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Carbon-11 labeled papaverine as a PET tracer for imaging PDE10A: radiosynthesis, in vitro and in vivo evaluation

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Abstract

Papaverine, 1-(3,4-dimethoxybenzyl)-6,7-dimethoxyisoquinoline, a specific inhibitor of phosphodiesterase (PDE) 10A with IC_{50} values of 36 nM for PDE10A, 1,300 nM for PDE3A and 320 nM for PDE4D, has served as a useful pharmaceutical tool to study the physiological role of PDE10A. Here, we report the radiosynthesis of [^{11}C]papaverine and the in vitro and in vivo evaluation of [^{11}C]papaverine as a potential positron emission tomography (PET) radiotracer for imaging PDE10A in the central nervous system (CNS). The radiosynthesis of papaverine with ^{11}C was achieved by *O*-methylation of the corresponding des-methyl precursor with [^{11}C]methyl iodide. [^{11}C]papaverine was obtained with ~70% radiochemical yield and a specific activity >10 Ci/μmol. In vitro autoradiography studies of rat and monkey brain sections revealed selective binding of [^{11}C]papaverine to PDE10A enriched regions: the striatum of rat brain and the caudate and putamen of rhesus monkey brain. The biodistribution of [^{11}C]papaverine in rats at 5 min demonstrated an initially higher accumulation in striatum than in other brain regions, however the washout was rapid. MicroPET imaging studies in rhesus macaques similarly displayed initial specific uptake in the striatum with very rapid clearance of [^{11}C]papaverine from brain. Our initial evaluation suggests that despite papaverine's utility for in vitro studies and as a pharmaceutical tool, [^{11}C]papaverine is not an ideal radioligand for clinical imaging of PDE10A in the CNS. Analogs of papaverine having a higher potency for inhibiting PDE10A and improved pharmacokinetic properties will be necessary for imaging this enzyme with PET.

Keywords: PDE10A; PET; Radiotracer; CNS; Phosphodiesterase 10A; C-11; Papaverine

1. Introduction

Phosphodiesterases (PDEs) are a class of intracellular enzymes involved in the hydrolysis of the nucleotides cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphates (cGMP) into their respective nucleotide monophosphates. cAMP and cGMP function as intracellular second messengers regulating many intracellular processes particularly in neurons of the central nervous system (CNS). A major mechanism for regulating cyclic nucleotide signaling is by phosphodiesterase-catalyzed cyclic nucleotide catabolism. There are 11 known families of phosphodiesterases encoded by 21 different genes [1–3]. PDE10A is a dual specificity phosphodiesterase that can convert both cAMP to adenosine monophosphate (AMP) and cGMP to guanosine monophosphate; PDE10A is uniquely localized in mammals relative to other PDE families. PDE10A mRNA is reported to be highly expressed in the brain, particularly in striatal medial spinal neurons, with variable expression seen in the testes [1,4]. In human brain, high expression of PDE10A was found in caudate nucleus and putamen of striatum. In other mammalian species PDE10A is enriched in the striatal complex including caudate nucleus, nucleus accumbens and olfactory tubercle [3,5–7]. Outside the brain, PDE10A distribution is limited to a few kinds of tissue, such as the testis, epididymal sperm and enteric ganglia. PDE10A is primarily membrane-bound and it is most often associated with membranes in dendrites and spines of medium spiny neurons, which suggests that PDE10A enables the regulation of intracellular signaling from glutamatergic and dopaminergic input to these neurons [3].

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Papaverine was identified as a specific inhibitor of PDE10A with an IC_{50} value of 36 nM for PDE10A and IC_{50} values of 1,300 nM for PDE3A and 320 nM for PDE4D [8]. Papaverine proved to be a useful pharmacological tool for investigations of the behavioral effects of PDE10A enzyme inhibition in rodents. Systemic administration of papaverine to genetically modified mice demonstrated inhibition of PDE10A activity and resulted in increased activation of the medial spinal neurons that led to suppression of behavioral responsiveness. The inhibition of PDE10A with papaverine increased the effectiveness of haloperidol-induced catalepsy in rats and inhibited conditioned avoidance responding in mice [8,9]. Papaverine also inhibits the locomotor hyperactivity induced by stimulants [6]. Furthermore, papaverine displayed anti-schizophrenic activity and anti-psychotic activity in different animal models of neurological disorders [8]. As an inhibitor of PDE10A, papaverine not only helped define the physiological role of PDE10A, but it also provided evidence to support the hypothesis that the inhibition of PDE10A mediated cyclic nucleotide hydrolysis might be an effective new approach for the treatment of schizophrenia and other disorders of basal ganglia function.

