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Studies on the Activities of Tannins and Related Compounds of Medicinal Plants and Drugs. II.[1] Effects of Various Tannins and Related Compounds on Adrenaline-induced Lipolysis in Fat Cells. (1)

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The effects of various tannins and related compounds on the adrenaline-induced lipolysis in fat cells isolated from rats were investigated. Hydrolyzable tannins such as geraniin, corilagin, tellimagrandin I, mallotusinic acid, chebulinic acid, alnusiin and pedunculagin inhibited the adrenaline-induced lipolysis. Condensed tannins such as Ss-tannin 1 and RSF-tannin H showed weak inhibitory effects on the adrenaline-induced lipolysis. Based on these results, the relationship between the structures and physiological actions of these tannins is discussed.

Keywords——hydrolyzable tannin; condensed tannin; adrenaline-induced lipolysis; fat cell; medicinal plant; rat

According to traditional Chinese and Japanese medicine, several medicinal plants which are rich in tannins have been used to treat diseases such as inflammation, liver injury and arteriosclerosis. We reported in the preceding paper[1] that several hydrolyzable tannins[2] (*e.g.* geraniin,[2d–f] corilagin,[2i] tellimagrandins I and II,[2a,b] alnusiin,[2c] penta-*O*-galloylglucose, *etc.*) strongly inhibit the adenosine 5′-diphosphate (ADP) and ascorbic acid-induced lipid peroxidation in mitochondoria, and the ADP and nicotinamide adenine dinucleotide phosphate (NADPH)-induced lipid peroxidation in microsomes.

A number of tannin-rich medicinal plants have been regarded as effective drugs for diseases induced by breakdown of homeostasis, which is known to be controlled by the nervous and hormonal systems. It is also well known that adrenaline has a lipolytic action in fat cells isolated from the rat epididymal adipose tissue, while insulin inhibits this lipolytic action in fat cells.

In the present paper, we report the results of our experiments carried out to examine the effects of various tannins in the medicinal plants on the adrenaline-induced lipolysis in fat cells, and to clarify the relationship between the structures and physiological actions of these tannins.

Materials and Methods

Materials——Tannins and related compounds examined in the present study were the same as those reported in part I of this series.[1] 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 the procedure of Rodbell.[3]

Estimation of Adrenaline-induced Lipolysis in Fat Cells——A mixture of fat cells suspension (equivalent to 100 mg of adipose tissue), 0.5 μg of adrenaline 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 phosphate buffer (pH 7.4) containing 2.5% albumin. Then the reaction was stopped by adding 5 ml of Dole's extraction mixture.[4] The mixture was shaken for 5 min, and the 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.[4] Lipolytic activity was expressed as μeq of free fatty acids per gram of adipose tissue per hour.

Results

When the fat cells were incubated with various tannins or related compounds, no lipolytic action was observed. We then studied the effects of these compounds on the adrenaline-induced lipolysis. When adrenaline was added to the medium and incubated at 37 °C for 2 h, 14.8 ± 0.21 μeq/h (mean ± standard error) of free fatty acids was released from 1 g of adipose tissue.

As shown in Table I, hydrolyzable tannins such as geraniin, corilagin, tellimagrandin I, chebulinic acid,[2j] alnusiin, penta-*O*-galloylglucose, isoterchebin,[2b] chebulagic acid,[2j] tellimagrandin II,[2b] agrimoniin[2k] and gemin A[2l] produced inhibition of over 50% at the concentration of 100 μg/ml on the lipolytic effect induced by adrenaline. Among them,

