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Effects of Saikosaponins on the Metabolic Actions of Adrenaline, ACTH and Insulin on the Fat Cells

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Saikosaponins a and d inhibited adrenaline-induced lipolysis in fat cells isolated from epididymal adipose tissue of rats. Saikosaponins a, b_1, b_2, b_4, c and d inhibited ACTH-induced lipolysis in the fat cells. Insulin-stimulated lipogenesis in adipose tissue was significantly reduced by saikosaponin d.

Propranolol (a β-blocker) inhibited not only lipolytic actions induced by adrenaline and ACTH but also lipogenesis stimulated by insulin. In contrast to propranolol, saikosaponins b_1, b_2, b_4 and c selectively inhibited ACTH-induced lipolysis without affecting the lipogenetic effect of insulin.

The structure-activity relationship of saikosaponins is discussed.

Keywords——saikosaponin; *Bupleurum falcatum*; adrenaline-induced lipolysis; ACTH-induced lipolysis; insulin-stimulated lipogenesis; fat cells; structure-activity relationship

The root of *Bupleurum falcatum* L. (Japanese name: Saiko) has been used for the treatment of inflammatory diseases in Chinese traditional medicine. The main constituents of the roots of *B. falcatum* are known to be saikosaponins,[2] which are oligoglycosides of oleanene-type triterpenes.

Various biological effects of saikosaponins have been reported. Takagi *et al.*[3] demonstrated that saikosaponins has central depressant action and other pharmacological activities.[4] It was also shown that saikosaponins a and d possessed anti-inflammatory[4,5] and plasma cholesterol-lowering actions.[6] Arichi *et al.*[7] examined the effects of saikosaponins on hepatic injury induced by D-galactosamine. The experimental pharmacological results with saikosaponins were consistent with the clinical applications of the crude drug "Saiko" in Chinese traditional medicine.

The present experiments were undertaken to investigate the effect of saikosaponins on the actions of adrenaline, adrenocorticotropic hormone (ACTH) and insulin, and to clarify the relationship between their chemical structures and biological actions.

Materials and Methods

Materials——The following saikosaponins were used in the present investigation: saikosaponins a, b_1, b_2, b_3, b_4, c, d and f (Figure). The saponins were dissolved in water and used for the experiments.

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2) *a)* T. Kubota and H. Hinoh, *Tetrahedron Lett.*, **1968**, 303; *b)* A. Shimaoka, S. Seo, and H. Minato, *J. Chem. Soc. Perkin I*, **1975**, 2043; *c)* K. Tori, S. Seo, Y. Yoshimura, M. Nakamura, Y. Tomita, and H. Ishii, *Tetrahedron Lett.*, **1976**, 4167; *d)* H. Ishii, S. Seo, K. Tori, T. Tozyo, and Y. Yoshimura, *Tetrahedron Lett.*, **1977**, 1227.
3) K. Takagi and M. Shibata, *Yakugaku Zasshi*, **89**, 712 (1969).
4) K. Takagi and M. Shibata, *Yakugaku Zasshi*, **89**, 1367 (1969).
5) M. Yamamoto, A. Kumagai, and Y. Yamamura, *Arzneim.-Forsch.*, **25**, 1021 (1975).
6) M. Yamamoto, A. Kumagai, and Y. Yamamura, *Arzneim.-Forsch.*, **25**, 1240 (1975).
7) S. Arichi, H. Konishi, and H. Abe, *Acta Hepato. Jap.*, **19**, 430 (1978).

