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EFFECTS OF FLAVONOIDS ON CYCLIC AMP PHOSPHODIESTERASE AND LIPID MOBILIZATION IN RAT ADIPOCYTES

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Abstract—Thirty-one flavonoids were tested for their effects on low K_m phosphodiesterase with cyclic AMP as the substrate. Quercetin, luteolin, scutellarein, phloretin and genistein showed inhibitory potencies comparable to or greater than 3-isobutyl-2-methylxanthine (EC_{50} 30–50 μM). Only four compounds namely, catechin, epicatechin, taxifolin and fustin stimulated the enzyme activity (stimulatory EC_{50} 130–240 μM). The most potent phosphodiesterase (PDE) inhibitors were aglycones that had a $C_{2,3}$ double bond, a keto group at C_4 and hydroxyls at $C_{3'}$ and/or $C_{4'}$. However, when the C-ring is opened then the requirement for the $C_{2,3}$ double bond is eliminated. The same series of flavonoids were also tested for their lipolytic activity. The structural features required for effective synergistic lipolysis (with epinephrine) were generally similar to that required for potent PDE inhibition except that, for lipolytic activity, an intact C-ring was necessary. Fisetin and quercetin having the above-mentioned structure showed a dose- and time-dependent increase in lipolysis which was synergistic with epinephrine. Only butein and hesperetin showed inhibition of epinephrine-induced lipolysis, and their effect was dose-dependent. A time-course study indicated that hesperetin was able to delay the lipolytic action of epinephrine. It is most likely that the lipolytic effects of these compounds were not a result of PDE inhibition, as the orders of potency for the two activities had poor correlation. Apparently, the effective lipolytic flavonoids were also potent PDE inhibitors but not all the PDE inhibitors were able to induce lipolysis.

Flavonoids, a group of polyphenolic natural products, are ubiquitous in the plant kingdom [1] and are found in many traditional herbal medicines [2, 3]. They exert various pharmacological effects [3–6] which are often mediated through inhibition of enzymes [5–11], including the cyclic AMP-hydrolysing enzyme, phosphodiesterase (PDE†) (EC 3.1.4.17) [3, 12–14].

Cyclic AMP has been established as an important intracellular second messenger to hormonal signals [15]. With the exception of cells which can actively extrude cyclic AMP [16], the action of PDE is the major pathway for the termination of the physiological effects of cyclic AMP [17]. Therefore, PDE inhibitors are useful compounds for drug development and some of them are currently being considered for the treatment of various clinical disorders [18].

In adipocytes, the action of lipolytic hormones such as catecholamines, glucagon and corticotropin on the mobilization of stored triglyceride to glycerol and free fatty acids is well known [19]. Adipocytes serve as a useful cell model for studying the mechanism of the action of drugs since these cells have a variety of inhibitory and stimulatory receptors. In addition, biological responses can readily be measured in these cells [20]. Knowledge of the mechanism of the action of drugs would allow a better understanding of their tolerance and provide a basis for application.

Many therapeutic agents have been shown to cause direct lipolysis or anti-lipolysis in adipocytes [21–24]; some of them induce lipolysis by inhibiting PDE [21, 22, 25, 26] and others cause anti-lipolysis by stimulating PDE [23]. Compounds that cause direct lipolysis may have a use in anti-obesity therapy through lipomobilization [24]. Although flavonoids have been reported to inhibit PDE from various tissues [3, 12–14], their effects on rat adipocyte PDE and lipid mobilization in these cells have not been investigated or reported. In view of the important role exerted by PDE inhibitors [18] and the clinical significance of lipid mobilization in biological systems [24], we report here the effects of 31 plant flavonoids on rat adipocyte PDE as well as on *in vitro* lipid mobilization. The structural requirements for both activities were also rationalized.

MATERIALS AND METHODS

Male Wistar rats (180–230 g) were obtained from the Animal Laboratory Centre, National University of Singapore. 2,8[^{3}H]Cyclic AMP was purchased from the Radiochemical Centre (Amersham, U.K.). Bovine serum albumin (Fraction V), bacterial collagenase (type II), snake venom (*Crotalus atrox*), cyclic AMP, theophylline, 3-isobutyl-1-methylxanthine (IBMX) and epinephrine were obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Flavonoids were purchased from

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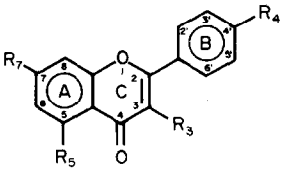
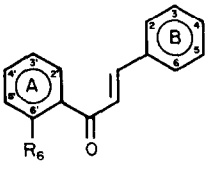
† Abbreviations: PDE, phosphodiesterase; IBMX, 3-isobutyl-1-methylxanthine; DMSO, dimethylsulfoxide; Hepes, *N*-(2-hydroxyethyl)piperazine-*N'*(2-ethanesulfonic acid).

