

In vivo demonstration of muscarinic receptor selectivity of fesoterodine active metabolite, 5-hydroxymethyl tolterodine (5-HMT), in rat tissue



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Aim of Study

Fesoterodine is now used clinically for the treatment of over active bladder (OAB). When administered orally fesoterodine is rapidly and extensively converted to its active metabolite, 5-hydroxymethyl tolterodine (5-HMT), which is also an active metabolite of tolterodine. In our previous in vitro study, 5-HMT binds to the muscarinic receptors with greater affinity in the human bladder mucosa and detrusor muscle than in the parotid gland in a competitive and reversible manner. Oral fesoterodine in rats significantly binds to muscarinic receptors in rat tissues with bladder selectivity. The present study was undertaken to characterize the in vivo muscarinic receptor binding of 5-HMT in rat tissues by using a tritiated ligand with high specific activity.

1. Identification of [³H]5-HMT binding sites

Methods

The homogenates of rat tissues (bladder, submaxillary gland, heart, colon, brain) were incubated with various concentrations of [³H]5-HMT (370 GBq/mmol, 10 nmol/kg) at 25°C for 60 min. The receptor binding assay was conducted by rapid filtration. Binding parameters of apparent dissociation constant (Kd) and maximal number of binding sites (Bmax) for [³H]5-HMT were estimated by nonlinear regression analysis using Graph Pad Prism.

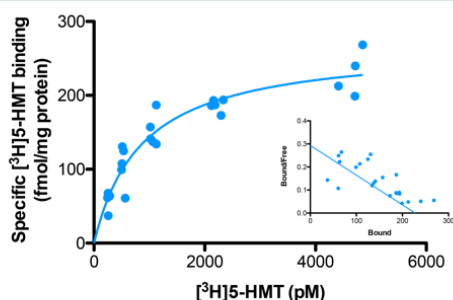


Fig.1 Saturation curves and Schatchard plot of specific binding of [³H]5-HMT to rat bladder.

Table.1 Kd and Bmax for specific binding of [³H]5-HMT in rat tissues.

	[³ H]5-HMT	
	Kd (nM)	Bmax (fmol/mg protein)
Bladder	0.868 ± 0.148	268.4 ± 16.1
Submaxillary gland	1.202 ± 0.270	166.8 ± 14.4
Heart	0.739 ± 0.176	192.7 ± 15.1
Colon	0.974 ± 0.230	377.4 ± 32.3
Cerebral cortex	0.767 ± 0.204	1188 ± 105

Values are means ± S.E. of five rats.

Specific binding of [³H]5-HMT at relatively low concentrations was detected at significant amount in the bladder and other tissues of rats, and it was saturable and of high affinity. Kd values of specific [³H]5-HMT binding displayed no significant difference among these tissues.

2. In vivo measurement of [³H]5-HMT binding

Methods

[³H]5-HMT was injected into the tail vein. The rats were sacrificed under anesthesia with isoflurane at 10, 30, 90 and 180 min. A blood sample was taken from the descending aorta, tissues (bladder, submaxillary gland, heart, colon, lung and cortex) were rapidly removed. After dissection on ice, each tissue was homogenized in ice-cold 50 mM Na⁺/K⁺ phosphate buffer to give a final tissue concentration of 20 mg/mL using Polytron homogenizer. Particulate-bound radioactivity was determined by rapid filtration of 0.5 mL of homogenate over Whatman CF/C filters, which were washed subsequently with 1 mL of ice-cold buffer. Radioactivity was measured in a liquid scintillation counter. Based on the data on pharmacological specificity, the particulate-bound radioactivity from vehicle- and atropine (14.8 μmol/kg i.v.)-pretreated rats was defined as total binding and nonspecific binding, respectively, and the difference was taken as the in vivo specific binding of [³H]5-HMT.

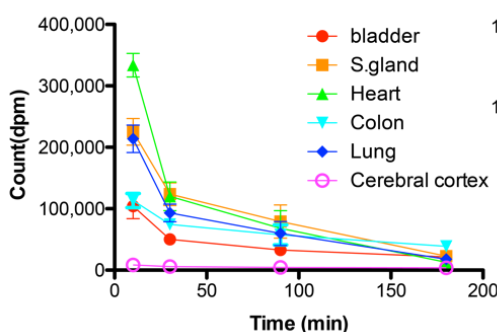


Fig.2 Time course of total radioactivity of [³H]5-HMT in rat tissues after i.v. injection of the ligand. Each point represents mean ± S.E. of 6 rats.

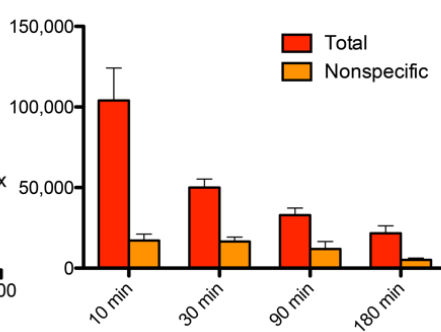


Fig.3 Effect of pretreatment with atropine on [³H]5-HMT binding in rat bladder.

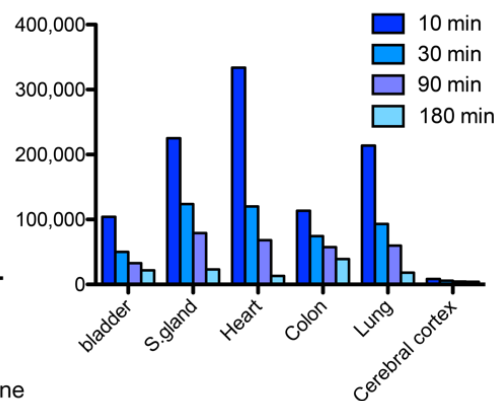


Fig.4 Time course of in vivo specific binding of [³H]5-HMT in rat tissues after i.v. injection of the ligand. Each column represents mean ± S.E. of 6 rats.

The specific binding in the bladder, submaxillary gland, heart, colon and lung was greatest at 10 min. The specific binding in the bladder, submaxillary gland, heart, colon and lung declined with the disappearance of [³H]5-HMT from the plasma. On the other hand, in the brain, the specific binding is a little.

Conclusion

[³H]5-HMT labels bladder muscarinic receptors in vivo. Thus, the present study may provide a rationale for the pharmacological usefulness of fesoterodine as therapeutic agent of overactive bladder. Also, this radioligand may be useful to characterize muscarinic receptors in tissues.