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POTENTIATION OF β-ADRENOCEPTOR AGONIST-MEDIATED LIPOLYSIS BY QUERCETIN AND FISETIN IN ISOLATED RAT ADIPOCYTES

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Abstract—Quercetin and fisetin, two naturally occurring bioflavonoids mobilized lipids and enzymes in the absence or presence of epinephrine in intact rat adipocytes. Dose-(0–250 μM) and time-(0–2 hr) course studies, showed that they stimulated phosphodiesterase (PDE) activity and simultaneously exert cyclic AMP accumulation. These bioflavonoids when present either singly or together with epinephrine stimulated the membrane-bound PDE but not the cytosolic PDE. The stimulation may act as a feed-back mechanism to terminate the cyclic AMP effects. The action of theophylline, a known lipolytic agent (exerting its effects through antagonism of adenosine A_1 receptor as well as PDE inhibition) was not potentiated by either fisetin or quercetin. However, the flavonoids potentiated epinephrine or isoproterenol- (a specific β-adrenoreceptor agonist) induced lipolysis. Their effects were inhibited by propranolol (a β-receptor antagonist). These results suggest that the flavonoids act synergistically with epinephrine on β-adrenergic receptor and not through phosphodiesterase inhibition to stimulate adipocyte lipolysis. Increase in membrane phospholipid methylation occurred as a consequence of the epinephrine and/or quercetin/fisetin actions, and it correlated with the cellular accumulation of cyclic AMP.

Key words: lipolysis, quercetin, fisetin, rat adipocytes

In adipocytes, it is well known that lipolytic hormones (β-adrenergic agonists) increase cyclic AMP content and activate a cyclic AMP-dependent protein kinase which subsequently phosphorylates and activates a hormone-sensitive lipase, resulting in the hydrolysis of stored triglyceride to glycerol and free fatty acids [1, 2]. PDE† plays an important role in the responsiveness or regulation of various effectors or drugs (especially phosphodiesterase inhibitors) that act via this second messenger [3–6]. Several other mechanisms control the β-adrenergic mediated stimulation of cyclic AMP. These include receptor binding sites, GTP-requiring proteins, direct activation of adenylate cyclase (cyclic AMP-synthesizing enzyme) and the change in membrane environment of these molecules [7]. The methylation of membrane phosphatidylethanolamine to form phosphatidylcholine has been shown to increase membrane fluidity in rat reticulocytes [8] and has also been implicated in the β-adrenoreceptor-mediated actions and regulations [7, 9].

Flavonoids are a group of phenolic compounds ubiquitously found in photosynthesizing cells. These compounds have been reported to exert a myriad of pharmacological effects [10–14], which are often mediated through the inhibition of enzymes [15–19]. Recently, quercetin was reported to be effective in blocking the insulin receptor tyrosine kinase-catalysed phosphorylation in rat adipocytes [20].

We have recently shown that several flavonoids can inhibit rat adipocyte phosphodiesterase activity and most of them could also increase lipid mobilization synergistically with epinephrine. Quercetin and fisetin were potent adipocyte PDE inhibitors in a cell free system. They were also effective in potentiating epinephrine-induced lipolysis [21]. These two plant flavonoids were therefore selected for further studies in an attempt to elucidate their mode of action.

Adipocytes have various stimulatory and inhibitory receptors and the biological effects can be measured readily [22]. Studies on compounds that exert lipomobilizing effects are useful as they may be exploited in the treatment of some lipid abnormality states.

In this report, the potentiation of epinephrine-induced lipolysis by fisetin and quercetin was shown to be associated with the β-adrenergic receptor and not a result of phosphodiesterase inhibition. Interestingly, an increase in phospholipid methylation was observed as a consequence of the epinephrine and/or quercetin/fisetin actions, and it correlated to the cellular accumulation of cyclic AMP.

MATERIALS AND METHODS

Male Wistar rats weighing 180–230 g were obtained from the animal holding unit, National University

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† Abbreviations: IBMX, 3-isobutyl-1-methylxanthine; Hepes, *N*-[2-hydroxyethyl] piperazine-*N'* [2-ethanesulphonic acid]; PDE, phosphodiesterase; BSA, bovine serum albumin.

of Singapore. They were maintained at 25° with free access to laboratory chow and water. Quercetin and fisetin were purchased from Extrasynthese, Genay, France. Cyclic AMP, theophylline, epinephrine, *Crotalus atrox* venom, collagenase (type II) and bovine serum albumin (BSA) fraction V were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). 2,8-[^{3}H]Cyclic AMP (250 μCi), cyclic AMP assay kit and [^{3}H]*S*-adenosyl methyl methionine (250 μCi) were obtained from Amersham (U.K.). Glycerol assay kit was obtained from Boehringer (Mannheim, Germany). Other chemicals used were of analytical grade.

