Central muscarinic receptor subtype regulating voiding in rats

橋排尿中枢を介する頻尿遺伝子の解明と遺伝子治療に関する実験的研究

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平成16年5月

研究代表者：小松和人　（金沢大学医学部附属病院・講師）

はしがき

本研究は，下記の科学技術研究費によって行われた研究の上に積み重ねられたものである．特に排尿中枢におけるムスカリンおよびオピオイドの役割について主に研究成果を得ることができた．

平成8年度ー平成9年度　科学技術研究費・基礎研究（C）90242552
研究課題：脳卒中後遺症としての神経因性膀胱の成因および治療法に関する実験的研究
研究者：横山　修，並木幹夫，
研究経費：2,540（千円）

平成9年度ー平成10年度　科学技術研究費・基礎研究（C）80291368
研究課題：脳卒中に伴う神経因性膀胱の成因解明および遺伝子治療に関する実験的研究
研究者：小松和人，並木幹夫，横山　修，高　栄哲
研究経費：2,500（千円）

平成11年度ー平成12年度　科学技術研究費・基礎研究（C）11671541
研究課題：アルツハイマー病に伴う排尿障害の遺伝子治療に関する実験的研究
研究者：小松和人，横山　修，高　栄哲，紺谷　仁
研究経費：3,400　（千円）

研究組織

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研究分担者：横山　修（福井大学医学部）
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交付決定額（千円）

	直接経費	間接経費
平成14年度	3,000	0
平成15年度	1,100	0
総計	4,100	0

研究発表

1）学会発表

河野政範ら，Central muscarinic receptor subtypes regulating voiding in rats
米国泌尿器科学会　2004年　5月8日～5月13日

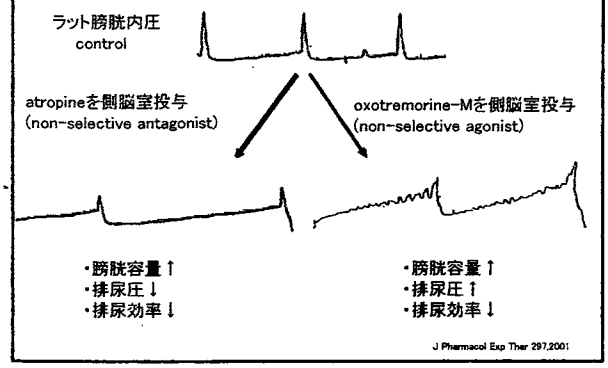
ラット脳内ムスカリン受容体サブタイプの排尿反射に対する機能的役割について

金沢大学医学部泌尿器科学教室
河野 眞範, 石浦 嘉之, 児玉 浩一
小松 和人, 並木 幹夫

秋田大学医学部生理学教室
中村 靖夫

北陸大学薬学部薬理学教室
紺谷 仁

Introduction



J Pharmacol Exp Ther 297,2001

Method

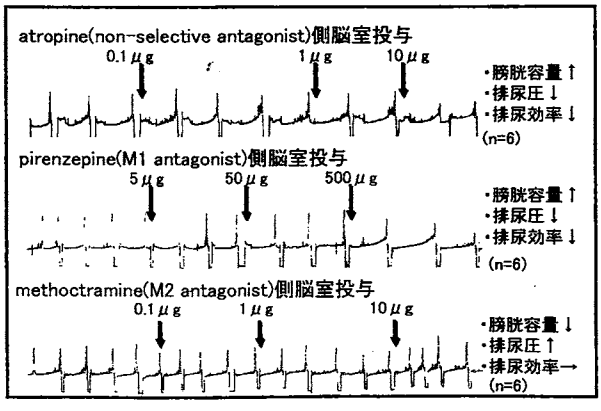
・SD種雌ラット(200-300g). 麻酔下に膀胱瘻を作成. 右側脳室に薬剤 注入用ステンレスパイプを留置.

