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EFFECT OF MYRICETIN AND OTHER FLAVONOIDS ON THE LIVER PLASMA MEMBRANE Ca^{2+} PUMP

KINETICS AND STRUCTURE–FUNCTION RELATIONSHIPS

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Abstract—Thirty-three different flavonoids were screened for their ability to influence ATP-dependent Ca^{2+} uptake by rat liver plasma membrane vesicles. Nine of the flavonoids, at a concentration of 100 μM inhibited Ca^{2+} uptake by more than 20%. The remaining 24 flavonoids exhibited little or no effect. The relative order of potency of the more biologically active flavonoids was myricetin > butein > phloretin = luteolin > eriodictyol = silybin. Myricitrin and phloridzin, the glycosides of myricetin and phloretin, respectively, had no effect. The degree of inhibition caused by myricetin was concentration dependent and was also affected by the preincubation time. After 10 min of preincubation, 52 μM myricetin lowered the initial rate of ^{45}Ca uptake by 50%. The inhibition by myricetin was non-competitive with respect to Mg-ATP and of a mixed type with respect to Ca^{2+}. At a concentration of 100 μM, myricetin had no effect on several plasma membrane enzymes such as 5′-nucleotidase, alkaline phosphatase and a Ca^{2+}-activated ATPase but inhibited K^+-dependent *p*-nitrophenyl phosphatase by 83%. The ATP-dependent Ca^{2+} transport systems located on the plasma membrane or endoplasmic reticulum derived from other tissues were also inhibited by myricetin. Analysis of the structure–activity relationship revealed that lipid solubility and polyhydroxylation particularly at positions 5,7,3′, and 4′ of the flavonoid ring structure enhanced the ability of the flavonoid to inhibit Ca^{2+} uptake. The results suggest that inhibition of Ca^{2+} transport activity probably involves the interaction of the phenolic groups of the flavonoid with the Ca^{2+} transporting protein.

The ATP-dependent Ca^{2+} transport system located on the plasma membrane represents the principal mechanism for the extrusion of Ca^{2+} ions from mammalian cells. It not only plays a fundamental role in regulating the total cellular Ca^{2+} concentration but also modulates or mediates the effects of Ca^{2+} mobilizing hormones and neurotransmitters [1–3]. In the rat liver, the plasma membrane Ca^{2+} pump has been well characterized [4–6]. The pump displays a high affinity for Ca^{2+} ions [3, 4] and is regulated by a variety of hormones including glucagon [7] and epinephrine [3]. Stimulation of the liver with Ca^{2+} mobilizing hormones such as epinephrine or vasopressin has been shown to inhibit the Ca^{2+} pumping activity. This inhibition is thought to serve as a mechanism for augmenting or sustaining the elevation of intracellular Ca^{2+} that occurs following the release of Ca^{2+} from an intracellular non-mitochondrial store [3].

In view of its important role in intracellular Ca^+ homeostasis and the hormonal mechanisms controlling liver metabolism, it was of interest to us to explore potential means of regulating the activity of the plasma membrane Ca^{2+} pump. The flavonoids are a class of naturally occurring plant compounds with wide ranging biochemical and pharmacological effects [8, 9]. Although their exact physiological function in plants is far from clear, some flavonoids are believed to regulate ion channels in the thylakoid membrane [8]. In mammalian tissues, the flavonoids have been shown to inhibit a variety of different enzymes including protein kinases [10], human lens aldolase [11], soybean lipoxygenase-1 [12] and mitochondrial succinoxidase [13]. In addition, inhibition of several ion-translocating ATPases such as the (Na^+, K^+)ATPase [14], erythrocyte Ca^{2+}-ATPase [15] and the mitochondrial proton translocating ATPase [16] by flavonoids, in particular quercetin, have also been reported. Our preliminary studies on the ATP-dependent Ca^{2+} transport system of liver plasma membrane however indicated that quercetin had only a small inhibitory effect. We therefore undertook a more extensive survey of the various classes of flavonoids with the aim of identifying potential inhibitors and rationalizing on the structural features necessary for inhibition to occur. The results obtained indicate that although a variety of different flavonoid classes inhibit the Ca^{2+} pump, those which are polyhydroxylated at rings A and B are particularly effective in preventing ATP-dependent Ca^{2+} translocation.