Adipocytes were isolated by the collagenase method of Rodbell [23], from the epididymal fat pad of male Wistar rats. A modified Krebs–Henseleit Hepes buffer (pH 7.4) supplemented with the 5.0% BSA and 2.5 mM $CaCl_2$ was used as the isolation buffer. The isolated fat cells were diluted appropriately (1 mL packed cells in 5 mL isolation buffer) before use. Aliquots (1 mL) of the cell suspension were taken for incubation with the various test compounds in a final volume of 2 mL. At the end of the incubation period, the glycerol, PDE activity and cyclic AMP levels were determined.

Glycerol assay. Glycerol content in the incubation mixture was used as an index for lipolysis [21]. Aliquots (1 mL) from each incubation mixture was deproteinized with 0.1 mL of cold perchloric acid (30% v/v) and chilled at 4°. Additional 0.5 mL of perchloric acid (3% v/v) was added and centrifuged for 15 min at 2500 *g*. The glycerol content in the supernatant (after neutralization with 1 M NaOH) was estimated using a glycerol assay kit (Boehringer).

Phosphodiesterase assay. Unless otherwise stated, the total low K_m cyclic AMP PDE activity (uncharacterized PDEs) was assayed in adipocytes, according to the method described by Bauer and Schwabe [24]. After incubation, the adipocytes were washed twice with Tris (10 mM)–EDTA (1 mM)–sucrose (0.25 M), disrupted by vortex mixing and centrifuged at 2000 *g* for 10 min as described in our previous report [21]. The lipid liberated from the broken cells surfaced and the aqueous phase (infranatant) was used for cyclic AMP PDE activity measurement. All procedures were carried out at 22–25° in order to minimize trapping of enzyme in the coalescing fat cake [25]. Protein content in the infranatant was also determined [26].

The assay was initiated by adding 50 μL of the infranatant solution into the incubation mixture containing 0.13 μM cyclic AMP, 6 mM Mg^{2+}, [^{3}H]-cyclic AMP (30,000 cpm) and 40 mM Tris buffer in a total volume of 200 μL and incubated for 10 min at 37°. At the end of the incubation period, 50 μL of cold cyclic AMP (5 mM) was added and the reaction was stopped by immersing the tubes in a boiling water bath for 3 min. The [^{3}H]AMP formed was degraded by snake venom 5′nucleotidase to [^{3}H]adenosine. The latter compound was isolated using column chromatography (QAE sephadex A25) and the radioactivity of each fraction was counted in the toluene–Triton X-100-based liquid scintillant.

Cyclic AMP determination. Cyclic AMP content in the cells was measured using a cyclic AMP assay kit (Amersham) at the end of the various incubations. Briefly, the cells were incubated and washed with Tris (50 mM)–EDTA (4 mM) (pH 7.4), resuspended in Tris (10 mM)–EDTA (4 mM) and disrupted by vortex mixing after freeze–thawing the cell suspension. The mixture was centrifuged at 2000 *g* for 10 min and the infranatant was heated in a boiling waterbath for 5 min in order to denature the proteins. The solution mixture was centrifuged at 12,000 *g* (4°) for 15 min and the clear supernatant was used for direct cyclic AMP estimation [27]. A range of concentrations of [^{3}H]cyclic AMP was added to the samples as internal standards. The cyclic AMP recovery in all cases was more than 95%.

Phospholipid methylation. The adipocytes were preincubated with [^{3}H]*S*-adenosyl-methyl-methionine (4 μCi) and 5 mM Mg^{2+} for 15 min at 37°. The fat cells were incubated for a further 15 min after the addition of flavonoids and/or epinephrine. The adipocytes were washed three times with Krebs–Hepes (pH 7.4) buffer and resuspended in the same buffer. The cells were disrupted and the lipids extracted with a mixture of chloroform : methanol (2 : 1 v/v) solvent. The chloroform extract was removed and evaporated to dryness over oxygen free nitrogen and the lipid residue weighed. The lipids were redissolved in 100 μL of chloroform : methanol (2 : 1 v/v) mixture and aliquots (0.1 mL) taken for separation on TLC plates (silica K_6 gel, 250 μM thickness, 20 × 20) using the solvent system: *n*-hexane: diethyl ether: acetic acid (80 : 20 : 1 by vol.). The developed TLC plates were sprayed with 2,7-dichlorofluorescein and the lipids appeared as orange-coloured bands. The phospholipid band (R_f = 0.0–0.3) was scraped off and transferred into scintillation vials. The radioactivity was measured after the addition of a toluene based scintillant. The counts per min (cpm)/g lipid was calculated and expressed as percentage of control.

