大白鼠體內麥夫胺酸受體之腎功能調整

Glutamate Receptor-mediated Regulation of Rebal Function in Rats

中文摘要

免疫組織化學研究顯示麩腰酸(Glutamate, Glu)大量分佈在腎小球和腎集尿管的上皮細胞而且被認爲是一種調節滲透平衡的物質。利用西方墨漬分析法，我們發現到 NMDA(N-methyl-D-aspartate)和 AMPA(α-amino-3-hydroxy-5-methyl isoxazole-4-propionic acid)功能性
受體的次單元存在於大白鼠的腎臟皮質和髓質中。NMDAR1 在相對分子量約 118-KDa 顯示出單一免疫反應帶，而組成 AMPA 受體的 GluR2 和 R3 則在相對分子量約 119 和 113-KDa 位置顯示出一個分裂的帶狀；爲了要闡明 Glu 在腎臟內的作用除了滲透平衡的調節作用外，是否會經由 Glu 受體(NMDA 或 AMPA)來調節腎功能，我們利用大白鼠麻醉動物模式探討 Glu 對腎臟血液動力學和尿液的形成是受體-調控的結果。腎動脈灌注 Glu(１０1?１０3 μg/kg/hr)會有劑量依存性的降低腎皮質微血管血流(cortical microvascular blood flow,CMBF)以及增加腎皮質血管阻力(renal cortical vascular resistance,RCVR)，但是並不會改變腎血流 (renal blood flow，RBF)和平均動脈血壓(mean arterial blood pressure，MABP)，顯示出 Glu 具有腎臟內血管收縮的作用。在劑量１００?１０3 μg/kg/hr 時，腎絲球過濾率(glomerular filtration rate, GFR)會大幅降低約 43%。在 Glu 的灌注速率爲１０1?１０3 μg/kg/hr 時亦會降低尿液排出量(urine output, UO)。尿液中鈉離子的排泄在１０1?１０3 μg/kg/hr 時也是減少。這些發現顯示出 Glu 在腎臟內是一個強力的血管收縮的胺基酸而且具有抗利尿和抗利鈉的作用。投與選擇性 NMDA 受體之拮抗劑(2-amino-5-phosphonovaleric acid, AP-5, 200 μg/50μL/treat)可部份但是有意義的防止在 Glu 實驗組所降低的 UO、GFR、CMBF 以及所增加的 RCVR。腎動脈灌注 NMDDA １０-1?１０3 μg/kg/hr 也和 Glu 有相似地降低 UO(在１０2、１０3 μg/kg/hr 時)和
CMBF(在１０3μg/kg/hr 時)以及增加的 RCVR，而且此現象亦可被 AP-5 所反轉。這些結果支持了 Glu 可作用在腎血管叢上的 NMDA 受體而調控腎臟的血管張力。在腎動脈內投與 AMPA 的實驗組我們也可以看到相同的趨勢；腎動脈灌注 AMPA １０-1?１０2 μg/kg/hr 和 Glu 一樣會降低 UO(在 102μg/kg/hr 時)和 GFR(在１０1、１０2μg/kg/hr 時)，但是對 RBF、CMBF 和 RCVR 並無影響。在 Glu 投與實驗組中投與競爭性 AMPA 受體之拮抗劑 6, 7-dinitroquinoxalme-2, 3-dione(DNQX,

