The role of pelvic nerve on the rat seminal vesicle

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利用電刺激骨盆腔神經所造成的老鼠精囊內壓的變化以探討骨盆腔神經在老鼠精囊所扮演的角色

使用成熟的雄性 Wister rats(12~14 **週大)作實驗. 對於骨盆腔神經電刺激所造成的老鼠精囊壓力的反應為電刺激頻率所依賴且為可重複的.最大的壓力反應為** 60HZ,**可達** 78.7±7.4mmHg **而最主要的分支來自** L6,**其最大的壓力反應為** 67.3±8.0mmHg **單側與雙側的骨盆腔神經電刺激其壓力相仿,無統計學上的意義.**
使用副交感神經受體抑制劑,經動脈注射 20 **分鐘後再電刺激骨盆腔神經發現** $3X10^{-2}$mmole/100gm 4-DAMP **可抑制其壓力反應達** 67.10±0.05%**與** atropine **抑制作用相仿**(68.03±0.07%), **但高於** pirenzepine (45.92±0.01%)**及** methoctramine (51.68±0.04%). **所以老鼠精囊的副交感神經主要是** M3 subtype.

利用切斷 LSN 或注射 DSP-4 以破壞交感神經系統並不會影響到電刺激骨盆腔神經所造成的精囊壓力反應其壓力反應分別是76.00±5.16**及**78.00±3.83 mmHg. **相反地切斷兩側骨盆腔神經電刺激 LSN 其壓力反應也沒改變為** 74.33±5.71 mmHg. **我們認為在老鼠精囊內其交感與副交感神經系統有其獨立的運動功能.**
使用 10^{-4}mmole/100g SIN-1 **及** L-NAME **只能部分抑制壓力反應其結果分別是**40.1±18.6%**及** 40.9±8.4%.
結論: 電刺激骨盆腔神經所造成的老鼠精囊壓力反應是頻率仰賴的且為可重複的其神經來源主要是 L6 **分支,而交感與副交感神經系統在老鼠精囊內有其獨立的運動功能.我們認為電刺激骨盆腔神經所造成的老鼠精囊壓力反應主要來自週邊的平滑肌或腺體.**

ABSTRACT

Objectives Using seminal vesicle (SV) pressure response to electric stimulation (ES) of pelvic nerve (PN) to investigate the role of the PN on the rat SV.

Materials and Methods Mature male Wistar rats (12-14 weeks) were used in this study. Various frequencies were tested to find out the best vesicle pressure response to PN stimulation. Comparing the results of the branches to the whole bunch and one side to both sides of PN stimulation, we are going to find out the major innervation and the distribution of PN on the rat SV. Pretreatment with muscurinic receptor antagonist is going to find out the subtype of muscurinic receptor of the rat SV. Ablation of the noradrenergic and cholinergic nervous system before stimulation the PN and the lesser splanchnic nerve (LSN) respectively will find out the isolated function of these two nervous systems. Pretreatment with SIN-1 and L-NAME is going to see the influence of nitric oxide system on the SV pressure response to PN stimulation.

Results Vesicle pressure response to ES of PN was frequency dependent and reproducible. The maximum pressure response was 78.7 ± 7.4 mmHg at 60 Hz. The major innervation of PN to the rat SV is coming from the branch of L6, the pressure response was 67.3 ± 8.0 mmHg, closed to that of whole bunch PN stimulation (75.3 ± 5.0 mmHg). (p=0.42) There was no difference between one side and both sides PN stimulation.

The mean maximum inhibition values were 67.10 ± 0.05 % by 4-DAMP at $3x10^{-2}$ mM/100gm, which is closed to that of atropine (68.03 ± 0.07 %) and higher than that of pirenzepine (45.92 ± 0.01 %) and methoctramine (51.68 ± 0.04 %). We believed that the subtype of muscarinic receptor in the rat SV was M3 subtype.