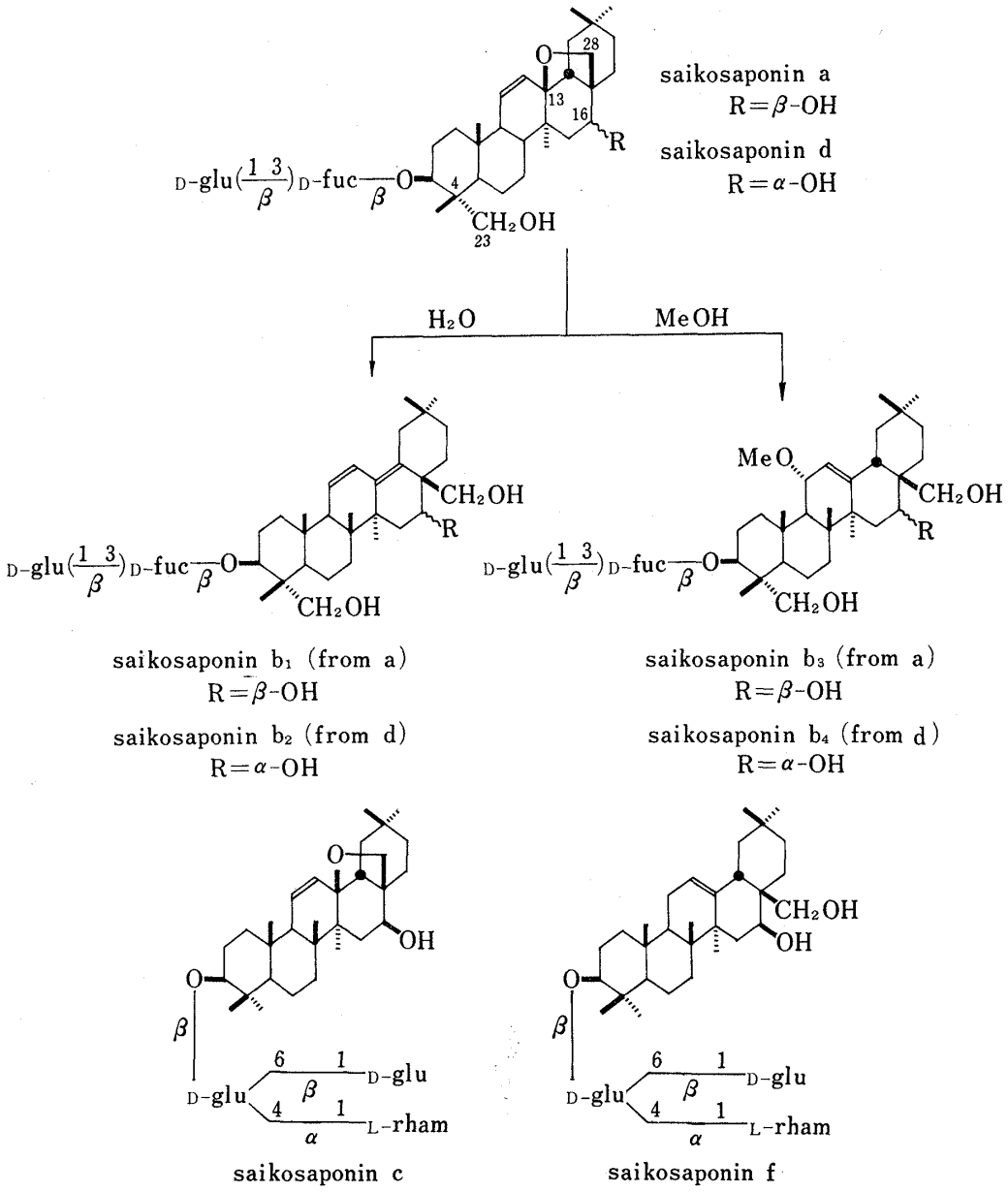

Fig. Structures of Saikosaponins

Animals——Young male Wistar King strain rats, weighing 180 to 220 g, were given standard laboratory diet and water *ad libitum*. They were sacrificed by means of a blow on the head, and their epididymal adipose tissue was quickly removed.

Preparation of Adipose Tissue and Fat Cells——Adipose tissues were cut into small slices from epididymal adipose tissue of rats. Fat cells were isolated from the adipose tissue by the method of Rodbell.[8]

Estimation of Adrenaline- and ACTH-induced Lipolysis——In a stoppered glass test tube, 0.25 ml of fat cell suspension (equivalent to 100 mg of adipose tissue) was incubated at 37° with shaking for 2 hr in 0.25 ml of Krebs-Ringer-phosphate buffer (pH 7.4) and 0.5 ml of Krebs-Ringer-phosphate buffer containing 5% albumin in the presence of 1 μg/ml of adrenaline and 20 μg/ml or 100/ μg/ml of saikosaponins. After incubation, free fatty acids (FFA) released from the fat cells were extracted and titrated with NaOH solution by the method of Dole.[9]

8) M. Rodbell, *J. Biol. Chem.*, **239**, 375 (1964).
9) V.D. Dole, *J. Clin. Invest.*, **35**, 150 (1965).

