Effect of CL316,243, a Highly Specific β_3-Adrenoceptor Agonist, on Sympathetic Nervous System Activity in Mice

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To examine whether long-term administration of a β_3-adrenoceptor agonist influences sympathetic nervous system (SNS) activity, norepinephrine (NE) turnover, a reliable indicator of SNS activity, in the interscapular brown adipose tissue (IBAT), the heart, and the spleen, as well as urinary excretion of NE, were measured using mice treated with CL316,243 (CL), a highly specific β_3-adrenoceptor agonist, at a dose that stimulated thermogenesis and reduced body weight. CL significantly decreased NE turnover in the IBAT, heart, and spleen and decreased urinary excretion of NE without affecting food intake over 1 to 4 weeks of treatment. These findings show that long-term administration of the β_3-adrenoceptor agonist decreases SNS activity and urinary excretion of NE.

MESSENGER RNA encoding the β_3-adrenergic receptor is present in human white and brown adipose tissue (BAT).[1] Because β_3-adrenoceptor agonists[2,3] that stimulate lipolysis in white adipose tissue and activate BAT could emerge as effective drugs for the treatment of obesity and diabetes in obese persons, the study and development of such substances is actively pursued.[4-6] However, it is not clear whether β_3-adrenoceptor agonists influence sympathetic nervous system (SNS) activity. To clarify this point, we measured norepinephrine (NE) turnover,[7,8] a reliable indicator of SNS activity, in the interscapular BAT (IBAT),[9,10] the heart, and the spleen, as well as urinary excretion of NE, using mice treated with CL316,243 (CL),[3,11] a highly specific β_3-adrenoceptor agonist, at a dose that stimulated thermogenesis and reduced body weight.[3,11]

MATERIALS AND METHODS

Chemicals

CL,[12] disodium (R,R)-5-[2-[[2-3-(3-chlorophenyl)-2-hydroxethyl]-amino]propyl]-1,3- benzodioxole-2,2-dicarboxylate, was provided by American Cyanamid (Pearl River, NY).

Animals

One hundred twenty-eight female Institute of Cancer Research (ICR) mice were obtained from Charles River (Osaka, Japan) at the age of 8 weeks. They were housed in plastic cages, six mice per cage, at 22° ± 2°C with a 12-hour light-dark cycle, and were given free access to laboratory chow and tap water. One week later, they were divided into two groups. One group was administered CL dissolved in distilled water at a dose of 0.1 mg/kg via a gastric tube once daily for 1, 2, or 4 weeks. The other group (controls) was administered plain distilled water once daily via a gastric tube for 1, 2, or 4 weeks. Body weight and daily food intake were measured at 1, 2, and 4 weeks of treatment. Urine for NE determinations from animals in individual metabolic cages was collected under oil with addition of 1.5 mL 1N HCl for each 24-hour collection. One hour after the last administration of CL or distilled water, NE turnover was determined in IBAT, heart, and spleen from the inhibition of NE biosynthesis by intraperitoneal administration of α-methyl-*p*-tyrosine (MPT),[8,13,14] the methyl ester of α-MPT (80 mg/kg; Sigma Chemical, St Louis, MO). After the 1-, 2-, or 4-week treatment, six animals in each group were killed by cervical dislocation before and at 3 and 6 hours after the injection. The IBAT, heart, and spleen were removed and homogenized in 0.1N perchloric acid. NE content of the tissues was determined by a high-performance liquid chromatograph (LC-6A; Shimadzu, Kyoto, Japan) using our previously described method.[15,16] The electrode potential was set at 0.5V versus Ag/AgCl reference electrode. NE levels were recorded by an integrator and calculated from the chromatographic peak area (LC-6A; Shimadzu). NE content in urine was measured using a minor modification of the above method[15,16] with Wakosil-II 3C18 HG (ϕ 4.6 mm × 50 mm; Wako Pure Chemical Industries, Tokyo, Japan) for the precolumn and Wakosil-II 5C18 HG (ϕ 4.6 mm × 250 mm; Wako Pure Chemical) for the main column. Urinary creatinine level was measured using a standard AutoAnalyzer (Hitachi-7450, Tokyo, Japan) technique.

