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SHORT COMMUNICATION

Binding of Phenylcyclohexylglycoloyl Ester of Hydroxyethyldimethylhydrazonium (Compound VUFB-3113) to Muscarinic Receptors in Rat Heart, Salivary Gland and Cerebral Cortex

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Summary

Phenylcyclohexylglycoloylester of hydroxyethyldimethylhydrazonium (compound VUFB-3113) has been shown earlier to have a strong antimuscarinic effect on smooth muscle. Its affinity to muscarinic binding sites in homogenates of rat heart ventricles (M2 subtype), submandibular salivary gland (M3 subtype) and brain cortex (predominantly M1 subtype) has now been investigated in radioligand displacement experiments using (^{3}H)quinuclidinyl benzilate ((^{3}H)QNB) as a relatively non-specific muscarinic ligand. VUFB-3113 inhibited the binding of (^{3}H)QNB with pK_i values of 8.17, 8.73, and 8.52 in the heart, salivary gland, and brain cortex, respectively. It is concluded that the compound has a high affinity for muscarinic binding sites without strong preference for any of the M1–M3 subtypes.

Key words

Muscarinic receptors – Heart – Salivary gland – Phenylcyclohexylglycoloyloxyethyldimethylhydrazonium – Brain cortex

Muscarinic receptor antagonists belong to the oldest known and most investigated compounds in human pharmacology. It has been recognized recently that muscarinic receptors are not homogeneous in their genetics, chemical structure, binding properties and the molecular mechanisms of action (Brown 1989). Five independently coded subtypes of muscarinic receptors (m1-m5) have been defined genetically (Bonner 1989), while only three subtypes (M1-M3) can be clearly defined pharmacologically (Nomenclature 1989).

Classical muscarinic antagonists like atropine or scopolamine do not distinguish between the subtypes and bind to all of them with similar affinities. It is important to find compounds acting selectively on only one or another subtype and thus interfering with only certain specific physiological consequences of muscarinic activation. Some subtype-specific muscarinic antagonists have already been described, like pirenzepine (anti-M1), AFDX-116 and methoctramine (anti-M2), hexahydrosiladifenidol and DAMP (anti-M3) (Hammer and Giachetti 1984, Buckley *et al.* 1989, Zeymal and Shelkovnikov 1989, Waelbroeck *et al.* 1990). Unfortunately, the subtype-specific antagonists described so far rarely show more than a tenfold preference for their particular subtype, which is too small compared to clinical needs. No generalizations can yet be made as to what structural features are required for muscarinic antagonists to become selective for one or another subtype.

Nearly three decades ago, Adlerová *et al.* (1964) described a series of more than 30 muscarinic antagonists, representing various esters of hexahydrobenzilic acid. Among these compounds was a substance in which the ammonium moiety of acetylcholine was replaced by hydrazonium, which is unusual in muscarinic pharmacology. The compound was the phenylcyclohexylglycoloyl ester of hydroxyethyldimethylhydrazonium (VUFB-3113, Fig. 1) and was found to surpass atropine and oxyphenonium in their antiacetylcholine spasmolytic activity on the rat duodenum. Although VUFB-3113 proved promising in initial clinical trials (Turek *et al.*

1964), its pharmacodynamics could not be investigated in the depth required by drug control regulations because sufficiently sensitive methods were not available to measure its low concentrations in body fluids (J. Metyš, personal communication).

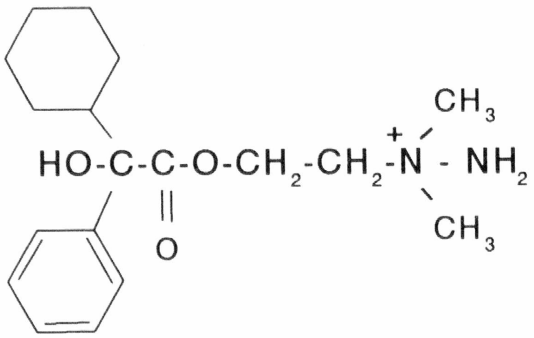

Fig. 1
Chemical formula of compound VUFB-3113.

In view of the high activity of VUFB-3113 on the smooth muscle and of its unusual chemical structure, we decided to investigate its affinity to muscarinic receptors in cardiac ventricles (M2), the salivary gland (M3) and the brain cortex (M1) in radioligand binding experiments, using (^{3}H)quinuclidinyl benzilate ((^{3}H)QNB) as a non-specific radiolabelled muscarinic ligand (Yamamura and Snyder 1974).