Krebs-Ringer-phosphate buffer (pH 7.4) containing 5% albumin was used for the estimation of ACTH-induced lipolysis, and the other preparations were the same as described above.

Lipolytic activity was expressed as microequivalents (μEq) of free fatty acid per gram of adipose tissue.

Estimation of Insulin-stimulated Lipogenesis from ^{14}C-Glucose in Adipose Tissue——A mixture of 100 mg of the epididymal adipose tissue slices, 0.5 μCi of ^{14}C-glucose, and 0.5 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) was incubated for 1 hr at 37° in the presence of 10 milli-international units (mI.U.) per ml of insulin and the indicated concentrations of saikosaponins. After incubation, the reaction was halted by the addition of 5 ml of Dole's extraction mixture.[9] The test tube was shaken vigorously for 5 min and then 3 ml of heptane and 2 ml of water were added, and the mixture was shaken again for 5 min. A 3 ml aliquot of the upper phase (heptane layer) was transferred to a stoppered glass test tube and shaken vigorously with an equal volume of alkaline ethanol (0.05 M NaOH in 50% ethanol) to remove free fatty acids, following the method of Borgstrom.[10] An aliquot of 1 ml of the heptane layer was used for the estimation of radioactivity. Stimulatory action of insulin of lipogenesis was expressed as a percentage compared with the control. The radioactivity of the heptane layer was analyzed by thin-layer chromatography following the method of Skipski *et al.*[11] More than 95% of the radioactivity was recovered in the triglyceride fraction, and the remainder was mainly diglyceride and phosphatidic acid.

Results

It is well known that adrenaline and ACTH stimulate lipolysis in isolated fat cells. When isolated fat cells prepared from rat epididymal adipose tissue were incubated with saikosaponins a, b_1, b_2, b_3, b_4, c, d, and f, no lipolytic action was observed, and studies were then carried out to clarify the effects of various saikosaponins on adrenaline- and ACTH-induced lipolysis.

As shown in Table I, 20 μg/ml of saikosaponin a or d solution inhibited the lipolytic action of adrenaline. Saikosaponins b_1, b_4 and c, as well as a and d, inhibited the lipolytic action of adrenaline at a concentration of 100 μg/ml. Saikosaponins b_2, b_3 and f did not affect the

Table I. Effects of Various Saikosaponins on Adrenaline-induced Lipolysis in Fat Cells

Additions (μg/ml reaction mixture)		Lipolysis (FFAμEq/g) M±S.E.	Significance
None		0.25±0.14	—
Adrenaline (1 μg/ml)		15.8 ±3.5	—
Adrenaline+saikosaponin a	(20 μg/ml)	9.6 ±1.6	*b*)
	(100 μg/ml)	0.0 ±0.0	*c*)
Adrenaline+saikosaponin b_1	(20 μg/ml)	13.8 ±2.7	N.S.
	(100 μg/ml)	9.3 ±2.4	*a*)
Adrenaline+saikosaponin b_3	(20 μg/ml)	11.9 ±2.3	N.S.
	(100 μg/ml)	14.5 ±3.1	N.S.
Adrenaline+saikosaponin c	(20 μg/ml)	12.7 ±2.5	N.S.
	(100 μg/ml)	5.6 ±2.4	*b*)
Adrenaline+saikosaponin d	(20 μg/ml)	4.2 ±0.7	*c*)
	(100 μg/ml)	1.9 ±0.4	*c*)
Adrenaline+saikosaponin b_2	(20 μg/ml)	13.1 ±2.5	N.S.
	(100 μg/ml)	12.1 ±1.0	N.S.
Adrenaline+saikosaponin b_4	(20 μg/ml)	11.4 ±1.8	N.S.
	(100 μg/ml)	3.9 ±1.0	*c*)
Adrenaline+saikosaponin f	(20 μg/ml)	12.7 ±2.7	N.S.
	(100 μg/ml)	10.1 ±0.8	N.S.
Adrenaline+insulin	(0.1 mI.U./ml)	8.7 ±1.0	*c*)
	(1.0 mI.U./ml)	6.3 ±1.0	*c*)

Significant: *a*) $p<0.05$, *b*) $p<0.02$, *c*) $p<0.001$.
N.S.: not significant.