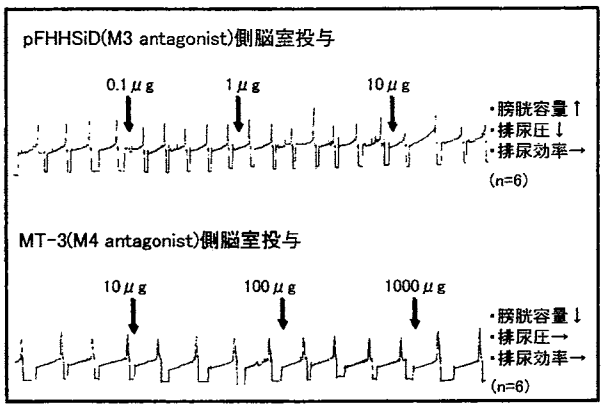
・覚醒後, 膀胱瘻より生理食塩水を0.1ml/min.の速度で注入. 膀胱内圧を連続測定.

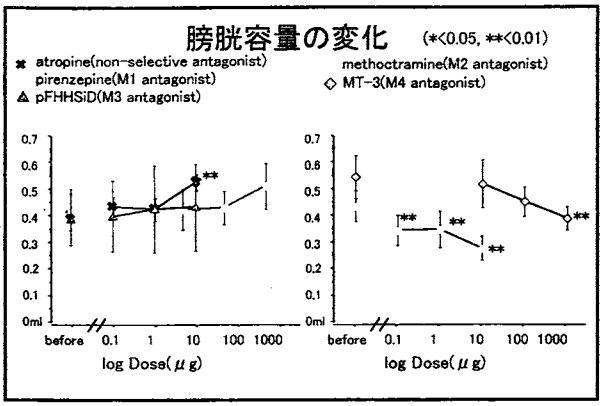
・排尿量を測定し, 適宜残尿を測定した.
膀胱容量=排尿量+残尿. 排尿効率=排尿量/膀胱容量X100とした.

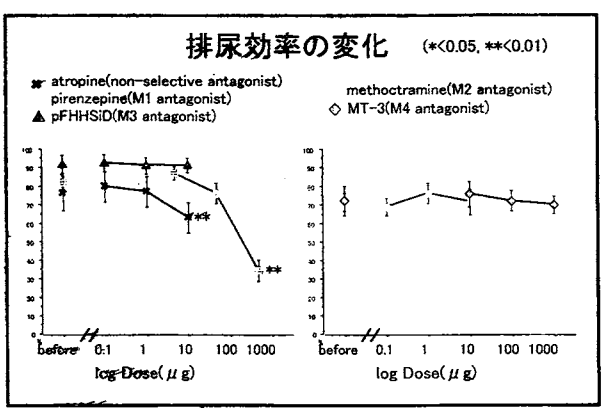
使用薬剤

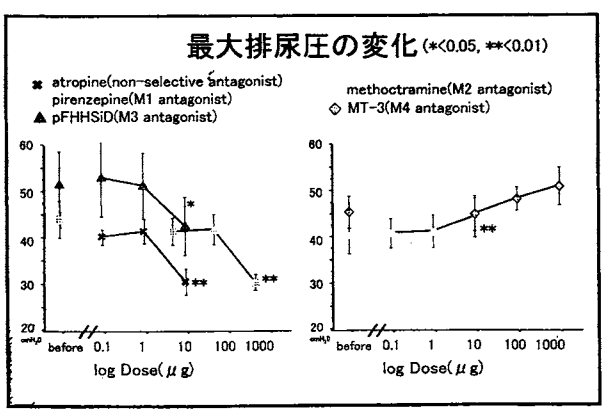
non-selective antagonist	atropine	(0.1, 1, 10 μg)
M1 antagonist	pirenzepine	(5, 50, 500 μg)
M2 antagonist	methoctramine	(0.1, 1, 10 μg)
M3 antagonist	p-F-HHSiD (p-Fluorohexahydro-sila-difenidol hydrochloride)	(0.1, 1, 10 μg)
M4 antagonist	MT-3 (muscarinic toxin-3)	(10, 100, 1000 μg)




pFHHSiD(M3 antagonist)側脳室投与
0.1 μg
1 μg
10 μg
・膀胱容量↑
・排尿圧↓
・排尿効率→
(n=6)
MT-3(M4 antagonist)側脳室投与
10 μg
100 μg
1000 μg
・膀胱容量↓
・排尿圧→
・排尿効率→
(n=6)


