Hints of Western Medicine from Chinese Medicine

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As a biochemist, I have been studying lipolytic and lipogenic pathways in fat cells since 1963. In 1966, I proposed a hormone-sensitive substrate theory in which catecholamines might not act on lipase but on substrate during their lipolytic processes. The lipolytic and lipogenic pathways are negative and positive processes in triglyceride content of fat cells. Insulin inhibits the negative process (lipolysis) and stimulates the positive process (lipogenesis from glucose). On the other hand, catecholamine stimulates the negative process and inhibits the positive one. These hormones discriminate the negative and positive rules and regulate opposite ways.

We tried to find these hormone-like substances in various natural products. We isolated tea saponins, chitosan, and others as insulin-like substances and dimethyl-xanthine as a catecholamine-like one. It is well known that extracellular fluid pH changes from 7.4 to 6.8. Reduction of the pH from 7.4 causes insulin resistance. Insulin failed to stimulate glucose uptake at pH 7.0 of the extracellular fluid. We found minus ions, which stimulated lipogenesis from glucose by raising extracellular fluid pH to 7.4. These are our approaches to find functional substances that prevent lifestyle-related diseases. Exp Biol Med 228:1250–1255, 2003

Key words: lipolysis; lipogenesis; catecholamines; fat cell; Chinese medicine

I have studied lipolytic and lipogenic pathways in fat cells since 1963 and proposed a hormone-sensitive substrate theory (1, 2). Fat cells are well known to contain positive and negative metabolic pathways, lipolysis and lipogenesis. Insulin inhibited lipolysis and stimulated lipogenesis from glucose. On the other hand, catecholamines stimulated lipolysis and inhibited lipogenesis. I found insulin-like substances, adenosine and pyroglutamic acid from *Panax ginseng* and catecholamine-like substances, eucryphin, bergenin, and astilbin, from the rhizomes of A. thunergii. Finally, I found that insulin-mediated 2-deoxy-D-glucose (2-DG) uptake by rat soleus muscle was inhibited by reduction in the pH of the extracellular fluid from 7.4 to 6.8 (3, 4). I named "water" in Chinese medicine the extracellular fluid and studied that insulin acted as a 2-DG uptake with Na^+/H^+ channel as its receptor.

Insulin-Like Substances in Korean Red Ginseng

Insulin is known to stimulate lipogenesis from glucose and inhibit catecholamine-induced lipolysis in fat cells. On the other hand, catecholamine inhibited insulin-mediated lipogenesis from glucose and stimulated lipolysis in fat cells. An important point on the actions of insulin and catecholamines is that these hormones discriminate lipogenesis and lipolysis in fat cells and regulate each metabolic pathway in the opposite direction.

Experiments were designed to identify such hormone-like substances in medicinal plants. *Panax ginseng* is a medicinal plant long used in the treatment of various pathological states including general complaints such as headache, shoulder ache, chilly constitution, anorexia, and diabetes. There have been many pharmacological studies on *Panax ginseng* roots. It was reported that oral administration of an aqueous alcoholic extract of ginseng roots decreased the blood sugar level of rabbits and that *Panax ginseng* suppressed hyperglycemia induced by epinephrine and high-carbohydrate diets. These findings suggest that *Panax ginseng* roots contain insulin-like substances. Insulin is well known to inhibit epinephrine-induced lipolysis and stimulate lipogenesis from glucose in fat cells.

One hundred grams of Korean red ginseng powder were mixed with water. The water extract of the red ginseng was subjected to dialysis against water. The outer dialysate was then subjected to gel-filtration on Bio-Gel P-2 column as shown in Figure 1. Anti-lipolytic activity was eluted mainly in fractions II and IV. Fraction IV was determined to be adenosine (5). Adenosine inhibited norepinephrine-induced lipolysis in rat fat cells and stimulated lipogenesis from glucose both in the presence and absence of insulin as shown in Figures 2 and 3.