The data are presented as the mean ± SEM. Statistical analyses used ANOVA analysis of covariance. In studies of NE turnover, data were plotted semilogarithmically. The slope (fractional NE turnover rate [K]) of the decline in endogenous NE after injection of α-MPT was calculated by the method of least squares. Comparison of fractional turnover rates was made with analysis of covariance. NE turnover rates were calculated as the product of the fractional turnover rate (k) and the endogenous NE concentration. Ninety-five percent confidence intervals were determined for NE turnover rates as previously described.[8,17]

RESULTS

Body weight in the CL-treated group was significantly ($P < .01$) less than in the control group treated with distilled water after 1, 2, and 4 weeks of treatment (Table 1). Judging from the two-way ANOVA on CL and duration of treatment, it is clear that CL had a significant effect ($P < .01$) but duration of treatment did not. Moreover, there was no significant interaction between CL and duration of treatment. CL did not affect food intake through 1 to 4 weeks of treatment. Urinary NE excretion in the CL-treated group was significantly ($P < .01$) lower than in the control group through 1 to 4 weeks of treatment. Results of the two-way ANOVA showed CL to have a significant effect

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Table 1. Body Weight, Food Intake, Urinary NE Excretion, and NE Content and Turnover by the NE Synthesis-Inhibition Technique in the IBAT, Heart, and Spleen of Mice Treated With CL

	Control			CL		
	1 Week	2 Weeks	4 Weeks	1 Week	2 Weeks	4 Weeks
Body weight (g)	31.2 ± 0.3	31.5 ± 0.3	31.9 ± 0.3	28.7 ± 0.2†	28.2 ± 0.2†	27.4 ± 0.2†
Food intake (g/d)	6.9 ± 0.5	6.9 ± 0.3	6.8 ± 0.4	7.3 ± 0.5	7.2 ± 0.5	7.2 ± 0.4
Urinary NE excretion (ng · NE/mg · creatinine)	191.2 ± 18.2	192.8 ± 13.4	196.4 ± 16.7	138.6 ± 9.4*	138.4 ± 10.2*	151.4 ± 7.1*
IBAT						
Weight (g)	0.76 ± 0.09	0.78 ± 0.07	0.81 ± 0.06	0.88 ± 0.06	0.89 ± 0.05	0.91 ± 0.06
NE (ng/IBAT)	113.7 ± 5.5	110.7 ± 3.8	113.0 ± 2.6	109.0 ± 4.2	108.2 ± 3.3	110.2 ± 4.1
NE k (%/h)	18.1 ± 2.6	17.9 ± 2.4	17.6 ± 1.7	5.1 ± 1.5†	4.8 ± 1.5†	4.9 ± 1.3†
NE turnover (ng · IBAT^{-1} · h^{-1})	20.6 ± 4.1	19.8 ± 3.4	19.9 ± 2.4	5.6 ± 1.9*	5.2 ± 1.8*	5.4 ± 1.7*
Heart						
Weight (g)	0.148 ± 0.005	0.151 ± 0.005	0.146 ± 0.005	0.148 ± 0.003	0.150 ± 0.005	0.150 ± 0.005
NE (ng/heart)	112.6 ± 2.4	111.0 ± 3.4	113.3 ± 3.0	110.7 ± 2.8	105.1 ± 5.4	108.2 ± 3.4
NE k (%/h)	18.1 ± 1.7	18.7 ± 1.4	18.8 ± 1.8	4.8 ± 1.2†	2.9 ± 1.2†	3.8 ± 1.0†
NE turnover (ng · heart^{-1} · h^{-1})	20.3 ± 2.4	20.8 ± 2.2	21.3 ± 2.7	5.3 ± 1.5*	3.0 ± 1.5*	4.1 ± 1.3*
Spleen						
Weight (g)	0.116 ± 0.008	0.118 ± 0.006	0.120 ± 0.010	0.107 ± 0.007	0.115 ± 0.006	0.125 ± 0.009
NE (ng/spleen)	97.1 ± 3.0	94.4 ± 3.3	94.1 ± 2.7	89.5 ± 3.8	92.7 ± 5.1	89.0 ± 4.3
NE k (%/h)	17.1 ± 2.9	18.2 ± 2.4	16.5 ± 2.5	2.9 ± 1.4†	4.3 ± 1.7†	5.4 ± 1.4†
NE turnover (ng · spleen^{-1} · h^{-1})	16.6 ± 3.4	17.2 ± 2.9	15.5 ± 2.9	2.6 ± 1.4*	4.0 ± 1.9*	4.8 ± 1.5*

