
原 著

Binding Assay for Muscarinic Cholinergic Receptors in Kaolin Induced Hydrocephalus

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Abstract

Pathophysiology of hydrocephalus has not been clarified completely. The changes in concentration of metabolites of neurotransmitters in the ventricular cerebrospinal fluid (CSF) in hydrocephalus have been reported. It is presumed that neurotransmitters may play a role in regulation of CSF dynamics.

The study of neurotransmitter receptors becomes possible with the development of ligands of a high specific radioactivity and a high affinity for the receptor.

The present study was undertaken to study muscarinic cholinergic binding in experimental hydrocephalus.

Hydrocephalus was induced in adult rats by intracisternal injection of kaolin. The brains of the acute to chronic stages after intracisternal injection of kaolin were used for the study of binding assay for muscarinic cholinergic receptors.

Specific binding of [³H] Quinuclidinyl benzilate (QNB) was assayed in homogenates of the brain after removal of the cerebellum and brainstem. The density of the receptors was determined from protein determination by the method of Lowry and others using bovine serum albumin as a standard.

The muscarinic cholinergic receptor density, i.e. binding sites per μ g tissue or per μ g protein was higher in hydrocephalic than in normal rat brain. This might represent supersensitivity to the muscarinic cholinergic agent or compensation for the decrease of the agent in hydrocephalic rat brain.

Muscarinic cholinergic receptor has a close relation to dementia. Consequently, the study of specific binding for this receptor may also contribute to clarify pathogenesis and treatment of normal pressure hydrocephalus which has many common aspects with hydrocephalus.

QNB autoradiography is useful for the evaluation of changes of distribution of muscarinic

key word: Binding assay, Hydrocephalus, Muscarinic cholinergic receptor, Neurotransmitter, Rat.

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cholinergic receptors in hydrocephalus. It is presumed that the present study may be applied to specific binding study by positron emission CT which will lead to a new diagnostic and therapeutic measures in the management of human hydrocephalus.

Introduction

Pathophysiology of hydrocephalus has not been clarified completely. The changes in concentration of metabolites of neurotransmitters in the ventricular cerebrospinal fluid (CSF) in hydrocephalus have been reported. It is presumed that neurotransmitters may play a role in regulation of CSF dynamics. The changes of biogenic amines, such as dopamine, noradrenaline, serotonin, etc. in the hydrocephalic brain have been studied. However, little is known about acetylcholine in hydrocephalus.

Neurotransmitters produce their biological effect by interacting with receptors in neurons and multiple receptors have been shown to exist for all neurotransmitters. The search for receptors is the most useful investigation of neurochemical involvement in hydrocephalus. The study of changes in neurotransmitter receptors may offer information about pathophysiology of hydrocephalus.

The study of neurotransmitter receptors become possible with the development of ligands of a high specific radioactivity and a high affinity for the receptor.

The present study was undertaken to study muscarinic cholinergic binding in experimental hydrocephalus.

Materials and Methods

1. Production of Experimental Hydrocephalus

Adult Wister/Fib rats, weighing 100–150 g, were used in this study. Under anesthesia with intraperitoneal administration of pentobarbital sodium (30 mg/kg), kaolin (0.2 ml of 30 mg/100 ml suspension) was injected intracisternally. The rat brains of the acute to chronic stages after intracisternal injection of kaolin were used to study binding assay for muscarinic cholinergic receptors (Fig. 1, 2 and 3).

2. Binding Assay for Muscarinic Cholinergic Receptors in Rat Brain

Specific binding of [^3H] Quinuclidinyl benzilate (QNB) was assayed in homogenates of rat brain after removal of the cerebellum and brainstem as shown in Fig. 4 and 5.

1) Crude membrane preparation of rat brain

Wister/Fib rats were decapitated and their brains were rapidly removed. After removal of the cerebellum and brain stem, the cerebrum was resuspended in 15 ml of ice-cold 0.23 M sucrose in a glass homogenizer fitted with a Teflon pestle. The whole homogenate was centrifuged for 10 min. at $1,000 \times g$. The supernatant was centrifuged for 20 min. at $10,000 \times g$. The pellet was homogenized in 15 ml of 0.05 M phosphate buffer and used for [^3H] QNB binding studies.

2) Assay for specific binding of [^3H] QNB of the receptor preparation



Fig. 1. A coronal section of normal rat brain. The ventricles are slit-like.



Fig. 2. A coronal section of hydrocephalic rat brain of the acute stage, three days after intracisternal injection of kaolin. The lateral ventricles are dilated and the periventricular white matter is edematous.