Fraction II was then applied to a Dowex-2 × 8 [Cl-

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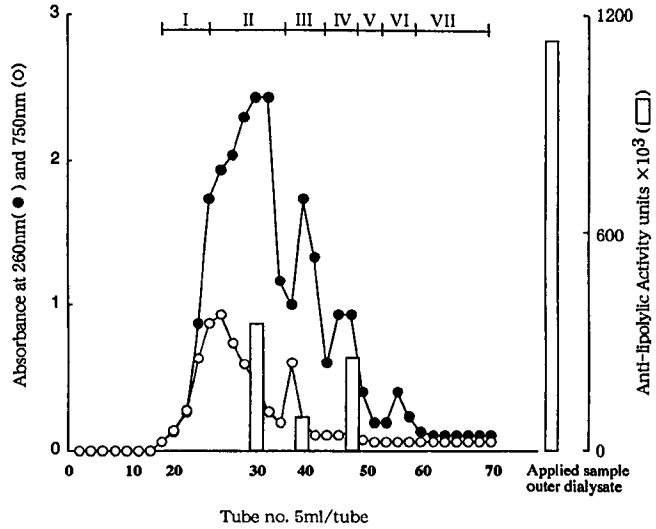

Figure 1. Gel filtration of the outer dialysate on a Bio Gel P-2 column. Column size, 2.2 x 43 cm. Elution was carried out with water. Absorbance of protein in the method of Lowry et al. (6).

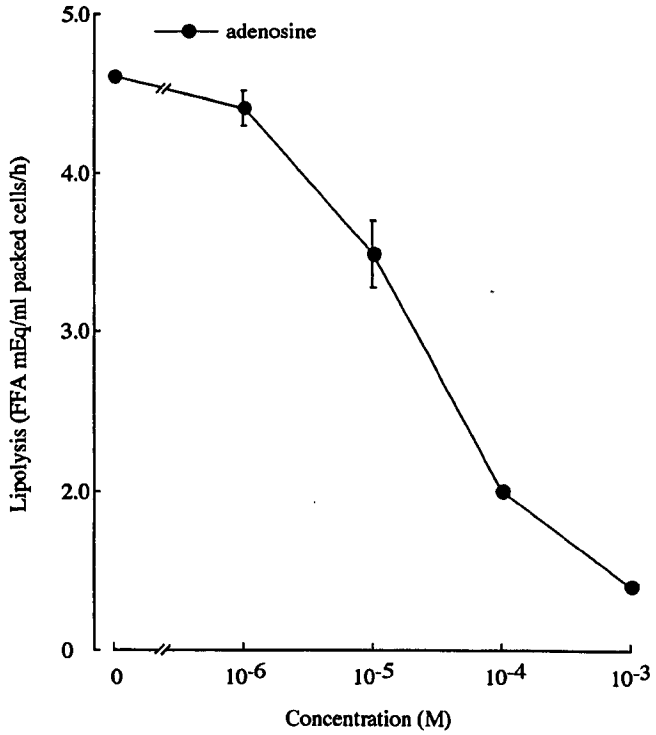

Figure 2. Effect of adenosine on norepinephrine-induced lipolysis in rat fat cells.

form] column (2.2 x 15 cm), washed with water and 0.01 N HCl, and then eluted with 0.5N HCl. The elute was subjected to dialysis with dialysis membrane to remove larger molecules with MW greater than 1000 dalton and outer dialysate was concentrated. The concentrated material was then applied to reverse-phase chromatography as shown in Figure 4. Each fraction was hydrolyzed (6 N HCl, 100°C, 24 h), and subjected to the ninhydrin reaction. Peaks at around tube No. 40 showed high ninhydrin reactions. The