NOTE. Data are presented as the mean ± SEM and were analyzed by one-way or two-way ANOVA and the Bonferroni *t* test. NE turnover rates are expressed as the mean with 95% confidence limits. Six mice were used at each time point to obtain turnover data. Endogenous NE is the value at time 0.

*$P < .05$, †$P < .01$: *v* corresponding control group.

($P < .01$). The weight and NE content of IBAT in the CL-treated group did not change as compared with those in the control group through 4 weeks. However, fractional NE turnover (k) and total NE turnover of IBAT in the CL-treated group were significantly ($P < .01$) lower than in the control group through 4 weeks of treatment (Fig 1). Judging from the two-way ANOVA with CL and duration of treatment, CL had a significant effect ($P < .01$). The weight and NE content of the heart did not change with CL treatment. However, fractional and total NE turnover rates in the heart in the CL-treated group were significantly ($P < .01$) lower than in the control group from 1 to 4 weeks of treatment (Fig 2). From the two-way ANOVA on CL and duration of treatment, CL was shown to have a significant effect ($P < .01$). The weight and NE content of the spleen were not affected by CL treatment. Fractional and total NE turnover rates in the spleen of the CL-treated group were significantly ($P < .01$) inhibited compared with rates in the control group from 1 to 4 weeks of treatment. Judging from the two-way ANOVA on CL and duration of treatment, CL had a significant effect ($P < .01$).

DISCUSSION

The present findings show that long-term administration of CL,[3,12] a specific β_3-adrenoceptor agonist, at a dose that stimulates thermogenesis and reduces body weight,[3,11] decreases NE turnover (a reliable indicator of SNS activity) in the BAT, heart, and spleen and decreases urinary NE excretion.

The finding that CL decreases body weight without affecting food intake is consistent with our previous reports using obese diabetic yellow KK mice[11] and MSG obese mice[3] and other reports.[12,18,19] However, had we reduced the body weight of mice from 31 g to 28 g by food restriction, the reduced animals would have eaten ravenously if food were available. Therefore, the fact that CL-treated mice did not eat indicates that treatment with CL restrained food intake. Furthermore, the present findings that the CL compound decreases NE turnover in the BAT, heart, and spleen and decreases daily urinary NE excretion are in accordance with the report of Yen et al[20] on acute injection of LY104119, a β-agonist that is a thermogenic weight-reducing compound, but are contrary to their data on the NE turnover of BAT in a 2-week administration of LY104119. Although the cause of the discrepancy between long-term administration of CL and LY104119[20] is not clear, the differences in β_3-receptor specificity between these drugs may explain this discrepancy. That is, CL has been reported[3,12] to be superior to conventional drugs in terms of β_3-receptor specificity (relative selectivity, 0, 1, and 100,000 for β_1-, β_2-, and β_3-receptors, respectively), although data on the relative selectivity for β_1-, β_2-, and β_3-receptors of LY104119 have not been reported.[20] Downregulation of the β_3-receptor has recently been reported[21-25] not to be caused by long-term administration of a β_3-adrenoceptor agonist, although there have been reports showing contradictory data.[26,27] Downregulation of β_1-, and β_2-receptors is induced by long-term administration of β_1- and β_2-adrenoceptor agonists.[28,29] Therefore, long-term administration of the CL compound may continuously stimulate β_3-receptors on peripheral organs without reducing its effect even in the chronic stage. In contrast, residual β_1 and β_2 actions of LY104119[20] may lead to downregulation of these β_1- and β_2-receptors, resulting in the increased SNS activity seen 2 weeks after treatment with this drug.