MATERIALS AND METHODS

^{45}Ca and (γ-[^{32}P]ATP) were purchased from the Radiochemical Centre (Amersham, U.K.). Flavonoids were from Extrasynthese (Genay, France). All other chemicals and biochemicals were from the Sigma Chemical Co. (St Louis, MO, U.S.A.).

Isolation of plasma membrane. Rat liver plasma membranes were prepared from male Wistar rats of

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about 200–250 g by the method of Pripic *et al.* [3] with slight modifications. EGTA was omitted from the isolation medium which consisted of 250 mM sucrose, 5 mM Hepes–KOH, pH 7.4. After centrifugation in Percoll and washing, the plasma membrane vesicles were resuspended in 0.11 M KCl, 10 mM Tris–HCl, pH 6.8, 0.1 mM DTT at a concentration of about 2 mg/mL protein and used immediately for measurement of Ca^{2+} uptake. Protein concentration was determined according to Lowry *et al.* [17].

^{45}Ca *Uptake.* ATP-dependent Ca^{2+} uptake was assayed in medium having the following composition: 100 mM KCl, 20 mM Hepes–KOH, pH 6.8, 4 mM Mg-ATP, approximately 0.1 mg/mL of membrane protein, a ^{45}Ca-EGTA buffer to give the required free Ca^{2+} concentration and flavonoids as indicated in the text. The free Ca^{2+} concentration was calculated as described previously [18]. For most experiments, the concentrations of EGTA and $CaCl_2$ used were 0.2 and 0.12 mM, respectively, giving a free Ca^{2+} concentration of 0.75 μM. Unless otherwise stated, ^{45}Ca uptake was initiated by the addition of Mg-ATP to membranes that had been preincubated at 37° for 10 min in the presence (test) or absence (control) of added flavonoid. After 1 min, suitable aliquots were filtered through Millipore membrane filters of 0.45 μm pore size, washed twice with 5 mL of ice-cold medium containing 0.11 M KCl, 1 mM EGTA and 5 mM Hepes–KOH, pH 6.8 and ^{45}Ca accumulated determined. Flavonoids were dissolved in DMSO (dimethylsuphoxide) at a concentration of 10 mM. The final concentration of DMSO in the assay mixture was maintained at 1% (v/v) for all incubations. All experiments were repeated at least three times and the values obtained for ATP-dependent ^{45}Ca uptake were corrected for ^{45}Ca binding to membranes incubated in the absence of ATP.

Other assays. 5′-Nucleotidase, alkaline phosphatase and K^+-dependent *p*-nitrophenyl phosphatase were determined as described previously [18]. The Ca^{2+}-activated ATPase of liver plasma membranes, which is believed to be distinct from the Ca^{2+}-transporting ATPase [19] was assayed according to Epping and Bygrave [20]. Parotid plasma membranes were isolated according to Low *et al.* [18] and endoplasmic reticulum according to Thiyagarajah and Lim [21]. Human erythrocyte ghosts were prepared by repetitive washing in 10 mM Tris–HCl pH 7.4 buffer containing 1 mM EGTA and the (Ca^{2+}, Mg^{2+})ATPase determined as described by Seals *et al.* [22] in the presence of excess calmodulin.