膀胱容量の変化 (*<0.05, **<0.01)
atropine(non-selective antagonist)
pirenzepine(M1 antagonist)
pFHHSiD(M3 antagonist)
methoctramine(M2 antagonist)
MT-3(M4 antagonist)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0ml
before 0.1 1 10 100 1000
log Dose(μg)


排尿効率の変化 (*<0.05, **<0.01)
atropine(non-selective antagonist)
pirenzepine(M1 antagonist)
pFHHSiD(M3 antagonist)
methoctramine(M2 antagonist)
MT-3(M4 antagonist)
before 0.1 1 10 100 1000
log Dose(μg)


最大排尿圧の変化 (*<0.05, **<0.01)
atropine(non-selective antagonist)
pirenzepine(M1 antagonist)
pFHHSiD(M3 antagonist)
methoctramine(M2 antagonist)
MT-3(M4 antagonist)
60
50
40
30
20
before 0.1 1 10 100 1000
log Dose(μg)

ムスカリン受容体

M1～M5のサブタイプがある.

中枢神経 ： M1～M5すべてが発現
平滑筋 ： M2, M3　　心臓 ： M2
分泌腺 ： M1, M3　　自律神経節： M1

作用機序

M1, M3, M5 ホスホリパーゼC(PLC)活性
M2, M4 アデニル酸シクラーゼ(AC)抑制

ラット大脳皮質におけるムスカリン受容体サブタイプの比率

Reference	M1	M2	M3	M4	M5	Method
Wall et al., 1991	34%					Immunological
Wall et al., 1991			10%			Immunological
Levey et al., 1991	40%	37%	0%	15%	0%	Immunological
Yasuda et al., 1993				24%	0.3%	Immunological
Li et al., 1991		20%				Immunological
Waelbroeck et al., 1990	34%	22%	10%	34%		Ligand binding

ラット排尿における中枢性ムスカリン受容体の役割

ラット等容量性律動性収縮モデルを使用，各種拮抗薬(M1～M3)を側脳室投与した．
M1とM3受容体拮抗薬は蓄尿に変化なし，排尿を抑制した．M2受容体拮抗薬は蓄尿を促進させ，膀尿も促進させた．(Nippon Hinyoukika Gakkai Zasshi 93:427,2002)

→実験形態が異なり，多少の差異はあるものの，我々の結果と類似するものである．

ラット側脳室にムスカリン受容体拮抗薬を投与した．atropine, tolterodineでは蓄尿促進、排尿抑制的に作用した．oxybutynin,darifenacin(M3拮抗薬)では変化を認めなかった．
ラット排尿において中枢性M3受容体は重要な役割を果たさない．(J Urol 168:2258,2002)

→我々の結果と異なるが，その原因として次のことが考えられる．
①darifenacinの投与量が少なかったため変化をおこさなかった．
②我々の用いた薬剤がM3受容体に対する選択性が低かったため，M1拮抗作用による変化が出現した可能性も否定できないが，M1拮抗薬であるpirenzepineが作用を示したのは500μg投与と高濃度であり，M3拮抗薬pFHHSiDは10μg投与で作用が出現しており，M3受容体は単独で作用があると考えられる．

結語

・ラット側脳室に各種ムスカリンサブタイプ拮抗薬を単独投与した．

・有意差を得られなかったものもあるが，M1,M3拮抗薬は蓄尿促進，排尿抑制的(つまりM1,M3作用は蓄尿抑制，排尿促進)に，M2,M4拮抗薬は蓄尿抑制，排尿促進的(M2,M4作用は蓄尿促進，排尿抑制)に作用することが示唆された．

