台灣款冬 (Petasites formosanus Kitamura) 成分 Petasins 在離體天竺鼠氣管的鬆弛作用

Relaxant effects of petasins, ingredients of Petasites formosanus Kitamura in isolated guinea-pig trachea

中文摘要

我們分析台灣款冬 (Petasites formosanus Kitamura) 所抽提的 petasins 類化合物，
包括 petasi、iso-petasin、S-petasin 與 iso-S-petasin 對氣管的鬆弛活性，上述 petasins 類化合物對 histamine (10 mM)、carbachol (0.2 mM)、KCl (30 mM) 及 leukotriene D4 (10 nM)預縮的離體天竺鼠氣管，產生濃度依存性的鬆弛作用，S-petasin 雖對四種收縮劑無特殊的選擇性，但 iso-S-petasin 對 carbachol 和 KCl 預縮的鬆弛作用較具有選擇性，它們的 IC50 都在 10 mM 左右，就構造-活性之關係而言，含有硫原子的petasins 對氣管的鬆弛活性強度 (potency) 比沒有硫原子的 petasins 要強。上述 petasins 類化合物中，除了 iso-S-petasin (50?200 mM) 使 carbachol 之對數濃度—反應曲線平行向右移動，且不改變其最大收縮，顯示具抗毒蕈素 (antimuscarinic effect)之競爭作用外，S-petasin (10?200 mM) 或 iso-S-petasin (10?200 mM)，均非競爭性地抑制 histamine、carbachol 或 KCl 累加引起之收縮，在無鈣環境中，S-petasin 或iso-S-petasin 預處理對 histamine (100 mM) 、carbachol (10 mM) 或 KCl (60 mM)去極化因累加外鈣引起的收縮能非競爭性地抑制；在完全無鈣 (含 0.02 mM EGTA)環境中，兩者預處理對 histamine 或 carbachol 累加引起之收縮亦能非競爭性地抑制，顯示兩者對細胞外鈣離子流入或者細胞內鈣離子釋放都有抑制作用。對 carbachol (0.2 mM) 預縮而 nifedipine (10 mM) 引起的最大鬆弛情況下，S-petasin 或 iso-S-petasin 也會產生更進一步的鬆弛，表示不管有無抑制 voltage 及/或 receptor operated calcium

c
hannels，一定尚有其他的鬆弛機轉。然而其鬆弛反應不受 Nw-nitro-L-arginine (20 mM
)、a-chymotrypsin (1 U/ml)、propranolol (1 mM)、glibenclamide (10 mM)、methylene blue (25 mM)及 2',5'-dideoxyadenosine (10 mM) 存在的影響，表示其鬆弛作用與
nitric oxide、vasoactive intestinal polypeptide、b-adrenoceptor 受體活化、ATP-敏感的鉀通道開啓、adenylate cyclase 或 guanylate cyclase 活化無關。S-petasin (10?20 mM) 或 iso-S-petasin (10?20 mM) 不能使 forskolin 及 sodium nitroprusside 的對數濃度—反應曲線濃度依存性地向左移動，亦不能增加 forskolin 及 sodium ni
troprusside 的 pD2 值，由 phosphodiesterase (PDE) 活性的直接測定，得知 S-petas
in (100?300 mM)能有意義地抑制 cAMP-dependent PDE 的活性，但最高只能抑制 33.94
±6.06 % (n=5)，顯示 S-petasin 只有輕微的抑制作用，而 iso-S-petasin (30?300 mM) 不能有意義地抑制此酵素，S-petasin 及 iso-S-petasin (3?300 mM) 亦不能有意義
地抑制 cGMP-dependent PDE 的活性。綜合以上結果，兩者對細胞外鈣離子流入或者細胞
內鈣離子釋放都有抑制作用，iso-S-petasin 具有較強的抗毒蕈素作用，而 S-petasin 對
cAMP-dependent PDE 只有輕微的抑制作用。