RESULTS

Thirty-three flavonoids belonging to different flavonoid classes including flavone, flavanone, flavanol, flavanonol, isoflavone, catechin and chalcone were tested for their ability to alter the rate of ATP-dependent Ca^{2+} accumulation by liver plasma membrane vesicles. The membrane vesicles were preincubated in the presence of 100 μM of the flavonoid before the initial rate of ^{45}Ca uptake was determined. As shown in Table 1, nine of the

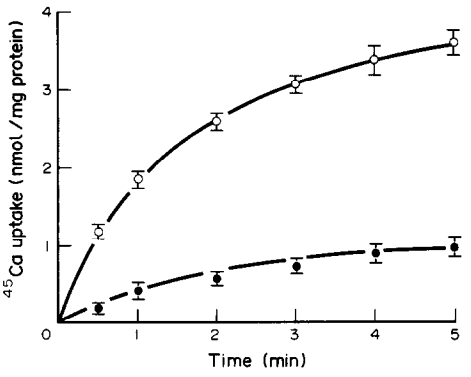


Fig. 1. Time course of ATP-dependent ^{45}Ca uptake. Liver plasma membrane vesicles were preincubated for 10 min in the presence (●) or absence (○) of 100 μM myricetin in assay medium lacking ATP. ATP-dependent ^{45}Ca uptake was then determined as described in Materials and Methods. Results are the mean ± SEM of triplicate determinations in a representative experiment.

flavonoids tested lowered the rate of Ca^{2+} uptake by more than 20%; six had a small inhibitory effect of between 10 and 20% and the remaining 18 exerted no significant effect. Myricetin and butein were the most effective in inhibiting ^{45}Ca accumulation and lowered the rate of uptake by more than 70%. The relative order of potency with which the flavonoids inhibited ^{45}Ca accumulation was myricetin ≥ butein > phloretin = luteolin > silybin = eriodictyol > chalcone, robinetine and naringenin. All the glycosides tested including myricitrin and phloridzin, rhamnosides of myricetin and phloretin respectively, had no effect. The catechins (catechin and epicatechin) and the isoflavanol (daidzein) also had little or no effect.

Since myricetin displayed the highest inhibitory activity, the myricetin induced inhibition was further characterized. The time course of ^{45}Ca uptake by plasma membranes incubated in the presence and in the absence of 100 μM myricetin is shown in Fig. 1. Inhibition by myricetin persisted throughout the 5 min period and lowered the rate of uptake by about 75%. The same concentration of myricetin had no effect on ^{45}Ca binding by the membranes incubated in medium lacking ATP (results not shown).

Inhibition of Ca^{2+} uptake by myricetin was dependent on the time for which membranes were preincubated with the flavonoid. Increasing the preincubation time caused a progressive diminuition in the rate of ^{45}Ca uptake in the presence of either 50 or 100 μM myricetin (Fig. 2). With 50 μM myricetin, the rate of uptake declined by 21% if there was no prior preincubation with the flavonoid. After 60 min of preincubation, the rate declined by more than 78%. When the effect of preincubation was tested on seven other flavonoids (flavone, chrysin, apigenin, luteolin, chalcone, phloretin and butein), only flavone and chalcone showed a slight increase in inhibitory potency with time. In the presence of 100 μM chalcone, ^{45}Ca uptake was inhibited by 14 and 24% after preincubation for 0

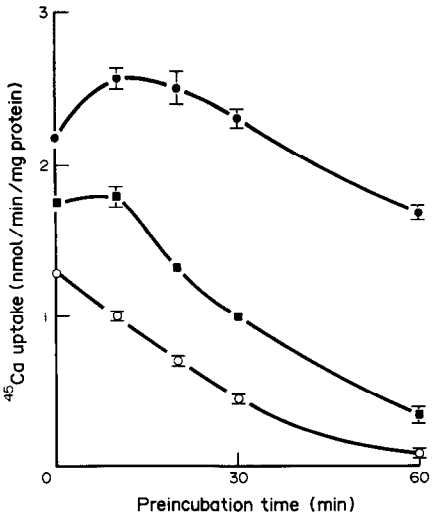


Fig. 2. Effect of preincubation time on inhibition of ^{45}Ca uptake by myricetin. Membrane vesicles were preincubated at 37° for 0 to 60 min in assay buffer lacking ATP and containing no added myricetin (●), 50 μM myricetin (■) or 100 μM myricetin (○). ^{45}Ca uptake was initiated by the addition of ATP and the initial rate determined. Results are the mean ± SEM of triplicate determinations in a representative experiment.