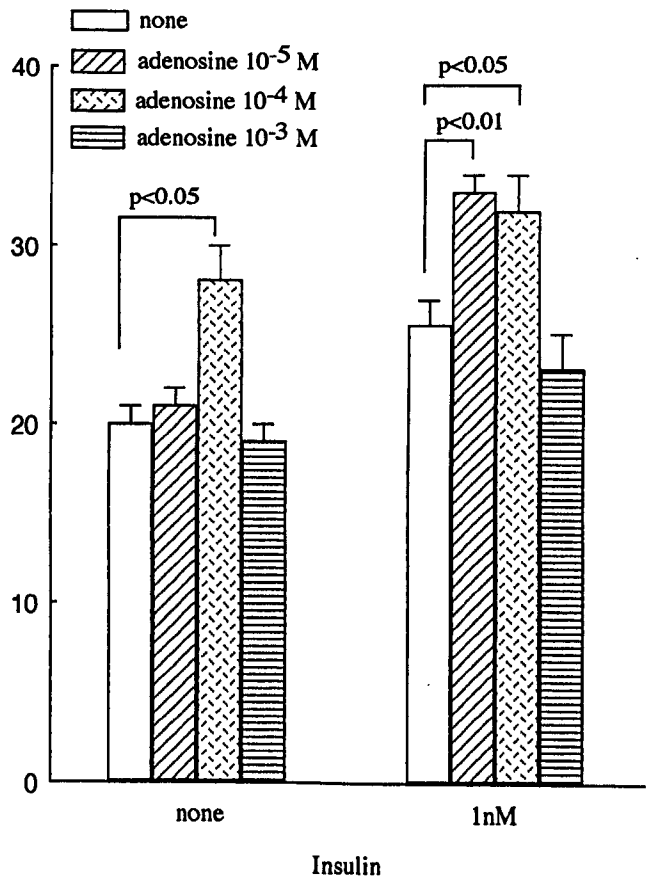

Figure 3. Effect of adenosine on insulin-induced lipogenesis in rat fat cells.

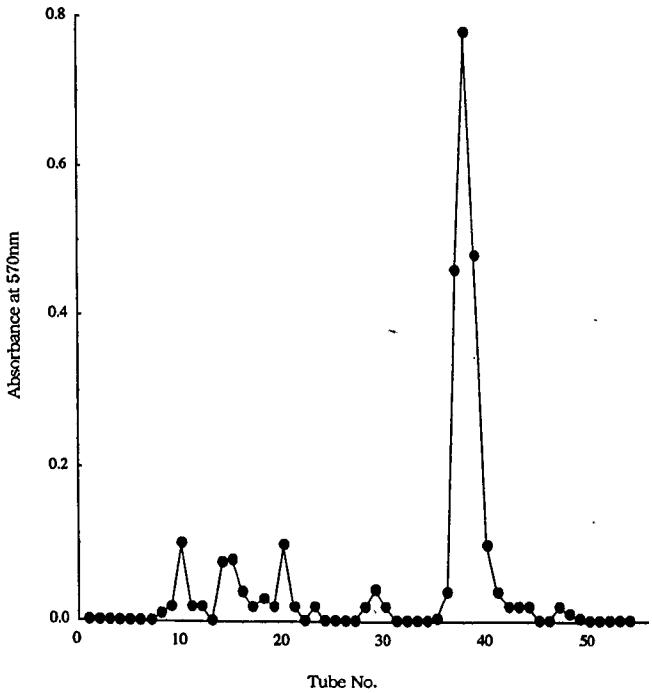

Figure 4. Reverse-phase chromatography of the elute from Dowex-2 column. Reverse-phase HPLC was done on a TSK gel ODS-80 TM column (TOSOH 4.6mm ID x 25 cm). Elution was carried out with 0.1% TFA in water. Each fraction (●) was hydrolyzed (6N HCl, 100°C 24 h) and subjected to ninhydrin reaction.

