

IN VIVO DEMONSTRATION OF MUSCARINIC RECEPTOR SELECTIVITY OF FESOTERODINE ACTIVE METABOLITE, 5-HYDROXYMETHYL TOLTERODINE (5-HMT), IN RAT TISSUE

Hypothesis / aims of study

Fesoterodine is now used clinically for the treatment of over active bladder (OAB)[1] 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[2]. In our previous *in vitro* study [3], 5-HMT bind 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 binding of 5-HMT in rat tissues by using a tritiated ligand with high specific activity.

Study design, materials and methods

In vitro study: 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 (K_d) and maximal number of binding sites (B_{max}) for [³H]5-HMT were estimated by nonlinear regression analysis using Graph Pa Prism.

In vivo study: [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.

Results

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. K_d values of specific [³H]5-HMT binding displayed no significant difference among these tissues.

After i.v. injection of [³H]5-HMT, radioactivity detected from each tissue (fig. 1). Pretreatment with atropine reduced the [³H]5-HMT binding in particulate fractions of the bladder, submaxillary gland, heart and colon. Therefore, there was a significant difference in particulate-bound radioactivity of [³H]5-HMT in the bladder. Submaxillary gland, heart and colon between vehicle- and atropine-pretreated 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 (fig. 2).

Interpretation of results

[³H]5-HMT was shown to bind pharmacologically relevant muscarinic receptors in rat tissues including the bladder with high affinity. The present study shows that 5-HMT binds to the muscarinic receptor except for brain.

Concluding message

It is concluded that [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.

Total radioactivity

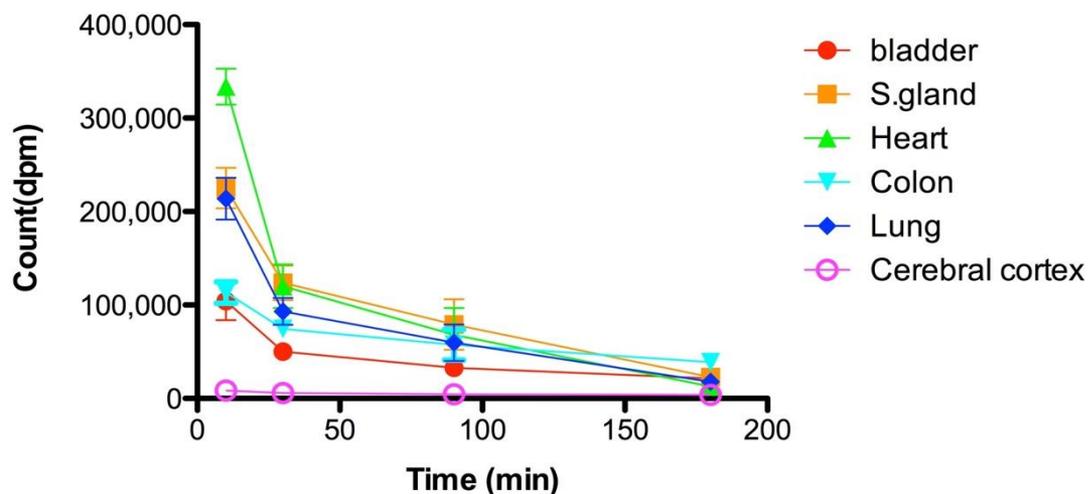


Fig. 1 Time course of total radioactivity of [³H]5-HMT in rat tissues after i.v. injection of the ligand. [³H]5-HMT (10 nmol/kg) was injected into the tail vein, and rats were sacrificed 10-180 min later. Total radioactivity of [³H]5-HMT in rat tissues was measured. Each point represents mean±S.E. of 6 rats.

Specific activity

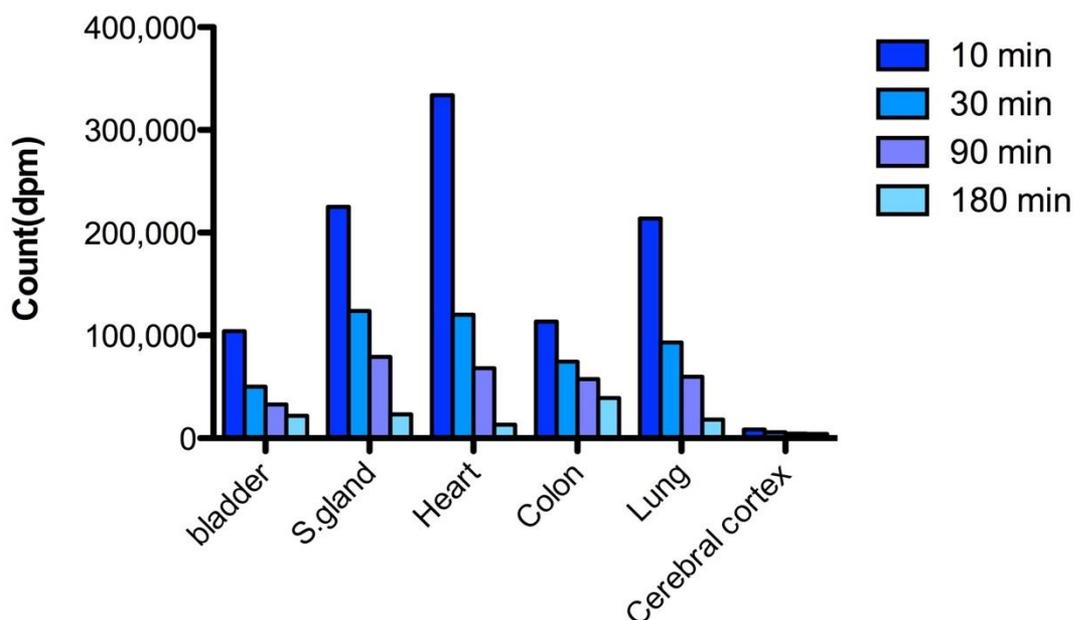


Fig. 2. Time course of *in vivo* specific binding of [³H]5-HMT in rat tissues after i.v. injection of the ligand. [³H]5-HMT (10 nmol/kg) was injected into the tail vein, and rats were sacrificed 10-180 min later. Specific [³H]5-HMT binding was experimentally defined as the difference in binding in particulate fractions of each tissue saline (total binding)- and atropine (14.8 μmol/kg, i.p.) (nonspecific binding)-pretreated mice. Each column represents mean±S.E. of 6 rats.

References

1. Expert Opin Pharmacother 9: 1787-1796, 2008
2. Curr. Med. Chem. 16: 4481-4489, 2009
3. Urology 78 : 920.e1-5, 2013

Disclosures

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