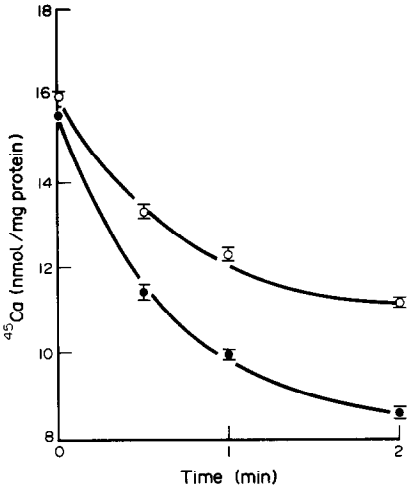


Fig. 3. Effect of myricetin on ^{45}Ca efflux from preloaded vesicles. Plasma membrane vesicles were loaded with ^{45}Ca by incubating them for 5 min in buffer of composition similar to that used for the determination of ^{45}Ca uptake (see Materials and Methods). ^{45}Ca efflux was induced by mixing the Ca^{2+}-loaded vesicles with an equal volume of medium of the following composition: 0.1 M KCl, 20 mM Hepes–KOH, pH 6.8, 20 mM EGTA, 5 mM glucose, 10 units/mL of hexokinase and 200 μM myricetin where indicated. At the times shown, ^{45}Ca retained in the vesicles was determined. Results are the mean of triplicate determinations in a representative experiment conducted in the presence (○) and absence (●) of 100 μM myricetin.

and 10 min, respectively; with 100 μM flavone, the inhibition was 8 and 15%, respectively. Control membranes showed optimal rates of ^{45}Ca uptake after 10 min of preincubation (Fig. 2); thereafter, the rates declined slowly suggesting that prolonged incubation of the membrane vesicles at 37°, leads to a slow but progressive loss of Ca^{2+} transport activity. In view of this slight loss of activity with prolonged incubation, most of the studies were done on membranes which had been preincubated for 10 min.

The observed decrease in the rate of ^{45}Ca uptake induced by myricetin and the other flavonoids could arise either from a direct inhibition of the transport protein or from an increase in the rate of ^{45}Ca efflux from the membrane vesicles. To distinguish between these two possibilities, the effect of myricetin on ^{45}Ca efflux from membrane vesicles preloaded with ^{45}Ca was determined. As shown in Fig. 3, the rate of ^{45}Ca efflux was significantly slower in the presence of 100 μM myricetin indicating that accelerated Ca^{2+} efflux does not account for the observed inhibition of ^{45}Ca uptake.

The dose–response to myricetin is shown in Fig. 4. After 10 min of preincubation with myricetin, the IC_{50} for myricetin was 52 μM. At a concentration of 200 μM, myricetin inhibited ^{45}Ca uptake by 85%. The myricetin-induced inhibition of Ca^{2+} uptake was predominantly due to a decrease in the V_{max} of the Ca^{2+} pump. As shown in Fig. 5, myricetin had no effect on the K_m for ATP but increased the K_m for Ca^{2+} from 0.35 to 0.53 μM. In both instances, the V_{max} was substantially reduced.

The effect of myricetin on the activities of several other liver plasma membrane enzymes was also studied (results not shown). At concentrations up to

