

Thesis Title Effects of Collagenase on Muscarine
Binding in Intact and Cultured Bovine
Adrenomedullary Chromaffin Cells

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ABSTRACT

The presence of muscarinic receptors in intact and culture bovine adrenomedullary chromaffin cells was investigated by a binding assay using the antagonist-specific high affinity ligand, [^3H] quinuclidinyl benzilate [(^3H)-QNB]. The kinetics of [^3H]-QNB binding to partially purified, mechanically isolated intact bovine chromaffin cells was examined. The rate constants at 37°C for association and dissociation of the [^3H]-QNB-receptor complex in intact cells were $1.2 \times 10^8 \text{ M}^{-1} \text{ min}^{-1}$ and $1.28 \times 10^{-2} \text{ min}^{-1}$, respectively. The dissociation equilibrium constant (K_d) can be derived from dissociation and association rate constants (k_{-1}/k_{+1}). The K_d value was calculated to be 0.106 nM.

The present study demonstrated that collagenase at 0.1% or higher concentrations decreased the specific binding of [³H]-QNB to muscarinic receptors in mechanically isolated intact bovine chromaffin cells. Scatchard analysis combined with computer program "LIGAND" was used for characterization of the specific [³H]-QNB binding. It was found that partially and Renografin purified, mechanically isolated intact bovine chromaffin cells had a single, high affinity class of binding sites with a K_d of 0.12 ± 0.02 nM and 0.09 ± 0.03 nM and a B_{max} of 3.1 ± 0.6 fmol/ 10^3 cells and 0.45 ± 0.16 fmol/ 10^3 cells, respectively. In addition, partially and Renografin purified, enzymatically (0.15% collagenase) isolated intact bovine chromaffin cells had a single, high affinity class of binding sites with a K_d of 0.14 ± 0.06 nM and 0.02 ± 0.004 nM and a B_{max} of 0.021 ± 0.0007 fmol/ 10^3 cells and 0.01 ± 0.003 fmol/ 10^3 cells, respectively. Interestingly the 7-day culture of bovine chromaffin cells exhibited a two class of binding sites with a K_d of 0.01 ± 0.003 nM and a B_{max} of 0.006 ± 0.001 fmol/ 10^3 cells for the high affinity site and a K_d of 17.0 ± 6.0 nM and a B_{max} of 0.13 ± 0.06 fmol/ 10^3 cells for the low affinity site, respectively.

These data demonstrate that 0.15% collagenase alters the receptor density (B_{max}) of mAChR in intact and culture bovine adrenomedullary chromaffin cells but the K_d remains unchanged.