The investigation of serotonergic system in the rat seminal vesicle

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大白鼠精囊血清素系統之探討

中文摘要

利用我們已建立的動物實驗模式: 電刺激小內臟神經及偵測精囊內壓力的變化作本實驗。使用不同受體的血清素促效劑包括 8-OH-DPAT(1A), 5-Nonyloxytryptamine(1B), BW723C86(2B)及 MK212(2C) 從 10^{-6} 到 10^{-4} M/kg 經由股動脈做累積性注射到 12~14 週大的大白鼠；所得的壓力變化使用 phasic tension 與對照比較以求得劑量-反應曲線圖。由劑量-反應曲線圖我們發現只有 8-OH-DPAT (5-HT_{1A})有劑量依賴的反應；而 10^{4} M/kg 的 8-OH-DPAT 能夠抑制 50%精囊內壓力的反應。所求得的 IC_{50} 爲 6.46 x 10^{-5} M/kg。

我們認爲 8-OH-DPAT (5-HT_{1A})調節大白鼠精囊的收縮,但是這些都需要做進一步的探討像免疫組織染色的研究。

英文摘要

Using our established animal model by stimulating the LSN and monitoring the rat seminal vesicle, we evaluated some of the subtypes of serotonergic agonists including 8-OH-DPAT (1A), 5-Nonyloxytryptamine (1B), BW723C86 (2B) and MK 212 (2C). Mature male Wistar rats (12-14 weeks) were used in this study. All of these test drugs from 10^{-6} to 10^{-4} M/kg were injected cumulatively via the catheter of femoral artery. The phasic tension (ΔmmHg) of each drug at different concentration will be compared to the control to obtain the dose-response curve of each subtype of 5-HT agonist. From the dose-response curve, only 8-OH-DPAT (5-HT_{1A}) had a dose-dependent inhibition on the seminal vesicle pressure response to the LSN stimulation. More than 50% of inhibition was noted at the concentration of 10^{4} M/kg

of 8-OH-DPAT, and the IC_{50} of 8-OH-DPAT was 6.46 x 10^{-5} M/kg.

We believed that 8-OH-DPAT (5-HT_{1A}) mediates the rat seminal vesicle contraction, not like 5-HT_{2C} receptors in the spinal cord reflex center. However, further investigation, as immuno-histochemical study is necessary to prove it.

Introduction

Some of the SSRIs appear effective in inhibiting the premature ejaculation both after acute and chronic administration. Our previous study showed that fluoxetine, serotonin and clomipramine reduced the pressure response of the seminal vesicle (SV) to electrical stimulation of lesser splanchnic nerve (LSN).

There are at least 16 different 5-HT receptors from 5-HT_1 to 5-HT_7 have been found, cloned and structure described. Only 5-HT_1 to 5-HT_3 receptor agonist have been used to investigate the sexual behavior. Subcutaneous injection of 8-OH-DPAT (5-HT_{1A} agonist) induced ejaculation with shortened latency, while 5-HTP (precursor of 5-HT) inhibited ejaculatory behavior, which is antagonized by treatment with the 5-HT_{1B} antagonist. 5-HTPwith TFMPP (5-HT_{1B} agonist) have synergistic effect on the rat sexual behavior. Stimulation of the 5-HT_2 receptors inhibited ejaculation and only slight effects on rat sexual behavior has been noted by activating 5-HT_3 receptor. A hypothesis of the role of 5-HT_{1A} and 5-HT_{2C} receptors in premature ejaculation is postulated.

All of these studies focused on the central serotonergic system. The peripheral serotonergic system in pelvic organ is still unclear. In this study, we would like to use different type of 5-HT agonist to investigate the serotonergic system of the rat seminal vesicle by using our established animal model.