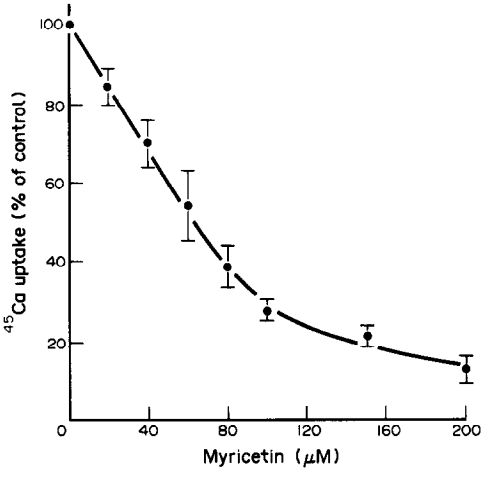
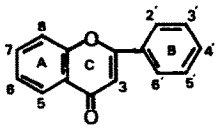
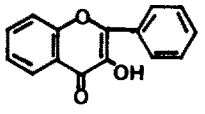
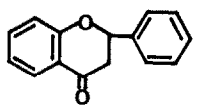
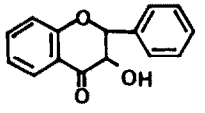
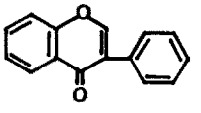
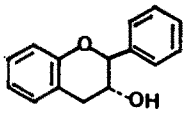
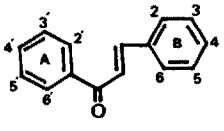


Fig. 4. Dose–response to myricetin. The initial rate of ATP-dependent ^{45}Ca uptake was determined as described in Materials and Methods in the presence of varying concentrations of myricetin. Results are the mean ± SEM of three experiments.

100 μM, myricetin had no effect on 5′-nucleotidase, alkaline phosphatase or Ca^{2+}-activated ATPase (an enzyme which is distinct from the Ca^{2+}-pump). K^+-dependent *p*-nitrophenyl phosphatase, often taken as an index of (Na^+, K^+)ATPase activity was

Table 1. Effect of different flavonoids on ATP-dependent ^{45}Ca uptake by liver plasma membrane vesicles

		R3	R5	R7	R2′	R3′	R4′	R5′	ATP-dependent ^{45}Ca uptake (% of control)
Flavones									
	Flavone	H	H	H	H	H	H	H	85 ± 1
	Chrysin	H	OH	OH	H	H	H	H	104 ± 3
	Techto chrysin	H	OH	O-me	H	H	H	H	100 ± 1
	Apigenin	H	OH	OH	H	H	OH	H	102 ± 3
	Luteolin	H	OH	OH	H	OH	OH	H	57 ± 4
	Diosmetin	H	OH	OH	H	OH	O-me	H	98 ± 8
	Diosmin	H	OH	O-rut	H	OH	O-me	H	103 ± 3
Flavanols									
	Fisetin	OH	H	OH	H	OH	OH	H	86 ± 4
	Robinetine	OH	H	OH	H	OH	OH	OH	77 ± 4
	Galangin	OH	OH	OH	H	H	H	H	03 ± 6
	Datiscetin	OH	OH	OH	OH	H	H	H	105 ± 2
	Morin	OH	OH	OH	OH	H	OH	H	95 ± 1
	Quercetin	OH	OH	OH	H	OH	OH	H	95 ± 1
	Quercitrin	O-rh	OH	OH	H	OH	OH	H	96 ± 5
	Myricetin	OH	OH	OH	H	OH	OH	OH	26 ± 2
	Myricetrin	O-rh	OH	OH	H	OH	OH	OH	96 ± 3
	Silybin	OH	OH	OH	H	H	-O-lign-O		69 ± 1
Flavanones									
	Flavanone	H	H	H	H	H	H	H	90 ± 2
	Naringenin	H	OH	OH	H	H	OH	H	76 ± 2
	Naringin	H	OH	O-rhg	H	H	OH	H	100 ± 3
	Eriodictyol	H	OH	OH	H	OH	OH	H	68 ± 3
	Hesperetin	H	OH	OH	H	OH	O-me	H	87 ± 3
	Hesperidin	H	OH	O-rhg	H	OH	O-me	H	104 ± 3
Flavanonols									
	Kaempferol	OH	OH	OH	H	H	OH	H	87 ± 4
	Taxifolin	OH	OH	OH	H	OH	OH	H	106 ± 3
	Fustin	OH	H	OH	H	OH	OH	H	100 ± 3
Isoflavone									
	Daidzein	—	H	OH	H	H	OH	H	84 ± 3
Catechins									
	Catechin	OH	OH	OH	H (B ring in *trans* position)	OH	OH	H	101 ± 1
	Epicatechin	OH	OH	OH	H (B ring in *cis* position)	OH	OH	H	99 ± 4
Chalcones			R3	R4	R2′	R4′	R6′		
	Chalcone		H	H	H	H	H		77 ± 1
	Butein		OH	OH	H	OH	OH		28 ± 2
	Phloretin		H	OH	OH	OH	OH		56 ± 2
	Phloridzin		H	OH	O-glu	OH	OH		102 ± 4