Table 1. General structures of flavonoids studied



Flavone



Chalcone

Flavonoids	Class	Hydroxylation pattern	Substitution
Flavone	Flavone		
Luteolin	Flavone	5,7,3′,4′	
Apigenin	Flavone	5,7,4′	
Scutellarein	Flavone	5,6,7,4′	
Rhamnetin	Flavone	3′,4′	$R_7 = OCH_3$
Diosmetin	Flavone	5,7,3′	$R_4 = OCH_3$
Chrysin	Flavone	5,7	
Luteolin-7-glucoside	Flavone	5,3′,4′	R_7 = glucose
Diosmin	Flavone	5,3′	R_7 = rutinose $R_4 = OCH_3$
Fisetin	Flavonol	3,7,3′,4′	
Quercetin	Flavonol	3,5,7,3′,4′	
Kaempferol	Flavonol	3,5,7,4′	
Myricetin	Flavonol	3,5,7,3′,4′,5′	
Myricitrin	Flavonol	5,7,3′,4′,5′	R_3 = rhamnoside
Morin	Flavonol	3,5,7,2′,4′	
Rutin	Flavonol	5,7,3′,4′	R_3 = rutinose
Catechin*	Flavan-3-ol	3,5,7,3′,4′	
Epicatechin*	Flavan-3-ol	3,5,7,3′,4′	
Daidzein	Isoflavone	7,4′	Ring B attached to C_3
Genistein	Isoflavone	5,7,4′	
Flavanone†	Flavanone		
Hesperetin†	Flavanone	5,7,3′	$R_4 = OCH_3$
Hesperidin†	Flavanone	5,3′	$R_4 = OCH_3$ R_7 = rhamno-glucose
Naringenin†	Flavanone	5,7,4′	
Naringin†	Flavanone	7,4′	R_5 = rhamno-glucose
Taxifolin†	Flavanonol	3,5,7,3′,4′	
Fustin†	Flavanonol	3,7,3′,4′	
Chalcone	Chalcone		
Butein	Chalcone	3,4,4′,6′	
Phloretin†	Chalcone	4,2′,4′,6′	
Phloridzin†	Chalcone	4,2′,4′	R_6 = −D-glucose

* Flavonoids without carbonyl group at C_4 and double bond between $C_{2,3}$.
† Flavonoids without double bond between $C_{2,3}$.

Extrasynthese (Genay, France). Glycerol assay kit was obtained from Boehringer Mannheim. Other chemicals used were of analytical grade.

Theophylline was dissolved in water whilst flavonoids and IBMX were dissolved in 100% dimethylsulfoxide (DMSO). Test compound solutions were prepared fresh for each experiment.

The adipocytes were isolated from epididymal adipose tissue of male Wistar rats (180–230 g) by the collagenase (2 mg/mL) digestion method of Rodbell [27]. The isolation buffer used was a modified Krebs–Henseleit–Hepes buffer (pH 7.4), supplemented with 5.0% bovine serum albumin and 2.5 mM $CaCl_2$.

Preparation of PDE enzyme and PDE assay method. The isolated adipocytes were washed twice with Tris (10 mM)–sucrose (0.25 M)–EDTA (1 mM) (pH 7.4) and were resuspended in Tris (10 mM)–EDTA (1 mM) (pH 7.4). The cells were disrupted by vortex mixing for 30 sec and then centrifuged at 2000 *g* for 10 min. The fat layer which was at the top phase was removed and the infranatant (turbid solution below the fat layer) was used as the PDE source.

Activity of PDE was assayed by the method of Bauer and Schwabe [28]. The assay mixture in a final volume of 200 μL contained the respective test compounds (0–250 μM), 6 mM Mg^{2+}, 0.13 μM cyclic AMP, 2,8 [^{3}H]cyclic AMP (30,000 cpm) and 40 mM Tris–HCl (pH 7.4). The assay was started by adding 50 μL (25 μg protein) of infranatant to achieve less than 25% cyclic AMP hydrolysis after 10 min incubation at 30°. The reaction was terminated by