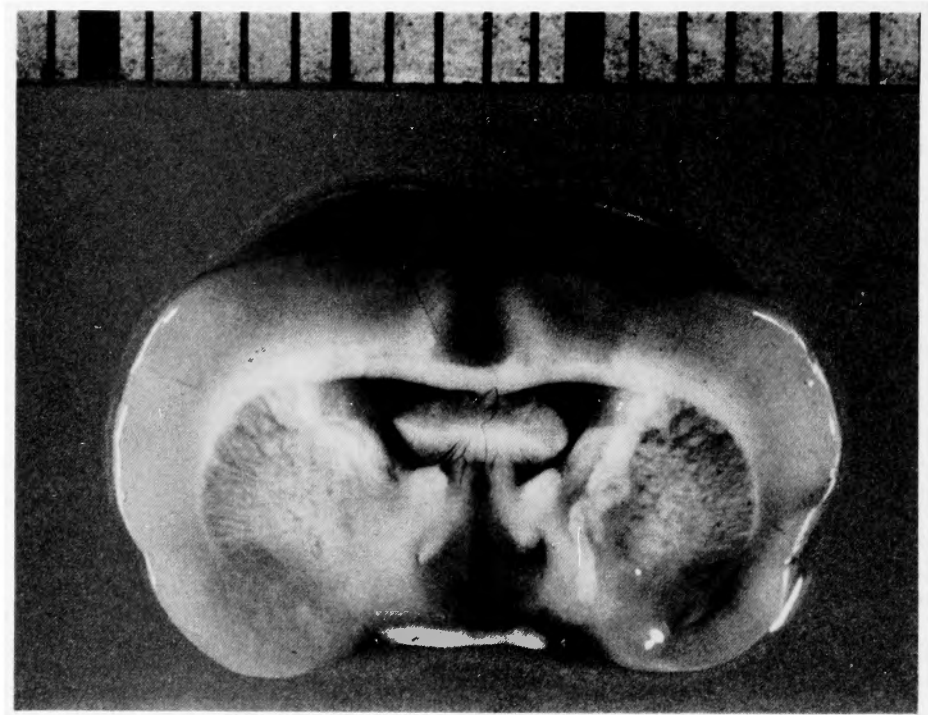


Fig. 3. A coronal section of hydrocephalic rat brain of the chronic stage, two weeks after intracisternal injection of kaolin. The lateral ventricles are still dilated, but edema in the periventricular white matter has almost disappeared.

[³H] QNB binding was performed by incubating the aliquots of the the tissue suspension at 25°C for 60 min. in 1 ml of the 0.05 M sodium phosphate buffer (pH 7.4) containing [³H] QNB in the absence or presence of 100 μM atropine sulfate. The assay was terminated by

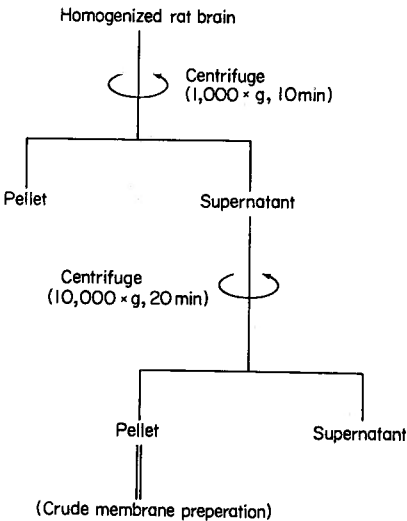


Fig. 4. Crude membrane preparation of rat brain.

Receptor preparation + [^3H]QNB
(with or without blocker buffer)

Incubation (25°, 1 hr)

Filtration

Assay of radioactivity

Fig. 5. Method of assay for muscarinic cholinergic binding in rat brain.

addition of 3 ml of the ice-cold buffer and rapid filtration through Whatman GF/B glass fiber filters under suction. The filters were dried with infra-red lamp for about 30 min. and placed in vials containing 10 ml of Triton-X + POPOP + DPO + toluene and the radioactivity then counted by liquid scintillation spectrometry. Specific binding is defined as the total minus the non-specific binding (i.e. binding in the presence of atropine). Protein was determined by the method of Lowry et al. using bovine serum albumin as a standard.

Results

Specific [^3H] QNB binding was saturable with increasing of the amount of ligand (Fig. 6).

The protein in the receptor preparation of both normal and hydrocephalic rat brain was determined by Lowry method². As the amount of protein increased, specific [^3H] QNB binding was higher in hydrocephalic than in normal rat brain (Fig. 7). Scatchard analysis of specific [^3H] QNB binding to normal rat brain indicated the presence of only 1 component of binding site. The dissociation constant (K_D) was about 0.2 nM while the maximal binding sites (B_{max})

Table 1. Dissociation constant (K_D) and maximal binding sites (B_{max}) in normal and hydrocephalic rat brains.

