

DEMONSTRATION OF MUSCARINIC AND NICOTINIC RECEPTOR BINDING ACTIVITIES OF ACETYLCHOLINESTERASE INHIBITORS TO TREAT IMPAIRED DETRUSOR CONTRACTILITY

Hypothesis / aims of study

Impaired bladder emptying can be caused by chronic conditions such as bladder outlet obstruction in men with benign prostatic hyperplasia and impaired detrusor contractility in patients of either sex [1]. Pharmacotherapy using cholinomimetic drugs, such as muscarinic agonists and acetylcholinesterase (AChE) inhibitors, has been used as effective way for impaired bladder emptying [2] since the drugs may improve detrusor contractility by activating the parasympathetic cholinergic system. However, despite *in vitro* experimental evidences indicating that AChE inhibitors increase detrusor contractility, the mechanism underlying pharmacological effects of these agents under *in vivo* conditions remains to be still clarified. Distigmine bromide, a long-acting carbamate AChE inhibitor, has been clinically used to treat patients with voiding dysfunction associated with impaired detrusor contractility [3]. The present study was undertaken to examine whether AChE inhibitors such as distigmine may bind directly to muscarinic and nicotinic receptors since these receptors play a significant role in the bladder voiding function, by radioreceptor binding assays using selective radioligands.

Study design, materials and methods

The muscarinic and nicotinic receptors in homogenates of rat tissues (bladder, brain, submaxillary gland) were measured by radioreceptor binding assays with [N-methyl-³H]scopolamine ([³H]NMS), [³H]oxotremorine-M and [³H]epibatidine as radioligands, and the abilities of AChE inhibitors to inhibit specific radioligand binding were estimated from IC₅₀ values, namely the molar concentrations of unlabeled drugs necessary to displace 50% of specific radioligand binding. The inhibition constant (K_i value) was calculated by using the equation, $K_i = IC_{50} / (1 + 1/K_d)$, where L is the concentration of radioligand. The K_i values express the potency of AChE inhibitors in competing for radioligand binding sites in rat tissues.

Results

Distigmine competed concentration-dependently with the binding sites for [³H]NMS, [³H]oxotremorine-M and [³H]epibatidine in rat tissues (Table 1). Based on K_i values, distigmine was the most potent inhibitor of specific binding sites for [³H]oxotremorine-M, followed by [³H]NMS and [³H]epibatidine. Thus, distigmine showed more than 10-fold higher affinity to muscarinic receptor sites than to nicotinic receptor sites. Compared with the case of distigmine, donepezil also showed similar binding characteristics of muscarinic and nicotinic receptor sites in rat tissues, while neostigmine, compared with other AChE inhibitors, showed much lower affinity to muscarinic receptor sites with roughly similar binding affinity to nicotinic receptors.

Interpretation of results

The major findings of this study are that 1) distigmine, widely used in the treatment of impaired detrusor contractility, exerts direct binding activities of muscarinic and nicotinic receptors in rat tissues including bladder with higher affinity to the former receptors and 2) donepezil showed similar binding activities to these cholinceptors as distigmine while neostigmine showed relatively lower affinity to muscarinic receptors.

Concluding message

The present study has shown that AChE inhibitors may improve voiding function by directly stimulating cholinceptors in the bladder. Thus, we believe that this work will contribute significantly to the further understanding of therapeutic effects of AChE inhibitors in patients with impaired detrusor contractility.

References:

- [1] J Urol (1996) 156; 1014-1018.
- [2] Urodynamics. Principles, Practice and Application. Churchill Livingstone, 1994 (43-70)
- [3] Urol (1977) 10; 83-89.

Table 1. *In vitro* inhibition by distigmine, neostigmine and donepezil of specific binding of [N-methyl-³H]scopolamine (NMS), [³H]oxotremorine (OXO)-M and [³H]epibatidine in rat tissues (bladder, brain, submaxillary gland: S.gland)

Drugs	Tissues	K _i (μM)		
		[³ H]NMS	[³ H]OXO-M	[³ H]Epibatidine
Distigmine	Bladder	1.13 ± 0.11	0.36 ± 0.12	-
	Brain	2.66 ± 0.29 *	0.17 ± 0.01	22.9 ± 3.3
	S.gland	2.92 ± 0.23 *	0.51 ± 0.16	
Neostigmine	Bladder	>50	17.4 ± 5.4 †	-
	Brain	>40	1.03 ± 0.25 *	18.8 ± 3.3

Mean \pm S.E. (n=3-4).

*: p<0.05 (vs. bladder value of each drug). †: p<0.05 (vs. bladder value of distigmine or donepezil).

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ANIMAL SUBJECTS: This study followed the guidelines for care and use of laboratory animals and was approved by Guidelines of the Experimental Animal Ethical Committee of the University of Shizuoka