Table 2. Effects of flavonoids, IBMX and theophylline on PDE and lipolysis

Test compound	PDE inhibition EC_{50} (μM)	Lipolysis Glycerol (nmol)	Lipolysis % increase over control
Control	—	124 ± 10	—
Flavone	122	171 ± 12	38
Luteolin	38	352 ± 30	184
Apigenin	64	345 ± 31	178
Scutellarein	40	340 ± 25	174
Rhamnetin	125	328 ± 22	164
Diosmetin	240	306 ± 20	147
Chrysin	>250 (45%)	180 ± 19	45
Luteolin-7-glucoside	250	152 ± 11	23
Diosmin	>250 (0%)	120 ± 9	0
Fisetin	62	412 ± 35	232
Quercetin	32	370 ± 26	198
Kaempferol	58	335 ± 24	170
Myricetin	125	311 ± 26	151
Myricitrin	250	115 ± 10	0
Morin	208	253 ± 26	104
Rutin	>250 (0%)	125 ± 9	0
Catechin	240*	118 ± 15	0
Epicatechin	136*	150 ± 10	21
Daidzein	222	191 ± 16	54
Genistein	50	163 ± 13	31
Flavanone	>250 (35%)	129 ± 14	0
Hesperetin	>250 (22%)	80 ± 8	−55
Hesperidin	>250 (5%)	118 ± 10	0
Naringenin	164	208 ± 20	68
Naringin	>250 (30%)	123 ± 13	0
Taxifolin	180*	219 ± 22	77
Fustin	202*	208 ± 23	68
Chalcone	>250 (0%)	129 ± 14	0
Butein	68	95 ± 10	−30
Phloretin	38	194 ± 19	56
Phloridzin	>250 (33%)	135 ± 14	0
IBMX	42	375 ± 12	203
Theophylline	114	329 ± 15	166

The disrupted adipocyte PDE activity in the presence of flavonoids was determined as described in Materials and Methods.

EC_{50} is the concentration of compound required to give 50% inhibition/stimulation of PDE activity determined using 0.13 μM substrate. * Denotes EC_{50} values for stimulation of PDE activity. Values in brackets show the per cent PDE inhibition obtained at 250 μM flavonoid.

Lipolytic activity of test compounds (250 μM) was determined in the presence of 0.1 μM epinephrine. The glycerol content in the incubation mixture was assayed after 1 hr of incubation.

Values are means ± SE of at least three separate experiments. The level of significance was $P < 0.05$, determined according to Student's t-test.

immersing the tubes in a boiling water bath for 3 min. The AMP formed was further hydrolysed to adenosine + P_i by the action of snake venom 5′ nucleotidase. For this step, 100 μg of crude snake venom (*Crotalus atrox*) were added to each tube and the tubes were incubated for 12 min before terminating the reaction with 20 mM EDTA (final concentration). The adenosine formed was separated using an anion exchanger (QAE-sephadex-A25) [28] and the radioactivity was counted after the addition of toluene–Triton X-100 based scintillant.

The EC_{50} (concentration of flavonoid resulting in 50% inhibition or stimulation of PDE activity) was calculated using the method reported by Beretz *et al.* [13]. However, we interpolated at least four values (instead of two) of inhibition/stimulation ranging from 0% to 100% against the logarithm of the dose of the test compound. The DMSO concentration present in the PDE assay mixture was <0.5% and, at this concentration, the enzyme activity was not altered. Preliminary experiments had been carried out to ensure that the test