31.3 μg/50μL/bolus)可減弱 Glu 所降低的 UO 和 GFR。以上結果顯示出 AMPA 受體可參與在 Glu 所調控的腎功能中。此外，在腎臟中有高親和力 Glu 回收／運送器的存在，Glu１０2 μg/kg/hr 與此運送器的抑制劑 DL-threo-3-hydroxyaspartic acid(T-HAA)400 μg/kg/hr 一起灌注，T-HAA 並不會改變 Glu 所降低的腎功能，但是可部份地恢復 Glu 所降低的 UO 和 GFR，顯示出腎臟內 Glu 的作用部份與其運送器無關。
Glu 所降低的 CMBF 和鈉離子排泄量意味著會改變腎絲球前血管的阻力以及在遠端小管內液體的組成，這兩者的變化會直接活化腎臟內小管與絲球體之間迴饋(tubuloglomerular feedback, TGF)機制。因此，Glu 是否與 TGF 的組成因素相關，本實驗即根據以下幾點來探討。(1)TGF 最終的調控是在腎傳入小動脈平滑肌上，鈣離子經由電位依存性的鈣離子通道(voltage-operated Ca2+ channels, VOC)內流。Glu 所降低的 UO、GFR 和 CMBF 可被 VOC 阻斷劑 nifidipine 10 μg/kg/hr 的灌注所減弱，示意著腎臟內 Glu 的作用與細胞內鈣離子的增加有關，而且是鈣離子依存性。(2)緻密斑(mnacula densa, MD)中環腺嘌呤單磷酸(adenosine 3', 5'-cyclic monophosphate, cAMP)的量。
投與環腺嘌呤酵素抑制劑，2'，5'-dideoxyadenosine (DDA) 200
μg/kg/hr，可反轉所有 Glu 對腎功能的影響。(3)乙型血管張力素(Angiotensin Ⅱ, Ang Ⅱ)在 TGF 機制中是一個啓始的調控者。預先靜脈內投與 Ang 轉換酵素抑制劑 captopril 2 mg/kg，可將 Glu 所降低的 CMBF 消除但是對於降低的 UO 和 GFR 只能部份反轉。(4)一氧化氮(Nitric oxide, NO)在 TGF 機制中扮演著組織間信號的傳遞。腎動脈內投與單一劑量的 NO 合成酵素(NO synthase, NOS)的抑制劑，ＮG-nitro-ι-arginine methyl ester(ι-NAME)5μg/50μL，會反轉 Glu 在劑
量１０1?１０3 μg/kg/hr 時所降低的 UO 和 GFR，對於降低的 CMBF 則沒有影響，顯示出選擇性神經型 NOS 的抑制劑可阻斷 Glu 對過濾率誘導的訊息傳遞。(5)當活化腎神經時會加強 TGF 的反應；電刺激腎神經會降低鈉離子的分泌、RBF 和 UO 以及經由乙型腎上腺素受體(β-adrenoceptor)加強腎素(renin)的釋放和 Ang Ⅱ的形成，經由急性的去腎神經可將 Glu 所減低的 CMBF 消除但是對降低的 UO 和 GFR 沒有影響，可推論出 Glu 血管收縮的作用與腎神經的活化有關。Glu １０2 μg/kg/hr 與選擇性乙型腎上腺素受體阻斷劑 propranolol(Pro)0.2 μg/kg/hr 一起灌注可將 Glu 所降低的 UO、GFR 和 CMBF 反轉。綜合以上結果可知，腎動脈內灌注 Glu 所改變的腎功能可經由自己的受體而與活化 TGF 有關，此種效應需要腎神經的存在、細胞內鈣離子

和環腺嘌呤單磷酸的增加，或是經由乙型血管張力素和乙型腎上腺素受體的活化改變腎臟血液動力學，其中部份作用與一氧化氮的傳遞有關。

英文摘要

Glutamate (Glu) has been suggested as an osmoregulatory agent as its considerable distribution in the renal corpuscles and in the epithelia of the collecting tubules, revealed by the immunohistochemical studies. Using Western blotting analysis, we found that the functional subunits of NMDA (N-methyl-D-aspartate) and AMPA (α -amino-3-hydroxy-5-methyl isoxazole-4-propionic acid) receptors were existed in the renal cortex and medulla of rat. NMDAR1 migrates approximately at Mr = 118-kDa as asingle band. GluR2 and GluR3 which compose AMPA receptor are showed as a splitted band at about Mr = 119 and 113-kDa. In order to elucidate the role of intrarenal Glu may via NMDA or non-NMDA receptors on the regulation of renal function except its osmotic regulation, we investigate the receptor-mediated effects of Glu on renal hemodynamics and urine formation in anesthetized rats. Intrarenal arterial (i.r.a.) infusion of Glu (１０1?１０3 μg/kg/hr) elicited a dose- dependent decrease in cortical microvascular blood flow (CMBF) and increase in renal cortical vascular resistance (RCVR) without changing renal blood flow (RBF) and mean arterial blood pressure (MABP), indicating an intrarenal vasoconstrictory action of Glu. The glomerular filtration rate (GFR) decrease marginally by 43% at １００?１０3 μg/kg/hr. Infusion of Glu at the rates of １０1?１０3 μg/kg/hr decrease urine output (UO). The urinary excretion of sodium were also decreased at １０1? １０3 μg/kg/hr. These findings indicate that Glu is a potent intrarenal vasoconstrictory amino acid with an antidiuretic and antinatriuretic actions. Treatment with selective NMDA receptor antagonist 2-amino-5- phosphonovaleric acid (AP-5, 200 μg/50 μL/treat) partially, but significantly prevent the decreases of UO、GFR、CMBF and increase of RCVR in Glu-treated group. I.r.a. infuision of NMDA １０-1?１０3 μg/kg/hr also caused reduction of UO (at １０2、１０3 μg/kg/hr) and CMBF (at １０3 μg/kg/hr) similar to Glu, and this phenomenon can be reversed by AP-5. These results support that Glu can act on MMDA receptors which may locate at renal vasculature to regulate renal vasomotor function. The same trend was also found in the AMPA-treated group. I.r.a. infusion of AMPA １０-1?１０2 μg/kg/hr decrease UO (at １０2 μg/kg/hr)、GFR (at １０1、１０2 μg/kg/hr) to the same extent as Glu, but not RBF、CMBF and RCVR. Treatment with AMPA receptor competitive antagonist 6, 7- dinitroquinoxaline-2, 3-dione (DNQX, 31.3 μg/50 μL/bolus) in Glu-treated group attenuate the declines of UO and GFR. These data indicate that AMPA receptors may be participated in the Glu-regulated renal function. In addition,

