 mRNA expression, which influences thermogenesis, is associated with the hyperpolarization of local sympathetic premotor neurons, via the 5-$HT_{1A}$ autoreceptor mechanism in the CNS, possibly in the ventromedial hypothalamus.

Furthermore, the selective 5-$HT_{2C}$ serotonin receptor agonist 1-(3-chlorophenyl) piperazine (mCPP) has been reported to lower food intake and reduce the body weight by regulating energy dissipation (Vickers et al. 2003) and to act as a 5-HT releaser itself (Bauman et al. 2001). It may be suggested that a rapid increase of the induction of UCP1 mRNA in the BAT is associated with the activation of $5HT_{1A}$ and/or $5HT_{2C}$ receptors following the EtOH-induced 5-HT releases in the hypothalamus. A $\beta$3-adrenergic receptor agonist, CL 316243, up-regulates BAT UCP1 mRNA expression or thermogenesis and reduces the body weight in mice (Kogure et al. 2002b). A possible explanation is that EtOH-induced release of norepinephrine and 5-HT in the hypothalamus activates the expression of the UCP1 gene in the BAT through the sympathetic nervous system.

In conclusion, the mRNA levels of BAT UCP1 increased following EtOH administration, although lower EtOH dose initially caused a decrease of the expression of BAT UCP1 mRNA. This suggests that the set point of thermogenesis and/or eating disorders are related to the EtOH-induced UCP1 mRNA levels.

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## References

Alkana, R.L., Davies, D.L. & Le, A.D. (1996) Ethanol's acute effects on thermoregulation : Mechanisms and consequences. In: *Pharmacological Effects of Ethanol on the Nervous System*, edited by R. A. Deitrich & V.G. Erwin, CRC Press, New York, pp. 291-328.

Bauman, M.H., Ayestas, M.A., Dersch, C.M. & Rothman, R.B. (2001) 1-(m-chlorophenyl) piperazine (mCPP) dissociate in vivo serotonin release from long term serotonin depletion in rat brain. *Neuropsychopharmacology,* **24**, 492-501.

Boss, O., Samec, S., Dullo, A., Seydoux, J., Muzzin, P. & Giacobino, J.P. (1997) Tissue dependent upregulation of rat uncoupling protein –2 expression in response to fasting or cold. *FEBS Lett.*, **412**, 111-114.

Boulant, J.A., Curras, M.C. & Deam, J.B. (1989) Neurophysiological aspects of thermoregulation. In: *Advances of Comparative and Emriroment physiology*, edited by L.C.H. Wang, Springer- Verlag, Berlin, pp. 117-134.

Cannon, B. & Nedergaard, J. (2004) Brown adipose tissue: function and physiological significance. *Physiol. Rev.*, **84**, 277-359.

Cinti, S., Cancello, R., Zingaretti, M.C., Ceresi, E., De Matteis, R., Giordano, A., Himme-Hagen, J. & Ricquier, D. (2002) CL 316,243 and cold stress induce heterogenous expression of UCP1 mRNA and protein in rodent brow adipocytes. *J. Histochem., Cytochm.,* **50**, 21-32.

Himms-Hagen, J. (1989) Brown adipose tissue thermogenesis and obesity. *Prog. Lipid Res.*, **28**, 67-115.

Hirvonen, J. (1976) Necropsy findings in fatal hypothermia cases. *Forens. Sci.*, **8**, 155-164.

Horvath, B., Spies, C., Horvath, G., Kox, W.J., Miyamoto, S., Barry, S., Warden, C.H., Bechmann, I., Diano, S., Heemskerk, J. & Horvath, T.L. (2002) Uncoupling protein 2 (UCP2) lowers alcohol sensitivity and pain threshold. *Biochem. Pharmacol.*, **64**, 369-374.

Huttunen, P. & Kortelainen, M.L. (1989) Chronic alcohol intake induces the oxdative capacity of brown adipose tissue in the rat. *Pharmacol. Biochem. Behav.,* **29**, 53-57.

Huttunen, P., Sampi, M. & Myllyla, R. (1998) Ethanol-induced hypothermia and thermogenesis of brown adipose tissue in the rat. *Alcohol,* **15**, 315-318.

Kalant, H. & Le, A.D. (1984) Effects of ethanol on thermoregulation. *Pharmacol. Ther.*, **23**, 313-364.