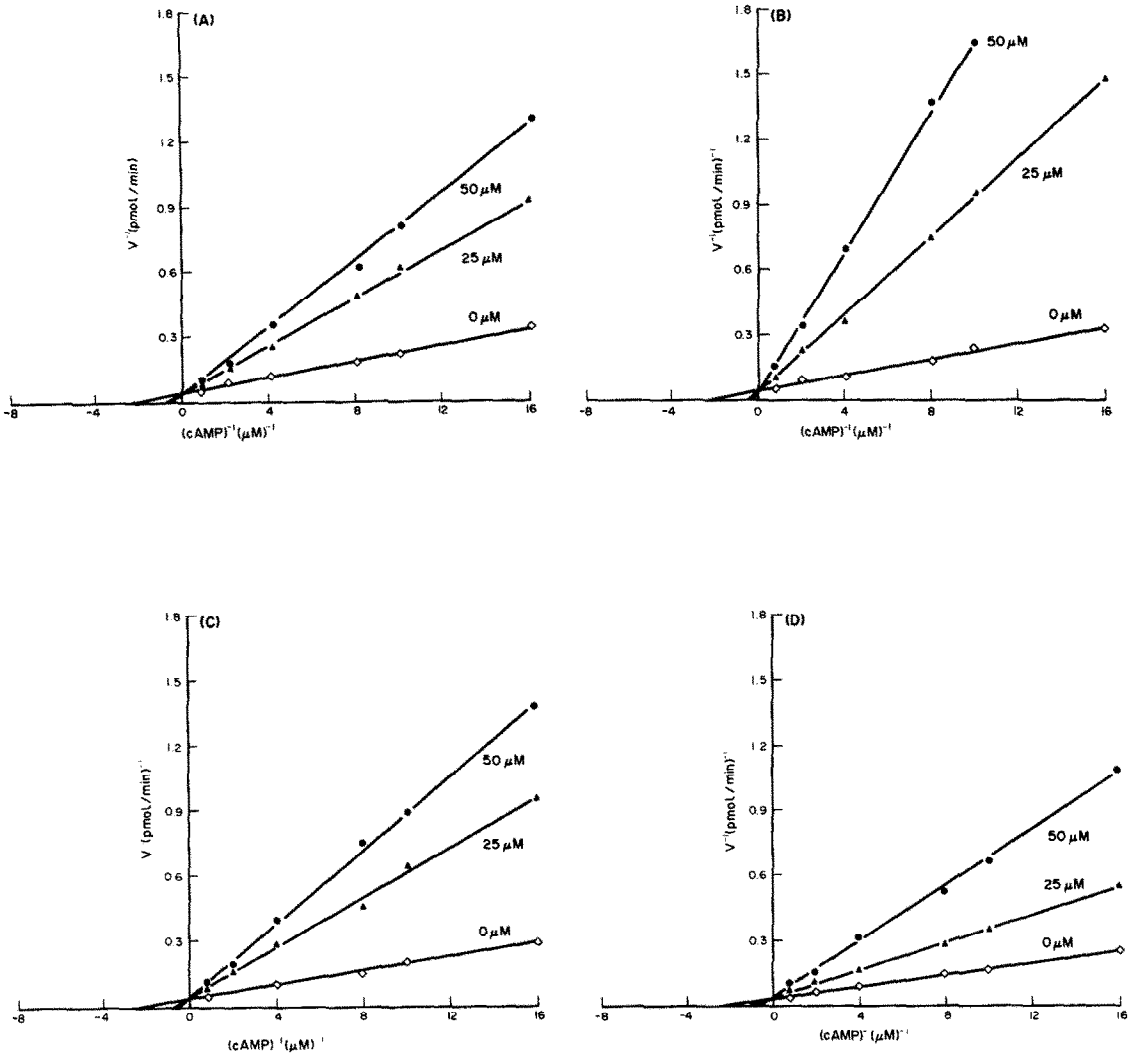


Fig. 1. Effects of (A) fisetin, (B) quercetin, (C) phloretin and (D) butein on kinetics of PDE. The assay was carried out as described in Materials and Methods with substrate concentrations ranging from 0.06 to 1.25 μM. The activity (V) is expressed as pmoles of cyclic AMP hydrolysed per minute. Data for a single experiment are represented graphically by the double reciprocal method. Similar results were obtained for three independent experiments.

compounds did not interfere with the assay condition used.

Incubation for lipolysis study and glycerol assay. The adipocytes in Krebs–Hepes buffer (pH 7.4) supplemented with 2.5% bovine serum albumin were incubated with test compounds and epinephrine. The DMSO concentration present in the incubation mixture was limited to 1% and, at this level, the lipolytic activity of the adipocyte was not affected. At the end of the incubation period, 0.1 mL of cold perchloric acid (30% v/v) was added to 1 mL aliquots of each incubation mixture which were then chilled at 4°. After 30 min, 0.5 mL of cold perchloric acid (3% v/v) was added to the precipitate which was then centrifuged at 2500 *g* for 15 min. The clear supernatant was neutralized with 1 M NaOH and the glycerol content was determined using Boehringer Mannheim assay kit. The test compounds were found not to interfere with the glycerol assay system.

Results are reported as the means ± SEM of at least three separate experiments. The level of significance was determined using Student's *t*-test.

