Mapping of cholinergic muscarinic receptor activation with pharmacological MRI

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Introduction

The central cholinergic system and cholinergic muscarinic receptor activation have long been associated with cognitive function. Recently, the cholinergic system has also been linked to traumatic brain injury, cerebrovascular disease and related disorders such as vascular dementia [1, 2]. Cerebral cholinergic neurons and their ascending projections are particularly vulnerable to acute and chronic traumatically mediated dysfunction [3]. They are mainly located in the basal forebrain and send their projections to different structures including the cortex. Acetylcholine (Ach) is synthesized in presynaptic nerve terminals, and exerts its action through binding to both nicotinic and muscarinic acetylcholine receptors (mAChR). It has been suggested that Ach and mAChR regulate growth, differentiation, and plasticity in the cortex of the developing central nervous system [4, 5], and thus, may also be involved in plastic changes after acquired brain damage. The aim of the current study is to assess the feasibility of pharmacological MRI (phMRI[6]) to detect *in vivo* cholinergic neuronal activity in rat brain.

Methods

Adult male Lewis rats (320-380g, n=10) were anesthetized with isoflurane and endotracheally intubated. A lateral tail vein was cannulated. Then the animals were placed in a stereotaxic head frame and mechanically ventilated with 3% isoflurane in O_2/N_2O (1/2). Vital functions were monitored by pulse oximetry and capnography. BOLD MRI was performed at 4.7 T (SISCO/Varian systems) using a gradient echo multi-slice sequence: TR= 300 ms; TE = 17.5 ms; pulse angle = 41°; data matrix = 64 x 64; field-of-view = 35 x 35 mm^2; 16 1.2-mm slices. After 10 minutes of baseline measurements, pilocarpine (2.5 mg/kg) was intravenously administered (n = 10). One animal received injection of saline beforehand. In two animals, the pilocarpine-induced peripheral muscarinic effects were blocked by injection of methyl-scopolamine (0.2 mg/kg, i.v.). MRI was continued up to 60 minutes following pilocarpine injection. Brain activation maps were generated using a Student's t-test that compared baseline measurements to the first 10 minutes after injection (threshold for statistical significance: p0.01 with Bonferroni correction). Arterial blood pressure response to pilocarpine was recorded off-line (n=5).

Results

Injection of pilocarpine was followed by a significant increase in BOLD signal intensity in several brain regions: basal forebrain, cortex, hippocampus and thalamus (Fig.1). Signal increase started immediately after injection and lasted for 15-60 minutes (i.e. the end of the experiment). Saline injection did not result in significant signal changes. After blocking of peripheral muscarinic effects with methyl-scopolamine, significant brain activation was observed only in the cerebral cortex.

Blood pressure measurements showed an increase in mean arterial blood pressure (MABP) from 70 mmHg (SD=1.7 mmHg) to a maximum of 145 mmHg (SD=6.5 mmHg) at 3 minutes after injection of pilocarpine. Thereafter, MABP gradually returned back to baseline values within 20-30 minutes. This increase in MABP was reduced to limits within cerebral vascular autoregulation after peripheral muscarinic receptor blockage (MABP = 70-100 mmHg). Blood pressure response was temporally different from BOLD signal response in activated regions.

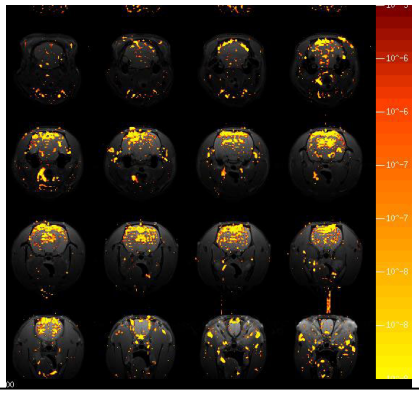

Fig.1: Activation map after pilocarpine injection

Discussion

After intravenous injection of pilocarpine, a non-selective muscarinic receptor agonist, phMRI demonstrated regional activation in basal forebrain, cortex, hippocampus and thalamus. This brain activation pattern corresponds to the cholinergic muscarinic receptor distribution in rat brain (Fig.2). Activation in these regions was reduced by blockage of the peripheral muscarinic receptors. Despite the pilocarpine-induced rise in blood pressure, the spatial and temporal pattern of the BOLD signal increases suggests a selective neuronal activation pattern. In conclusion, this study demonstrates the feasibility of phMRI to assess cholinergic neuronal activation *in vivo*, which provides a basis for future studies on plasticity of cholinergic networks in relation to brain injury.

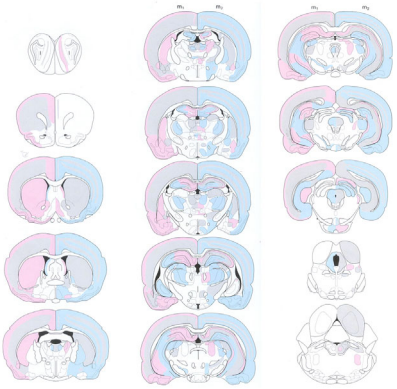

Fig.2: Muscarinic m1/m2-receptor binding sites. (Atlas of Neuroactive Substances and Their Receptors in the Rat. Tohyama & Takatsuji,

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