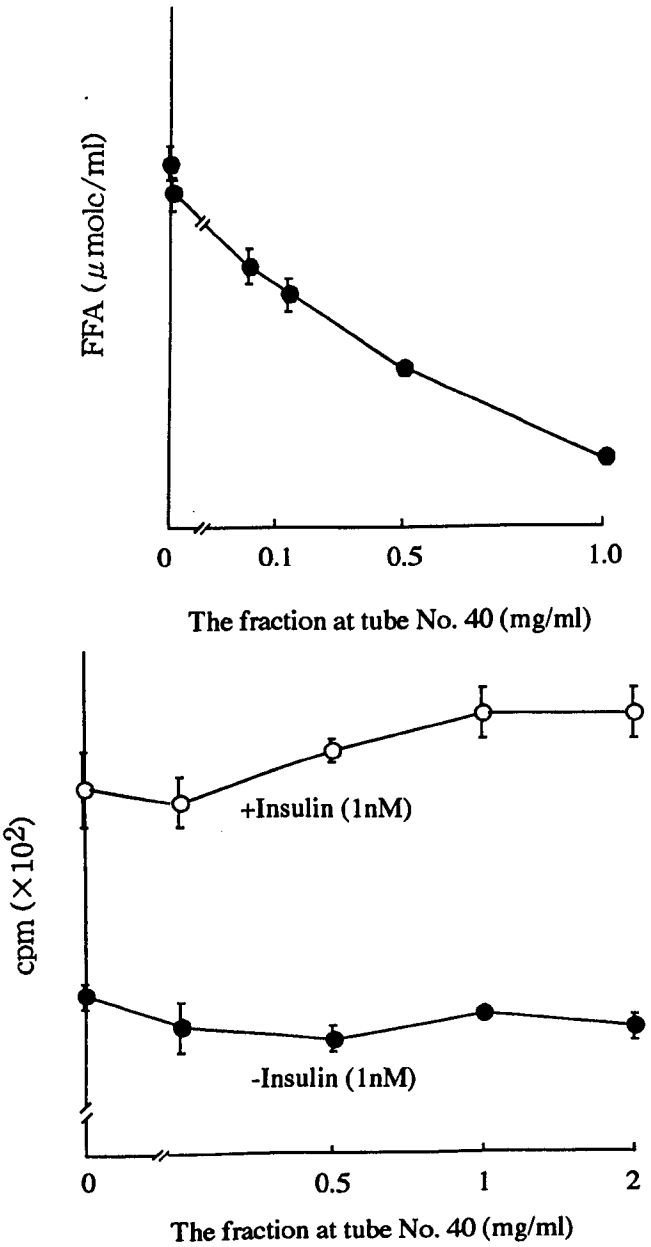

Figure 5. Effect of the fraction at tube No.40 on epinephrine-induced lipolysis and lipogenesis from glucose in fat cells. Lipogenesis was examined in the presence (O) and absence (●) of insulin (1nM).

peaks at around tube No.40 (the fraction at tube No.40) did not contain any amino acids. On the other hand, only glutamic acid was demonstrated after acid hydrolysis, suggesting-that the active principle may be a derivative of glutamic acid. The fraction at tube No.40 was found to inhibit epinephrine-induced lipolysis in fat cells as shown in Figure 5. In addition to anti-lipolytic activity, the fraction at peak No.40 stimulated lipogenesis from glucose in the presence of insulin (Fig. 5).

Therefore, we concluded that this fraction is the insulin-like substance. The chemical structure of this substance was determined to be pyroglutamic acid. The yields of pyroglutamic acid and adenosine are 0.3% and 0.03% from Korean red ginseng powder, respectively.