10) B. Borgstrom, *Acta Physiol. Scand.*, **25**, 111 (1952).
11) V.P. Skipski, A.F. Smolowe, R.C. Scullivan, and M. Barclay, *Biochem. Biophys. Acta*, **106**, 386 (1965).

Table II. Effects of Various Saikosaponins on ACTH-induced Lipolysis in Fat Cells

Additions (μg/ml reaction mixture)		Lipolysis (FFAμEq/g) M±S.E.	Significance
None		0.38±0.38	—
ACTH (1 μg/ml)		13.8 ±0.4	—
ACTH+saikosaponin a	(20 μg/ml)	4.5 ±1.1	*b*)
	(100 μg/ml)	0.8 ±0.3	*b*)
ACTH+saikosaponin b_1	(20 μg/ml)	10.9 ±1.0	*b*)
	(100 μg/ml)	2.6 ±1.1	*b*)
ACTH+saikosaponin b_3	(20 μg/ml)	12.3 ±0.7	N.S.
	(100 μg/ml)	8.3 ±1.0	*b*)
ACTH+saikosaponin c	(20 μg/ml)	12.0 ±0.6	*a*)
	(100 μg/ml)	7.0 ±1.5	*b*)
ACTH+saikosaponin d	(20 μg/ml)	0.3 ±0.3	*b*)
	(100 μg/ml)	0.8 ±0.4	*b*)
ACTH+saikosaponin b_2	(20 μg/ml)	10.4 ±0.8	*b*)
	(100 μg/ml)	10.4 ±0.4	*b*)
ACTH+saikosaponin b_4	(20 μg/ml)	3.0 ±1.0	*b*)
	(100 μg/ml)	0.6 ±0.2	*b*)
ACTH+saikosaponin f	(20 μg/ml)	12.0 ±1.0	N.S.
	(100 μg/ml)	4.3 ±0.9	*b*)
ACTH+insulin	(0.1 mI.U./ml)	8.0 ±0.7	*b*)
	(1.0 mI.U./ml)	7.5 ±0.6	*b*)

Significant: *a*) $p<0.05$, *b*) $p<0.001$.
N.S.: not significant.

Table III. Effects of Various Saikosaponins on Insulin-stimulated Lipogenesis from Glucose in Adipose Tissue

Additions (μg/ml reaction mixture)		Lipogenesis (% activity) M±S.E.	Significance
None		100	—
Insulin (10 mI.U./ml)		163± 5.7	—
Insulin+saikosaponin a	(20 μg/ml)	146± 6.3	N.S.
	(100 μg/ml)	38± 2.7	*c*)
Insulin+saikosaponin b_1	(20 μg/ml)	163± 3.3	N.S.
	(100 μg/ml)	124± 9.4	*a*)
Insulin+saikosaponin b_3	(20 μg/ml)	167±21.9	N.S.
	(100 μg/ml)	156± 6.3	N.S.
Insulin+saikosaponin c	(20 μg/ml)	163±16.5	N.S.
	(100 μg/ml)	130± 5.8	*b*)
Insulin+saikosaponin d	(20 μg/ml)	76± 4.6	*c*)
	(100 μg/ml)	31± 1.8	*c*)
Insulin+saikosaponin b_2	(20 μg/ml)	157± 4.7	N.S.
	(100 μg/ml)	131± 9.1	*a*)
Insulin+saikosaponin b_4	(20 μg/ml)	148±16.6	N.S.
	(100 μg/ml)	61± 6.4	*c*)
Insulin+saikosaponin f	(20 μg/ml)	140± 8.6	N.S.
	(100 μg/ml)	156± 6.9	N.S.

Significant: *a*) $p<0.02$, *b*) $p<0.01$, *c*) $p<0.001$.
N.S: not significant.

lipolytic action of adrenaline. Insulin also showed an antilipolytic activity against adrenaline-induced lipolysis.[12]

As shown in Table II, ACTH-induced lipolysis was inhibited by saikosaponins a, b_1, b_2, b_4, c and d at a concentration of 20 μg/ml and by all saikosaponins at a concentration of 100 μg/ml.