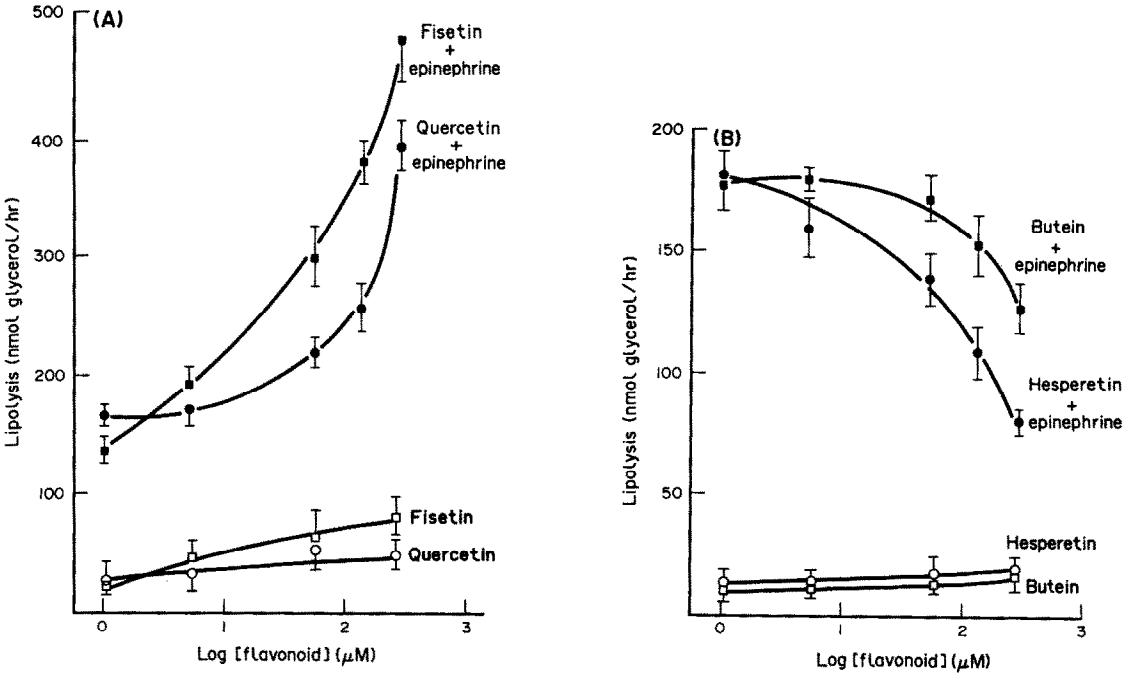


Fig. 2. Dose–response effects of fisetin, quercetin, hesperetin and butein on lipolysis. Panels A and B show the dose–response effects of flavonoids (concentration range of 0–250 μM) on lipolysis in the presence (closed symbols) or absence (open symbols) of 0.1 μM epinephrine. The glycerol released after an incubation period of 1 hr was determined as described in Materials and Methods. Each point represents the mean ± SE of three separate experiments performed in duplicate.

RESULTS AND DISCUSSION

Effects of flavonoids on PDE activity

Thirty-one flavonoids (at concentrations of 0–250 μM) were examined for their effects on the PDE activity of disrupted adipocytes in the presence of 0.13 μM cyclic AMP at 30°, pH 7.4. Our preliminary kinetic studies with substrate concentrations ranging from 0.06 to 1.25 μM showed an apparent K_m value of 0.3 ± 0.1 μM. The hormone-sensitive PDE activity in rat adipocytes has been reported to have a K_m value of approximately 0.3 μM [29]. For routine assays of low K_m cyclic AMP PDE, a substrate concentration of 0.1–0.2 μM has been commonly used [30, 31]. Owing to the low solubility of flavonoids in water, the maximum flavonoid concentration used was limited to 250 μM. Preliminary studies showed that the flavonoids did not interfere with the PDE assay system (results not shown).

The flavonoids tested (Table 1) showed variable inhibitory potencies with the exception of the flavonoids catechin, epicatechin, taxifolin and fustin which exhibited stimulatory effects on the enzyme activity (Table 2). Out of the 31 flavonoids studied only five compounds showed inhibitory potencies (EC_{50} 32–50 μM) comparable to IBMX, which is a well-known potent PDE inhibitor (Table 2). The flavonoid glycosides (Table 1) were generally inactive or exerted very low inhibitory potencies (Table 2). Absence of the $C_{2,3}$ double bond reduces the flavonoid inhibitory activity as indicated by comparison of flavone and flavanone; apigenin and naringenin, or diosmetin and hesperetin (Tables 1 and 2). However, the comparison of quercetin and taxifolin, or fisetin and fustin (Table 1) shows that the absence of $C_{2,3}$ results in stimulation of PDE activity (Table 2). The catechins which lack a $C_{2,3}$ double bond and a C_4 keto group also stimulated PDE activity. Several studies [3, 12–14] on the effects of flavonoids on PDE enzymes from various sources have indicated that the absence of a $C_{2,3}$ double bond only resulted in reduced inhibitory potencies and not stimulation of the enzyme. With the limited data obtained we are unable to speculate on the mechanism through which these flavonoids stimulate adipocyte PDE.