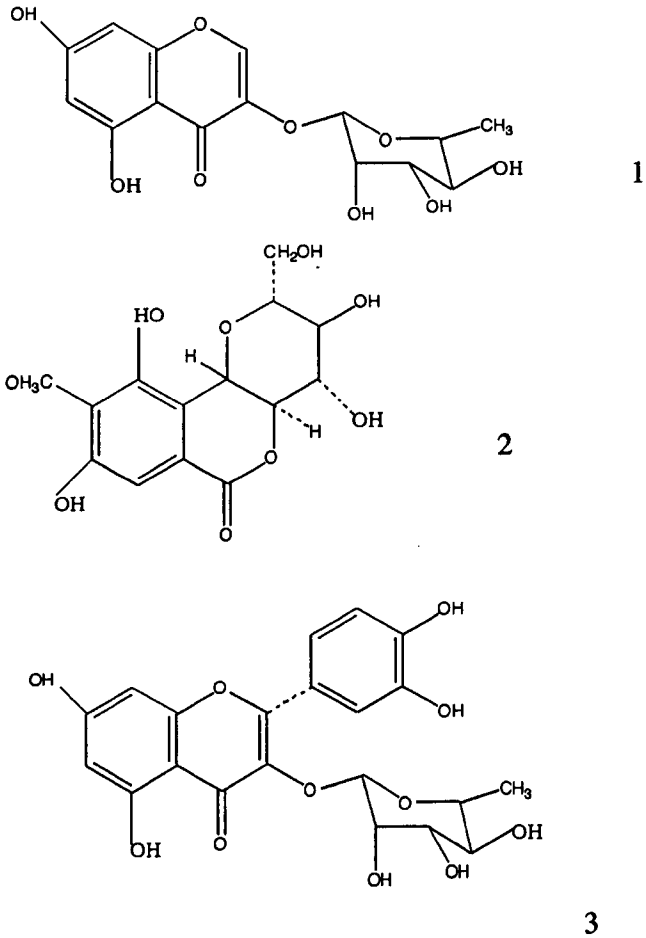

Figure 6. The chemical structures of the compounds found in the rhizomes of *A. thunbergii*.

Isolated fat cells are well known to possess opposite pathways of lipid metabolism; lipolysis, and lipogenesis, which will be postulated to be negative and positive metabolic pathways in traditional medicine. Lipolysis is stimulated by catecholamines and lipogenesis is activated by insulin. In various pathological conditions, the balance between lipolysis and lipogenesis is often broken. For example, lipolysis is accelerated in diabetes and lipogenesis is enhanced in obesity.

From ancient times, *Panax ginseng* is believed to improve pathological conditions of diabetes mellitus. If so, *Panax ginseng* should contain inhibitions toward lipolysis and stimulators toward lipogenesis, because lipolysis is accelerated and lipogenesis is inhibited in diabetes.

It is well known that propranolol, a β-blocker, inhibits epinephrine-mediated lipolysis in fat cells. In addition to the anti-lipolytic activity, propranolol also inhibits insulin-stimulated lipogenesis in fat cells (unpublished data). In contrast to propranolol, pyroglutamic acid and adenosine selectively inhibit the epinephrine-induced lipolysis and stimulate lipogenesis.