Statistical analysis. The results obtained from the various experiments were analysed using the Student's *t*-test [28].

RESULTS

Dose- and time-course studies

The PDE activity in intact adipocytes was stimulated in a dose-dependent manner by fisetin or quercetin. This effect was enhanced when epinephrine was present simultaneously (Fig. 1A and B). The PDE activity measured represents the total low K_m PDE activity in the adipocytes. The cellular cyclic AMP accumulated exerted by fisetin or quercetin was also dose-dependent. The stimulation of the cellular cyclic AMP accumulation was found to be more enhanced when either fisetin or quercetin was present alone (Fig. 1A and B). Preliminary studies showed that the two bioflavonoids in the range of 1–250 μM did not interfere with the cyclic AMP assay (Table 1) and therefore, the cyclic AMP levels measured in the incubation mixtures cannot be artefacts.

The intracellular cyclic AMP levels increased very rapidly during the early part of incubation (0–15 min) and decreased gradually with time for the remaining period of study (1 hr). These features occurred for incubations that contained either epinephrine alone,

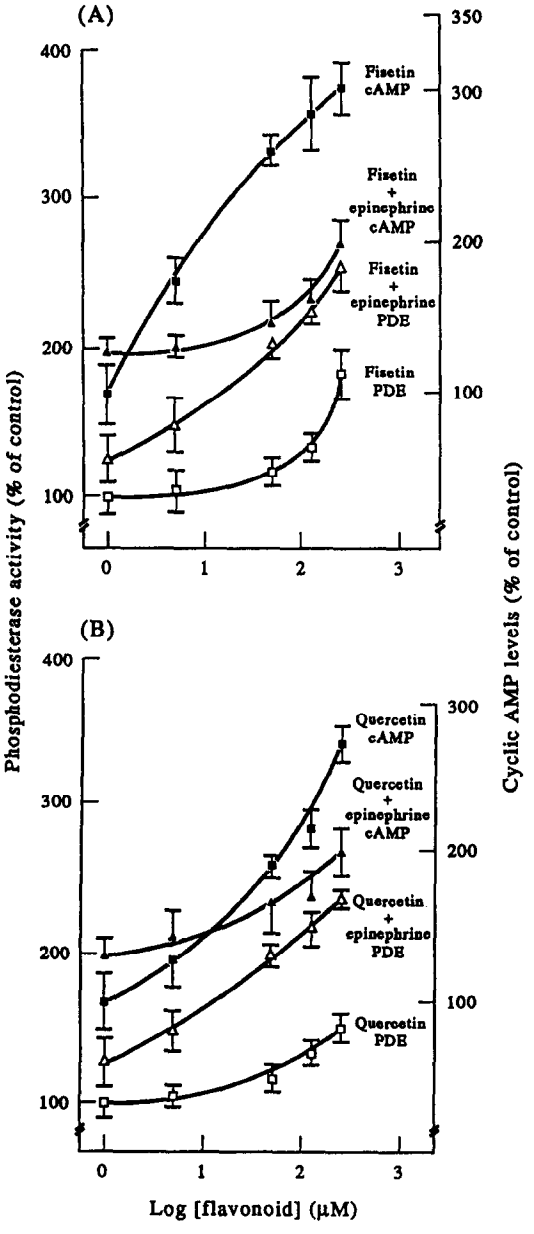

Fig. 1. Effects of increasing concentration of (A) fisetin and (B) quercetin on PDE activity and cyclic AMP levels in the presence or absence of epinephrine. The adipocytes were incubated at 37° for 15 min with the various test compounds. The PDE activity and cyclic AMP levels were measured as described in Materials and Methods. Epinephrine concentration used was 0.1 μM. The control activity was taken as 100%. Each value represents the mean ± SE of three separate experiments determined in duplicate.

fisetin/quercetin alone or fisetin/quercetin plus epinephrine (Fig. 2A and B). The enhanced accumulation of cyclic AMP in adipocytes was more pronounced for fisetin (Fig. 2A) than quercetin (Fig. 2B). The cyclic AMP accumulation caused by either epinephrine or epinephrine plus fisetin/quercetin were not significantly different (Fig. 2A and B).