Plasma membrane vesicles were preincubated as described in Materials and Methods for 10 min at 37° in assay buffer containing 100 μM of flavonoid. Uptake was initiated by the addition of Mg-ATP and ATP-dependent ^{45}Ca uptake determined after 1 min. Results are expressed as a percent of ATP-dependent ^{45}Ca uptake in the absence of added flavonoid and are the mean ± SEM of at least three separate experiments.

Substituents in flavonoid basic structures are as follows: ru = rutin; rh = rhamnose; rhg = rhamnoglucose; glu = glucose; me = methyl; lign = lignan.

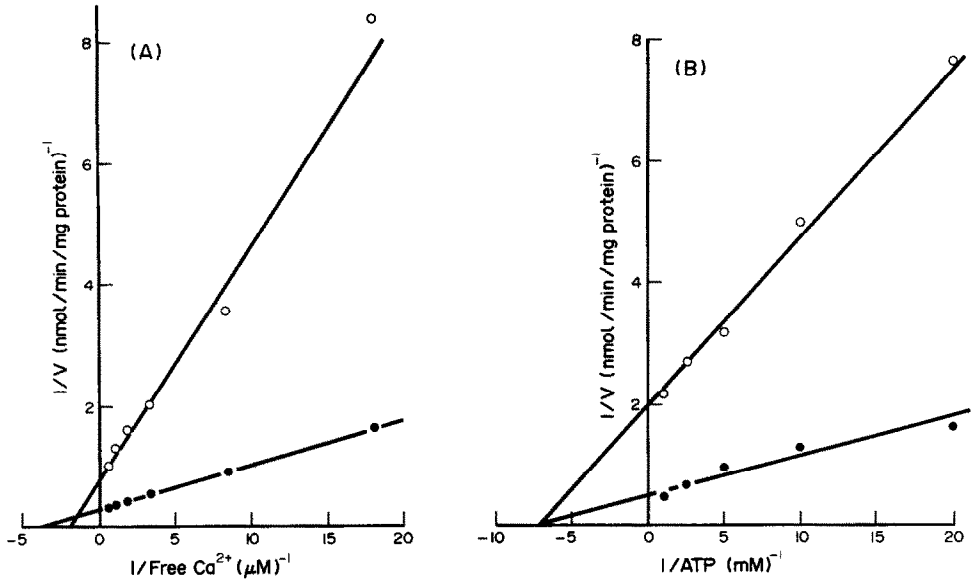


Fig. 5. Kinetics of inhibition by myricetin. Membranes were preincubated in the absence (●) or in the presence (○) of 100 μM myricetin and the initial rate of ^{45}Ca uptake then determined under the following conditions: (A) 4 mM Mg-ATP and varying concentrations of free Ca^{2+}, (B) 0.75 μM free Ca^{2+} and varying concentrations of ATP. Results of a representative experiment are shown. The mean ± SEM of the K_m values obtained from three separate experiments were as follows: K_m for Ca^{2+}, 0.35 ± 0.03 and 0.53 ± 0.06 μM, respectively, in the absence and in the presence of myricetin; K_m for Mg-ATP, 0.15 ± 0.01 and 0.17 ± 0.02 mM, respectively, in the absence and in the presence of myricetin.