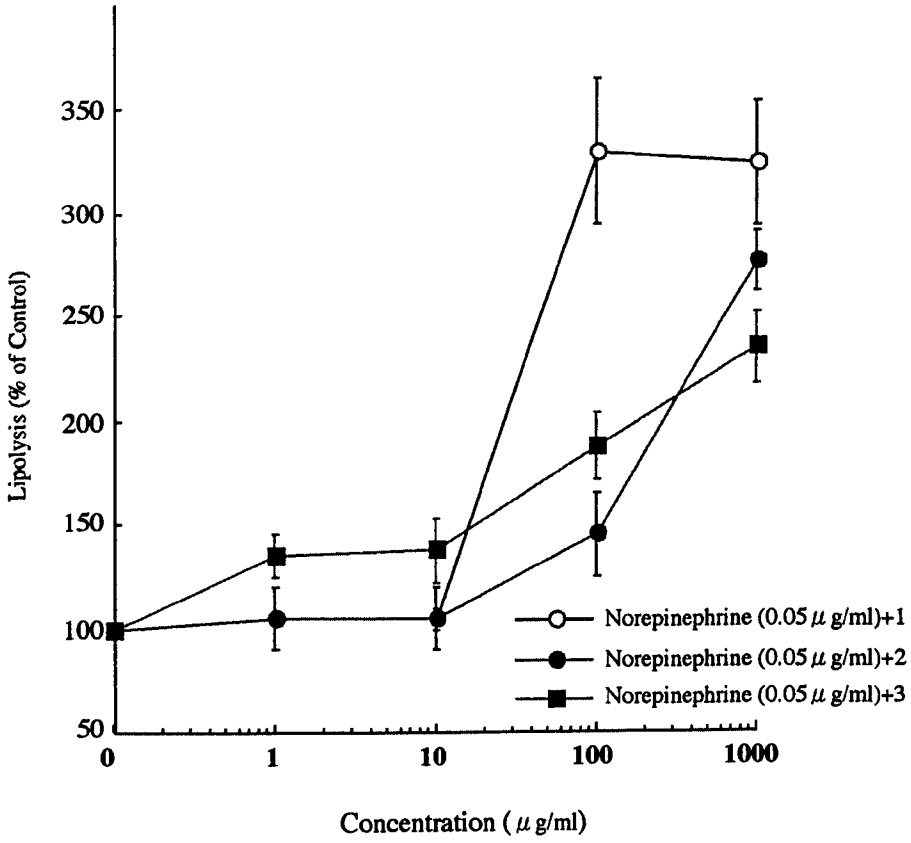

Figure 7. Effects of compounds 1 to 3 isolated from the rhizomes of A. *thunbergii* on norepinephrine-induced lipolysis in fat cells. Values are expressed as the mean ± SE of 3 experiments. The activity of norepinephrine-induced lipolysis is expressed as 100%.

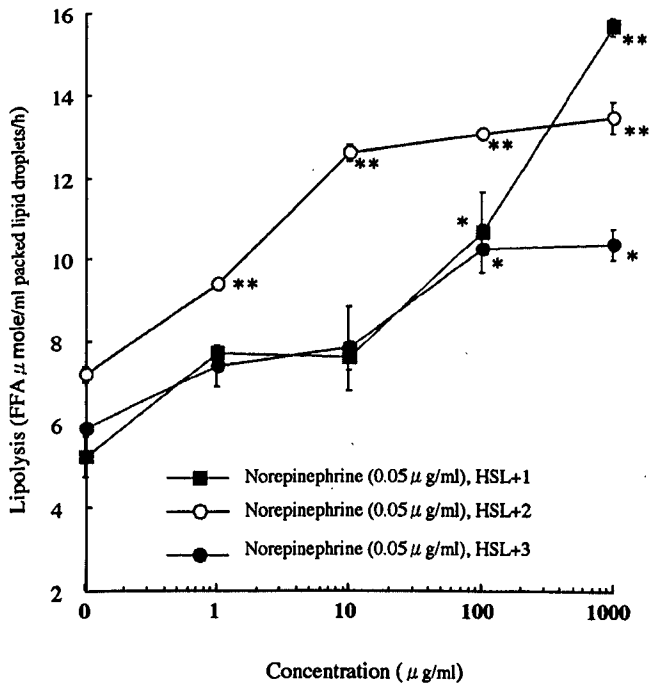

Figure 8. Effects of compounds 1 to 3 on norepinephrine-induced lipolysis in a cell-free system consisting of intact lipid droplets and HSL solution. Values are expressed as the mean ± SE of 3 experiments. Significantly different from norepinephrine alone. $^{*}P < 0.05$; $^{**}P < 0.01$.

Based on these experimental results, we suggest that pyroglutamic acid and adenosine should be called selective modulators to discriminate negative and positive metabolic pathways.

Catecholamine-Like Substances in *Astilbe Thunbergii*

The dried rhizomes of species such as *Astilbe chinensis* (Maxim.) Franch. et Savat., A. *revularis* Buch.-Ham. ex D. Don, var. *rivularis* Buch.-Ham. ex D. Don, A. *japaonica* (Morr. et Decne.) A. Gray, and A. *thunbergii* (Sieb. et Zucc.) Miq., known as "Hong-Sheng ma" (Chinese name) and "Aka-Shouma" (Japanese name), are used as substitute drugs for "Shengma". The latter drug is extracted from the rhizomes of *Cimicifuga* species such as C. *heracleifolia* Komarov, C. *dahurica* (Turxz.) Max., and C. *foetida* L. in the People's Republic of China and Japan. The rhizomes of A. *thubergii* are known to contain eucryphin, bergenin, and astilbin as shown in Figure 6.

We first found that these compounds (1–3 in Fig. 6) enhanced norepinephrine-induced lipolysis in fat cells, whereas they did not stimulate lipolysis in the absence of the hormone (Fig. 7).