The present experiment involved administration of CL by gastric tube, and measurement of NE turnover started 1 hour later. Therefore, the short-term effect of the drug

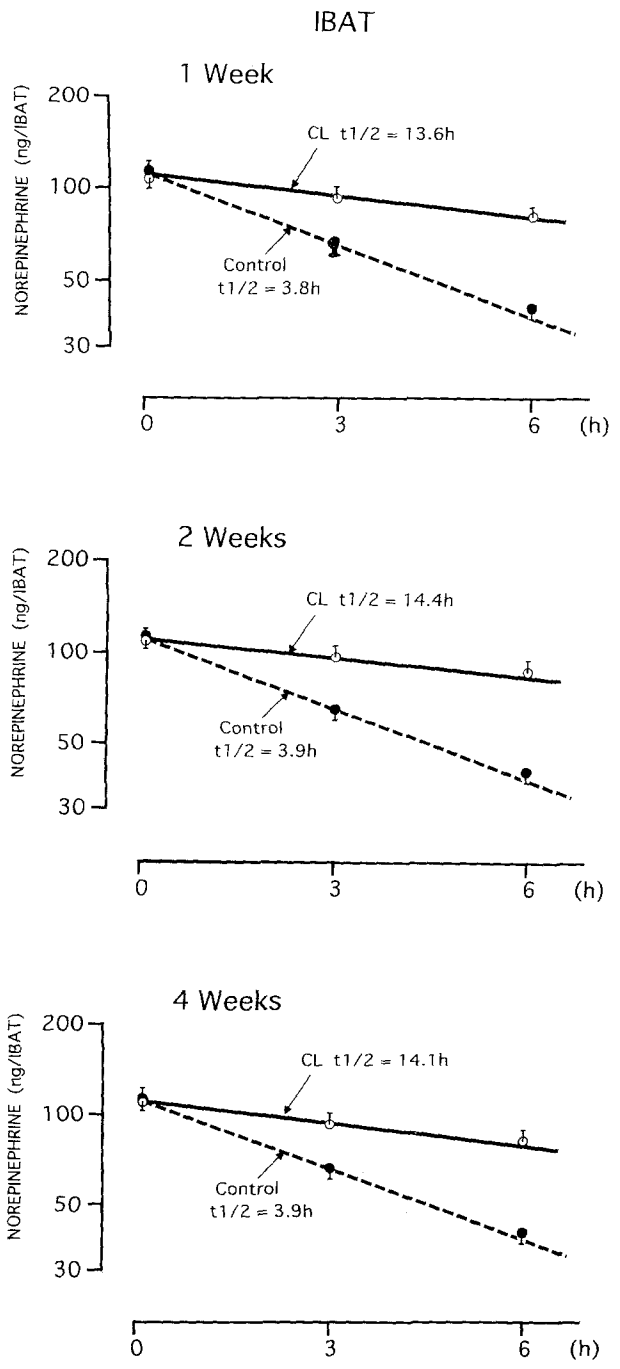

Fig 1. NE turnover determined by the NE biosynthesis-inhibition technique in IBAT of mice treated with CL (○) for 1, 2, or 4 weeks versus control mice (●). All data are plotted as the mean ± SEM for endogenous NE in IBAT of 6 animals in each group at 0, 3, and 6 hours after injection of α-MPT (80 mg/kg). The null hypothesis that the two regression lines could be represented by a common one was rejected for 1 week [$F(1, 32) = 18.99$, $P < .01$], 2 weeks [$F(1, 32) = 20.66$, $P < .01$], and 4 weeks [$F(1, 32) = 33.90$, $P < .01$].