Ferrel *et al.* [3] reported that the opening of the C-ring as in the case of butein completely destroyed the inhibitory potency exerted on liver fluke PDE. However, in our present study butein was found to be a more potent inhibitor (EC_{50} = 68 μM) than various other flavonoids that have an intact C-ring (Tables 1 and 2). In addition, the flavonoid phloretin (a dihydrochalcone), which lacks the $C_{2,3}$ double bond and the C-ring (Table 1) showed an even greater inhibitory potency (EC_{50} = 38 μM) (Table 2). Thus, it would be pertinent to suggest that when the C-ring is absent in the flavonoid molecule, the requirement for the $C_{2,3}$ double bond for PDE inhibition no longer holds.

The attachment of ring B to the chromone structure (Table 1) did not affect the inhibitory potency since genistein, an isoflavone (Table 1), was one of the most potent flavonoids (Table 2).

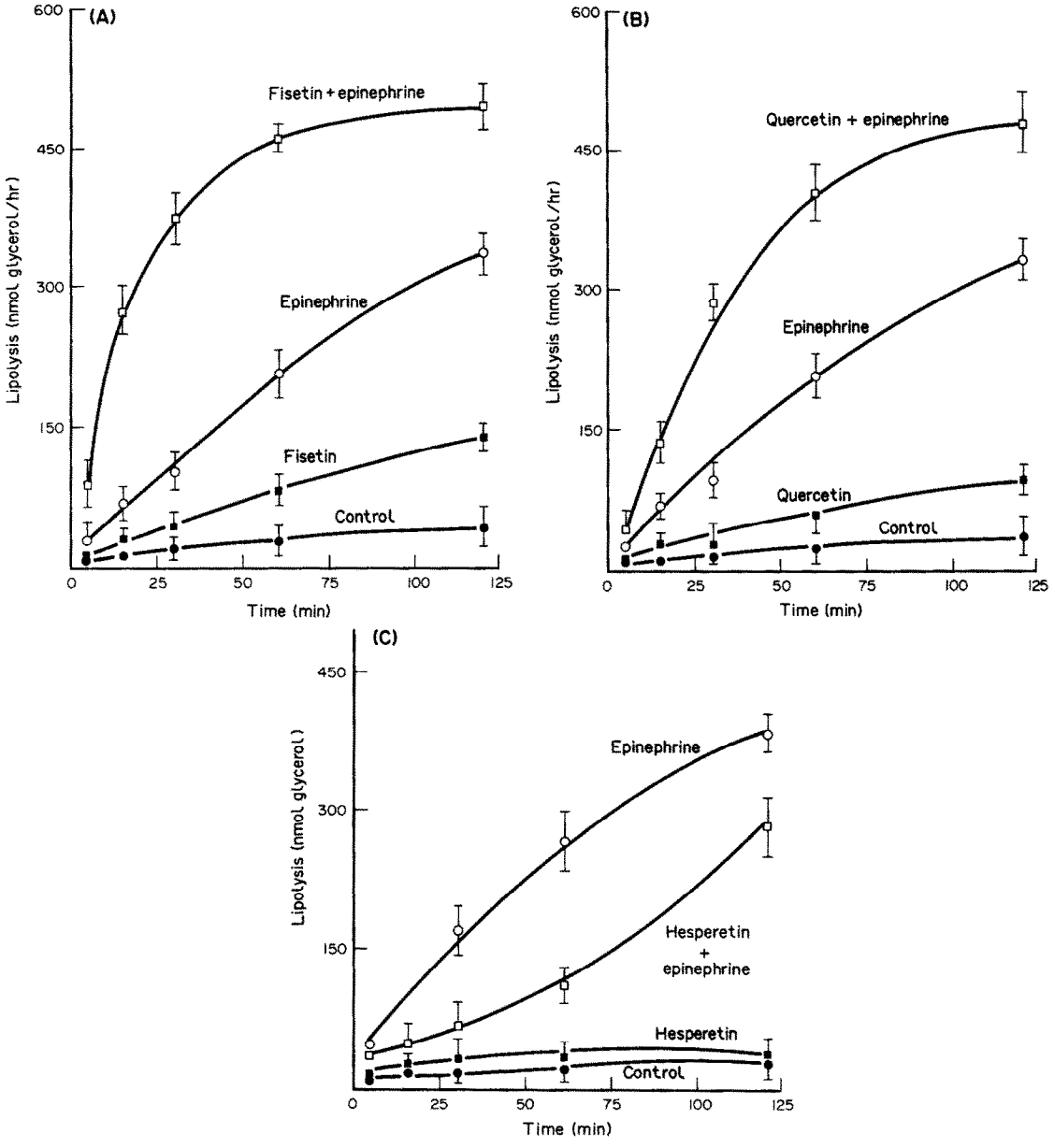


Fig. 3. Time-course effects of fisetin, quercetin and hesperetin on lipolysis. Panels A, B and C show the time effect of fisetin, quercetin and hesperetin. The concentrations of epinephrine and flavonoids used were 0.1 and 250 μM, respectively. Glycerol released was determined (see Materials and Methods) at the indicated times as a measure of lipolysis. Each point represents the mean ± SE of three separate experiments performed in duplicate.