Generally, lipolytic action in fat cells plays an important role in energy metabolism in animals. It is well known that lipolytic action in fat cells is stimulated by various pharmacological lipolytic hormones, such as epinephrine, norepinephrine, ACTH, and growth hormone. It is postu-

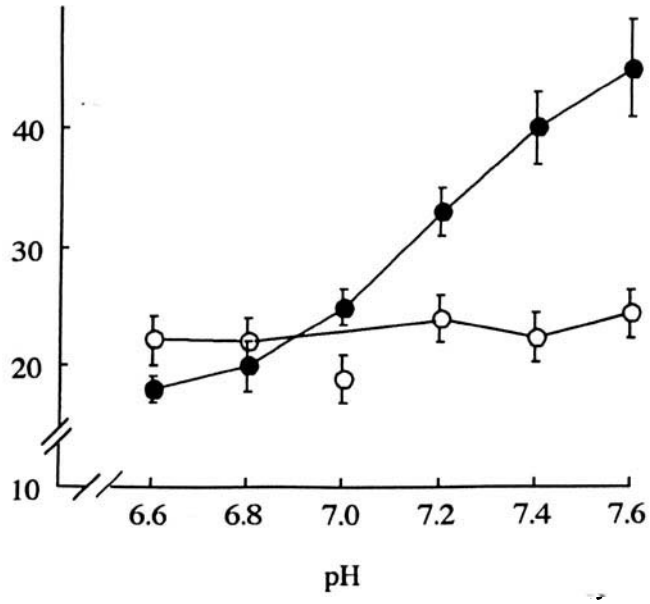

Figure 9. pH-dependence of basal and insulin-stimulated 2-deoxy-D-glucose uptakes by rat soleus muscle. Rat soleus was incubated in Hanks buffer solution at different pH values in the absence (○) or presence (●) 10 nM insulin for 15 min. Then 2-deoxy-D-glucose uptake was initiated by adding radioactive tracer. Bars show standard errors of means (n = 6–10).

lated that cyclic AMP (cAMP) plays a key role in the lipolysis stimulated by the above lipolytic hormones. Catecholamines such as epinephrine and norepinephrine are thought to stimulate adenylate cyclase in the membranes of fat cells and to increase the cAMP level of the cells. This increased level of cAMP stimulates protein kinase A activity, which in turn activates hormone-sensitive lipase (HSL), and the activated HSL catalyzes the hydrolysis of triglyceride in fat cells (7). However, Okuda *et al.* (8) found that cAMP-dependent activation of HSL stimulated lipolysis of [^{3}H-] triolein emulsified with gum arabic, but not that of endogenous lipid droplets prepared from fat cells. The endogenous lipid droplets were found to show lipolysis in response to catecholamines, theophylline, and p-aminophenol in the presence of HSL (9–11). Previously, we suggested that phospholipids in the endogenous lipid droplets were important in catecholamine-mediated lipolysis (2).

In the present experiments, compounds 1 to 3 enhanced norepinephrine-induced lipolysis in both fat cells and a cell-free system consisting of HSL and endogenous lipid droplets, but not in the sonicated lipid droplets and HSL (Fig. 7 and Fig. 8) (Table I), indicating that the site of the stimulatory actions of these substances was not HSL but the endogenous lipid droplets. It is suggested that compounds 1 to 3 stimulate the binding to the phospholipids of norepinephrine and, consequently, elicit a greater degree of lipolysis than norepinephrine alone. In addition, compounds 1 to 3 at a higher concentration (100 μg/mL) stimulated ACTH-induced lipolysis. Moreover, they inhibited insulin-induced lipogenesis from glucose (12).

Therefore, encryphin, bergenin, and astilbin were identified to be catecholamine-like substances that stimulated lipolysis and inhibited lipogenesis in fat cells. In other words, these substances are selective modulators that discriminate negative and positive metabolic pathways.

A New Hypothesis on Insulin Action

In traditional medicine, water is supposed to be an important factor that maintains good health. I would like to suggest that the water is extracellular fluid. In 1990, we found that insulin-mediated 2-DG uptake by rat soleus muscle was inhibited by reduction in the pH of the medium from 7.4 to 6.8 (4) (Fig. 9).