Immediate stimulation of PDE (uncharacterized PDEs) activity occurred upon the addition of either epinephrine or fisetin/quercetin or epinephrine plus fisetin/quercetin (Fig. 2C and D). The immediate increase exerted by epinephrine was about 50% over control and it reached approximately 100% after 30 min incubation. The activity declined gradually to the basal level at the end of the 2 hr time-course (Fig. 2C and D). When fisetin or quercetin was present alone (250 μM) the immediate PDE activity increase was about 80% over control and was maintained at about 100% over the control throughout the 2 hr period of incubation (Fig. 2C and D). However, when fisetin and epinephrine were present simultaneously in the incubation mixture the maximum increase of PDE activity over control was >200% at approximately 30 min of incubation. The activity began to decline gradually but did not reach basal level at the end of the 2 hr incubation period (Fig. 2C). On the other hand, quercetin plus epinephrine showed PDE activity increases of 100% and 200% for the periods of 3 and 30 min, respectively. The activity declined gradually and reached the basal level at the end of the 2 hr incubation period (Fig. 2D).

Flavonoids stimulate membrane-bound phosphodiesterase

Low K_m cyclic AMP PDE has been reported to be present in cytosolic as well as the membrane fraction of adipocyte [1]. In order to locate the enzyme that is stimulated by the flavonoids, the PDE (uncharacterized PDEs) activity in both the cytosolic and membrane fractions were assayed. Earlier reports have also shown that the hormone sensitive cyclic AMP PDE can be located both in the plasma membrane fraction [29] and in the microsomal fraction [27]. Therefore, in the present study, the 100,000 *g* pellet was taken to represent the total membrane and microsomal fractions. The supernatant was taken to represent the cytosolic fraction. The flavonoids when present singly or with epinephrine were able to stimulate the membrane-bound PDE but not the cytosolic PDE (Table 2).

Membrane phospholipid methylation

Cellular receptor regulation by methylated membrane phospholipid can be affected by hormone and/or drug [7, 9]. In order to investigate the effects of fisetin or quercetin on adipocyte phospholipid methylation, [^{3}H]methyl-labelled *S*-adenosyl-methyl-methionine was used as the substrate. Epinephrine alone or together with either fisetin or quercetin exerted about 30% increase in phospholipid methylation (Fig. 3). Fisetin by itself caused >70% increase in methylation over the control but quercetin showed only about 55% increase (Fig. 3). Phosphatidylcholine fraction had higher radioactive counts ([^{3}H]methyl group incorporation) than the phosphatidylethanolamine fraction. The radioactive counts of the other phospholipid fractions did not significantly differ from the controls (results not shown).

Interaction with β-adrenoreceptors

Propranolol, the β-receptor antagonist, was able

Table 1. Effects of quercetin and fisetin on cyclic AMP assay system*

Flavonoid added	No cyclic AMP		Cyclic AMP (1.0 pmol)	
	Av. radioactive (cpm)	% Decrease of cpm over zero dose of flavonoid	Av. radioactive (cpm)	% Decrease of cpm over zero dose of flavonoid
(No dose)	3332	—	1972	—
Fisetin (1 μM)	3216	3.48	1889	4.21
Fisetin (10 μM)	3147	5.55	2002	−1.52
Fisetin (100 μM)	3155	5.31	1954	0.91
Fisetin (250 μM)	3236	2.88	1855	4.41
Quercetin (1 μM)	3162	5.10	1980	−0.40
Quercetin (10 μM)	3231	3.03	1986	−0.70
Quercetin (100 μM)	3225	3.21	1870	5.17
Quercetin (250 μM)	3248	2.52	1884	4.46

* The flavonoid concentration used was in the range of 1–250 μM. The cyclic AMP assay was carried out as described in Materials and Methods. Each assay was carried out in triplicate and the average range ± SD was <10%.

to inhibit the epinephrine (0.1 μM)-induced lipolysis or epinephrine plus fisetin/quercetin (250 μM each)-induced lipolysis in a dose-dependent manner (Fig. 4A). Maximum inhibition of the epinephrine-induced lipolysis was achieved with 1 μM propranolol. The synergistic lipolysis exerted by epinephrine (0.1 μM) plus fisetin/quercetin (over a concentration range of 0–250 μM) was effectively inhibited by 10 μM propranolol (Fig. 4A and B). Fisetin or quercetin potentiated the isoproterenol (specific β-receptor agonist)-induced lipolysis. This effect can be inhibited by propranolol in a dose-dependent manner (Fig. 4C).