・今回の実験では排尿における尿道圧の影響を検討することは困難であり，今後は同一薬剤同一デザインでの，中枢性ムスカリン受容体の尿道圧に及ぼす影響を検討予定である．

・受容体サブタイプに対しさらに選択性の高い薬剤の登場が望まれる．

EFFECTS OF OPIOID SUBTYPES ON BLADDER OVERACTIVITY IN RATS WITH CEREBRAL INFARCTION

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INTRODUCTION

The opioid receptors mediate the analgestic and other pharmacological actions of opioid drugs, such as respiratory, cardiovasucular functions. However opioid drugs are not currently used in the treatment of overactive bladder, because of their range of side effects and because their actions have been largely unrecognized. Opioids exert their diverse physiological effects through three distinct membrane-bound receptor subtypes mu (μ), delta (δ) and kappa (κ) in the central nervous system and periphery. The different receptors have diverse behavioral characteristics for example, euphoria, physical dependence and respiratory depression are mainly associated with μ and δ receptors. In contrast, opioids acting κ receptors produce dysphoric rather than euphoric effects which limits their physical dependence liability. A number of pharmacological studies indicated that δ opiate receptors are primarily responsible for opiate inhibition of micturition reflexes in the spinal cord, whereas μ and δ opiate receptors mediate inhibition in the central nervous system.

We therefore evaluated the effect of different opioid receptor subtypes on bladder overactivity after left MCA (the middle cerebral artery) occlusion following intracerebroventricular administration.

MATERIALS AND METHODS

In this study we used 76 female Sprague-Dawley rats (Japan SLC, Inc., Hamamatsu, Japan) weighting between 200 and 270 gm.

Cerebral infarction (CI) was induced by left middle cerebral artery occlusion (MCAO) in SD rats under halothane anesthesia (Am J Physiol 273: R1900, 1997). Bladder activity was monitored with continuous infusion cystometrography in awake rats. In sham operated (SO) animals, the left carotid bifurcation was exposed through a midline incision in the neck, but no further procedures were performed (SO rats). Cystometry was recorded by infusing physiological saline into the bladder at 0.04 ml. per minute, followed by collecting and measuring saline voided from the urethral meatus to determine voided volume. Evacuating the bladder through the cystometry catheter enabled us to measure post-voided residual urine volume after the micturition reflex. The bladder capacity of each rat before drug administration was assumed to represent 100% for that rat. The post-administration volume was expressed as a percentage of the pre-administration volume, and micturition threshold pressure and bladder contraction pressure were similarly expressed. Drug administration was performed intracerebroventriculaly.

Evaluation of effects of drugs

Two hours after MCA occlusion or sham operation and after control cystometry recording, we examined the effects of drugs on bladder activity. A single drug was administered intracerebroventricularly at graded doses (0.1-1000 μg., respectively) to conscious rats. Drugs were administered in intracerebroventricular fashion as a single 1 μl. dose in conscious rats. The drug dosage was increased at 60 minutes intervals. Bladder capacity, residual urine volume, micturition threshold pressure, and bladder contraction pressure were determined from each of the CMGs.

Drugs

Drugs used in this study were [D-Ala^2,Phe^4,Gly^5]-enkephalin (DAGO, μ agonist; Research Biochemicals International, Natick, MA), [D-Pen^{2,5}]-enkephalin (DPDPE, δ_1 agonist; Research Biochemicals International), deltorpin II (δ_2 agonist Research Biochemicals International), and U-50488 (κ agonist; Research Biochemicals International). All drugs were dissolved in artificial cerebrospinal fluid for intracerebroventricular administration.

fig. 1 Effect of DAGO (μ agonist) on bladder capacity, bladder contraction pressure and micturition threshold pressure

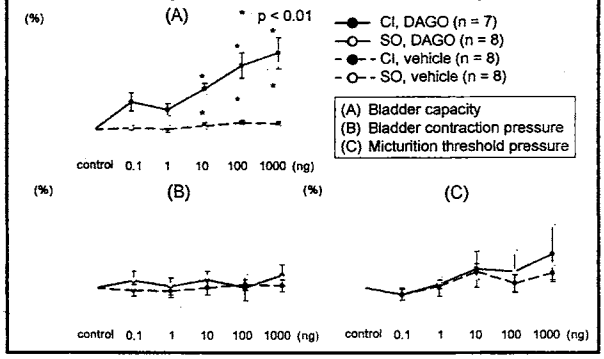


fig. 2 Effect of DPDPE (δ_1 agonist) on bladder capacity, bladder contraction pressure and micturition threshold pressure