Kogure, A., Sakane, N., Takakura, Y., Umekawa, T., Yoshioka, K., Nishino, H., Yamamoto, T., Kawada, T., Yoshikawa, T. & Yoshida, T. (2002a) Effects of cafferin on the uncoupling protein family in obese yellow KK mice. *Clin. Exp. Pharm. Physiol.*, **29**, 391-394.

Kogure, A., Yoshida, T. & Yoshikawa, T. (2002b) Upregulation of the uncoupling protein family by chronie treatment with $\beta_3$-adrenergic receptor agonist in obese yellow KK mice. *J. Clin. Biochem. Nut.*, **32**, 1-9.

Le, A.D., Khanna, J.M. & Kalant, H. (1981) The effect of lesions in the dorsal median and magnus raphe nuclei on the development of toler-

ance to alcohol. *J. Pharmacol. Exp. Ther.*, **218**, 525-529.

Lomax, P., Bajork, J.G., Chesarek, W.A. & Chaffee, R.R.J. (1980) Ethanol induced hypothermia in the rat. *Pharmacology*, **21**, 288-294.

Malcolm, R.O. & Alkana, R.L. (1981) Temperature dependence of ethanol depression in mice. *J. Pharmacol. Exp. Ther.*, **217**, 770-775.

Morrison, S.F. (2004) Activation of 5-HTIA receptors in raphe pallidus inhibits leptin-evoked increases in brown adipose tissue thermogenesis. *Am. J. Physiol. Regul. Interg. Comp. Physiol.*, **286**, R832-R837.

Morrison, T.B., Weis, J.J. & Witwer, C.T. (1998) Quantification of low-copy transcripts by cantinuous SYBR Green monitoring during amplification. *Biotechniques*, **24**, 952-962.

Muradidhara, D.V. & Desautels, M. (1996) Effects of ethanol consumption on brown adipose tissue thermogenic capacity in mice. *Physiol. Behav.*, **60**, 639-644.

Nicholls, D.G. & Locke, R.M. (1984) Thermogenic mechanisms in brown fat. *Physiol. Rev.*, **64**, 1-61.

O'Connor, C.S., Crawshaw, L.I., Kosobud, A., Bedichek, R.C. & Crabbe, J.C. (1989) The effect of ethanol on behavioral temperature regulation in mice. *Pharmacol. Biochem. Behav.*, **33**, 315-319.

Rezazadeh, S.M., Praather, P.L. & Lal, H. (1993) Sensitization to 5-$HT_{1c}$ receptor agonist in rats observed following withdrawal from chronic ethanol. *Alcohol,* **10**, 281-283.

Ricquier, D., Bouillaud, F., Toumelin, P., Mory, G., Bazin, R., Arch, J. & Penicaud, L. (1986) Expression of uncoupling protein mRNA in thermogenic or weakly thermogenic brown adipose tissue. *J. Biol. Chem.*, **261**, 13905-13910.

Vickers, S.P., Easton, N., Webster, L.J., Wyatt, A., Bickerdike, M.L., Dourish, C.T. & Kennett, G.A. (2003) Oral administration of 5-$HT_{2c}$ receptor agonist, mCPP, reduces body weight gain in rats over 28 days as a result of maintained hypophagia. *Psychopharmacology (Berl.),* **167**, 273-280.

Yoshida, T., Hiraoka, N., Yoshioka, K., Hasegawa, G. & Kondo, M. (1991) Anti-obesity and anti-diabetic actions of a beta 3-adrrenoceptor agonist, BRL 26830A, in yellow KK mice. *Endocrinol. Jpn.*, **38**, 397-403.

Yoshimoto, K., McBride, W.J., Lumeng, L. & Li, T.K. (1991) Alcohol stimulates the release of dopamaine and serotonin in the nucleus accumbens. *Alcohol,* **9**, 17-22.

Yoshimoto, K., Komura, S., Hattori, H., Yamori, Y., Miura, A., Yoshida, T., Hioki, C., Kato, B., Fukuda, F., Tanaka, S., Hirai, A., Nishimura, A., Sawai, Y. & Yasuhara, M. (2003) Pharmacological studies of alcohol susceptibility and brain monoamine function in stroke-prone spontaneously hypertensive rats (SHRSP) and stroke-resistant spontaneously hypertensive rats (SHRSR). *Tohoku J. Exp Med.*, **201**, 11-22.