It is well known that insulin increases triglyceride synthesis from glucose in adipose tissue. As shown in Table III, 20 μg/ml of saikosaponin d solution inhibited triglyceride synthesis from glucose in adipose tissue in the presence of insulin. At a concentration of 100 μg/ml, saikosaponins a, b_1, b_2, b_4, c and d were also found to inhibit triglyceride synthesis in the presence of insulin.

Table IV shows the effects of phentolamine (an α-blocker) and propranolol (a β-blocker) on adrenaline- and ACTH-induced lipolysis and insulin-stimulated lipogenesis from glucose in isolated fat cells. It was found that phentolamine inhibited both ACTH-induced lipolysis and insulin-stimulated lipogenesis, and propranolol inhibited adrenaline- and ACTH-induced lipolysis, as well as insulin-stimulated lipogenesis from glucose.

Table IV. Effects of Phentolamine (an α-blocker) and Propranolol (a β-blocker) on Adrenaline- and ACTH-induced Lipolysis and Insulin-stimulated Lipogenesis from Glucose in Fat Cells

Additions (μg/ml reaction mixture)	Lipolysis (FFAμEq/g) M±S.E.	Significance
None	0.25±0.25	—
Adrenaline (1 μg/ml)	17.5 ±1.3	—
Adrenaline+phentolamine (100 μg/ml)	13.6 ±1.9	N.S.
Adrenaline+propranolol (100 μg/ml)	0.0 ±0.0	*a*)
None	0.7 ±0.4	—
ACTH (1 μg/ml)	15.1 ±0.6	—
ACTH+phentolamine (100 μg/ml)	4.9 ±0.4	*a*)
ACTH+propranolol (100 μg/ml)	3.8 ±0.4	*a*)
	Lipogenesis (%activity) M±S.E.	
None	100	—
Insulin (0.1mI.U./ml)	135±7.8	—
Insulin+phentolamine (100 μg/ml)	70±2.4	*a*)
Insulin+propranolol (100 μg/ml)	79±8.6	*a*)

a) Significant ($p<0.001$).
N.S.: not significant.

Discussion

In the course of the experiments it was found that the actions of 20 μg/ml solutions of saikosaponins on adrenaline- and ACTH-induced lipolysis and insulin-stimulated lipogenesis (Table V) depended on the structures of the saponins.

The aglycone moieties of the saikosaponins are as follows: a 13β,28-epoxy-oleanene system (saikogenins F and G) in saikosaponins a and d; an 11,13(18)-heteroannular diene system (saikogenins A and D) in saikosaponins b_1 and b_2; an 11α-methoxy-olean-12-ene system in saikosaponins b_3 and b_4; and an olean-12-ene system(longispinogenin) in saikosaponin f. It was reported that the 4α-CH_2OH function in the aglycone moiety of saikosaponins a and d was essential for the anti-inflammatory[5] and plasma cholesterol-lowering actions.[6] Further-

12) D. Steinberg, J.C. Khoo, and S.E. Mayer, "The Regulation of the Adipose Tissue Mass," ed. by J. Vague and J. Boyer, Academic Press, New York, 1974, pp. 61—69.

Table V. Summary of the Actions of Various Saikosaponins

Saikosaponin	Adrenaline-induced lipolysis	ACTH-induced lipolysis	Insulin-stimulated lipogenesis
Saikosaponin a	↓	↓	→
Saikosaponin b_1	→	↓	→
Saikosaponin b_3	→	→	→
Saikosaponin c	→	↓	→
Saikosaponin d	↓	↓	↓
Saikosaponin b_2	→	↓	→
Saikosaponin b_4	→	↓	→
Saikosaponin f	→	→	→

Decrease, ↓, No change →.

more, it was shown that the 13β,28-oxide ring in the aglycones of saikosaponins a and d was important for hemolytic activity and that the 16α-OH and 4α-CH_2OH groups were also significant for hemolysis.[13] Saikosaponins b_1, b_2, b_3 and b_4 are artifact derived from saikosaponins a and d,[2b] but the saikosaponin b group is important in view of the facts that they have effective anti-allergic action[14] and that they are major saponin components of decoctions of prescriptions containing "Saiko" in Chinese traditional medicine ("Saiko-zai" in Japanese).[15]

Adrenaline-induced lipolysis was inhibited by saikosaponins a and d. The other saikosaponins did not show any inhibitory action on adrenaline-induced lipolysis at a dose of 20 μg/ml. This finding is in accord with the reported relationship between the chemical structure and biological activities[5,13] of saikosaponins.