Positron emission tomography (PET) imaging with appropriate tracers is a highly sensitive non-invasive imaging modality that can measure the densities of neuronal receptors in CNS and thus provide neurological information regarding molecular and cellular function in living subjects. Up to now, no suitable PET tracer for imaging PDE10A enzyme activity has been reported, thus the measurement of PDE10A activity is currently limited to the use of ex vivo immunohistochemistry techniques in tissue. Although radioactive [^{3}H]papaverine [10] and [^{14}C]papaverine [11] have been made for pharmacologic studies, the radiosynthesis of [^{11}C]papaverine and the evaluation of [^{11}C]papaverine as a PET tracer for imaging PDE10A have not yet been reported. Here, we report the radiosynthesis of [^{11}C]papaverine and the initial in vitro and in vivo evaluation of [^{11}C]papaverine as a PET tracer for imaging PDE10A. Papaverine possesses four *O*-methyl groups that can be readily labeled with carbon-11 via *O*-alkylation of the *des*-methyl precursors. We chose 1-(4-(benzyloxy)-3-methoxybenzyl)-6,7-dimethoxyisoquinoline, **4**, as precursor for radiosynthesis of [^{11}C]papaverine, which is based on this position is easy to be metabolized. Metabolic demethylation from this labeling position will result that the metabolite lose the radioactivity carbon-11, which can avoid the formation of radiolabeled metabolites that might cross the blood brain barrier and interrupt measurement of [^{11}C]papaverine in the brain. In this paper, we report the synthesis of precursor **4** and the radiolabeling conditions used to prepare [^{11}C]papaverine via *O*-alkylation of **4** with [^{11}C]methyl iodide. In vitro autoradiographic studies on both rat and macaque brain sections were conducted with [^{11}C]papaverine; the biodistribution and regional brain uptake studies of [^{11}C]papaverine was evaluated in mature male Sprague–Dawley rats. MicroPET brain imaging was carried out using male rhesus macaques. The metabolite studies were performed on rat blood and rat brain post injection of [^{11}C]papaverine. The results of these studies indicated that although [^{11}C]papaverine binds to PDE10A-rich regions of the brain in vitro, the rapid washout of the tracer limits its utility as PET radiopharmaceutical for in vivo measurements of PDE10A.

2. Materials and methods

2.1. Chemistry

2.1.1. General

All analytical grade chemicals and reagents were purchased from Sigma-Aldrich (Milwaukee, WI, USA) and were used without further purification unless otherwise stated. Flash column chromatography was conducted using Scientific Adsorbents, Inc. silica gel, 60a, "40 Micron Flash" (32–63 μm). ^{1}H NMR spectra were recorded at 300 MHz on a Varian Mercury-VX spectrometer. All chemical shift values are reported in ppm (δ).

*2.1.2. 2-(4-(benzyloxy)-3-methoxyphenyl)-N-(2-(3,4-dimethoxyphenyl)-2-hydroxyethyl)-acetamide (**2**)*

A mixture of 2-(4-(benzyloxy)-3-methoxyphenyl)acetic acid, **1** (0.5 g, 1.83 mmol) in 3 ml of thionyl chloride was refluxed for at least 3 h, then the excess thionyl chloride was evaporated under reduced pressure. The residue was dissolved in 8 ml of chloroform, then added dropwise into a pre-cooled mixture of 8 ml chloroform ($CHCl_3$) with 2-amino-1-(3,4-dimethoxylphenyl)ethanol hydrochloride (0.42 g, 1.80 mmol) and 10 ml of 10% NaOH aqueous solution. The reaction mixture was stirred overnight at room temperature. The organic layer was separated out and the aqueous layer was extracted with dichloromethane (3×10 ml). The dichloromethane extraction solutions were combined and washed sequentially with saturated sodium bicarbonate brine and then dried over sodium sulfate (Na_2SO_4). After removal of the solvent by evaporation under reduced pressure, the crude product was purified on a silica gel column with a mobile phase of hexane/ethyl acetate/methanol (50/50/10) to give a brown solid, **2** (0.60 g, 72%). The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the purified product was 3.20–3.40 (m, 1H), 3.50–3.52 (s, 2H), 3.52–3.61 (m, 1H), 3.85–3.86 (t, 9H), 4.60–4.80 (s, 1H), 5.14–5.15 (d, 2H), 5.80–6.00 (s, 1H), 6.60–6.90 (m, 6H), 7.20–7.50 (m, 5H), 8.02 (s, 1H).

*2.1.3. 1-(4-(benzyloxy)-3-methoxybenzyl)-6,7-dimethoxyisoquinoline (**3**)*

A mixture of compound **2** (0.5 g, 1.1 mmol) and phosphorous oxychloride ($POCl_3$) (0.34 g, 2.2 mmol) in 30 ml acetonitrile was refluxed for at least 2 h. The solvent was evaporated under reduced pressure. 30 ml chloroform was added into the residue and then additional 10 ml of pre-cooled 50% NaOH aqueous solution was added into the reaction flask. The organic layer was separated, and