	K_D	B_{max} (p mol/mg-protein)
Normal	0.200	5.610
Acute	0.172	7.240
Chronic	0.208	6.249

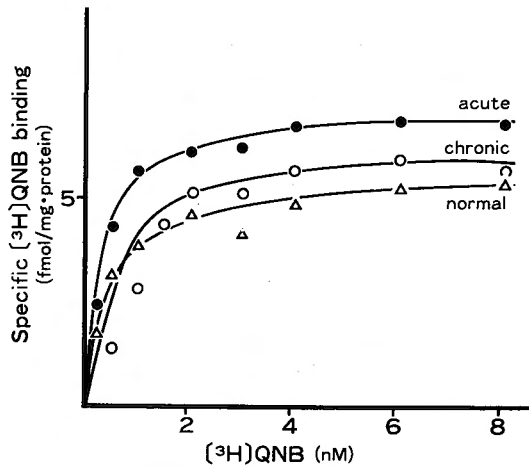


Fig. 6. Specific $[^3\text{H}]\text{QNB}$ binding to crude membrane prepared from rat brain.

was about 5.61 pmol/mg. protein (Table 1).

Discussion

Binding sites with high affinity and specificity for $[^3\text{H}]\text{QNB}$ are known to be present in homogenates of rat and mouse brains and they resemble muscarinic cholinergic receptors^{1,30}. The specific binding of $[^3\text{H}]\text{QNB}$ to homogenates of rat brain might be expected of interactions with muscarinic cholinergic receptors in the brain.

Displacement of $[^3\text{H}]\text{QNB}$ binding is greatest with muscarinic cholinergic antagonists and the relative affinity of muscarinic agonists tends to parallel their pharmacological potency. The selective high affinity of muscarinic anticholinergic drugs for muscarinic cholinergic receptors and for QNB-binding sites is useful for the identification of the binding sites with muscarinic cholinergic

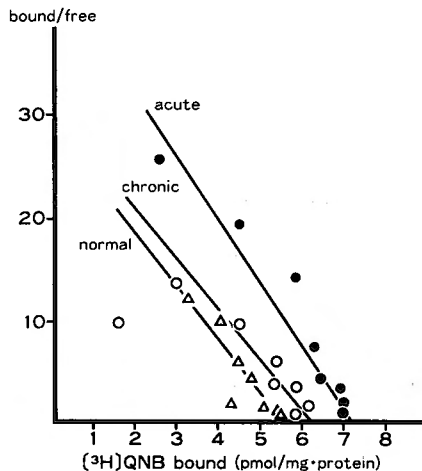


Fig. 7. Scatchard plot of $[^3\text{H}]\text{QNB}$ binding to crude membrane prepared from rat brain.

gic receptors in the brain.

The muscarinic cholinergic receptor density, i.e. binding sites per μg protein was higher in hydrocephalic than in normal rat brain in the present study. Changes of K_D and B_{max} in normal and hydrocephalic rat brains were observed as shown in Table 1. This might represent supersensitivity of hydrocephalic rat brain to muscarinic cholinergic agents or compensation for the decrease of muscarinic cholinergic agents in hydrocephalic rat brain. Conclusions about the changes of muscarinic cholinergic receptors in hydrocephalus should await further and more detailed experiments.

Muscarinic cholinergic receptor has a close relation to dementia. Consequently, the study of specific binding for this receptor may also contribute to clarify pathogenesis and treatment of normal pressure hydrocephalus which has many common aspects with hydrocephalus.

QNB autoradiography is useful for the evaluation of changes of distribution of muscarinic cholinergic receptors in hydrocephalus. It is presumed that the present study may be applied to specific binding study by positron emission CT which will lead to a new diagnostic and therapeutic measures in the management of human hydrocephalus.

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和文抄録

水頭症ラットにおける Muscarinic Cholinergic
Receptors の Binding Assay

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最近，neurotransmitter に対して high specific radioactivity と high affinity を有する ligand が開発され，receptor の測定が容易となったことより，水頭症病態における neurotransmitter の関与を直接調べられるようになった。本研究においては，実験的に作成したラット水頭症脳における muscarinic cholinergic receptor の binding assay を行ったので，その結果を報告する。

水頭症脳においては receptor が増加する傾向を示

し，その傾向はとくに急性期に著しく，慢性期になると次第に正常に近づいた。

これは水頭症病態において，アセチルコリンに対する supersensitivity あるいはアセチルコリン減少に対する代償作用を示すものと考えられる。Neurotransmitter receptor の specific binding は，positron emission CT による水頭症，正常圧水頭症，痴呆の診断・治療の基礎となるものとする。