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MUSCARINIC RECEPTOR BINDING CHARACTERISTICS OF ANTICHOLINERGIC AGENTS IN THE URINARY BLADDER AND OTHER TISSUES

Aims of Study

Anticholinergic agents such as oxybutynin are widely used for the treatment of detrusor instability (DI) or detrusor hyperreflexia which is characterized by symptoms of increased frequency of micturition and urge urinary incontinence [1]. However, the major problems with its use are uncomfortable systemic side effects such as dry mouth that can lead to the discontinuation of treatment. The therapeutic effect and dry mouth by anticholinergic agents in patients with DI are mainly based on the blockade of muscarinic receptors in the urinary bladder and salivary gland, respectively. Thus, the bladder-selective anticholinergic agents receive a great deal of attention, in terms of the development of effective therapeutic agents with less side effects. Tolterodine is a potent and competitive antagonist of muscarinic receptor antagonist, used in the treatment of overactive bladder with symptoms of frequency, urgency and urge urinary incontinence. Further, one of most interesting features of tolterodine is that this compound shows selectivity for the bladder over salivary glands *in vivo* [2]. Currently, our studies have highlighted the usefulness of *in vivo* analysis of drug-receptor binding in relation to the pharmacokinetics for characterizing pharmacological specificity (potency, duration of action and tissue selectivity) of the drug [3-5]. To clarify the *in vivo* blockade by tolterodine of muscarinic receptors, we measured specific binding of [N-methyl-^{3}H]scopolamine (NMS) in various tissues of mice including the urinary bladder after oral administration of this agent, compared with that of oxybutynin.

Methods

At 0.5 to 24 hr after the oral administration of tolterodine or oxybutynin, mice were sacrificed by exsanguination from the descending aorta, and the bladder, prostate, submaxillary gland, heart, colon and lung were dissected. The mucarinic receptor in each tissue was measured by a radioreceptor binding assay with [^{3}H]NMS as a radioligand, and binding constants of apparent dissociation constant (K_d) and maximal number of binding sites (B_{max}) for [^{3}H]NMS were estimated by Scatchard analysis. The *in vitro* inhibitory effects of tolterodine and oxybutynin on specific [^{3}H]NMS binding in the bladder, submaxillary gland and heart of mice were also examined.

Results

Tolterodine (0.1~30 nM) reduced specific [^{3}H]NMS binding in the bladder, submaxillary gland and heart in a concentration-dependent manner, and it exhibited a similar inhibitory effect in the bladder and submaxillary gland. The *in vitro* inhibitory effect of tolterodine in the heart tended to be slightly weaker than in other tissues. Similar order of inhibitory effect of specific [^{3}H]NMS binding in each tissue was seen by oxybutynin. Further, the inhibitory effects of tolterodine in the bladder, submaxillary gland and heart of mice were significantly greater than those of oxybutynin. Following the oral administration of tolterodine (21.0 mol/kg), there was a significant increase in K_d values for specific [^{3}H]NMS binding in the bladder, prostate, submaxillary gland, heart, colon and lung of mice compared with each control value. Such increase in K_d value in each tissue attained to the maximal level 2 hr after the oral administration of tolterodine, and it was maintained up to 6 and/or 12 hr. The increased rate of K_d value in the bladder due to the tolterodine administration was similar to that in the prostate, submaxillary gland, heart and colon. On the other hand, there was a significant reduction of B_{max} value for [^{3}H]NMS binding in the lung with the largest increase in the K_d value, 0.5 to 6 hr after the tolterodine administration.

The oral administration of oxybutynin at the dose of 76.1 mol/kg brought about a significant increase in K_d value for specific [^{3}H]NMS binding in the bladder, prostate, submaxillary gland, heart, colon and lung of mice. The increment of K_d value by the oxybutynin administration was maximal at 0.5 hr later and it was disappeared at 6 hr. The enhancement in the K_d value produced by the oxybutynin administration was considerably greater in the submaxillary gland than in other tissues.

Conclusions

These data suggest that tolterodine binds more selective to the muscarinic receptors in the mouse urinary bladder than oxybutynin does under *in vivo* condition, and that the muscarinic receptor blockade after the oral administration of tolterodine, compared with that of oxybutynin, develops more slowly and it is of a longer duration. Thus, it is speculated that such slow kinetics of muscarinic receptor binding by the orally administered tolterodine may contribute to the slow onset and prolonged duration of pharmacological effects with less incidence of side effects. These findings support the advantage of tolterodine over oxybutynin as a

therapeutic agent for overactive bladder with symptoms of frequency, urgency and urge urinary incontinence.

References

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