Ablation of the noradrenergic nervous system by excision of LSN or by DSP-4 injection didn't change the vesicle pressure response to PN stimulation. The pressure responses were 76.00 ± 5.16 and 78.00 ± 3.83 mmHg of LSN excision and DSP-4 injection respectively. In addition, the vesicle pressure response to ES of LSN remained the same after ablation of the cholinergic nervous system. The pressure response was 74.33 ± 5.71 mmHg. The sympathetic and parasympathetic innervation should have independent motor function on the rat SV.

Only partially suppression were noted by pretreatment of SIN-1 and L-NAME. The inhibitions of pressure response were 40.1 ± 18.6 % and 40.9 ± 8.4 % by 10^{-4} mM/100mg of SIN-1 and L-NAME respectively.

Conclusions SV pressure response to ES of PN was frequency dependent and reproducible. The major innervation of PN to the rat SV is coming from the branch of L6. The sympathetic and parasympathetic innervations have independent motor function on the rat SV. The vesicle pressure response to ES of PN is resulting from the contraction of the peripheral smooth muscle or the glandular smooth muscle, not via

glandular secretion.

INTRODUCTION

Electric stimulation (ES) of the pelvic nerve (PN) induced erection, seminal vesicle (SV) contraction and emission of the rat.[4-8] The rat SV is a secretomotor organ, which is composed of outer smooth muscle layer and subepithelial glandular tissue.[9-10] The major innervation to smooth muscle layer is by noradrenergic and cholinergic nerves, to glandular tissue by cholinergic nerve terminal.[9] From our established animal model, ES of lesser splanchnic nerve (LSN) induced good pressure response of the rat seminal vesicle.[11-13] Hypogastric nerve and the PN join together in the major pelvic ganglia to become a mixed nerve and innervated the pelvic organs.[8] All of the acetylcholine, norepinephrine and dopamine induced good contraction of the rat SV in organ bath study.[14] Intra-arterial injection of acetylcholine through a catheter from femoral artery to the bifurcation of common iliac artery also induced good pressure response of the rat SV in our pilot study. We believe that PN should have motor function as LSN in the rat SV. What is the innervation to control the secretory function of the rat SV? From NOS activity study, nitric oxide control the secretion of the rat SV, but inhibit the process of emission.[15-19] The participation of nitric oxide has close relationship to the PN. The role of the PN in the rat SV may influence both of the motor and the secretory functions and need to do further investigation. The purposes of this study are 1. To establish an experimental animal model “SV pressure response to ES of PN”, 2. To understand the relationship between the PN and the LSN in the rat SV, 3. To realize the influence of nitric oxide system on the SV pressure response to ES of PN.

Materials and Methods

Mature male Wistar rats, aged 12-14 weeks were obtained from the animal center of National Taiwan University Medical College. Rats were housed four per cage at 23 ℃ with a 12-h light-dark cycle (07.00-19.00 hours), and free access to food and water. Rats anesthetized with pentobarbital (40 mg/kg, intraperitoneal) were used as the *in vivo* animal model, as described previously. The trachea was intubated to keep the airway patent. Catheters were placed both in the common carotid artery for continuous blood pressure monitoring, in the femoral artery and vein for test drugs and anesthetic agent administration respectively. A polyethylene catheter (PE-60) filled with normal saline was placed via the tail into the main lumen of the SV to record pressure. The tubes for monitoring blood pressure and intraluminal pressure of

the SV were connected to a pressure transducer (Viggo-Spectramed P23VL-1) and the responses recorded by a Gould RS 3400 polygraph. It is easy to identify the major pelvic ganglion on either side of the dorsolateral lobes of the prostate with the aid of a Zeiss dissecting microscope (OPMI 1-DFC) (magnification = X10). It receives innervation of PN via L6 and S1 and hypogastric nerve from the thoracolumbar outflow. Dissection the branches of PN on both sides and the LSN in the aorto-caval space were performed carefully. A bipolar stainless-steel electrode and a Grass SD 9 stimulator were used for ES.

