**255**

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# PROPIVERINE AND METABOLITES: BINDING TO MUSCARINIC RECEPTORS, EFFECTS ON INTRACELLULAR CALCIUM-RELEASE AND HUMAN DETRUSOR CONTRACTION

Hypothesis / aims of study

Propiverine is a commonly used antimuscarinic drug for therapy of overactive bladder, which is extensively metabolized. Recently we showed that three of the main metabolites (M-5: 2,2-diphenyl-2-propoxy-acetic acid [1-methyl-piperid-4-yl]-ester-N-oxide-trans; M-6: 2,2-diphenyl-2-hydroxy-acetic acid [1-methyl-piperid-4-yl]-ester-N-oxide-trans; M-14: 2,2-diphenyl-2-propoxy-acetic acid [piperid-4-yl]-ester) have different potencies and efficacies reducing detrusor contractions in various species. They also affect the concentration-response curves for carbachol and impair L-type $Ca^{2+}$-current.

Here we have investigated the affinity of the metabolites to human muscarinic receptor subtypes in comparison to their potency and efficacy in models of detrusor function. The aim of our study was to provide further experimental evidence for the contribution of the metabolites to the therapeutic action of propiverine.

Study design, materials and methods

Muscarinic receptor binding was measured as displacement of [$^3$H]-NMS in CHO cells stably transfected with one of the human muscarinic receptor subtypes $M_1$ to $M_5$. Fura-II loading of cells was used for measuring intracellular calcium-concentration $[Ca^{2+}]_i$ in cell suspensions of either cultivated human detrusor smooth muscle cells (hDSMC) or HEK-293 cells stably transfected with human $M_3$ receptors. Detrusor contractility was measured in biopsy samples obtained from patients undergoing transurethral resection of bladder cancer. The samples were denuded of mucosa and force of contraction was determined in response to electric field stimulation (40 Hz; 1 ms pulse duration; 90 mA for 10 s every 2 min).

Results

Propiverine, M-5, M-6 and M-14 bind to muscarinic receptors with different affinity in the following order: M-6 > propiverine > M-14 > M-5. The 4 compounds exhibited a slightly higher affinity for the human $M_3$ than for the $M_2$ subtype, both of which are involved in detrusor contraction. The $pK_i$ value for binding of M-6 to $M_3$ receptors was one order of magnitude higher than of the $pK_i$ value of propiverine, whereas the affinities of M-5 and M-14 were 1-2 orders of magnitude lower (Table 1).

Table 1: $pK_i$ ($\pm$ SD) values of propiverine and its three metabolites for binding to human muscarinic receptor subtypes expressed in CHO cells.

| Compound | n | $hM_1$ | $hM_2$ | $hM_3$ | $hM_4$ | $hM_5$ |
|---|---|---|---|---|---|---|
| Propiverine | 3 | 6.58 $\pm$ 0.02 | 5.79 $\pm$ 0.03 | 6.39 $\pm$ 0.02 | 6.46 $\pm$ 0.03 | 6.43 $\pm$ 0.03 |
| M-5 | 3 | 4.66 $\pm$ 0.14 | < 4.0 | 4.50 $\pm$ 0.14 | 4.57 $\pm$ 0.13 | 4.32 $\pm$ 0.13 |
| M-6 | 3 | 7.22 $\pm$ 0.02 | 6.67 $\pm$ 0.03 | 7.03 $\pm$ 0.02 | 6.88 $\pm$ 0.04 | 6.80 $\pm$ 0.03 |
| M-14 | 3 | 5.96 $\pm$ 0.01 | 4.74 $\pm$ 0.04 | 5.94 $\pm$ 0.03 | *not determined* | *not determined* |

Propiverine concentration-dependently inhibited carbachol-induced increase of intracellular calcium concentration $[Ca^{2+}]_i$ in hDSMC and in HEK-293 cells expressing $hM_3$. The concentration-response curve for carbachol was shifted to higher concentrations. In addition, propiverine suppressed the maximum of the carbachol-induced elevation of $[Ca^{2+}]_i$. In HEK-293 cells the three metabolites M-5, M-6 and M-14 concentration-dependently inhibited carbachol-induced $Ca^{2+}$ release. At higher concentrations propiverine and M-14, but not M-5 and M-6 directly elevated $[Ca^{2+}]_i$.

Propiverine and its three metabolites concentration-dependently decreased detrusor contractions evoked by electric field stimulation. The calculated potencies and efficacies of propiverine, M-5, M-6 and M-14 were comparable with the order of affinity to muscarinic receptors and the order of potency in reducing intracellular $Ca^{2+}$ release.

Interpretation of results

Loss of the aliphatic side chain (M-6) is associated with high binding affinity to all human muscarinic receptor subtypes. This is reflected by the high potency of M-6 with respect to reduction of carbachol-induced $Ca^{2+}$ release and of electrically-evoked contractions. Changing the tertiary to a secondary amine (M-14) results in lower binding affinity and loss of potency compared to the parent compound. Oxidation of the nitrogen (M-5) amplifies the loss of binding affinity and further lowers the potency for functional changes.

Concluding message

Our results suggest that the main metabolite of propiverine, M-5, probably contributes little to the overall effect of propiverine, whereas M-6, which is detected in urine, should contribute significantly because of its higher affinity to muscarinic receptors.

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