Flavonoids and theophylline-induced lipolysis

Theophylline, a well known PDE inhibitor and a lipolytic agent [25], showed a sigmoidal curve for lipolysis over a concentration range of 0–1000 μM in the rat adipocytes (Fig. 5A). A synergistic increase in lipolysis was also observed when epinephrine and theophylline were simultaneously present (Fig. 5A). Propranolol did not antagonize the theophylline-

Table 2. Effects of fisetin, quercetin and epinephrine on PDE activity in the subcellular fractions of rat adipocytes

Incubation*	PDE activity (pmol/min/mg protein)			
	Supernatant (cytosolic) (S^n)	Pellet (membrane) (P)	$S^n + P$	Infranatant†
Control	21.1 ± 1.0	21.7 ± 0.9	42.8	42.3 ± 2.6
Fisetin	23.7 ± 0.9	40.8 ± 2.8‡	64.5	65.2 ± 4.9‡
Quercetin	23.1 ± 1.6	39.8 ± 2.9‡	62.9	66.7 ± 3.9‡
Epinephrine	23.7 ± 1.8	38.1 ± 3.0‡	61.8	57.4 ± 4.3‡
Fisetin + epinephrine	24.7 ± 1.0	59.5 ± 3.6‡	84.2	88.9 ± 7.1‡
Quercetin + epinephrine	22.6 ± 1.1	48.3 ± 4.1‡	70.9	73.8 ± 5.2‡

* The adipocytes were incubated with or without epinephrine (0.1 μM) and fisetin/quercetin (250 μM) at 37° for 15 min. The PDE activity in the 100,000 *g* particulate and cytosolic fractions was determined as described in Materials and Methods. Each value represents mean ± SE of at least three separate experiments, determined in duplicate. The level of significance was ‡$P < 0.01$ compared to the control (Student's *t*-test).

† Mixture of the membrane as well as the cytosolic fractions.