Frequency-response curve of SV pressure response to ES of PN

Various frequencies (20, 40, 60 and 80 Hz) were studied by 1 min ES to PN under the conditions of 10 V and 1 ms duration. The phasic tension of the SV pressure response will be used for analyze. The frequency to reach the maximum pressure response will be used in the following studies.

Comparing the results of ES the branches (L6 and S1) to the whole bunch of the PN is going to see the contribution of these branches. Comparing the results of one side to both sides of PN stimulation is going to see " is there any difference between one side and both sides stimulation". Anyway, both-sides stimulation is more physiological.

Muscarinic receptors of the rat SV

Various concentrations from 10^{-4} to $3x10^{-2}$ mM/100gm of muscarinic receptor antagonist injected cumulatively (i.a.) 20 min before ES of PN. Before the drug administration, we stimulated the PN in each rat as the control data of this study. The inhibitory dose-response curves were obtained by comparing the SV contractile response before and after pretreatment. The muscarinic antagonists include atropine, pirenzepine (M1), methoctramine (M2) and 4-DAMP (M3).

The relationship between PN and LSN in the rat SV

Ablation of noradrenergic nervous system of the rat SV was planned by excision of LSN 7 days before or by DSP-4 (100 mg/kg) intraperitonerl injection 3 days before. The vesicle pressure response to ES of PN on one side is going to see the motor function of the PN on the rat SV.

Ablation of cholinergic nervous system of the rat SV was used by excision of both sides of PN 7 days before. The vesicle pressure response to ES of LSN is going to see the effect of without PN on the rat SV. In this study, we used cutaneous cystostomy for urinary diversion when PN was excised.

The influence of nitric oxide (NO) in the vesicle pressure response to ES of PN

Various concentrations of SIN-1 (NO donor) and L-NAME (NO inhibitor) were injected intra-arterially cumulatively 20 min before ES of PN to see the influence of NO system in the vesicle pressure response.

Results

Various frequencies (20, 40, 60 and 80 Hz) were studied by 1 min ES to PN under the conditions of 10 V and 1 ms duration. The SV pressure response to ES of PN was frequency dependent and reproducible. The maximum pressure response was 78.7 ± 7.4 mmHg at 60 Hz of stimulation. (Fig. 1)

Using 60 Hz as the standard frequency, we stimulated the branches (L6 and S1) and the whole bunch of the left side PN and the vesicle pressure response were 67.33 ± 7.99, 28.50 ± 8.24 and 75.33 ± 5.00 mmHg respectively. The vesicle pressure response to ES of L6 was closed to the whole bunch of PN (p=0.42), while the vesicle pressure response of S1 was much lower than the whole bunch of PN (p =0.0006). When we stimulated PN on one side and both sides concomitantly, the vesicle pressure response were 75.33 ± 5.00, 70.67 ± 4.49 and 86.00 ± 7.49 mmHg on the left, right and both sides respectively. There was no significant change when comparing the data of one side to both sides of ES. (p> 0.05)

The inhibitory dose-response curves of muscarinic receptor antagonists: atropine, pirenzepine, methoctramine and 4-DAMP were shown in Figure 2. The mean maximum inhibition values were 68.03 ± 0.07 % by atropine, 45.92 ± 0.01 % by pirenzepine, 51.68 ± 0.04 % by methoctramine and 67.10 ± 0.05 % by 4-DAMP at $3x10^{-2}$ mM/100gm. There was no significant change when comparing the data of pirenzepine, methoctramine and 4-DAMP to atropine. (p>0.05) Among them, the inhibitory potency of 4-DAMP was similar to that of atropine and higher than that of pirenzepine and methoctramine.