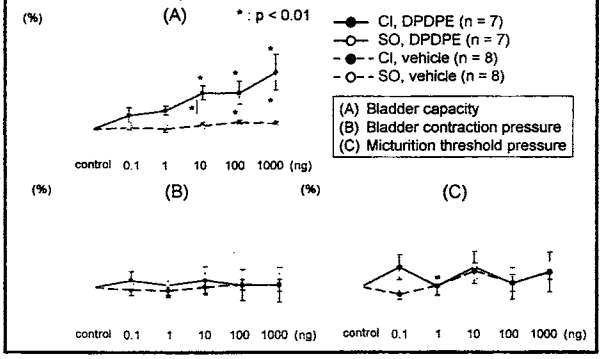


fig. 3 Effect of deltorpin II (δ_2 agonist) on bladder capacity, bladder contraction pressure and micturition threshold pressure

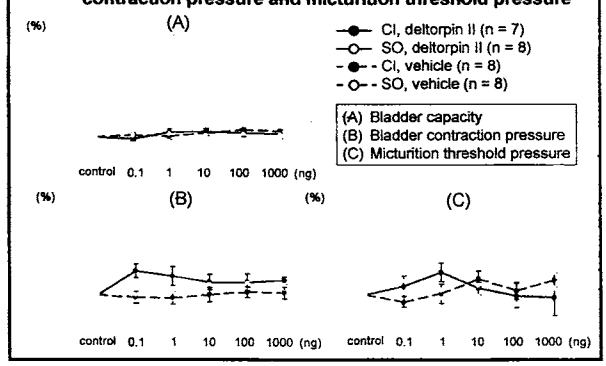


fig. 4 Effect of U-50488 (κ agonist) on bladder capacity, bladder contraction pressure and micturition threshold pressure

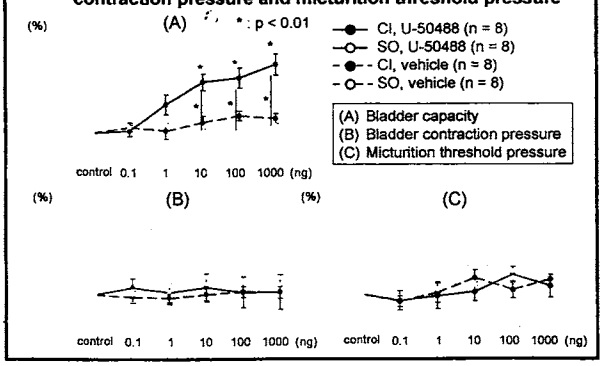
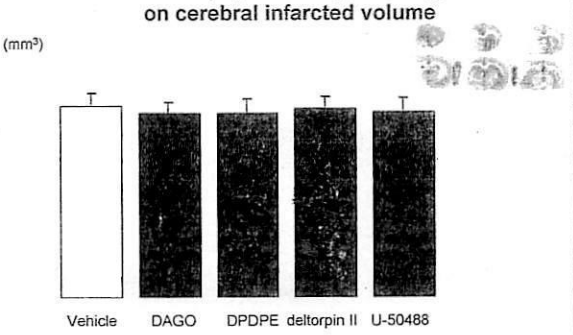


fig. 5 **Influence of opioid receptor subtypes on cerebral infarcted volume**



RESULTS

Effects of intracerebroventricular administration of opioid receptor agonists in sham operated and cerebral infarcted rats (fig. 1-4)

In both sham operated and cerebral infracted rats, the intracerebroventricularly administration of DAGO increased the bladder capacity in dose-dependent fasion (fig. 1). However the effects of DAGO on bladder capacity were not significantly different in sham operated and cerebral infarcted rats. DAGO at any dose produced insignificant increases in bladder contraction pressure and micturition threshold pressure. Any dose of DAGO produced small and insignificant increases in post-void residual urine volume in sham operated and cerebral infarcted rats (data not shown).

