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Studies on the Activities of Tannins and Related Compounds of Medicinal Plants and Drugs. III.[1] Effects of Various Tannins and Related Compounds on Adrenocorticotropic Hormon-induced Lipolysis and Insulin-induced Lipogenesis from Glucose in Fat Cells. (2)[1]

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The effects of various tannins and related compounds on the actions of adrenocorticotropic hormone (ACTH) and insulin in fat cells isolated from rats were investigated. Hydrolyzable tannins such as geraniin, mallotusinic acid, chebulinic acid and chebulagic acid enhanced the ACTH-induced lipolysis at the concentration of 20 μg/ml or 5 μg/ml. On the other hand, these hydrolyzable tannins had no effect on the insulin-stimulated lipogenesis from glucose. Condensed tannins such as Ss-tannin 1 and RSF-tannin H showed weak inhibitory effects on the ACTH-induced lipolysis, while they enhanced the insulin-stimulated lipogenesis from glucose. Based on these results, the relationship between the structures and physiological actions of these tannins is discussed.

Keywords——hydrolyzable tannin; condensed tannin; ACTH-induced lipolysis; insulin-induced lipogenesis; fat cell; medicinal plant; rat

In the previous papers[1] we reported that several hydrolyzable tannins[2] (*e.g.* geraniin,[2d-f] corilagin,[2i] tellimagrandins I and II,[2a,b] alnusiin[2c] and gemin A[2l]) strongly inhibit the adrenaline-induced lipolysis in fat cells isolated from rat epididymal adipose tissue.

In the present paper, we examined the effects of various tannins in the medicinal plants on the actions of ACTH and insulin in fat cells, in order to clarify the physiological effects of these tannins and related compounds.

Materials and Methods

Materials——Tannins and related compounds examined in the present study were the same as those reported in part I[3] and part II[1] of this series. The twenty-five tannins or related compounds were each dissolved or suspended in Krebs–Ringer phosphate buffer (pH 7.4).

Animals——Young male Wistar King strain rats weighing 150—160 g were housed in a room at 25±1 °C with 60% relative humidity and given free access to food and water. Light was provided for 12 h a day starting at 7:00 a.m.

Preparation of Fat Cells——Rats were sacrificed by means of a blow on the head, and their epididymal adipose tissue was quickly removed. Fat cells were isolated from the adipose tissue by procedure of Rodbell.[4]

Estimation of ACTH-induced Lipolysis in Fat Cells——A mixture of fat cell suspension (equivalent to 100 mg of adipose tissue), 0.5 μg of ACTH and the indicated amount of various tannins or related compounds was incubated at 37 °C for 2 h in a final volume of 1 ml of Krebs–Ringer bicarbonate buffer (pH 7.4) containing 2.5% albumin. Then the reaction was stopped by adding 5 ml of Dole's extraction mixture.[5] The mixture was shaken for 5 min, and then 3 ml of heptane and 2 ml of water were added, and shaking was continued for 5 min. The upper heptane layer was transferred to a test tube and titrated with 0.008 N NaOH solution by Dole's method.[5] Lipolytic activity was

expressed as μeq of free fatty acids per 1 g of adipose tissue per hour.

Estimation of Insulin-induced Lipogenesis from [U-^{14}C] glucose in Fat Cells——A mixture of 0.25 ml of fat cell suspension (equivalent to 100 mg of adipose tissue) and 0.5 ml of Krebs–Ringer bicarbonate buffer containing 1.25 mM calcium ion, 5% albumin and 2 mM glucose, was pre-incubated at 37 °C for 30 min, and then incubated at 37 °C for 1 h in the presence of 0.5 μCi of [U-^{14}C] glucose, 0.1 milli-international unit (m I.U.) of insulin and the indicated concentrations of various tannins or related compounds. The reaction was stopped by adding 5 ml of Dole's extraction mixture.[5] The test tube was shaken vigorously for 5 min. Then 3 ml of heptane and 2 ml of water were added, and the mixture was shaken vigorously 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 N NaOH in 50% ethanol) to remove free fatty acids, according to Börgstrom's method.[6] An aliquot of 1 ml of the heptane layer was placed in a counting vial containing 10 ml of scintillation fluid (ACS II, Amersham Co.). The samples were subjected to radioactivity estimation with a Packard liquid scintillation counter. The radioactivity of the heptane layer was analyzed by thin layer chromatography according to Skipski *et al.*[7] More than 95% of the heptane extract was the triglyceride fraction, and the remainder was mainly diglyceride and phosphatidylic acid. Lipogenetic activity was expressed as cpm per gram of adipose tissue.