In accordance with a previous report that 4'-methoxy substitution may produce steric hindrance in the structural feature of the flavonoid molecule that is required for inhibitory activity [7], diosmetin showed reduced inhibitory potency when compared with luteolin (Table 2). Generally, the potent PDE inhibitors had hydroxyl groups at $C_{3'}$ and/or $C_{4'}$. Hydroxylation at other carbon positions appeared not to influence the inhibitory potency (Tables 1 and 2).

The kinetics of enzyme inhibition were carried out for only a selected number of flavonoids namely, fisetin, quercetin, butein and phloretin. This selection was based on the following criteria: quercetin and fisetin were potent PDE inhibitors which were also among the more active lipolytic compounds (Table 2). Fisetin was only half as potent, as a PDE inhibitor, when compared to quercetin but showed greater lipolytic activity than quercetin. Phloretin with an inhibitory potency comparable to quercetin showed low lipolytic activity (56%), whilst butein with an inhibitory potency comparable to fisetin

showed inhibition of lipolysis (−30%) (Table 2).

Lineweaver–Burk plots (Fig. 1A–D) were used in order to determine the mode of inhibition with least-square lines fitted to the data. The results were consistent with Michaelis–Menten kinetics and the maximal enzyme velocities (V_{max}) determined at each inhibitor concentration did not show statistically significant differences. This suggests that the inhibition was in all cases competitive. This conclusion was also confirmed on the basis of Hofstee plots (data not shown).

Various factors could be affecting the potency of flavonoids as PDE inhibitors. The competitive nature of the kinetics suggests that the flavonoids could compete for the same cyclic AMP binding site in the adipocyte PDE. Ferrel *et al.* [3] suggested that flavonoids can mimic cyclic AMP in a stacking interaction mainly because of the resemblance between the charge distributions of the pyronone rings of the inhibitors and the pyrimidine ring of cyclic AMP, and/or the resemblance between substrate and the inhibitors in their propensity to accept electrons in a π orbital [3]. Our present work shows that the flavonoids butein and phloretin could exert potent inhibition in a competitive manner despite the absence of a pyronone structure in the molecule. This discrepancy could be due to the difference in the source of the PDE used. It is evident from our observation that PDE inhibitors having the same mode of inhibition can exert a wide range of activities on epinephrine-induced lipolysis.

Effects of flavonoids on lipolysis

Many PDE inhibitors have been shown to cause lipid mobilization either on their own or in the presence of a β-adrenergic agonist [21, 22, 25], and adipocytes have been used widely as a cellular model to study the mechanism of action of hormones and drugs [19, 20]. Thus, we investigated the effects of flavonoids on lipolysis in intact adipocytes and we attempted to rationalize the correlation between PDE inhibition and lipolysis. In the present study, glycerol release was determined as a measure of lipolysis.

Epinephrine concentration study showed a biphasic curve for lipolysis with maximal responses at 1 and 250 μM (data not shown). The response at low concentrations has been termed "Lipolysis I" and that at high concentrations "Lipolysis II" [32]. The sub-maximal Lipolysis I dosage of 0.1 μM was used in this study, as it was closer to physiological concentrations. Our preliminary study with selected flavonoids that were either PDE inhibitors or stimulators indicated that flavonoids (at 250 μM) on their own were inactive or could only marginally stimulate lipid mobilization. However, in the presence of epinephrine (0.1 μM), a synergistic increase in lipolysis occurred. The flavonoids which stimulated PDE activity did not cause inhibition of lipolysis (results not shown). Thus, the whole series of 31 flavonoids (each at 250 μM) were screened for lipolytic activity in the presence of epinephrine (0.1 μM). Theophylline and IBMX (each at 250 μM) were also included in the study for comparison. Based on our preliminary dose–response studies on lipolysis, 250 μM was found to be the ideal concentration at which a wide spectrum of lipolytic activities could be observed (result not shown).