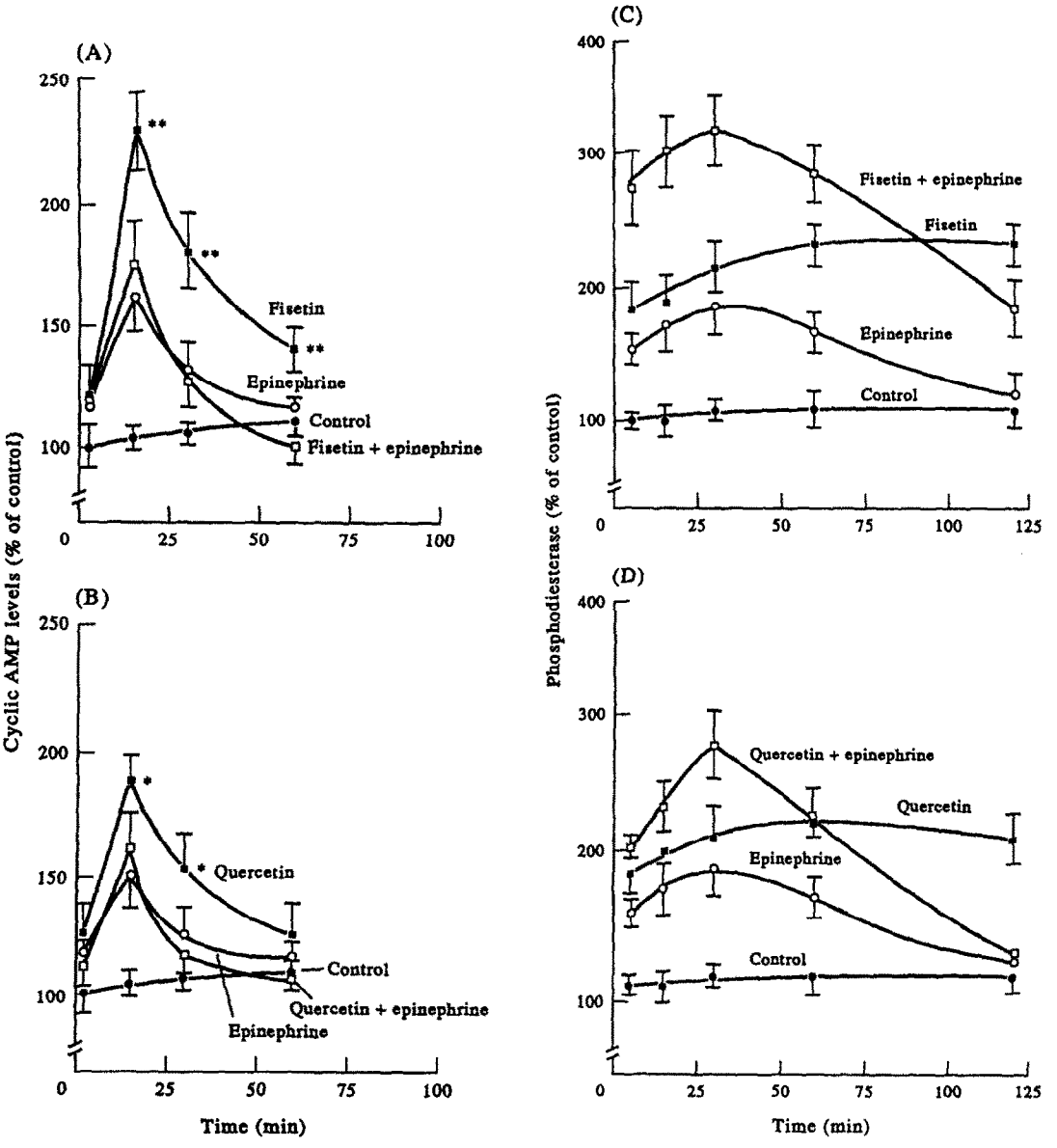

Fig. 2. Time-course effect of fisetin, quercetin and epinephrine (A and B) on cyclic AMP levels and (C and D) PDE activity. The adipocytes were incubated at 37° with 250 μM fisetin/quercetin in the presence or absence of 0.1 μM epinephrine for the indicated period. The cyclic AMP levels and PDE activity were measured as indicated in Materials and Methods. The control activity was taken as 100%. Each value represents mean ± SE of three separate experiments determined in duplicate. **$P < 0.01$, compared to cyclic AMP accumulation caused by either epinephrine alone or fisetin plus epinephrine. *$P < 0.05$, compared to cyclic AMP accumulation caused by either epinephrine alone or quercetin plus epinephrine (Student's t-test).

induced lipolysis (Fig. 5A). Fisetin or quercetin did not stimulate the theophylline-induced lipolysis (Fig. 5B).

DISCUSSION

Fisetin and quercetin were found to exert potent PDE inhibition on disrupted fat cell preparation [21]. Interestingly, the present study showed that these bioflavonoids increased the total PDE activity in intact rat adipocytes (Figs 1A, B, 2C, D), suggesting that the stimulation of epinephrine-induced lipolysis by flavonoids could occur through mechanism(s) other than PDE inhibition. This observation is not surprising for other workers have also reported that PDE inhibitors in a cell-free system need not necessarily inhibit this enzyme in intact cells and that the cAMP levels may not correlate with the extent of PDE inhibition [4].

There are reports to suggest that the activation of

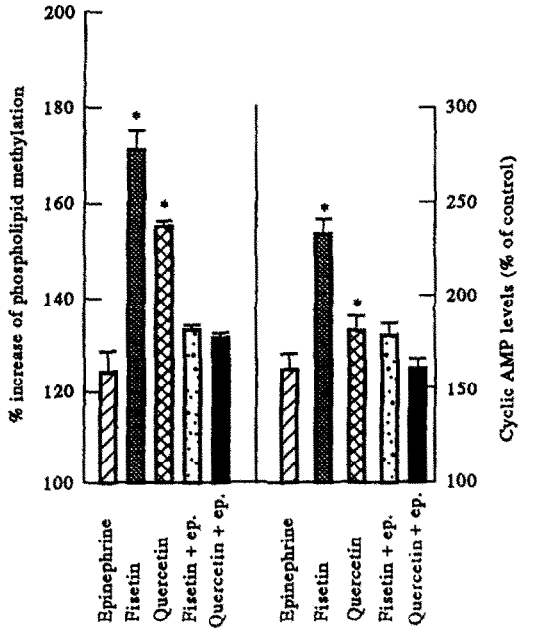

Fig. 3. Effects of epinephrine, fisetin and quercetin on phospholipid methylation and corresponding cyclic AMP levels in rat. Adipocytes were preincubated with *S*-adenosyl [^{3}H]methyl methionine at 37° for 15 min. The cells were incubated for a further 15 min with the various test compounds. The [^{3}H]methylated phospholipids formed were quantified after separation by TLC as described in Materials and Methods. The methylation caused by control incubation (without any additions) is taken as 100%. The corresponding levels of cyclic AMP at the end of 15 min incubations with the respective test compounds were also determined as indicated in Materials and Methods. The control activity was taken as 100%. *$P < 0.01$, compared with incubations carried out in the presence of epinephrine alone.

cyclic AMP PDE and lipolysis were secondary to changes in cyclic AMP [31] which is mediated through a cyclic AMP-dependent protein kinase [2, 5]. Apparently in intact adipocytes, a close relationship (presumably a functional coupling via activation of protein kinase A) between activation of adenylate cyclase (cyclic AMP synthesis) and of PDE activity exists.