Results

Effects on ACTH-induced Lipolysis in Fat Cells

When ACTH was added to the medium and incubated at 37 °C for 2 h, 10.5 μeq/h of free fatty acids was released from 1 g of the adipose tissue. As shown in Table I, ACTH-induced lipolysis was enhanced by the addition of 20 μg/ml or 5 μg/ml of a hydrolyzable tannin such as

Table I. Effects of Various Tannins and Related Compounds on ACTH-induced Lipolysis in Fat Cells

Reaction mixture (/ml)		Lipolysis (FFA μeq/g)	Activity (%)	Significance
None		0.54±0.38	—	—
ACTH	(0.5 μg)	10.5±0.17	100	—
ACTH (0.5 μg)+geraniin	(100 μg)	11.0±0.72	105	N.S.
	(20 μg)	14.7±0.92	140	e)
	(5 μg)	15.1±1.12	144	e)
ACTH (0.5 μg)+corilagin	(100 μg)	12.2±0.67	116	b)
	(20 μg)	14.2±0.73	135	e)
	(5 μg)	13.4±0.69	128	d)
ACTH (0.5 μg)+tellimagrandin I	(100 μg)	11.5±0.56	110	N.S.
	(20 μg)	13.9±0.79	132	e)
	(5 μg)	14.0±1.03	133	d)
ACTH (0.5 μg)+mallotusinic acid	(100 μg)	9.4±0.55	90	N.S.
	(20 μg)	15.2±1.15	145	d)
	(5 μg)	14.3±1.15	136	d)
ACTH (0.5 μg)+chebulinic acid	(100 μg)	12.2±0.85	116	a)
	(20 μg)	16.2±1.25	154	e)
	(5 μg)	15.5±1.18	148	e)
ACTH (0.5 μg)+alnusiin	(100 μg)	11.3±0.43	108	N.S.
	(20 μg)	13.9±0.97	132	d)
	(5 μg)	14.2±1.17	135	d)
ACTH (0.5 μg)+3,3′-di-*O*-methylellagic acid	(100 μg)	11.1±0.46	106	N.S.
	(20 μg)	10.6±0.39	101	N.S.
	(5 μg)	11.3±0.79	108	N.S.
ACTH (0.5 μg)+methyltetramethylluteate	(100 μg)	11.3±0.57	108	N.S.
	(20 μg)	11.0±0.33	105	N.S.
	(5 μg)	11.0±0.34	105	N.S.
ACTH (0.5 μg)+methyl gallate	(100 μg)	11.4±0.70	109	N.S.
	(20 μg)	12.9±0.85	123	c)
	(5 μg)	11.7±0.44	111	b)

TABLE I. (continued)