we further investigate whether the intrarenal actions of Glu is via its uptake system in the kidney. Coinfusion of Glu １０２ μg/kg/hr with Glu transporter inhibitor, DL-threo-3-hydroxyaspartic acid (T-HAA) 400 μg/kg/hr, insignificantly reverse the Glu-mediated decreases of UO and GFR but not CMBF, suggest that the intrarenal actions of Glu is independent of uptake/transport system.

Decrease of CMBF and sodium excretion imply changes of the preglomerular resistance and fluid compositions in the distal tubules by Glu, may activate intrarenal tubuloglomerular feedback (TGF). Therefore, components of TGF mechanism were studied as following. (1) Calcium influx via voltage-dependent Ca2+ channels (VOC) in TGF effector site, afferent arterioles smooth muscle. Glu-mediated decreases in UO、GFR and CMBF caused by elevated intracellular Ca2+ are also markedly attenuated during coinfusion of VOC blocker, nifedipine 10 μg/kg/hr. This suggests that the intrarenal actions of Glu is Ca2+ dependent. (2) Macula densa (MD) adenosine 3', 5'-cyclic monophophate (cAMP) singal levels. Administration of adenylate cyclase inhibitor, 2',5'-dideoxyadenosine (DDA) 200 μg/kg/hr, reverse the changes of renal function by Glu. (3) Angiotensin Ⅱ(Ang Ⅱ) as an initialmediator in TGF. Pretreatment with Ang-convertmg enzyme inilibitor, captopril (Cap, iv 2 mg/kg), abolish the decrease of CMBF by Glu １０２ μg/kg/hr infusion but partially reverse the declines of UO、GFR. (4) Nitric oxide (NO) as an interstitial signal in TGF. I.r.a. bolus NO synthase (NOS) inhibitor, Ｎ G-nitro-ι-arginine methyl ester (ι-NAME) 5 μg/50 μL, reverse the decrease ofUO and GFR by Glu １０1?１０3 μg/kg/hr but not CMBF. Selective neuronal NOS inhibition blocks Glu-stimulated transduction between the MD and vasculature. (5) Increased renal nerve activity may enhance TGF responses. Electrical stimulation on renal nerves decreases sodium excretion、RBF and UO, enhances the release of renin via β-adrenoceptor and formation of Ang Ⅱ. After acute renal denervation, the decrease of CMBF by Glu infusion was blunted but not UO、GFR. Therefore the vasoconstrictory action of Glu correlates with the activation of renal nerve. Coinfusion of Glu １０２ μg/kg/hr with β- adrenergic blocker, propranolol (Pro) 0.2 μg/kg/hr, also reverse the declines of UO、GFR and CMBF by Glu. Taken together, these data suggest that the renal effects of i.r.a. Glu infusion is due to activation of TGF mechanism by its own receptors, and this effect requires the presence of the renal nerves, elevation of intracelluar Ca2+、cAMP in MD or dymamics of renal vasculature via Ang Ⅱ and β-adrenoceptor, or partially dependent on NO.