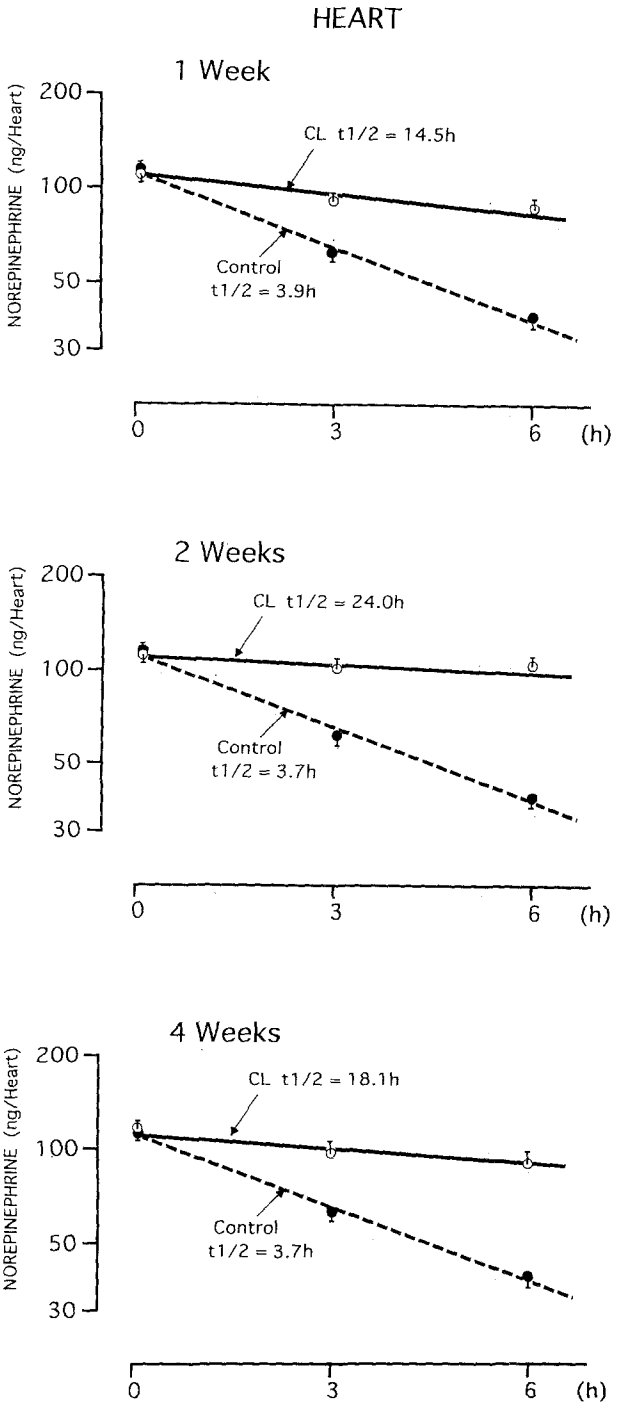

Fig 2. NE turnover determined by the NE biosynthesis-inhibition technique in the heart of mice treated with CL (○) for 1, 2, or 4 weeks versus control mice (●). All data are plotted as the mean ± SEM for endogenous NE in IBAT of 6 animals in each group at 0, 3, and 6 hours after injection of α-MPT (80 mg/kg). The null hypothesis that the two regression lines could be represented by a common one was rejected for 1 week [$F(1, 32) = 39.87$, $P < .01$], 2 weeks [$F(1, 32) = 72.29$, $P < .01$], and 4 weeks [$F(1, 32) = 50.37$, $P < .01$].

perhaps played a role in suppressing the rate of NE turnover, as well as the consequences of its long-term administration. However, because Yen et al[20] administered LY104119 in food, the mice in their study did not receive it as a single daily dose. Furthermore, it should be noted that they reported the increase in NE turnover rate was per gram of tissue, and when the total is calculated, the difference was much smaller. Therefore, it would be interesting to follow the time course of the increase in heat production and to study the correlation between heat

production and NE turnover after gastric administration of the drug.

