3,7,4'-Trihydroxyflavones 在離體天竺鼠氣管的鬆弛作用與結構-活性關係

Relaxant effects of 3,7,4'-Trihydroxyflavones in Isolated Guinea Pig Trachea and Their Structure-Activity Relationship

中文摘要

Fisetin、kaempferol、quercetin、myricetin 及 morin 是 3,7,4'-trihydroxyflavone 的衍生物。Fisetin、kaempferol及 quercetin 對 histamine (30μM)、carbachol (0.2μM)、及 isotonic KCl (17.5mM) 預縮的離體天竺鼠氣管，產生劑量依存性的鬆弛作用。由其 IC50 之大小，可知其作用強度爲 fisetin> kaempferol、quercetin > myricetin、morin，顯示於第 5、2'、5'位置以 OH group 取代則活性下降，可能因增加極性降低油溶性之故。然而 kaempferol與 quercetin 鬆弛作用類似，顯示 3'位置以 OH group 取代，活性不受影響。

Fisetin、kaempferol 或 quercetin 的鬆弛作用不會被 propranolol(1μM)所拮抗，表示並非活化β受體而來，它們的鬆弛作用也不受 2',5'-dideoxyadenosine (10μM)或 methyleneblue (25 μM)所拮抗，表示並非活化 adenylate 或 guanylatecyclase 而來，亦不會被 glibenclamide (100μM)所反轉，表示並非使鉀通道開啓而來 。將上皮細胞去除會增加其鬆弛反應，那是因爲除去後減少屏障，藥物可直接與平滑肌上之受體接觸，因此其鬆弛作用可能與 epithelium -derived relaxing factor的釋放無關。

由於-logIC50 有意義地大於 pD2'値，顯示 quercetin 抑制外鈣流入的能力要大於抑制內鈣釋放的能力，然而 fisetin 與 kaempferol對兩者並無選擇性。在高鉀(60 mM)無鈣溶液中，能抑制外加鈣離子引起的收縮，表示會抑制 voltage-operatedcalcium channel(VOC)，但對 nifedipine (10 μM)處理過後的氣管能產生更進一步地鬆弛，表示尚有其他的鬆弛機轉。

Fisetin、kaempferol或 quercetin 類似 protein kinase C (PKC)抑制劑 staurosporene 對 phorbol12-myristate 13-acetate (PMA, 10μM) 引起的氣管收縮能劑量依存性地使其鬆弛。Fisetin 或 quercetin 亦能對 staurosporine (1 μM)或 nifedipine (10μM)及 staurosporine (1μM)處理過的氣管產生更進一步地鬆弛，kaempferol則否，顯示 fistin 或 quercetin 可能尚有其他鬆弛機轉，而 kaempferol的鬆弛作用是否主要來自 PKC 活性的抑制，尙須進一步證實。

因 fisetin (25 及 50μM)類似 3-isobutyl-1-methylxanthine (IBMX，3 及 6μM)能劑量依存性地使 forskolin 及 sodium nitroprusside 的對數劑量-

反應曲線向左平行移動，而 quercetin 65 μM 僅對 forskolin 的對數劑量-反應曲線向左平行移動，但對 sodium nitroprusside 則否，至於 kaempferol 60 μM 對二者皆無作用。由以上結果顯 fisetin 的鬆弛作用可能主要來自於 phosphodiesterase (PDE) 抑制，又由於在 25 或 50 μM 的劑量下對 sodium nitroprusside 的對數劑量-反應曲線移動的劑量比倍數分別有意義地大於對此 forskolin 移動的倍數，因此它似乎對 cyclic GMP-PDE 的抑制較有選擇性，至於 quercetin 似乎對 cyclic AMP-PDE 的抑制較有選擇性，而 kaempferol 似乎全無作用。

英文摘要

Fisetin, kaempferol, quercetin, myricetin and morin are 3,7,4'-trihydroxyflavone derivatives. Fisetin, kaempferol and quercetin dose-dependently relaxed histamine (30 μM)-, carbachol(0.2 μM) -and isotonic KCl(17.5 mM)-induced precontractions in isolated guinea-pig trachea. It showed the ability of kaempferol or quercetin on tracheal relaxation was less than that of fisetin. but larger than that of myricetin or morin, as a result of comparison among their IC50 values. It shows if the proton at position of 5, 2' or 5' is substituted by OH group, the relaxant activity will decrease because the substitution results the higher polarity and the lower lipophilic solubility. However, if the substitution occurs at the position of 3', the relaxant activity will not change owing to the relaxant activity of kaempferol was similar to that of quercetin.

The relaxant effect of fisetin, kaempferol or quercetin was not antagonized by propranolol (1 μM), 2', 5'-dideoxyadenosine (10 μ M) or methylene blue (25 μM), and also not reversed by glibenclamide (100 μM). It suggests that the relaxant effects of these flavonoids may be not due to the activation of β-adrenoceptor, adenylate cyclase or guanylate cyclase. The relaxant effect of these flavonoids become larger in epithelium denuded than in epithelium- intact trachealis. It may be due to these compounds became easier to contact with their receptors on the smooth muscle cells, in the lack of epithelium as a barrier. Therefore, their relaxant effects may not be correlated to the release of epithelium-derived relaxing factor.

The ability of quercetin to inhibit influx of extracellular calcium may be larger than that to inhibit calcium release from intracellular calcium stores, because its -logIC50 value was significantly larger than its pD2' value. However, the ability of fisetin or kaempferol to inhibit both may be similar each other. They could inhibit cumulative calcium-induced contractions in the trachealis preincubated in high potassium (60 mM), calcium-free medium. It shows they may inhibit the voltage-operated calcium channels (VOC). In addition to inhibiting VOC channels, they may have another relaxing mechanism on the basis of observation that they

produced further relaxation after nifedipine (10 μM)-treatment.
Fisetin, kaempferol or quercetin, similar to staurosporine, an inhibitor of protein kinase C (PKC), dose-dependently relaxed the precontraction induced by phorbol 12-myristate 13-acetate (PMA, 10 μM). Fisetin or quercetin, but not kaempferol, produced further relaxation after staurosporine (1 μM)-treatment, or after nifedipine (10 μM)- and staurosporine (1 μM)-treatments. It suggests that fisetin or quercetin may have other relaxing mechanism in addition to inhibiting VOC and PKC activity. Whether the relaxant effect of kaempferol is predominantly due to inhibiting the activity of PKC needs more evidences.
Fisetin (25 and 30 μM), similar to 3-isobutyl-1-methylxanthine (IBMX, 3 and 6 μM), dose-dependently. and parallelly left-ward shifted the log dose-response curves of forskolin and sodium nitroprusside. Quercetin (65 μM) parallelly left-ward shifted that of forskolin only, but not that of sodium nitroprusside. However, kaempferol (60 μM) shifted neither. The above results suggest that the relaxant effect of fisetin may be predominantly due to inhibiting phosphodiesterase (PDE). It appears to more selectively inhibit cyclic GMP-PDE, because that the dose ratio for log dose-response curves of sodium nitroprusside parallelly left-ward shifted by 25 or 50 μM of fisetin was significantly greater than that of forskolin, respectively. In the opposite, quercetin appears to more selectively inhibit cyclic AMP-PDE. However, kaempferol appears to inhibit neither.