Experiments were performed on tissues from female Wistar rats of 150–200 g body weight which had been killed by cervical dislocation and exsanguination. The heart ventricles, submandibular glands and brain cortices (with underlying white matter) were homogenized in a Polytron homogenizer in a medium consisting of (mmol/l): NaCl 136, KCl 5, $MgCl_2$ 1, Na_2HPO_4 1, Na-HEPES (N-2-hydroxyethylpiperazine-N'-2-ethane-sulfonate) 10, EGTA 0.1, and protease inhibitor phenylmethylsulfonyl fluoride 0.1. The homogenates were filtered through medical gauze and kept frozen at −40 °C until the day of experiment.

Radioligand binding experiments were performed according to generally accepted rules (Yamamura *et al.* 1990, Tuček *et al.* 1990). To investigate the displacement of (^{3}H)QNB binding by VUFB-3113, identical portions of the homogenates (corresponding to 4 mg, 2.5 mg, and 0.5 mg of original tissue per incubation tube for the ventricles, salivary glands and brain cortex, respectively) were incubated in the presence of 100 pmol/l (^{3}H)QNB (produced by Amersham International, Amersham; specific radioactivity 1.05 Bq/fmol) and of increasing concentrations of VUFB-3113. In all experiments, the effect of each VUFB-3113 concentration was determined in three tubes incubated in parallel. The composition of the incubation medium corresponded to that of the solution used for homogenization; its total volume was 2 ml per tube, and the incubation lasted 2 h at 38 °C. It was arrested by vacuum filtration on Whatman GF/C glass fibre filters and the bound radioactivity retained on filters was measured by scintillation counting in Bray's solution. Non-specific binding of (^{3}H)QNB was determined in the presence of 5 μmol/l atropine. The binding parameters of (^{3}H)QNB (K_d and B_{max}) were determined in saturation binding (Scatchard-type) experiments investigating the binding of (^{3}H)QNB at concentrations in the range of 12.5–400 pmol/l, with incubations performed in triplicates. Data on the displacement of (^{3}H)QNB binding and on the saturation of (^{3}H)QNB binding were analyzed using non-linear regression with the Enzfitter computer programme. Concentrations of VUFB-3113 causing 50 % inhibition of radioligand binding (IC_{50} values) were transformed to K_i values in the way proposed by Cheng and Prusoff (1973).

Table 1
Negative decadic logarithms of the inhibition constants (pK_i) and Hill slope factors (nH) for the inhibition of the binding of (^{3}H)QNB to homogenates of rat heart ventricles, submandibular salivary gland and brain cortex by the compound VUFB-3113

Tissue	pK_i	nH
Heart ventricles	8.17 (8.07,8.27)	0.74 (0.67,0.80)
Submandibular gland	8.73 (8.58,8.88)	0.94 (0.93,0.95)
Cerebral cortex	8.52 (8.43,8.62)	0.83 (0.76,0.89)

Data are means of two independent experiments for each tissue, performed with incubations in triplicates. Individual results are given in parentheses.

Under the conditions used, the K_d values for the binding of (^{3}H)QNB to the cardiac, glandular and cerebrocortical homogenates were found to be 124, 83, and 52 pmol/l, respectively. The results of experiments with radioligand displacement have been summarized in Table 1. VUFB-3113 inhibited (^{3}H)QNB binding with high efficiency, corresponding to K_i values in the range of 1.3–8.5 nmol/l, with only small differences between its affinities to the three tissues examined. Hill slope factors were very close to unity in the case of the salivary gland and somewhat lower than unity in the cortex and heart. The reason for the observed deviation from unity in the latter two tissues (which might be due to negative cooperativity in the binding, heterogeneity of the receptor population to which the compound

binds, or some unknown factors - see Limbird 1986) has not been investigated.

The high affinity of VUFB-3113 for muscarinic receptors corresponds to the general observation made by Abramson *et al.* (1969) that the simultaneous presence of a phenyl and a cyclohexyl group in muscarinic antagonists increases their affinity for the receptor. The presence of hydrazonium in the polar part of antagonist molecule is compatible with its high affinity. Our results indicate, however, that the hydrazonium moiety does not provide any substantial degree of specificity with regard to any of the M1–M3 muscarinic subtypes.

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