The flavonoid glycosides were inactive (Tables 1 and 2). The aglycone fisetin exhibited the most effective lipolysis (232%) which was even greater than the effects of IBMX (203%) and theophylline (166%), well-known lipolytic agents. The flavonoids which exerted lipolytic activities that were comparable to or greater than theophylline were from the flavone and flavonol groups (Tables 1 and 2). Saturation of the $C_{2,3}$ double bond reduced the lipolytic activity of the flavonoid as can be seen by comparison of apigenin and naringenin, flavone and flavanone, fisetin and fustin, or quercetin and taxifolin (Tables 1 and 2). However, hesperetin and diosmetin, two compounds with very similar structural features (Table 1), exerted contrasting effects on epinephrine-induced lipolysis. Diosmetin exerted 147% stimulation whilst hesperetin (lacking the $C_{2,3}$ double bond) showed 55% (Tables 1 and 2). The absence of hydroxyl groups at $C_{3'}$ and $C_{4'}$ reduces the lipolytic activity as in the case of chrysin and luteolin. The most effective lipolytic flavonoids (>100% lipolysis) possessed a hydroxyl group at $C_{4'}$ (Tables 1 and 2). From our data it is evident that flavonoids having the $C_{2,3}$ double-bond, a C-ring, a keto group at C_4 and hydroxyl groups at $C_{4'}$, and lacking a sugar molecule stimulated most effectively the epinephrine-induced lipolysis.

Dose–response and time-course studies

Fisetin and quercetin, the two most effective lipolytic compounds, were chosen to represent the activities of lipolytic flavonoids in the dose–response and time-course studies. The effects of hesperetin and butein, the only two compounds that showed inhibition of epinephrine-induced lipolysis, were also investigated. The compounds on their own did not exert significant lipolysis within the dose range tested (0–250 μM). However, in the presence of 0.1 μM epinephrine, fisetin and quercetin showed a dose-dependent synergistic increase in lipolysis (Fig. 2A) while hesperetin and butein showed a dose-dependent decrease in lipolysis (Fig. 2B). Many drugs used in the treatment of various clinical disorders have been shown to exert a synergistic increase in lipolysis in the presence of β-adrenergic agonist(s) but not alone [22, 25, 32]. Sulfonylureas, drugs widely used in the treatment of type II or non-insulin-dependent diabetes mellitus, have been shown to inhibit hormone-induced lipolysis in rat adipocytes [23]. It must be noted that in the study reported here, the concentration used was limited to 250 μM due to poor solubility of the compounds and one should not rule out the possibility that at higher concentrations these compounds (even in the absence of epinephrine) may be able to induce lipolysis.

Epinephrine (0.1 μM) exerted a time-dependent increase in lipolysis and a linear relationship was observed for the 2-hr period of study (Fig. 3A, B and C). Fisetin and quercetin caused a synergistic increase in lipolysis in the presence of epinephrine over the same period of study (Fig. 3A and B). When present alone, these compounds showed a significant increase in lipolysis after 60 min.

Hesperetin appears to delay the lipolytic action of epinephrine (Fig. 3C). The time-course effect of butein was not investigated, since this compound exhibited only a low anti-lipolytic effect at 250 μM (Table 2, Fig. 2B).

Flavonoids were found to exert a wide spectrum of activities on adipocyte low K_m cyclic AMP PDE in a dose-dependent manner. The flavonoids having the following structures were found to be the most potent PDE inhibitors: absence of a sugar moiety, $C_{2,3}$ double bond and C_4 keto group, and presence of $C_{3'}$ and/or $C_{4'}$ hydroxyl group(s). When the C-ring is absent, then the requirement of a $C_{2,3}$ double bond for inhibitory potency is eliminated. In fact, the saturation of the $C_{2,3}$ double bond in some cases could stimulate the PDE activity. Fisetin, quercetin, phloretin and butein, potent PDE inhibitors that exerted a competitive mode of inhibition, showed a wide range of effects on lipolysis. The structural features of flavonoids required for a synergistic increase in epinephrine-induced lipolysis are generally similar to the features required for the most potent PDE inhibition, except that the basic flavone structure with a C-ring is necessary for lipolytic activity. PDE stimulators did not cause anti-lipolysis as would be expected but two structurally unrelated compounds, the flavanone hesperetin (poor PDE inhibitor) and the chalcone butein (potent PDE inhibitor), did show anti-lipolysis. Thus, it is pertinent to suggest that the effects of the flavonoids on lipolysis were not mediated through PDE activity.

In conclusion, we are able only to rationalize that generally the flavonoids which showed active lipolysis were also potent PDE inhibitors but not all PDE inhibitors were able to induce lipolysis. It is possible to speculate (based on the information we have obtained) that some of the flavonoids could be exploited for beneficial use as therapeutic agents.

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