Reaction mixture (/ml)		Lipolysis (FFA μeq/g)	Activity (%)	Significance
ACTH (0.5 μg)+Ss-tannin I	(100 μg)	8.3±0.60	79	d)
	(20 μg)	9.9±0.63	94	N.S.
	(5 μg)	12.0±0.60	114	b)
ACTH (0.5 μg)+RSF-tannin H	(100 μg)	8.6±0.45	82	d)
	(20 μg)	10.7±0.58	102	N.S.
	(5 μg)	12.0±0.70	114	a)
ACTH (0.5 μg)+pedunculagin	(100 μg)	9.6±0.44	91	N.S.
	(20 μg)	12.1±0.56	115	b)
	(5 μg)	12.6±0.51	120	d)
ACTH (0.5 μg)+isoterchebin	(100 μg)	6.5±1.11	62	d)
	(20 μg)	14.8±0.12	141	e)
	(5 μg)	15.0±0.29	143	e)
ACTH (0.5 μg)+ellagic acid	(100 μg)	10.4±0.66	99	N.S.
	(20 μg)	8.8±0.54	84	c)
	(5 μg)	8.3±1.59	79	N.S.
ACTH (0.5 μg)+gallic acid	(100 μg)	8.3±1.47	79	N.S.
	(20 μg)	8.6±0.79	82	a)
	(5 μg)	8.8±0.46	84	d)
ACTH (0.5 μg)+(+)-catechin	(100 μg)	16.3±0.64	155	e)
	(20 μg)	12.3±1.94	117	N.S.
	(5 μg)	10.9±1.65	104	N.S.
ACTH (0.5 μg)+(−)-epicatechin	(100 μg)	10.9±0.58	104	N.S.
	(20 μg)	11.1±1.21	106	N.S.
	(5 μg)	10.8±0.88	103	N.S.
ACTH (0.5 μg)+(−)-epigallocatechin gallate	(100 μg)	8.2±0.12	78	e)
	(20 μg)	10.0±0.29	95	N.S.
	(5 μg)	10.8±1.53	103	N.S.
ACTH (0.5 μg)+penta-*O*-galloylglucose	(100 μg)	11.3±0.62	108	N.S.
	(20 μg)	13.8±0.62	131	e)
	(5 μg)	13.4±0.64	128	e)
ACTH (0.5 μg)+chebulagic acid	(100 μg)	8.2±0.93	78	b)
	(20 μg)	14.8±0.43	141	e)
	(5 μg)	14.6±0.54	139	e)
ACTH (0.5 μg)+tellimagrandin II	(100 μg)	7.1±0.73	68	e)
	(20 μg)	13.2±0.27	126	e)
	(5 μg)	14.2±0.68	135	e)
ACTH (0.5 μg)+agrimoniin	(100 μg)	2.3±0.50	22	e)
	(20 μg)	12.7±0.36	121	e)
	(5 μg)	13.8±0.53	131	e)
ACTH (0.5 μg)+furosinin	(100 μg)	10.8±2.16	103	N.S.
	(20 μg)	12.6±0.56	120	d)
	(5 μg)	13.0±0.34	124	e)
ACTH (0.5 μg)+dehydrogeraniin	(100 μg)	7.7±0.53	73	e)
	(20 μg)	12.8±0.50	122	e)
	(5 μg)	13.4±0.10	128	e)
ACTH (0.5 μg)+gemin A	(100 μg)	3.4±0.61	32	e)
	(20 μg)	12.6±0.50	120	d)
	(5 μg)	13.0±0.27	124	e)
ACTH (0.5 μg)+insulin	(0.1 m I.U.)	7.2±0.25	69	e)

Results are mean±standard errors for 4—6 replicate experiments.

Significance of difference from ACTH-only value: *a)* $p<0.05$, *b)* $p<0.02$, *c)* $p<0.01$, *d)* $p<0.005$, *e)* $p<0.001$. N.S.: not significant.