In both sham operated and cerebral infracted rats, lower doses of DPDPE (0.1 and 1 ng.) did not change bladder capacity compared with vehicle administered rats. On the other hands, higher doses of DPDPE (100 and 1000 ng.) increased bladder capacity in a dose dependent manner in both, but the percentage increase in bladder capacity in cerebral infarcted rats at 10 ng. of DPDPE was 84.4 ± 42.5 % ($p < 0.05$), whereas no change was observed in sham operated rats. DPDPE at any dose produced insignificant increases in bladder contraction pressure and micturition threshold pressure (fig. 2).

In both sham operated and cerebral infarcted rats deltorpin II did not significantly increase bladder capacity compared with vehicle, nor did the effects of deltorpin II on bladder contraction pressure and micturition threshold pressure differ from those in vehicle treated rats (fig. 3).

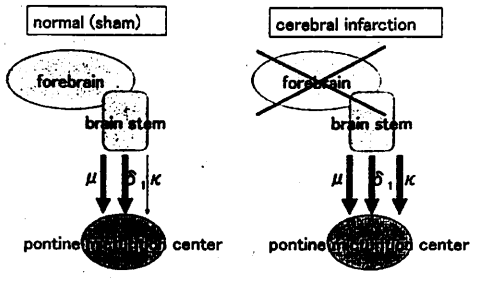
In sham operated rats U-50488 did not significantly increase bladder capacity compared with vehicle, nor did the effects of U-50488 on bladder contraction pressure and micturition threshold pressure in sham operated rats differ from those in vehicle treated rats. U-50488 increased bladder capacity in a dose dependent manner in cerebral infarcted rats (fig. 4). Bladder capacity increased just after the administration of 10, 100 and 1000 ng./body U-50488, while the 1000 ng./rats dose induced the maximum response in cerebral infarcted rats. U-50488 at any dose produced insignificant increase in post-void residual urine volume (data not shown). Furthermore, the effects of U-50488 on bladder contraction pressure and micturition threshold pressure in cerebral infarcted rats did not differ from those of vehicle.

The intracerebroventricular administration of opioid receptor subtypes did not have any effect on the hemiparesis.

Influence of opioids on cerebral infarcted volume (fig. 5)

All of the rats used for this study had an infarction in the frontoparietal cortex and subcortical basal ganglia. The mean infarcted volumes (DAGO: 202.1 ± 10.4 mm^3, DPDPE: 201.9 ±15.7 mm^3, deltorpin II: 207.0 ±12.9 mm^3, U-50488: 203.1 ±15.2 mm^3) were not statistically different from those of vehicle treated rats (209.6 ± 13.2 mm^3). No evidence of infarction was found in any of the sham operated rats.

Discussion



DISCUSSION

Previous studies described that urinary bladder was inhibited by activation of spinal and supraspinal μ and δ opioid receptors, but was not influenced by the stimulation of κ opioid receptors. Since then, the possibility of modulating bladder function using κ receptor agonists has not been considered. However, in recent years it was found that the κ agonists injected into the sacral level of cats inhibited bladder contractions due to the stimulation to the sacral dorsal root. Furthermore it was pointed out that in the antinociceptive effect of κ agonists administered systemically, the κ opioid receptors in the spinal cord were more important than those located supraspinally. Several studies indicated that the selective non-peptide κ agonists U-50488, U-62066 and their analogues reduced mortality and hippocampal CA1 neuronal necrosis following transient bilateral carotid occulusion in the Mongolian gerbil and in Fischer 344 rats. We administered opioid receptor agonists on two hours after MCA occlusion or sham operation, so the neural protection from the cerebral ischemia was not occurred. Our results suggested that U-50488 increased bladder capacity in a dose dependent manner in cerebral infarcted rats, not in sham operated rats. Based on the increased sensitivity of infarcted rats to U-50488, it seems reasonable to conclude that this pathway is upregulated after cerebral infarction and may contribute to the bladder overactivity in infarcted rats.

Conclusions

Intracerebroventricular administration of U-50488 significantly increased bladder capacity in CI rats but not in SO rats. This result indicates that the kappa opioid receptor on the supraspinal central nervous system may play a role and may be a target for medication in bladder overactivity after cerebral infarction.