On the one hand, there is little possibility that the CL compound works on neurons in the central nervous system, because this agent did not cross the blood-brain barrier in an experiment using rats (personal communication, September 1995, T.H. Claus, American Cyanamid, Pearl River, NY), and furthermore, β_3-receptor was not detected in the mouse brain.[30] NE concentrations in the brain were not affected by LY104119.[20]

On the other hand, long-term administration of the CL compound decreased plasma thyroid hormone levels.[19] However, thyroxine administration decreased NE turnover in BAT, but not in the heart or pancreas.[31] Furthermore, hypothyroidism causes an increase in urinary excretion of NE and in NE turnover in rat hearts.[32-34] Therefore, decreased NE turnover may not depend on the reduced thyroid hormone level that may be observed in mice treated with the CL compound.

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REFERENCES

1. Krief S, Lönnqvist F, Raimbault S, et al: Tissue distribution of β_3 adrenergic receptor mRNA in man. J Clin Invest 91:344-349, 1993

2. Arch JRS, Ainsworth AT, Cawthorne MA, et al: Atypical β-adrenoceptor on brown adipocytes as target for antiobesity drugs. Nature 309:163-165, 1984

3. Yoshida T, Sakane N, Wakabayashi Y, et al: Anti-obesity effect of CL316,243, a highly specific β_3 adrenoceptor agonist, in mice with monosodium-L-glutamate–induced obesity. Eur J Endocrinol 131:97-102, 1994

4. Arch JRS, Cawthorne MA, Coney KA, et al: β-Adrenoceptor–mediated control of thermogenesis, body composition and glucose homeostasis, in Rothwell NJ, Stock MV (eds): Obesity and Cachexia. Chichester, UK Wiley, 1991, pp 241-268

5. Holloway BR: Selective β-agonist of brown fat and thermogenesis, in Lardy HA, Statman FW (eds): Hormones, Thermogenesis and Obesity. New York, NY, Elsevier, 1989, pp 477-484

6. Meier MK, Blum-Kaelin D, Gerold M, et al: Ro40-2148, a novel thermogenic β-agonist with anti-obesity activity, in Bjorntorp P, Rossner S (eds): Obesity in Europe 88. London, UK, Libbey, 1989, pp 329-338

7. Young JB, Landsberg L: Stimulation of the sympathetic nervous system during sucrose feeding. Nature 269:615-617, 1977

8. Yoshida T, Kemnitz J, Bray GA: Lateral hypothalamic lesions and norepinephrine turnover in rats. J Clin Invest 72:919-927, 1983

9. Foster DO: Quantitative role of brown adipose tissue in thermogenesis, in Trayhurn P, Nicholls DG (eds): Brown Adipose Tissue. London, UK, Arnold, 1986, pp 31-51

10. Himms-Hagen J: Neural control of brown adipose tissue thermogenesis, hypertrophy and atrophy. Front Neuroendocrinol 12:38-93, 1991

11. Yoshida T, Sakane N, Wakabayashi Y, et al: Anti-obesity and anti-diabetic effects of CL316,243, a highly specific β_3-adrenoceptor agonist, in yellow KK mice. Life Sci 54:491-498, 1994

12. Bloom JD, Dutia MD, Johnson BD, et al: Disodium (R,R)-5-[2-[[2-(3-chlorophenyl)-2-hydroxyethyl]-amino]propyl]1,3-benzodioxole- 2,2-dicarboxylate (CL316,243). A potent β-adrenergic agonist virtually specific for β_3-receptors. A promising antidiabetic and antiobesity agent. J Med Chem 35:3081-3084, 1992

13. Vander Tuig JG, Ohshima K, Yoshida T, et al: Adrenalectomy increases norepinephrine turnover in brown adipose tissue of obese (ob/ob) mice. Life Sci 34:1423-1432, 1984

14. Yoshida T, Nishioka H, Nakamura Y, et al: Reduced norepinephrine turnover in mice with monosodium glutamate–induced obesity. Metabolism 33:1060-1063, 1984

15. Yoshimoto K, Komura S: Reexamination of the relationship between alcohol preference and brain monoamines in inbred strains of mice including senescence-accelerated mice. Pharmacol Biochem Behav 27:317-322, 1987