Table 2. Inhibition of different Ca^{2+} transport systems by flavonoids

Ca^{2+}-transport system	% Inhibition of activity		
	Myricetin (100 μM)	Luteolin (100 μM)	Quercetin (100 μM)
Liver plasma membrane ^{45}Ca uptake	74 ± 2	43 ± 4	5 ± 1
Parotid plasma membrane ^{45}Ca uptake	69 ± 1	65 ± 5	21 ± 4
Parotid endoplasmic reticulum ^{45}Ca uptake	71 ± 2	64 ± 4	13 ± 5
Erythrocyte ghosts (Ca^{2+} + Mg^{2+})ATPase	92 ± 1	68 ± 1	44 ± 2

The activities of the different Ca^{2+} transport systems was assayed either as ATP-dependent ^{45}Ca uptake or as (Ca^{2+} + Mg^{2+})ATPase as described in Materials and Methods, after preincubating membranes for 10 min in the presence of different flavonoids. Results are expressed as the percentage decrease in activity compared to control membranes assayed in the absence of flavonoid and are the mean ± SEM of at least three experiments.

however inhibited 83% by 100 μM myricetin. When tested on other ATP-dependent Ca^{2+}-translocating systems, myricetin proved to be a potent inhibitor of all the Ca^{2+} transport systems tested (Table 2). The Ca^{2+}-pumping ATPase of erythrocyte ghosts as well as the parotid plasma membrane and endoplasmic reticulum associated Ca^{2+} pumps were inhibited by about 70% or more by 100 μM myricetin. For all the Ca^{2+} transport systems, the relative order of potency with which the three flavonoids inhibited Ca^{2+} transport were similar (myricetin > luteolin > quercetin), although there were some differences in the absolute amount of inhibition caused by individual flavonoids.

DISCUSSION

The results presented in this paper provide evidence that several naturally occurring flavonoids

when present in micromolar amounts inhibit ATP-dependent Ca^{2+} uptake by the liver plasma membrane vesicles. There was no simple or direct correlation between structure and inhibitory activity but certain features that appeared to enhance the inhibitory potency could be identified. Of the 33 different flavonoids tested, the most potent inhibitors were the polyhydroxylated flavonoids myricetin (flavanol) and butein (chalcone). A common structural feature shared by these two compounds was the presence of hydroxyl groups corresponding to position 5 and 7 of ring A and 3′ and 4′ of ring B of the flavonoid structure (note that the numbering of the positions in ring A and B are different for chalcones). This hydroxylation pattern may be an important determinant of inhibitory potency. Support for this view also comes from our observation that luteolin and eriodictyol, both of which have similar hydroxylation patterns were also inhibitory. Quercetin and taxifolin were however notable exceptions to this generalization. These two flavonoids differ from luteolin and eriodictyol by the presence of a single additional 3-hydroxyl group on ring C. Their significantly lower ability to interfere with Ca^{2+} transport may therefore be ascribed to some unfavourable characteristic, either electronic or structural, imparted by this 3-hydroxyl function.

Flavonoids lacking hydroxyl groups in ring B such as flavone, flavanone, chrysin or those in which these groups were methylated (diosmetin and hesperetin) generally had little or not effect on Ca^{2+} transport. Similarly, absence of the 5-hydroxyl group (robinetine) also rendered the flavonoid less effective. There was no significant difference in the inhibition caused by flavones and flavonones having similar substituent groups indicating that the presence of a high electron density between C-2 and C-3 has little effect. The two catechins used in the study, catechin and epicatechin had no effect on the Ca^{2+} uptake.

The ability of the flavonoids to depress Ca^{2+} transport activity was also influenced by their lipophilicity. Glycones of the flavonoids with inhibitory activity such as myricitrin and phloridzin failed to affect Ca^{2+} transport, presumably due to their inability to dissolve in the membrane. In their studies on the synaptosomal Ca^{2+}-transport ATPase, Barzilai and Rahamimoff [23] made a similar observation that the inhibitory potency of the three flavonoids quercetin, morin and rutin paralleled their oil-water partition coefficient. The increase in the degree of inhibition of the Ca^{2+} pump caused by myricetin, chalcone and flavone, upon preincubation with the liver membranes, is also compatible with this view that solubilization of the flavonoid in the membrane is a prerequisite for inhibition. However, other flavonoids such as chrysin, apigenin, luteolin, phloretin and butein failed to show a similar enhancement in inhibitory potency following preincubation. Thus, a multiplicity of factors besides solubility, are likely to be involved in the molecular mechanisms by which inhibition is achieved.