geraniin, corilagin, tellimagrandin I, mallotusinic acid,[2g] chebulinic acid,[2j] alnusiin, pedunculagin, penta-*O*-galloylglucose, isoterchebin,[2b] chebulagic acid,[2j] tellimagrandin II,[2b] agrimoniin,[2k] furosinin,[2h] dehydrogeraniin[2h] or gemin A.[2l] Several hydrolyzable tannins such as isoterchebin, chebulagic acid, tellimagrandin II, agrimoniin, dehydrogeraniin and gemin A inhibited the ACTH-induced lipolysis in fat cells at the concentration of 100 μg/ml, while the other hydrolyzable tannins had no effect at the concentration of 100 μg/ml. Condensed tannins such as Ss-tannin 1[2m] and RSF-tannin H[2m] weakly inhibited the ACTH-induced lipolysis in fat cells at the concentration of 100 μg/ml. (−)-Epigallocatechin gallate was also inhibitory at the dose of 100 μg/ml, while (+)-catechin enhanced the ACTH-induced lipolysis. 3,3′-Di-*O*-methylellagic acid, methyl tetramethylluteate, methyl gallate, ellagic acid and (−)-epicatechin had no effect on the ACTH-induced lipolysis at the concentration of 100 μg/ml.

Effects on Insulin-induced Lipogenesis from [U-^{14}C] Glucose in Fat Cells

It is well known that insulin increases triglyceride synthesis from glucose in fat cells isolated from epididymal adipose tissue. When the fat cells were incubated with various tannins or related compounds, no lipogenetic activity from glucose was observed. We then studied the effects of these compounds on the insulin-induced lipogenesis from glucose in the

TABLE II. Effects of Various Tannins and Related Compounds on Insulin-induced Lipogenesis from [U-^{14}C] Glucose in Fat Cells

Reaction mixture (/ml)		Lipogenesis (^{14}TG cpm × 10^3/g)	Activity (%)	Significance
None		33.8 ± 2.48	—	—
Insulin	(0.1 m I.U.)	114.8 ± 6.26	100	—
Insulin (0.1 m I.U.) + alnusiin				
	(100 μg)	135.1 ± 1.93	118	b)
	(20 μg)	99.3 ± 2.57	86	a)
	(5 μg)	114.0 ± 2.48	99	N.S.
Insulin (0.1 m I.U.) + 3,3′-di-*O*-methylellagic acid				
	(100 μg)	93.7 ± 1.43	82	b)
	(20 μg)	111.5 ± 4.33	97	N.S.
	(5 μg)	115.1 ± 3.07	100	N.S.
Insulin (0.1 m I.U.) + methyl tetramethylluteate				
	(100 μg)	81.3 ± 4.40	71	d)
	(20 μg)	106.8 ± 2.89	93	N.S.
	(5 μg)	116.9 ± 3.76	102	N.S.
Insulin (0.1 m I.U.) + methyl gallate				
	(100 μg)	124.8 ± 6.81	109	N.S.
	(20 μg)	128.0 ± 4.92	111	N.S.
	(5 μg)	130.0 ± 1.32	113	a)
Insulin (0.1 m I.U.) + Ss-tannin 1				
	(100 μg)	134.1 ± 2.17	117	b)
	(20 μg)	133.4 ± 3.67	116	a)
	(5 μg)	136.8 ± 2.91	119	b)
Insulin (0.1 m I.U.) + RSF-tannin H				
	(100 μg)	141.0 ± 4.15	123	c)
	20 μg)	138.9 ± 0.99	121	a)
	(5 μg)	131.7 ± 4.87	115	N.S.
Insulin (0.1 m I.U.) + pedunculagin				
	(100 μg)	139.2 ± 6.08	121	b)
	(20 μg)	145.4 ± 2.45	127	d)
	(5 μg)	125.1 ± 7.29	109	N.S.

Results are mean ± standard errors for 4—6 replicate experiments.
Significance of difference from insulin-only value: *a*) $p<0.05$, *b*) $p<0.02$, *c*) $p<0.01$, *d*) $p<0.005$. N.S.: not significant.

fat cells. As shown in Table II, 3,3′-di-*O*-methylellagic acid and methyl tetramethyllutate inhibited the insulin-induced lipogenesis from glucose, while the other hydrolyzable tannins except for alnusiin and polyphenols such as (+)-catechin, (−)-epicatechin, (−)-epigallocatechin gallated did not show such inhibitory action. Condensed tannins such as Ss-tannin 1 and RSF-tannin H, and hydrolyzable tannins such as alnusiin and pedunculagin enhanced the insulin-induced lipogenesis from glucose at the concentration of 100 μg/ml.