ACTH-induced lipolysis was inhibited by saikosaponins a, b_1, b_2, b_4, c and d. Saikosaponins b_3 and f did not show any inhibitory effect on ACTH-induced lipolysis.

The difference of inhibitory action between saikosaponins a and d, and the finding that saikosaponin c inhibits ACTH-induced lipolysis relatively ineffectively might be due to the presence of 4α-CH_2OH in saikosaponins a and d, and 4α-CH_3 in saikosaponin c in the aglycone moiety. The inhibitory effect of the saikosaponin b_2 and b_4 group possessing the 16α-OH function on ACTH-induced lipolysis is higher than that of the saikosaponin b_1 and b_3 group, possessing 16β-OH. Thus, it seems likely that 4α-CH_2OH, 16α-OH, and an appropriate sugar moiety are essential structural requirements for the inhibition of lipolysis induced by ACTH.

Insulin-stimulated lipogenesis in adipose tissue was only inhibited by saikosaponin d.

It is well known that phentolamine (an α-blocker) and propranolol (a β-blocker) have strong antilipolytic action. In the present investigation, phentolamine inhibited ACTH-induced lipolysis and insulin-stimulated lipogenesis. Propranolol inhibited lipolytic action induced by adrenaline and ACTH, and lipogenesis stimulated by insulin. In contrast with these catecholamine blockers, saikosaponins b_1, b_2, b_4 and c did not affect adrenaline-induced lipolysis or insulin-stimulated lipogenesis, but inhibited only ACTH-induced lipolysis. Saikosaponin a did not show any inhibitory action on insulin-stimulated lipogenesis, but inhibited both adrenaline- and ACTH-induced lipolysis. Therefore, it seems likely that the mechanism of the inhibitory effects on lipolysis and lipogenesis of these saikosaponins may be different from those of α- and β-blockers of adrenaline.

As shown in Table V, saikosaponins b_1, b_2, b_4 and c selectively inhibited ACTH-induced lipolysis. Saikosaponin a also selectively inhibited both adrenaline- and ACTH-induced

13) H. Abe, M. Sakaguchi, H. Konishi, T. Tani, and S. Arichi, *Planta medica*, **34**, 160 (1978).
14) S. Arichi, M. Kubo, K. Komatsu, and S. Toda, *proc. symp. WAKAN-YAKU*, **10**, 103 (1977).
15) *a*) A. Akahori and K. Kagawa, *proc. symp. WAKAN-YAKU*, **10**, 61 (1977); *b*) S. Arichi, T. Tani, and M. Kubo, *Med. J. Kinki Univ.*, **4**, 59 (1979).

lipolysis. The selectivity of inhibitory effects of these saikosaponins is in good accord with those of ginsenosides.[16]

In Chinese traditional medicine, prescriptions containing "Saiko" are said to be effective for diseases induced by unbalanced control of the sympathetic and parasympathetic autonomic nervous systems. A medicine which acts selectively on the sympathetic or parasympathetic system is required to ameliorate such disturbances. Saikosaponins a, b_1, b_2, b_4 and c may be candidates for such medicines, because they may inhibit selectively the actions of the sympathetic system, having inhibitory effects on adrenaline- and ACTH-induced lipolysis. These saikosaponins may not affect the actions of the parasympathetic system, which involve insulin-stimulated lipogenesis. On the other hand, α- and β-blockers inhibit both the sympathetic and parasympathetic systems.

Experiments are now in progress to clarify the mechanism of the selective inhibition by these saikosaponins, and to examine their applicability as medicines for the treatment of diseases induced by disturbances of the autonomic nervous system.

16) H. Ohminami, Y. Kimura, H. Okuda, T. Tani, S. Arichi, and T. Hayashi, *Planta medica*, in press.