Central nervous system innervation of the seminal vesicle as revealed by the transneuronal transport of pseudorabies virus

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利用 pseudorabies virus 病毒做透過神經運輸以探討中樞神經至精囊的神經支配

中文摘要

HRP 分別注射到大白鼠精囊的壁上 8-12 處，大白鼠則在 72 小時後殺掉。我們使用 PBS 做心臟灌流然後使用 1.25% glutaldehyde + 1% paraformaldehyde 做固定。取出脊髓節 T10-L2, L5-S3 及 major pelvic ganglia 固定在之前的固定液中並且浸泡在 phosphate buffer (30% PBS, 0.1 M, pH 7.4, 4 ^{o}C)3 天。冰凍的組織在做 HRP 組織化解反應之前用 OCT 包埋及 TMB 染色。經過酵素反應後的組織切片用 1 % neutral red 染色以做顯微鏡下觀察。

我們使用 10 隻大白鼠做實驗；其中最後 6 隻大白鼠中有 2 隻大白鼠發現有模糊的棕紅色顆粒物在 major pelvic ganglia；但是在脊髓節 T10-L2, L5-S3 責未發現。

英文摘要

After exposing the seminal vesicle of the mature male Wistar rats, horseradish peroxidase (HRP) was injected into the wall of the seminal vesicle in 8-12 separated areas. The rats were sacrificed 72 hours later. We used phosphate buffered saline (PBS) for transcardial perfusion and followed by fixatives (1.25% glutaldehyde + 1% paraformaldehyde). The tissues of spinal segments T10-L2, L5-S3 and the major pelvic ganglia were removed and fixed in previous fixatives (1.25% glutaldehyde +

1% paraformaldehyde) and immersion in phosphate buffer (30% PBS, 0.1 M, pH 7.4, 4 ^{o}C) for another 3 days. The frozen specimens were cut into slices with OCT frozen medium and followed with tetramethylbenzidine (TMB) staining before HRP histochemical reaction. After treated with enzymatic reaction solution, the specimens were dried and stained with 1 % neutral red for 3 minutes and followed with light microscopic examination.

10 mature male Wistar rats were used in this study. Only 2 of the final six rats showed faint brownish red staining particles on the major pelvic ganglia. In the segments of spinal cord from T10 toL2 and L5 to S3, all of them did not show any evidence of horseradish peroxidase tracing.

Introduction

In the process of emission, seminal vesicle is an important organ. As our established animal model by using electrical stimulation of lesser splanchnic nerve and monitor the intraluminal pressure of seminal vesicle. We can evaluate the efficacy of medicine for premature ejaculation by suppressing the seminal vesicle pressure response well. I believe the seminal vesicle is a good candidate to do the neurological studies. From our previous neurophysiological and neuropharmacological studies on the rat seminal vesicle, noradrenergic, cholinergic, dopaminergic and serotonergic nerve systems are all involved in this pelvic organ. Kimura Y et al demonstrated that dopaminergic and serotonergic systems of the CNS regulated the ejaculation. Trying to link the central and the peripheral nerve system, we are going to use horseradish peroxidase (HRP) technique on the rat seminal vesicle.

Materials and Methods

Mature male Wistar Rats (400 gm) were used in this study. The rats were anesthetized with pentobarbital. After exposing the seminal vesicle, horseradish peroxidase (HRP) (0.0125 gm HRP in 50 micro liter of distilled water) was injected into the wall of the seminal vesicle in 8-12 separated areas from the place near ejaculatory duct to the body of the seminal vesicle. The rats were sacrificed 72 hours later. We used phosphate buffered saline (PBS) for transcardial perfusion and followed by fixatives (1.25% glutaldehyde + 1% paraformaldehyde). The tissues of spinal segments T10-L2, L5-S3 and the major pelvic ganglia were removed and fixed in previous fixatives (1.25% glutaldehyde + 1% paraformaldehyde) and immersion in phosphate buffer (30% PBS, 0.1 M, pH 7.4, 4 ^{o}C) for another 3 days. The frozen specimens were cut into slices with OCT frozen embedding medium and followed

with tetramethylbenzidine (TMB) staining before HRP histochemical reaction.

HRP retrograde histochemical reaction:

1) Pre-reaction soaking solution

Solution A: 92.5 ml distilled water
100 mg sodium nitoprusside
5 ml 0.2M acetate buffer, pH 3.3

Solution B: 5 mg TMB warmed in 40 ^{o}C with 2.5 ml ethanol absolute

* The mixed solution containing solution A and B must not be reserved over 2 hours, and it has to be prepared before the desired reaction.

2) Enzymatic reaction solution

Put the obtained specimen in 0.1M phosphate buffer (pH 7.4), and then wash it three times with distilled water to remove the remnant PBS on it. Then soaked the specimens in 100 ml pre-reaction soaking solution and shake well for 20 minutes. Add 3.5 ml 0.3% hydrogen peroxide and keep shaking for 30 minutes. After the reaction is finished, wash the specimens several times with diluted acetate buffer (pH 3.3, 1 ml 0.2M acetate buffer + 19 ml distilled water). Then spread the specimens on slides with chrome alum gelatin coating and dry them. The dried specimens were stained with 1 % neutral red for 3 minutes for light microscopic examination.

Results

10 mature male Wistar rats were used in this study. Only 2 of the final six rats showed faint brownish red staining particles on the major pelvic ganglia. After discussing with the professor of Anatomy Department, it cannot indicate the faint staining particles as a positive finding of HRP study. In the segments of spinal cord from T10 toL2 and L5 to S3, all of them did not show any evidence of horseradish peroxidase tracing. We believed that our study by using HRP for tracing the neural transport of the seminal vesicle is failed.

Discussion

According to the study of LR Mata, who used isolated seminal vesicle and injected HRPase into the lumen of the seminal vesicle to see the dynamic changes of the HRPase absorption in the epithelial layer. The secretory epithelium of the Hamster had the capacity to absorb products present in the lumen of the seminal vesicle. The secretory epithelium of the Hamster seminal vesicle presented a transcellular transport process behaving as an open system for absorbed HRPase and the tracer then migrated to the subepithelial blood vessels. And the cyclic absorption of the HRPase could eventually returned to the lumen.

People used HRP for studying the pelvic nerve innervation of the external sphincter of urethra successfully. Seminal vesicle is a secretory-motor organ. If the secretory epithelium play the major role to absorb the HRPase, the injected HRPase will secrete to the lumen. As our design in this study, we injected 8-12 separated areas on the surface of the seminal vesicle from ejaculatory duct to the body. If HRPase was absorbed by secretory epithelium and secreted into the lumen, tracing of the HRPase in the nerve system like major pelvic ganglia and the spinal cord should be scanty. The faint brownish red staining particles on the major pelvic ganglia might be the scanty tracing of HRPase. The innervation of the rat seminal vesicle by tracing the HRPase should be very difficult. Unfortunately, we failed to find the commercialized pseudorabies virus as the protocol we designed before. However, we still don’t know the dynamic absorption and the tracing of the pseudorabies virus on the rat seminal vesicle.