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

Reaction mixture (/ml)		Lipolysis (FFA μeq/g)	Activity (%)	Significance
None		0 ± 0	—	—
Adrenaline	(0.5 μg)	14.8 ± 0.21	100	—
Adrenaline (0.5 μg) + geraniin	(100 μg)	6.1 ± 0.24	41	e)
	(20 μg)	10.5 ± 1.17	71	d)
	(5 μg)	13.6 ± 0.59	92	N.S.
Adrenaline (0.5 μg) + corilagin	(100 μg)	4.8 ± 0.54	32	e)
	(20 μg)	10.4 ± 0.83	70	e)
	(5 μg)	13.3 ± 1.01	90	N.S.
Adrenaline (0.5 μg) + tellimagrandin I	(100 μg)	3.5 ± 0.58	24	e)
	(20 μg)	5.8 ± 0.35	39	e)
	(5 μg)	10.9 ± 0.59	74	e)
Adrenaline (0.5 μg) + mallotusinic acid	(100 μg)	7.9 ± 0.47	53	e)
	(20 μg)	12.0 ± 0.41	81	e)
	(5 μg)	13.9 ± 0.13	94	a)
Adrenaline (0.5 μg) + chebulinic acid	(100 μg)	6.0 ± 0.46	41	e)
	(20 μg)	10.3 ± 0.52	70	e)
	(5 μg)	14.5 ± 0.20	98	N.S.
Adrenaline (0.5 μg) + alnusiin	(100 μg)	6.7 ± 0.45	45	e)
	(20 μg)	8.0 ± 0.46	54	e)
	(5 μg)	12.5 ± 0.46	84	d)
Adrenaline (0.5 μg) + 3,3′-di-*O*-methyl-ellagic acid	(100 μg)	14.9 ± 0.11	101	N.S.
	(20 μg)	15.0 ± 0.25	101	N.S.
	(5 μg)	14.0 ± 0.41	95	N.S.
Adrenaline (0.5 μg) + methyl tetramethyl-luteate	(100 μg)	15.2 ± 0.37	103	N.S.
	(20 μg)	15.0 ± 0.27	101	N.S.
	(5 μg)	15.2 ± 0.28	103	N.S.
Adrenaline (0.5 μg) + methyl gallate	(100 μg)	11.4 ± 0.51	77	e)
	(20 μg)	14.8 ± 0.44	100	N.S.
	(5 μg)	15.1 ± 0.11	102	N.S.

TABLE I. (continued)