Klip, Ramlal, and Cragoe (13) reported that in addition to increase in 2-DG uptake, activation of Na^+/H^+ antiport is one of the earliest responses of muscle cells to insulin. To confirm this activation of Na^+/H^+ antiport, we examined amiloride-sensitive ^{22}Na uptake into rat soleus muscles. Amiloride is known to be an inhibitor of Na^+/H^+ antiport. Amiloride-sensitive ^{22}Na uptake was 8.50 nmol mg^{-1} tissue in the absence of insulin and 48.13 nmol mg^{-1} tissue in its presence, suggesting that insulin stimulates Na^+/H^+ antiport in the muscles. When medium Na^+ was replaced by other ions such as K^+, Rb^+, and choline$^+$, insulin failed to stimulate 2-DG uptake into the soleus muscles. On the other hand, in medium with Li^+ in place of Na^+, 2-DG uptake was 1.79 pmol mg^{-1} tissue in the absence of insulin and 3.66 pmol mg^{-1} tissue in its presence. Thus insulin increased 2-DG uptake in the presence of Na^+ or Li^+, which are known to be substrates of Na^+/H^+ antiport (14). All these results suggest that Na^+/H^+ antiport is closely related to the stimulatory action of insulin on 2-DG uptake.

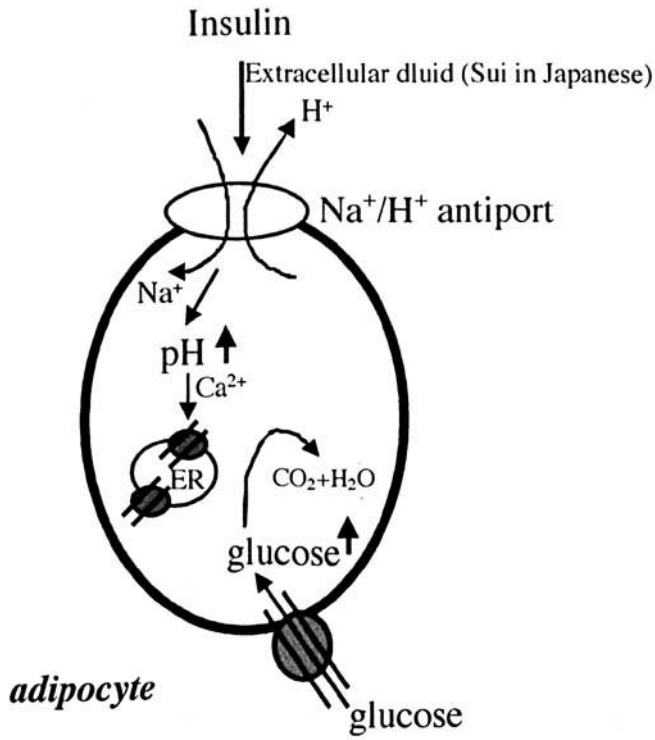

Figure 10. Hypothetical scheme on insulin-stimulated glucose metabolism.

Stimulation of Na^+/H^+ antiport by insulin causes alkaline shift of cytoplasmic pH, which may accelerate translocation of glucose transporter type 4 from microsomal fraction to plasma membrane possibly through increase in free Ca^{++} of cytoplasm (15). The increase in cytoplasmic pH is sufficient to activate glycolytic enzyme including phosphofructokinase (15). Therefore, it seems likely that the increase in cytoplasmic pH may be a messenger of insulin actions on glucose uptake and postreceptor glucose metabolism (Fig. 10).

The effect of medium pH on insulin-stimulated 2-DG uptake may be explained by assuming that Na^+/H^+ antiport is involved in insulin-mediated glucose uptake. Reduction of the extracellular pH implies increase in the H^+ content of extracellular medium, which would reduce efflux of intracellular H^+ and thus inhibit Na^+/H^+ antiport with consequent decrease of insulin-mediated 2-DG uptake. The medium used in the experiment of Figure 9 corresponds to extracellular fluid *in vivo*. We found that the pH value of extracellular fluid surrounding rat skeletal muscle was easily reduced by hemorrhage, endotoxin shock, interception of blood flow, and/or CO_2 inhalation (16). Based on these results, I suggest that water in traditional medicine may involve extracellular fluid and the reduction of its pH may cause insulin resistance corresponding to water toxicity ("suidoku" in Japanese) in traditional medicine.

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