16. Yoshimoto K, Komura S, Kawamura K: Occurrence in vivo of 5-hydroxytryptophol in the brain of rats treated with ethanol. Alcohol Alcohol 27:131-136, 1992

17. Zar JH: Biostatistical Analysis. Englewood Cliffs, NJ, Prentice Hall, 1974

18. Largis EE, Burns MG, Muenkel HA, et al: Antidiabetic and antiobesity effects of a highly selective β_3-adrenoceptor agonist (CL316,243). Drug Dev Res 32:69-76, 1994

19. Himms-Hagen J, Cui J, Danforth E Jr, et al: Effect of CL316,243, a thermogenic β_3-agonist, on energy balance and brown and white adipose tissues in rats. Am J Physiol 266:R1371-R1382, 1994

20. Yen TT, Fuller RW, Hemrick-Luecke SK, et al: Effects of LY104119, a thermogenic weight-reducing compound, on norepinephrine concentrations and turnover in obese and lean mice. Int J Obes 12:59-67, 1988

21. Thomas RF, Holt BD, Schwinn DA, et al: Long-term agonist exposure induces upregulation of β_3-adrenergic receptor expression via cAMP response elements. Proc Natl Acad Sci USA 89:4490-4494, 1992

22. Nantel F, Bonin H, Emorine LJ, et al: The human β_3-adrenergic receptor is resistant to short term agonist-promoted desensitization. Mol Pharmacol 43:548-555, 1993

23. Liggett SB, Freedman NJ, Schwinn DA, et al: Structural basis for receptor subtype-specific regulation revealed by a chimeric β_3/β_2 adrenergic receptor. Proc Natl Acad Sci USA 90:3665-3669, 1993

24. Susuki T, Nguyen CT, Nantel F, et al: Distinct regulation of β_1, β_2 adrenergic receptors in Chinese hamster fibroblasts. Mol Pharmacol 41:542-548, 1992

25. Carpene C, Galitzky J, Collon P, et al: Desensitization of beta-1 and beta-2, but not beta-3, adrenoceptor–mediated lipolytic responses of adipocytes after long-term norepinephrine infusion. J Pharmacol Exp Ther 265:237-247, 1993

26. Granneman JG, Lahners KN: Differential adrenergic regulation of β_1- and β_3-adrenoceptor messenger ribonucleic acids in adipose tissues. Endocrinology 130:109-114, 1992

27. Granneman JG: Effects of agonist exposure on the coupling of beta1- and beta3-adrenergic receptors to adenyl cyclase in isolated adipocytes. J Pharmacol Exp Ther 261:638-642, 1992

28. Benovic JL, Bouvier M, Caron MG, et al: Regulation of adenylyl cyclase–coupled β-adrenergic receptors. Annu Rev Cell Biol 4:405-427, 1988

29. Dohlman HG, Thorner J, Caron MG, et al: Model systems for the study of seven-transmembrane-segment receptors. Annu Rev Biochem 60:653-688, 1991

30. Nahmias C, Blin N, Elalouf JM, et al: Molecular characterization of the mouse β_3-adrenergic receptor: Relationship with the atypical receptor of adipocytes. EMBO J 10:3721-3727, 1991

31. Knehans AW, Romsos DR: Effects of thyroxine on Na^+,

K-ATPase and norepinephrine turnover in brown adipose tissue of obese (ob/ob) mice. Metabolism 33:652-657, 1984

32. Tedesco JL, Flattery KV, Sellers EA: Effects of thyroid hormones and cold exposure on turnover of norepinephrine in cardiac and skeletal muscle. Can J Physiol Pharmacol 55:515-522, 1977

33. Sellers EA, Flattery KV, Steiner G: Cold acclimation of hypothyroid rats. Am J Physiol 226:290-294, 1974

34. Tu T, Nash CW: The influence of prolonged hyper- and hypothyroid states on the noradrenaline content of rat tissues and on the accumulation and efflux rates of tritiated noradrenaline. Can J Physiol Pharmacol 53:74-80, 1975