Reaction mixture (/ml)		Lipolysis (FFA μeq/g)	Activity (%)	Significance
Adrenaline (0.5 μg)+Ss-tannin 1	(100 μg)	10.2±0.53	69	e)
	(20 μg)	12.2±0.37	82	e)
	(5 μg)	13.5±0.51	91	a)
Adrenaline (0.5 μg)+RSF-tannin H	(100 μg)	11.8±0.28	80	e)
	(20 μg)	13.1±0.37	89	d)
	(5 μg)	13.6±0.20	92	c)
Adrenaline (0.5 μg)+pedunculagin	(100 μg)	7.9±0.28	53	e)
	(20 μg)	11.9±0.56	80	e)
	(5 μg)	14.5±0.35	98	N.S.
Adrenaline (0.5 μg)+ellagic acid	(100 μg)	11.1±2.25	75	N.S.
	(20 μg)	13.7±1.50	93	N.S.
	(5 μg)	14.2±1.28	96	N.S.
Adrenaline (0.5 μg)+gallic acid	(100 μg)	8.7±1.28	59	e)
	(20 μg)	11.9±1.08	80	b)
	(5 μg)	15.2±1.56	103	N.S.
Adrenaline (0.5 μg)+(+)-catechin	(100 μg)	17.5±1.46	118	N.S.
	(20 μg)	16.7±1.43	113	N.S.
	(5 μg)	16.2±1.21	109	N.S.
Adrenaline (0.5 μg)+(−)-epicatechin	(100 μg)	17.9±1.10	121	c)
	(20 μg)	16.5±1.36	111	N.S.
	(5 μg)	15.4±1.84	104	N.S.
Adrenaline (0.5 μg)+(−)-epigallocatechin gallate	(100 μg)	7.1±1.24	48	e)
	(20 μg)	13.2±1.06	89	N.S.
	(5 μg)	14.6±1.97	99	N.S.
Adrenaline (0.5 μg)+penta-*O*-galloyl-glucose	(100 μg)	5.5±0.71	37	e)
	(20 μg)	14.0±1.12	95	N.S.
	(5 μg)	16.5±0.84	111	a)
Adrenaline (0.5 μg)+isoterchebin	(100 μg)	1.1±0.22	7	e)
	(20 μg)	3.8±0.38	26	e)
	(5 μg)	14.2±1.72	96	N.S.
Adrenaline (0.5 μg)+chebulagic acid	(100 μg)	1.7±0.34	12	e)
	(20 μg)	13.4±1.10	91	N.S.
	(5 μg)	15.7±1.67	106	N.S.
Adrenaline (0.5 μg)+tellimagrandin II	(100 μg)	1.1±0.22	7	e)
	(20 μg)	2.2±0.22	15	e)
	(5 μg)	15.0±1.42	101	N.S.
Adrenaline (0.5 μg)+agrimoniin	(100 μg)	5.9±1.46	40	e)
	(20 μg)	12.4±0.61	84	d)
	(5 μg)	15.5±0.26	105	N.S.
Adrenaline (0.5 μg)+furosinin[2h)]	(100 μg)	11.5±1.15	78	c)
	(20 μg)	14.1±0.63	95	N.S.
	(5 μg)	14.2±0.73	96	N.S.
Adrenaline (0.5 μg)+dehydrogeraniin[2h)]	(100 μg)	11.5±1.07	78	c)
	(20 μg)	14.9±0.32	101	N.S.
	(5 μg)	15.0±0.32	101	N.S.
Adrenaline (0.5 μg)+gemin A	(100 μg)	4.5±1.84	31	e)
	(20 μg)	12.5±0.91	84	b)
	(5 μg)	14.6±0.47	99	N.S.
Adrenaline (0.5 μg)+insulin	(0.1 mI.U.)	8.3±0.41	56	e)

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

Significance of difference from adrenaline-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.

tellimagrandin I, isoterchebin and tellimagrandin II exhibited inhibition of over 50% even at the concentration of 20 μg/ml. Mallotusinic acid,[2g] pedunculagin and gallic acid produced 40—50% inhibition of the adrenaline-induced lipolysis in fat cells at the concentration of 100 μg/ml. Methyl gallate and condensed tannins such as Ss-tannin 1[2m] and RSF-tannin H[2m] exhibited 20—30% inhibition, while 3,3′-di-*O*-methylellagic acid and methyl tetramethylluteate (derived from ellagic acid and luteic acid) did not show any inhibition at the concentration of 100 μg/ml. (−)-Epigallocatechin gallate also strongly inhibited the adrenaline-induced lipolysis, while (−)-epicatechin enhanced the adrenaline-induced lipolysis at the concentration of 100 μg/ml.

Discussion

The present investigations demonstrated that various tannins and related compounds from medicinal plants affect lipid metabolism induced by adrenaline in fat cells isolated from epididymal adipose tissue of rats.

The inhibitory effects of the ellagitannins and the gallotannins on the adrenaline-induced lipolysis in fat cells are generally higher than those of the condensed tannins such as Ss-tannin 1 and RSF-tannin H. Low molecular polyphenols such as gallic acid, methyl gallate and (−)-epigallocatechin gallate also showed inhibition, while ellagic acid, 3,3′-di-*O*-methylellagic acid, methyl tetramethylluteate, (+)-catechin and (−)-epicatechin had no effect on the adrenaline-induced lipolysis. Thus, it seems likely that galloyl, hexahydroxydiphenoyl (HHDP) and dehydrohexahydroxydiphenoyl (DHHDP) groups of the tannins are essential structural requirements for the inhibition of lipolysis caused by adrenaline.

Experiments are in progress to clarify the mechanism of the actions of various tannins on the adrenaline-induced lipolysis in fat cells.

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