EFFECTS OF BETAHISTINE ON THE BINDING OF NICOTINE TO NICOTINIC CHOLINERGIC RECEPTORS IN RAT BRAIN SYNAPTOSOMES

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Bethahistine is effective in the treatment of labyrinthine vertigo (1). It has been reported also that bethahistine improves mental function in patients with arteriosclerosis-related dementia (2–5). Although the mechanism of action of bethahistine has not been fully elucidated, it is known to have a potent cerebral and peripheral vasodilator action, the pharmacological properties of histamine (6). However, the effect of this antidiabetic on the cerebral blood flow is not blocked by anti-histaminic drugs.

We previously demonstrated the existence of nicotinic cholinergic receptors in brain synaptosomes utilizing ^3H -nicotine as a ligand (7). The binding of ^3H -nicotine is competitively inhibited by D-tubocurarine and carbamylcholine, but not by atropine. These receptors seem to exist in presynaptic nerve endings, since nicotine enhanced the release of noradrenaline from synaptosomes (8). Since a portion of the structure of bethahistine is stereochemically similar to that of nicotine, we studied the effect of bethahistine on the binding of ^3H -nicotine to brain nicotinic receptors.

Male Wistar rats weighing approximately 200 g were used. Subcellular fractionation was done by the conventional techniques of Gray and Whittaker (9), and the synaptosomal fraction was further washed by a slight modification of the method of Bradford et al. (10). Briefly, 10% (W/V) homogenate of the brain excluding the cerebellum in 0.32 M

sucrose was centrifuged in a refrigerated centrifuge at $1,000\times g$ for 10 min. The supernatant was then centrifuged at $17,000\times g$ for 55 min. The resultant precipitate (P2) was further subfractionated into myelin (A), synaptosomal (B) and mitochondrial (C) fractions by centrifugation at $75,000\times g$ for 60 min on a discontinuous sucrose gradient. The synaptosomal pellet was obtained by further centrifugation of the diluted intermediate layer (B) of the gradient tube at $55,000\times g$ for 20 min in 0.45 M sucrose, and the synaptosomal supernatant including microsomes and myelin fragment was discarded.

Synaptosomes obtained in the form of a pellet were resuspended in 50 mM Tris-HCl buffer (pH 7.7) to give a concentration of 170 μg protein/ml. To determine the binding of ^3H -nicotine to synaptosomes, a 0.5 ml aliquot of this suspension was incubated with 0.5 ml of Tris-HCl buffer containing 3 pmol of ^3H -nicotine (S.A. 25.0 Ci/mmol, (G- ^3H)-nicotine-D-bitartrate, Radiochemical Centre, Amersham) with or without increasing quantities of cold nicotine, bethahistine or related substances, at 25°C for 30 min, as described previously (7). Then, the reaction mixture was filtered (Whatman GF/B) to remove the unbound fraction and the preparation washed with Tris-HCl buffer. The radioactivity retained on the filter paper was counted in 10 ml of toluene scintillation cocktail using a Packard Tri-Carb scintillation

Fig. 1. Structural formulae of the drugs examined.

spectrometer. Protein contents of suspensions were determined by the method of Lowry et al. (11). The reported values are means of quadruplicate determinations.

The structural formulae of two antidinics examined, betahistine and dyphylline are shown in Fig. 1 which includes two other betahistine derivatives, A and B. All these drugs are synthesized and provided by Eisai Co., Ltd., Tokyo.

The binding of ^3H -nicotine to synaptosomes was displaced by the addition of cold nicotine. Cold nicotine at 10^{-5} M inhibited ^3H -nicotine binding by 90%. The remaining 10% was regarded as non-specific binding.

The specific binding of ^3H -nicotine to synaptosomes was reduced by betahistine as shown in Fig. 2. Betahistine inhibited 50% of ^3H -nicotine specific binding at the concentration of 7×10^{-8} M, but at the concentration below 10^{-6} M, dyphylline or two betahistine derivatives had little effect on the nicotine binding. The addition of 100 mM sodium reduced nicotinic binding by about 50%. Furthermore, with the addition of 100 mM NaCl, betahistine inhibited half of the ^3H -nicotine specific binding at the concentration of 2×10^{-7} M. From the double-reciprocal plot, ^3H -nicotine specific binding was reduced in the presence of betahistine competitively, but it was inhibited

Fig. 2. Inhibition of ^3H -nicotine binding to synaptosomes by betahistine and other related agents. The effect of sodium chloride on ^3H -nicotine binding is shown by Data, expressed as a percent of the control ^3H -nicotine total binding to synaptosomes are plotted on the ordinate against the drug concentration (log M) on the abscissa. ^3H -nicotine specific binding is regarded as the binding in the absence of cold nicotine minus the binding in the presence of 10^{-5} M cold nicotine.

by 100 mM NaCl in a non-competitive fashion.

The present study demonstrates that betahistine inhibits the specific binding of ^3H -nicotine to synaptosomes competitively, whereas two betahistine derivatives and dyphylline have, if any, little effect. The present study also shows that 100 mM sodium chloride inhibits the specific binding of ^3H -nicotine to synaptosomes by about 50%, indicating that a kind of 'sodium effect' exists in the binding of nicotinic agonists to their receptors. The fact that sodium reduces ^3H -nicotine specific binding non-competitively suggests that this compound may alter the configuration of nicotinic cholinergic receptors.

It is concluded that betahistine may play a pharmacological role *via* nicotinic cholinergic receptors in the brain.

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