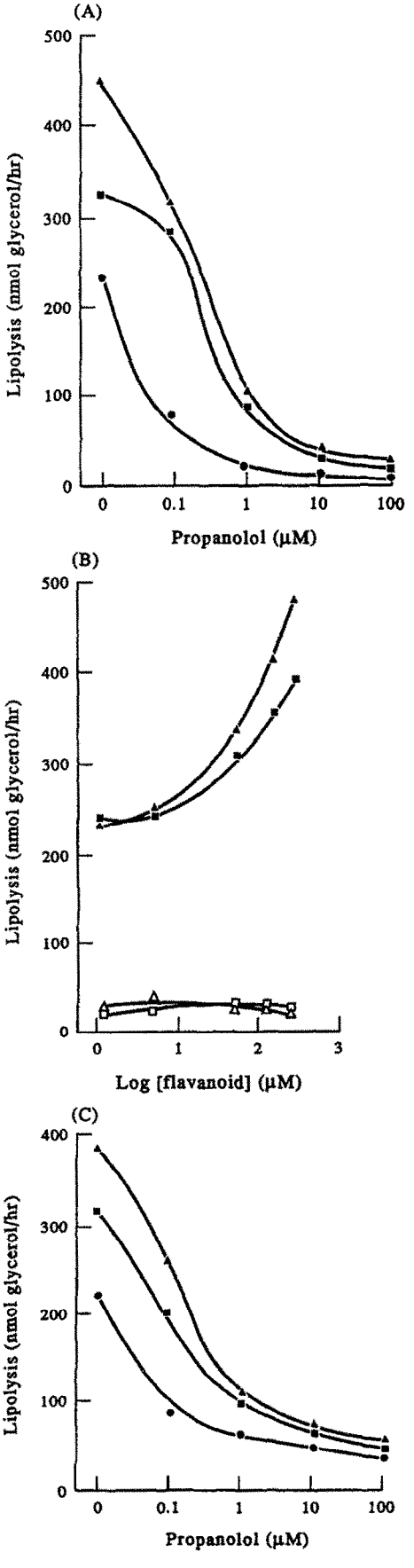

Fig. 4. Effects of propranolol on (A and B) epinephrine- and (C) isoproterenol-induced lipolysis in the presence of fisetin or quercetin. (A) Adipocytes were incubated with 0–100 μM concentrations of propranolol in the presence of 0.1 μM epinephrine (●), epinephrine plus 250 μM fisetin (▲) and epinephrine plus 250 μM quercetin (■). (B) Adipocytes were incubated with epinephrine plus fisetin (triangles) or quercetin (squares) (concentration range of 250 μM) in the absence (closed symbols) or presence (open symbols) of propranolol (10 μM). (C) Adipocytes were incubated with 0–100 μM propranolol in the presence of 0.02 μM isoproterenol (●), fisetin (250 μM) plus isoproterenol (▲) or quercetin (250 μM) plus isoproterenol (■). Glycerol estimation was carried out as described in Materials and Methods. Data presented are the average of at least two separate experiments determined in duplicate.

Fatemi [31] has suggested that the total cyclic AMP pool may not be affected even though there is a high cyclic AMP turnover and this does not affect the lipolytic activity which is the end result. Our data showed that both the flavonoids and/or epinephrine stimulated the membrane-bound PDE (uncharacterized PDEs, 100,000 *g* particulate fraction, Table 2) but the data could not indicate whether the activity was exerted by the same PDE isoform. Fisetin or quercetin when present singly exerted greater stimulation of the cyclic AMP level than epinephrine. However, a larger rise in PDE activity was observed when the flavonoid was present simultaneously with epinephrine (Fig. 2A–D). Thus, the data may show effects arising from the stimulation of different PDE isoforms. A direct effect of the flavonoids on the cascade enzyme(s) can also be considered as an explanation for the poor induction of lipolysis during the cyclic AMP elevation.

Other reports [17, 32, 33] have suggested that a direct activation of plasma membrane PDE could be manifested via the alteration of membrane phospholipid. The effect of β-agonist has also been reported to be associated with the membrane phospholipid methylation [9]. Phospholipid methyl transferase (catalysing the methylation of phospholipids) are widely distributed in cell membranes [7]. Hirata *et al.* [34] have reported the presence of two forms of methyltransferases.

If flavonoid and epinephrine were acting on different pools of methyltransferase enzymes, each associated with one receptor type, a synergistic methylation reaction would be expected to occur when they were present simultaneously. However, it was found that the simultaneous presence of epinephrine and flavonoid did not increase significantly the extent of methylation when compared to the methylation caused by epinephrine alone (Fig. 3). Therefore, it is likely that these two compounds compete for the same methyl transferase pool.

