Changes in Muscarinic Acetylcholine Receptor Binding in Skin Slices of Cholinergic Urticaria

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Muscarinic acetylcholine receptors were studied in slices of unaffected skin and in lymphocyte membranes from 3 patients with cholinergic urticaria and 5 healthy subjects using receptor autoradiography and the radioligand [3H]quinuclidinyl benzilate binding technique. Autoradiography revealed that muscarinic acetylcholine receptors were located in regions with sweat glands. The distribution of receptors did not differ qualitatively between patients and controls. However, the maximum number of muscarinic acetylcholine receptors was reduced in skin from patients with cholinergic urticaria, by 44% as compared with the controls, whereas the binding affinity was not affected. Muscarinic cholinergic binding in lymphocyte membrane fractions obtained from patients with cholinergic urticaria did not differ statistically from that of healthy control persons. Key words: [3H]quinuclidinyl benzilate binding; Lymphocyte.

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Cholinergic urticaria is one of the physical urticarias with local and systemic manifestations. In laboratory investigations, only a rare leukocytosis was reported in the peripheral blood (1). Histological sections from cholinergic wheals exhibit an upper dermal, perivascular infiltrate which consists of mononuclear cells, neutrophils and occasionally eosinophils (2). In addition, dermal edema and endothelial swelling of the smaller dermal vessels were found (3). No relationship between nerves and mast cells was observed (4). Pathogenetically, the stimulation of the neuroglandular junction of the sweat glands via cholinergic effrent fibres, which receive their impulse from the central nervous system, seems to be involved.

Shelley et al. (3) reported an increase in muscarinic acetylcholine receptors (mAChR) in skin lesions, but after the lesion had subsided, and on rechallenge at previously involved sites, 4 h later, the level of [3H]quinuclidinyl benzilate (QNB) binding to mAChR was found to be decreased in the seemingly healthy skin, suggesting an important role of mAChR in the pathogenesis of this disease. Using in vitro autoradiography, mAChR were also detected physiologically in skin areas containing eccrine sweat glands (5).

Lymphocyte function can be modulated by cholinergic agonists in vitro, possibly through the presence of cholinergic receptors demonstrated in both intact and lysed lymphocyte membranes (6, 7). The number of cholinergic receptors on lymphocytes is altered in certain diseases affecting the cholinergic system (see e.g. 7, 8).

The content of mAChR in lymphocyte membrane fractions and in slices of unaffected skin from patients with cholinergic urticaria was therefore studied to reveal possible relationships between the cholinergic system of skin and lymphocytes in the pathogenesis of cholinergic urticaria.

MATERIALS AND METHODS

Materials

[3H]-L-quinuclidinyl benzilate ([3H]-QNB, specific activity 1.11 TBq/mmol) was obtained from the Radiochemical Centre Amersham, Bucks, England. Atropine sulphate (VEB Arzneimittelwerk Dresden, GDR) was used as unlabelled displacer. All other chemicals were commercial products of highest purity available.

Patients

The diagnosis of 3 patients (2 male, one female, mean age 25.6 years) with cholinergic urticaria was based on the typical history, clinical picture, and lesions provoked by appropriate procedures (4). Five healthy age-matched subjects were used as controls.
Fig. 1. (a) Autoradiogram of \(^{3}H\)-OQB binding to muscarinic acetylcholine receptors in tissue sections of human skin. (b) Same section as in (a) shows darkly stained sweat glands (hematoxylin stain).

Skin tissue preparation
The frozen piece of skin tissue was trimmed into a block in order to obtain sections of similar size, and then cut into slices of 10 \(\mu\)m thickness by means of a cryocut. Slices were thaw-mounted onto glass slides and stored at \(-20^\circ\)C until used (normally not longer than 2 weeks). No loss of specific binding due to storage for this length of time was noted.

Muscarinic acetylcholine receptor binding to skin tissue
For muscarinic cholinergic receptor binding, the incubation of four slide-mounted skin sections was carried out in humid chambers at room temperature by flooding the sections with 50 mM sodium-potassium phosphate buffer, pH 7.4, containing \(^{3}H\)-OQB at a final concentration ranging between 0.7 and 11 nM. After 3 h of incubation, slides were given two rinses of 5 min each in ice-cold buffer before drying in a weak nitrogen stream. Non-specific binding was determined in adjacent skin sections, co-incubated with 100 
\(\mu\)M atropine sulphate and ranged between 2\% and 4\% of total binding.

The amount of radioligand bound to the tissue section was assessed by scraping the tissue slices from the slides and measuring the radioactivity via liquid scintillation counting.

Specific binding reached equilibrium during the incubation time indicated and was completely displaced by the concentration of the corresponding displace indicated above. The washing conditions of the incubated slices were selected so that the non-specific binding was greatly reduced, while the specific binding remained nearly unchanged.

The binding parameters, such as equilibrium dissociation constant (\(K_d\)) and maximum receptor number (\(B_{max}\)), were obtained from saturation curves by fitting the experimental data to the theoretical curve (one binding site model) using a non-linear least-squares regression analysis (computer program DATABFIT2, see e.g. 9). Specific binding was calculated as moles of radioligand specifically bound per mg of protein content (fig. 2). The protein content of the skin slices was measured in parallel experiments using the method of Lowry et al. (10).

Receptor autoradiography
For autoradiographic experiments, the labelled and dried tissue sections were apposed to tritium-sensitive film (Ultrafilm, LKB) at 4\(^\circ\)C. After exposure for 3 months, the films were developed with a Kodak D19 developer for 5 min at 20\(^\circ\)C, fixed, rinsed and dried.

