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-methylisoxazole-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(10^{1}?10^{3} 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10^{0}?10^{3} 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10^{1}?10^{3} 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10^{1}?10^{3} 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}?10^{3} μg/kg/hr也和Glu有相似地降低UO(在10^{2}、10^{3} μg/kg/hr時)和CMBF(在10^{3}μg/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}?10^{2} μg/kg/hr和Glu一樣會降低UO(在10^{2}μg/kg/hr時)和GFR(在10^{1}、10^{2}μg/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 monophophate, 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 methylester(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已的受體而與活化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 compeptitive 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.