Interestingly, the stimulation of membrane phospholipid methylation by the epinephrine and/or flavonoids correlated with the accumulation of the intracellular cyclic AMP levels (Fig. 3) for the first 15 min of incubation. Hirata *et al.* [34] have reported that the extent of membrane phospholipid methylation was proportional to the activation of adenylate cyclase and the consequent cyclic AMP levels. The cyclic AMP levels in intact cells would depend not only on adenylate cyclase (the synthesis process) but also the PDE activity (the degrading process). Thus, it is pertinent to conclude that increase in membrane phospholipid methylation may be indicative of the extent of adenylate cyclase activation as well as PDE activity in rat adipocytes. However, the increase in cAMP induced by quercetin/fisetin alone did not correlate with an enhancement of lipolysis. Therefore, the role of cAMP as a "second messenger" in adipocytes needs to be re-examined.

Adenosine has been implicated in the decrease in cyclic AMP synthesis which inhibits hormone-stimulated lipolysis [35]. Several workers have shown that the elimination of inhibitory influences caused by adenosine was sufficient for adipocytes to achieve

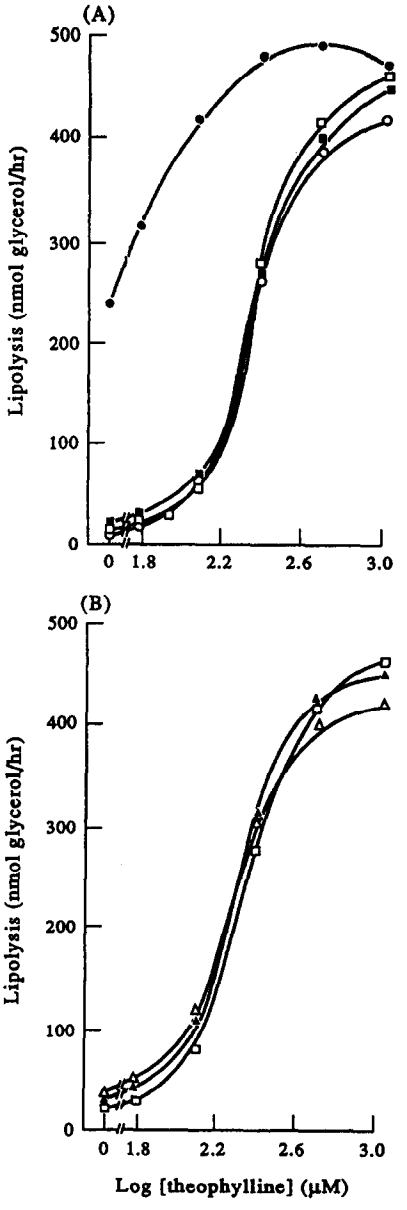

Fig. 5. Effects of (A) epinephrine and propranolol (B) fisetin and quercetin on theophylline-induced lipolysis. Adipocytes were incubated at 37° with 0–1000 μM theophylline (A) in the absence (□) or presence of epinephrine (●), 10 μM propranolol (■), propranolol plus epinephrine (○), and (B) fisetin (▲) and quercetin (△). Glycerol estimation was carried out as described in Materials and Methods. Data presented are averages of at least two separate experiments, determined in duplicate.

lipolytic activities comparable to the lipolytic hormone effect [36, 37]. The use of adenosine deaminase was recommended to eliminate the adenosine effects [36]. But adenosine deaminase can cause the accumulation of adenosine breakdown products such as inosine and subsequently hypoxanthine. These metabolites are known to stimulate lipolysis when added exogeneously into the incubation medium [38]. Therefore, this enzyme was excluded from our studies.

Xanthines, theophylline and IBMX are known to cause lipolysis in rat adipocytes mainly by their adenosine A_1 receptor antagonism with the concurrent accumulation of cyclic AMP via the inhibition of PDE [25, 39]. In order to elucidate whether fisetin and quercetin could potentiate epinephrine-induced lipolysis via the antagonism of adenosine A_1 receptor their effects on theophylline-induced lipolysis were tested. They were found not to potentiate the theophylline-induced lipolysis (Fig. 5B) indicating that their mode of action was dissimilar.

The *in vitro* lipolytic activity of fisetin or quercetin in the simultaneous presence of epinephrine was inhibited by propranolol (β-receptor antagonist) (Fig. 4A and B). Additional studies showed that fisetin/quercetin were able to increase isoproterenol-(specific β-receptor agonist) induced lipolysis but could be completely blocked by propranolol (Fig. 4C). Thus, the two bioflavonoids, quercetin and fisetin are able to potentiate adrenoceptor agonist-mediated lipolysis in rat adipocytes. These findings suggest that the flavonoids should be studied further for lipomobilization in obesity.

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