血根鹼對骨骼肌血管平滑肌以及心肌之作用機制探討

Studies of sanguinarine on skeletal, vascular smooth, and cardiac muscle

中文摘要

血根鹼是屬於苯菲里啶類(benzophenanthridine)之生物鹼，其成分乃源自於血根、白屈菜以及薊罌粟的種子。就已報導的文獻指出，血根鹼中毒與人類流行性水腫的爆發有其極密切的關係，其臨床症狀諸如：下肢水腫、呼吸困難、充血性心衰竭、心搏過速、心律不整、肝臟腫大以及青光眼等。雖然，在血根鹼中毒方面的研究已趨於完整，但其作用的機轉目前仍不清楚。因此，在本論文針對血根鹼作用於骨骼肌、血管平滑肌以及心肌之毒性機轉作深入研究。

首先，在骨骼肌方面，將觀察血根鹼對小鼠之離體橫膈膜肌以及兔子之肌漿網做深入研究。結果顯示，血根鹼會誘導小鼠的離體橫膈膜產生攣縮作用，此作用是屬於肌原性並且與神經傳遞無關。若移去細胞外鈣離子則會部分的抑制血根鹼所誘導的攣縮作用；若預先以 ryanodine 將細胞內鈣離子儲存槽之鈣離子排盡時，血根鹼所誘導的橫膈膜肌攣縮作用則被大幅的抑制。此外，血根鹼誘導鈣離子由肌漿網釋放之作用會被 ruthenium red 以及 DTT 所抑制，綜合上述結果顯示，血根鹼之作用部位是位於 ryanodine-受體。此外，血根鹼在低濃度時會加強 [3H]ryanodine 與肌漿網之 ryanodine -受體的結合，而高濃度時則會抑制此作用；然而，DTT 將會反轉這些作用。推測其機轉主要是歸因於血根鹼會氧化其 ryanodine-受體上的硫醇基，以致進一步促使肌漿網內之鈣離子的釋放。

其次，在血管平滑肌方面，將以大白鼠之胸主動脈來探討血根鹼與平滑肌之作用。根據結果顯示，血根鹼會對於預先以 phenylephrine (PE)所誘導收縮作用產生一非內皮細胞依賴型的鬆弛作用。再者，我們也發現血根鹼會抑制 PE 所誘導的收縮，其中包括了瞬間性收縮以及持續性收縮；此一結果更可進一步透過以 PE 所誘導肌醇三磷酸鹽(IP3)的生成以及 45Ca2+的內流皆會被血根鹼所抑制而得到證明。血根鹼之抑制作用也會被 DTT 所反轉，此意味著血根鹼造成硫醇基的氧化作用在調節血管平滑肌的收縮上將是一個關鍵性的角色。總之，血根鹼對於血管平滑肌的作用機制乃是透過抑制 IP3 的生成以及阻斷鈣離子通道所致。

最後，將以大鼠離體之右心房、左心房以及右心室來探討血根鹼對於心肌的作用。如本研究的結果所示，血根鹼會使右心房、左心房以及右心室產生攣縮作用；若前處理氯化鑭(La3+)或以 EGTA 將細胞外鈣離子移去後，此攣縮作用將會完全的被抑制。此外，血根鹼也會增加其心肌細胞的鈣離子內流；然而，一些鈣離子通道的阻斷劑卻不能抑制血根鹼所導致的鈣離子內流作用，但氯化鑭卻可以。因此，我們推測其攣縮機制乃是血根鹼會藉由提昇細胞膜對鈣離子之通透性，進而促使鈣離子大量流入細胞內所致。

綜合由本論文之研究的結果顯示，其血根鹼對於骨骼肌、血管平滑肌以及心肌的作用都與鈣離子之調控有著密切的關係。

英文摘要

Sanguinarine (SANG) belongs to the benzophenanthridine alkaloid analog, and is

derived mainly from the root of Sanguinaria Canadensis L., Chelidonium majus L. and the seeds of Argemone mexicana L. SANG has been implicated in outbreaks of human poisoning known as epidemic dropsy characterized by leg edema, breathlessness, congestive heart failure, tachycardia, gallop rhythm, hepatomegaly, and glaucoma. Although much work with respect to the toxic effects has been carried out, the mechanism of SANG action is still unclear. In this study, SANG was examined for its effects on the skeletal, vascular smooth and cardiac muscle in vitro. First, SANG was studied on an isolated mouse phrenic-nerve diaphragm and rabbit sarcoplsmic reticulum (SR). The SANG-induced contracture might be myogenic and independent of innervation. The SANG-induced contracture was partially inhibited when extracellular Ca2+ was removed. Most importantly, the contracture was largely prevented when the internal SR Ca2+ pool was depleted by pretreatment with ryanodine. SANG induced Ca2+ release from the actively loaded SR vesicles was blocked by ruthenium red and dithiothreitol (DTT), consistent with the ryanodine receptor as the site of SANG action. In addition, SANG potentiated [3H]ryanodine binding to the ryanodine receptor of isolated SR vesicles at lower concentration and inhibited binding at higher concentrations. All these effects were reversed by DTT. This mechanism was assumed directly due to the SANG effect on the oxidation of critical SH groups of the ryanodine receptor Ca2+ SR release channel.
Secondly, SANG was studied for its effects on smooth muscle in the thoracic aorta isolated from rats. As our data shows, SANG relaxed the phenylephrine (PE)-precontracted with an endothelium-independent manner. In addition, we also found that SANG could inhibit the PE-induced vasocontraction including both phasic and tonic contraction. This was further supported by the fact that inositol trisphosphate (IP3) formation and 45Ca2+ influx induced by PE in denuded aorta were inhibited by SANG concentration-dependently. The inhibitory effects of SANG were reversed by DTT and that the oxidation of critical SH groups on key molecules that regulate the vascular smooth muscle contraction were involved. These data suggested that the SANG mechanisms on the aorta are due to the inhibition of IP3 formation and blockade of the Ca2+ channel.
Third, SANG was studied on the right ventricle, left and right atria isolated from rat hearts. As our data revealed, we found that SANG induced ventricle and atria contracture, and inhibited the spontaneous beat of the right atria in a dose-dependent fashion. The SANG-induced contracture was completely suppressed by pretreating with La3+ or when the extracellular Ca2+ was removed with EGTA. In addition, SANG pretreatment on cardiomyocytes enhanced 45Ca2+ influx, however, this SANG-induced 45Ca2+ influx was unable to be inhibited by pretreating with Ca2+ channel blocker. Pretreatment with La3+ could inhibit the SANG action. We therefore

assumed that the SANG mechanisms on the heart are due to the enhancement of membrane permeability that promotes Ca^{2+} influx.

In conclusion, results from these studies showed that SANG could interfere with Ca^{2+} regulation in skeletal, vascular smooth and cardiac muscle.