Materials and Methods

Mature male Wistar rats (12-14 weeks) were obtained from the animal center of National Taiwan University Medical College. Rats are 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 were anaesthetized with pentobarbital (40 mg/kg, intraperitoneally); the experimental procedure was described previously.[27] Briefly, the trachea of the rat was intubated to maintain a patent airway, and catheter place in the common carotid artery for continuous blood pressure monitoring. The anaesthetic agent was injected through the femoral vein and the test drugs were injected via a catheter from the femoral artery the bifurcation of common iliac artery. A polyethylene catheter (PE-60) filled with normal saline was placed via the tail into the main lumen of the seminal vesicle to record pressure. The tubes for monitoring blood pressure and intraluminal pressure of the seminal vesicle were connected to pressure transducer (Viggo-Spectramed

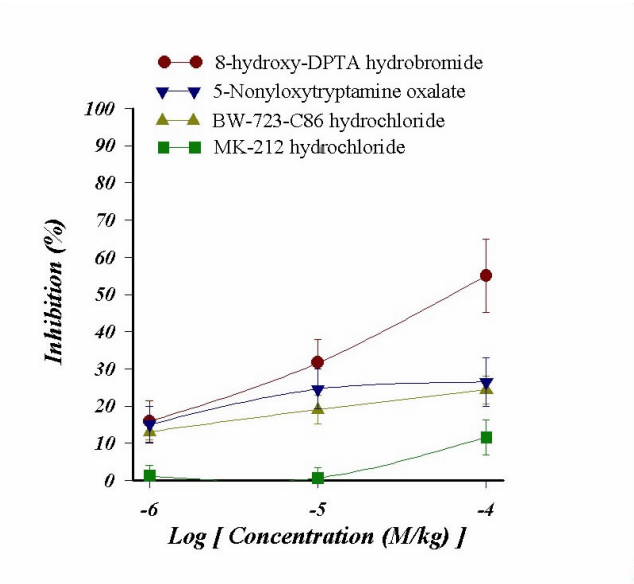
P23XL-1) and record by a Gould RS 3400 polygraph.

Dose-response curve of 5-HT agonists

Several types of 5-HT agonists were used in this study, including 8-OH-DPAT (1A), 5-Nonyloxytryptamine (1B), BW723C86 (2B) and MK 212 (2C). All of these test drugs from 10^{-6} to 10^{-4} M/kg were injected cumulatively via the catheter of femoral artery. Before the test drugs injection, we stimulated the lesser splanchnic nerve (LSN) for 1 min and record the intraluminal pressure of the seminal vesicle as the baseline control. Electrical nerve stimulation was applied at 10 V, 80 Hz and 1 ms duration. After each injection, 30 min of observation was allowed to stabilize the blood pressure and the seminal vesicle pressure. The pressure response to electrical LSN stimulation after each dosage of the test drug injection will be recorded. The phasic tension (△mmHg) of each drug at different concentration will be compared to the control to obtain the dose-response curve of each subtype of 5-HT agonist. The number of the rats in each test drug should be more than 6.

Results

From the figure, only 8-OH-DPAT (5-HT_{1A}) had a dose-dependent inhibition on the seminal vesicle pressure response to the LSN stimulation. More than 50% of inhibition was noted at the concentration of 10^4 M/kg of 8-OH-DPAT and the IC_{50} of 8-OH-DPAT were 6.46 x 10^{-5} M/kg.



Discussion

Dopamine released from medial preoptic area (MPOA) and nucleus accumbens, which is facilitative to copulation; while 5-HT released from lateral hypothalamic area (LHA) at the time of ejaculation, which increase the latency to copulation and the latency of the first ejaculation. Neurons in the region of the rostral nucleus paragigantocellularis (nPGi) mediate the inhibition of spinal sexual reflexes. Following pseudo-rabies virus trans-synaptic retrograde labeling from the corpus cavernosum, all virus-labelled neurons exhibited 5-HT_{2C} receptors. Intrathecal injection of 5,7-DHT to abolish the serotonergic neurons of spinal cord enhances the SV pressure response to LSN stimulation. I believe 5-HT_{2C} receptors in the spinal cord reflex center may mediate the transfer between erection and ejaculation.

From this study, 8-OH-DPAT (5-HT_{1A}) had a dose-dependent inhibition on the seminal vesicle pressure response to the LSN stimulation. More than 50% of inhibition was noted at the concentration of 10^{-4} M/kg of 8-OH-DPAT. The IC_{50} of 8-OH-DPAT was 6.46 x 10^{-5} M/kg. It's higher to our previous study that 3 mg/kg of serotonin could inhibit 60% of seminal vesicle pressure response to electrical stimulation of the lesser splanchnic nerve. It's possible that 8-OH-DPAT (5-HT_{1A}) mediates the rat seminal vesicle contraction, not like 5-HT_{2C} receptors in the spinal cord reflex center. However, further investigation, as immuno-histochemical study is necessary to prove it.