英文摘要

Four petasins, including petasin, iso-petasin, S-petasin and iso-S-petasin, were isolated from Petasites formosanus Kitamura. They concentration-dependently relaxed histamine (10 *M)-, carbachol (0.2 *M)-, KCl (30 mM)- or leukotriene D4 (10 nM)-induced precontractions of isolated guinea-pig trachealis. Iso-S-petasin selectively relaxed carbachol- and KCl-induced precontractions, although S-petasin non-selectively relaxed the precontractions induced by these contractile agents. Their IC50s were approximately about 10 mM. It seems that the relaxant effects of sulfur containing petasins, S-petasin and iso-S-petasin, were more potent than those of non-sulfur containg petasins, petasin and iso-petasin.The preincubation of S-petasin or iso-S-petasin non-competitively inhibited contractions induced by cumulatively adding histamine, carbachol and KCl in isolated guinea-pig trachealis, with an exception that the preincubation of iso-S-petasin (50~200 *M) competitively inhibited cumulative carbachol-induced c

ontractions, suggesting that iso-S-petasin had an antimuscarinic effect. Both S-petasin and iso-S-petasin had a selectively inhibitory effect on cumulative KCl-induced contractions. In Ca2+-free medium, preincubation of S-petasin or iso-S-petasin non-competitively inhibited cumulative Ca2+-induced contractions in histamine (100 mM)-, carbachol (10 mM)- or KCl (60 mM)-depolarized trachealis. In Ca2+-free medium containing 0.02 mM EGTA, the incubations of S-petasin and iso-S-petasin also non-competitively inhibited cumulative histamine- or carbachol-induced contractions. The above results suggest that S-petasin and iso-S-petasin may inhibit Ca2+-influx from extracellular space and Ca2+-release from intracellular Ca2+ stores. In normal Ca2+-medium, S-petasin was significantly more potent than iso-S-petasin on the inhibition of Ca2+-influx from extracellular space and Ca2+-release from intracellular Ca2+ stores induced by histamine. However, iso-S-petasin was significantly more potent than S-petasin on the inhibition of Ca2+-influx from extracellular space via receptor (ROC) and/or voltage operated calcium channels (VOC), which were opened by carbachol-depolarization in Ca2+-free medium. After a maximal inhibition on carbachol (0.2 *M)-induced precontraction by nifedipine (10 *M), S-petasin or iso-S-petasin caused a further relaxation of the trachealis. The result suggests S-petasin and iso-S-petasin may have other relaxant mechanisms regardless of whether inhibiting VOC in the trachealis. However, their relaxant effects were not affected by the presence of propranolol (1 *M), 2*,5*-dideoxyadenosine (10 *M), methylene blue (25 *M), glibenclamide (10 *M), Nw-nitro-L-arginine (20 *M) or *-chymotrypsin (1 U/ml). It suggests their relaxing effect may be unrelated to activation of *-adrenoceptor, adenylate cyclase or guanylate cyclase, the opening of ATP-sensitive potassium channels and the liberation of nitric oxide (NO) or vasoactive intestinal polypeptide (VIP).S-petasin and iso-S-petasin (10~20 *M) did not produce a parallel leftward shift of the log concentration-response curves of forskolin and sodium nitroprusside. They did not affect pD2 values of forskolin and sodium nitroprusside. Neither cAMP- nor cGMP-dependent phosphodiesaterase (PDE) activity was inhibited by S-petasin and iso-S-petasin, except that S-petasin (100~300 *M) had a slightly inhibitory effect on cAMP-dependent PDE activity. The maximal inhibition on the enzyme was only 33.94 * 6.06 % (n=5). In conclusion, S-petasin and iso-S-petasin inhibited both Ca2+-influx from extracellular space and Ca2+-release from intracellular stores. In addition, iso-S-petasin had an antimuscarinic effect and S-petasin slightly inhibited cAMP-dependent PDE.