Ablation of noradrenergic nervous system by excision of LSN 7 days before or by DSP-4 (100 mg/kg) intraperitonerl injection 3 days before, the vesicle pressure response to ES of the left side PN were 76.00 ± 5.16 and 78.00 ± 3.83 mmHg respectively. There was no significant change when comparing the data of without ablation of noradrenergic nervous system. (p>0.05) Ablation of cholinergic nervous system by excision of both sides of PN 7 days before, the vesicle pressure response to ES of LSN was 74.33 ± 5.71 mmHg. It was similar to our previous data. (p> 0.05) [11]

Pretreatment of SIN-1 and L-NAME could suppress the vesicle pressure response to ES of PN. The mean maximum inhibition values were 40.08 ± 18.65 % by SIN-1 at 10^{-4} mM/100gm and 40.98 ± 8.36 % by L-NAME at 10^{-4} mM/100mg.

Discussion

In rat, each side of PN has two branches coming from L6 and S1.[8] The vesicle pressure response to ES of L6 and S1 branches were 67.3 ± 8.0 and 28.5 ± 8.2 mmHg respectively. Comparing to the value of whole bunch PN stimulation, the vesicle pressure response was 75.3 ± 5.0 mmHg, closed to that of L6 stimulation. (p=0.4)

We believed the major innervation of PN to the rat SV is coming from L6 branch.

When ES of PN on one side and both sides concomitantly, the vesicle pressure response were 75.3 ± 5.0, 70.7 ± 4.5 and 86.0 ± 7.5 mmHg of the left, right and both sides respectively. There was no significant change when comparing the data of one side to both sides ES. ($p > 0.05$) The PN innervation of the rat SV can cross over to the opposite side and result in a similar pressure response. We proposed that one side of PN stimulation was enough to represent the cholinergic influence on the rat SV.

From the inhibitory dose-response curves of muscarinic receptor investigation, the mean maximum inhibition value of 4-DAMP (67.10 ± 0.05 %) was similar to that of atropine (68.03 ± 0.07 %), and much higher than that of pirenzepine (45.92 ± 0.01 %) and methoctramine (51.68 ± 0.04 %) at $3x10^{-2}$ mM/100gm. We believed that the subtype of muscarinic receptor in the rat SV was M3 subtype.

Ablation of noradrenergic nervous system by excision of LSN or by DSP-4 injection didn't interfere the vesicle pressure response to ES of PN. Ablation of PN by excision of both sides of PN, the vesicle pressure response to ES of LSN remained the same. The sympathetic and parasympathetic innervation should have the independent role on the motor function of the rat SV. Most of the smooth muscle, located in the peripheral zone, was innervated major by sympathetic and partial by parasympathetic nerve. While the glandular tissue, located in the central area near lumen, was innervated major by parasympathetic nerve.[9] The vesicle pressure response to ES of PN and LSN resulting from glandular tissue secretion, smooth muscle of the glandular tissue contraction or from the peripheral smooth muscle contraction is still unknown.

Nitric oxide increased by SIN-1 or decreased by L-NAME pretreatment, all can suppress the vesicle pressure response to PN stimulation partially. The suppression of the vesicle pressure response to PN stimulation may result from the drug itself, not by the glandular secretion via nitric oxide system. Nitric oxide influences the secretion of the rat SV, but inhibit the process of emission.[15-19] We don't think glandular tissue secretion contribute the major role of vesicle pressure response to ES of PN. The inhibition of emission process may not come from the direct effect of nitric oxide on the glandular tissue.

From the sympathetic and parasympathetic nerve ablation study and the nitric oxide investigation, we can conclude that the SV pressure response to the sympathetic and parasympathetic nerve stimulation should be resulting from the peripheral smooth muscle or the glandular smooth muscle contraction.

In conclusion, the SV pressure response to ES of PN was frequency dependent and reproducible. The maximum pressure response was 78.7 ± 7.4 mmHg at 60 Hz. The major innervation of PN to the rat SV is coming from L6 branch. One side of PN stimulation was enough to represent the cholinergic influence on the rat SV. The

sympathetic and parasympathetic innervations have independent motor function on the rat SV. The vesicle pressure response to ES of PN or LSN is resulting from the contraction of the peripheral smooth muscle or the glandular smooth muscle, not via glandular secretion.

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