Muscarinic acetylcholine receptor binding to lymphocytes
Lymphocytes were separated from heparinized peripheral venous blood by means of a density gradient centrifugation as described elsewhere (11).

Lymphocytes obtained from about 10 ml blood were homogenized in 250 \(\mu\)l 50 mM sodium-potassium phosphate buffer, pH 7.4, and stored at \(-20^\circ\)C overnight. For the binding assay, samples were rehomogenized and centrifuged at 48,000 \(g\) for 20 min. The pellet was washed in 250 \(\mu\)l buffer, again centrifuged, and the resulting pellet rehomogenized in 250 \(\mu\)l buffer. \(^{3}H\)-OQB binding to muscarinic acetylcholine receptors was measured using a centrifugation method. 50 \(\mu\)l of the tissue preparation containing about 50 \(\mu\)g protein was added to 150 \(\mu\)l 50 mM sodium-potassium phosphate buffer, pH 7.4. The reaction was initiated by adding 20 \(\mu\)l \(^{3}H\)-QNB at a final concentration of 4 nM. The incubation was continued for 1 hour at 25\(^\circ\)C followed by centrifugation at 70,000 \(g\) for 10 min. The supernatant was discarded and the pellet was rinsed twice superficially with 2 ml of ice-cold buffer to remove unbound radioligand. The rinsed pellet was solubilized in 200 \(\mu\)l 1 N hydroxide, and the radioactivity bound to the pellet was determined by liquid scintillation counting. Each assay was run in duplicate. Non-specific binding was present.
brane preparation from control persons could be detected, indicating a marked amount of mACHR located on lymphocyte membranes (534 ± 40 fmol/mg protein). 3H-QNB binding in lymphocyte membrane fractions obtained from patients with cholinergic urticaria (505 ± 45 fmol/mg protein) was not statistically different from that of healthy control persons.

DISCUSSION

Cholinergic receptors represent the target structures which receive cholinergic input and might therefore be involved in the pathogenesis of cholinergic urticaria. This is emphasized by investigations from Shelley et al. (3), showing a transient increase in mACHR in cholinergic wheals from patients with cholinergic urticaria, whereas on rechallenge of previously involved sites 4 h later, the number of receptors was decreased. These observations suggest a transient increase in mACHR expression in cholinergic wheals and could also explain the temporary refractoriness of the tissue in some patients (4).

Our data, obtained from unaffected skin from patients with cholinergic urticaria, revealed a decreased maximum mACHR count in comparison with that from control donors, suggesting reactive changes in the responsiveness and/or expression of mACHR, depending on the mediator action and the time course of the formation of cholinergic wheals.

Our measurements of mACHR on lymphocytes from patients with cholinergic urticaria revealed no difference in 3H-QNB binding vis-à-vis controls. Obviously, the changes in mACHR binding in skin from patients with cholinergic urticaria are not accompanied by corresponding alterations of cholinergic receptors in lymphocytes as was found in other diseases (6–8). However, acetylcholine is principally able to influence mACHR located on lymphocytes and thus to modify the regulatory activities of the mediator releasing cells (7).

Regardless of possible explanations, strategies for pharmacological treatment have to be established according to a more precise insight into the pathogenesis, which warrants further elucidation.

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RESULTS

Autoradiographic experiments revealed that 3H-QNB binding to muscarinic acetylcholine receptors in human skin slices is located mainly in areas of the skin containing sweat gland tissue (Fig. 1a), as can be seen by comparing the autoradiogram with a tissue section stained with hematoxylin visualizing sweat glands in the tissue sections (Fig. 1b).

Muscarinic acetylcholine receptors are present in the normal human skin with a concentration of 586 ± 60 fmol/mg protein (mean ± SEM) and a binding constant of 4.4 ± 1.1 nM. In patients with cholinergic urticaria, a reduced amount of muscarinic cholinergic receptor density (by about 44%, compared with untreated controls, p < 0.01, two-tailed Student’s t-test) was found, whereas the binding affinity did not differ significantly from that detected in normal skin (the corresponding figures in patients were 329 ± 39 fmol/mg protein, and 5.0 ± 0.6 nM).

Assayed at a single ligand concentration of 4 nM, a specific binding of 3H-QNB to lymphocyte mem-

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REFERENCES
1. Warin RP, Champion RH. Urticaria. London: Saun-
ders, 1974.
   1. Klinische Beobachtungen und histologische Unter-
247.
3. Shelley WB, Shelley ED, Ho AKS. Cholinergic urticari-
a: acetylcholine-receptor-dependent immediate-type
hypersensitivity reaction to copper. Lancet 1983, 1:
843–846.
4. Czarnetzki B. Cholinergic urticaria. In: Urticaria. Hei-
5. Ryer HJ, Nock B, Serby M, Katz S, McEwen BS. In
vitro autoradiography of muscarinic acetylcholine re-
ceptors in human skin. Eur J Pharmacol 1986; 120:
379–380.
6. Adem A, Nordberg A, Slanina P. A muscarinic recep-
tor type in human lymphocytes: a comparison of
H QNB binding to intact lymphocytes and lysed lympho-
7. Bering B, Moises HW, Müller WE. Muscarinic cholin-
ergic receptors on intact human lymphocytes. Prop-
ties and subclass characterization. Biol Psychiatry
ural cholinergic markers in Alzheimer’s and Parkin-
son’s disease. Prog Neuropsychopharmacol Biol Psy-
9. Rothe T, Schlebs R. Laminar distribution of benzodia-
zipine receptors in visual cortex of adult rat. Gen
10. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ.
Protein measurement with Folin phenol reagent. J Biol
11. Bayum A. Isolation of lymphocytes, granulocytes and