In order to verify that the flavonoid induced inhibition of Ca^{2+} uptake was a result of specific interference of the Ca^{2+} transport system and not due to a general disruption of membrane structure, the effect of myricetin was also tested on several other plasma membrane enzymes. Concentrations of myricetin that caused more than 70% inhibition of Ca^{2+} uptake only inhibited K^+-dependent *p*-nitrophenyl phosphatase activity; several other enzymes such as alkaline phosphatase, 5′-nucleotidase and Ca^{2+}-activated ATPase were completely unaffected. The observed inhibition of ^{45}Ca uptake was also not due to an increase in permeability of the membrane to Ca^{2+} ions since myricetin decreased rather than increased the rate of ^{45}Ca efflux from preloaded plasma membrane vesicles.

Inhibition of Ca^{2+} uptake by myricetin was predominantly due to a decrease in the V_{max} of the Ca^{2+}-pump. In addition, myricetin also caused a small increase in the K_m for Ca^{2+} suggesting that it may at least partially interfere with the binding of Ca^{2+} to the Ca^{2+}-binding site. A plausible mechanism of inhibition could therefore involve an interaction between the phenolic groups of myricetin and the Ca^{2+}-transporting channel of the transport protein. Alternative mechanisms such as the binding of myricetin to other sites on the transport protein leading to conformational changes at the Ca^{2+} binding site, cannot however be ruled out. In their studies on the ATP-dependent Ca^{2+} transport system of erythrocyte ghosts, Wuthrich and Schatzmann [15] reported that quercetin had no effect on the K_m for Ca^{2+}. The reasons for this apparent difference in the mechanism of inhibition by quercetin and myricetin are not clear at present but differences in preparative procedures which were noted to induce marked differences in sensitivity to inhibition by quercetin [15] could be a contributing factor.

Although the flavonoids inhibit a variety of different ATP-utilizing enzymes, there appears to be no common mechanism by which this is achieved. Thus, myricetin was found to be competitive with ATP in inhibiting tyrosine protein kinase $pp130^{fps}$ and myosin light chain kinase but non-competitive in inhibiting the insulin receptor kinase and cAMP-dependent protein kinase [10]. Similarly, quercetin is non-competitive with respect to ATP in inhibiting the erythrocyte Ca^{2+}-pump [15] but affects both the K_m and the V_{max} of the platelet Ca^{2+}-ATPase [24]. In the present study, myricetin failed to have any effect on the K_m for ATP indicating that myricetin does not interfere with the ATP-binding site.

Inhibition of Ca^{2+} transport by the flavonoids was not confined to the liver plasma membrane alone. The ATP-dependent Ca^{2+} transport systems of the parotid plasma membrane and endoplasmic reticulum as well as the Ca^{2+}-pumping ATPase of erythrocyte ghosts displayed similar patterns of sensitivity to myricetin, quercetin and luteolin (inhibition by myricetin > luteolin > quercetin) suggesting that the molecular mechanism by which each bioflavonoid inhibited the various Ca^{2+} transport systems was essentially the same. In addition to the Ca^{2+} transport systems investigated in the present study, the Ca^{2+}-transporting ATPase of the sarcoplasmic reticulum, platelet microsomal membrane [24] and synaptosomal membrane [23] are also inhibited by flavonoids at micromolar concentrations. Thus, the flavonoids found to have inhibitory potential in the present study are likely to be general inhibitors of

most mammalian ATP-dependent Ca^{2+} transport systems. Many of these, including the more potent inhibitors such as myricetin and butein also inhibit a wide spectrum of other enzymes. This lack of specificity is likely to limit their potential for pharmacological applications as Ca^{2+} transport inhibitors. But it is envisaged that a detailed knowledge of their structure–activity relationships could aid in the development of more specific inhibitors of the Ca^{2+} transport systems.

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