Discussion

In the previous paper,[1] we reported that various tannins inhibited the adrenaline-induced lipolysis in fat cells. The present investigations demonstrated that various tannins and related compounds from medicinal plants affect lipid metabolism induced by the hormones ACTH and insulin in fat cells isolated from epididymal adipose tissue of rats.

The dimeric structures of agrimoniin and gemin A are important for the inhibitory action on the ACTH-induced lipolysis. Dehydrogeraniin and furosinin, having two dehydrohexahydroxydiphenoyl (DHHDP) groups, inhibited the ACTH-induced lipolysis at the concentration of 100 μg/ml, whereas geraniin, having a hexahydroxydiphenoyl (HHDP) group, a galloyl group and a DHHDP group, did not show any inhibitory action on the ACTH-induced lipolysis. Therefore, the difference of inhibitory actions between geraniin and the other two tannins might be due to the difference in the number of DHHDP groups in the molecules. Chebulagic acid, having an HHDP group, a galloyl group and a monocarboxyllactone group, inhibited the ACTH-induced lipolysis at the concentration of 100 μg/ml, while chebulinic acid (having three galloyl groups and a monocarboxyllactone group) enhanced the hormone-induced lipolysis at the concentration of 100 μg/ml. Therefore, the difference of inhibitory actions between chebulagic acid and chebulinic acid seems to be due to the presence of an HHDP group in the former, in place of two galloyl groups in the latter. Most of the hydrolyzable tannins (geraniin, corilagin, tellimagrandin I, mallotusinic acid, chebulinic acid, alnusiin, pedunculagin, isoterchebin, penta-*O*-galloylglucose, chebulagic acid, tellimagrandin II, agrimoniin, furosinin, dehydrogeraniin and gemin A) enhanced the ACTH-induced lipolysis at the dose of 20 μg/ml or 5 μg/ml. Among them, geraniin, mallotusinic acid, chebulinic acid, isoterchebin and chebulagic acid increased the ACTH-induced lipolysis in fat cells to 140% or more of the control level. These results suggest that the DHHDP groups (which contain carbonyl groups) and the carboxyl group in the above hydrolyzable tannins play an important role in the enhancement of lipolysis induced by ACTH.

Low molecular polyphenolic compounds such as 3,3′-di-*O*-methylellagic acid, methyl tetramethylluteate, ellagic acid and (−)-epicatechin had no effect on the ACTH-induced lipolysis, while gallic acid, (−)-epigallocatechin gallate, Ss-tannin 1 and RSF-tannin H inhibited the ACTH-induced lipolysis at the concentration of 100 μg/ml or 20 μg/ml. Therefore, it seems likely that the galloyl group is an essential structural requirements for inhibitory action in polyphenolic compounds or condensed tannins. Condensed tannins such as Ss-tannin 1 and RSF-tannin H, and hydrolyzable tannins such as alnusiin and pedunculagin enhanced the insulin-induced lipogenesis from glucose in fat cells at the concentration of 100 μg/ml, while the other hydrolyzable tannins had no effect. It seems likely that a polymeric structure composed of (−)-epicatechin gallate is important for the enhancement of lipogenesis induced by insulin. It is noteworthy that pedunculagin has the greatest activity on insulin-induced lipogenesis among the tannins, though the fundamental activity of pedunculagin as a tannin, as indicated by the relative astringency (RA) value[8] (0.22), is very low. A similar correlation of potent biological activity and weak fundamental activity of pedunculagin was recently observed in a study on the inhibition of lipid peroxidation.[3]

Tannins is well known to bind to protein. Therefore, the possibility exists that various tannins used in the present study bind with peptide hormones such as ACTH and insulin, and thus modify the actions of these hormones on lipolysis and lipogenesis. Experiments are in progress to clarify the mechanisms of action of these tannins on the hormonal actions.

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