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# Rapid increase in inositol pentakisphosphate accumulation by nicotine in cultured adrenal chromaffin cells

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When [$^3$H]inositol-prelabeled cultured bovine adrenal chromaffin cells were stimulated with nicotine (10 $\mu$M), a large and transient increase in [$^3$H]-inositol pentakisphosphate ($InsP_5$) accumulation was observed. The accumulation reached the maximum level at 15 s, then declined to the basal level at 2 min. Nicotine also induced [$^3$H]inositol tetrakisphosphate ($InsP_4$) and [$^3$H]inositol hexakisphosphate ($InsP_6$) accumulation with a slower time course and a lesser magnitude than [$^3$H]$InsP_5$. The peaks of [$^3$H]$InsP_4$, [$^3$H]$InsP_5$ and [$^3$H]$InsP_6$ coincided with those of $^{32}$P radioactivity, when cells were doubly labeled with [$^3$H]inositol and inorganic $^{32}$P. These results suggest that inositol pentakisphosphate is rapidly increased by nicotine, a cholinergic agonist, in cultured adrenal chromaffin cells.

Inositol pentakisphosphate; Inositol tetrakisphosphate; Inositol hexakisphosphate; Nicotine; Adrenal chromaffin cell

## 1. INTRODUCTION

Inositol trisphosphates ($InsP_3$) are well-established second messengers for $Ca^{2+}$ release from the endoplasmic reticulum [1–3]. Inositol tetrakisphosphates ($InsP_4$) appear to mediate $Ca^{2+}$ sequestration [4], $Ca^{2+}$ influx [5,6] and membrane current activities [7,8]. Inositol pentakisphosphates ($InsP_5$) exist in mammalian cell types [6,9–12], but have been considered as a 'housekeeping' inositol phosphate rather than an acute second messenger [2]. Therefore, the regulation of biosynthesis and the function remain to be explored. Vallejo et al. [12] have shown that exogenously added $InsP_5$ regulates blood pressure via the central nervous system. This raises the possibility that $InsP_5$ biosynthesis is finely regulated by stimulations of hormones and neurotransmitters. We report here that nicotinic receptor stimulation induces a rapid and large increase in $InsP_5$ in adrenal chromaffin cells.

## 2. EXPERIMENTAL

### 2.1. *Primary culture of bovine adrenal chromaffin cells*

Chromaffin cells were isolated from fresh bovine adrenal medulla as described previously [14,15]. The chromaffin cells were purified by differential plating [15] and were plated on 35-mm diameter dishes ($2.5 \times 10^6$ cells/dish) in 3 ml of minimum essential medium (Gibco; Gland Island, NY), supplemented with 10% fetal calf serum (Hyclone; Logan, UT). The purity of chromaffin cells was over 95%. They were cultured at 37°C in an atmosphere of 95% air/5% $CO_2$. The chromaffin cells were used for experiments 5 days after plating.

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### 2.2. *Measurement of inositol phosphate accumulation*

The cells were labeled with [$^3$H]inositol (50 $\mu$Ci/ml) in 35-mm diameter dishes for 2 days, as described previously [14–17,20]. In some experiments, they were double-labeled with [$^{32}$P]$H_3PO_4$ (50 $\mu$Ci/ml) and [$^3$H]inositol (50 $\mu$Ci/ml). The culture medium was aspirated and was replaced by Locke's solution, consisting of (in mM): 154 NaCl, 5.6 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 3.6 $NaHCO_3$, 5.6 glucose, 10 Hepes and 0.1% bovine serum albumin (pH 7.4). The cell sheets were washed 3 times with Locke's solution. The cells were preincubated at 37°C for 20 min in 10 mM LiCl-containing Locke's solution. Nicotine solution was added to the incubation mixture and the cells were incubated for various periods. At the end of the incubation, the Locke's solution was aspirated and inositol phosphates were extracted as described previously [14–17,20]. Isomers of inositol polyphospates were separated by high-performance anion exchange chromatography according to the method of Balla et al. [18]. In brief, the samples were subjected to HPLC with an Adsorbosphere SAX 5-$\mu$m column (Alltech Applied Science Labs) and a linear gradient (1% increase/min) of ammonium phosphate (pH 3.35, with phosphoric acid at 25°C), at a flow rate of 1 ml/min. The effluent radioactivity was monitored by an on-line flow detector (Packard Japan, Tokyo, Japan).

### 2.3. *Chemicals*

The following chemicals were obtained from the companies indicated: myo-[2-$^3$H]inositol (17 Ci/mmol), [$^{32}$P]$H_3PO_4$ from Amersham International, Buckinghamshire, England; myo-[1-$^3$H]inositol 1,4,5-trisphosphate (17 Ci/mmol), myo-[1-$^3$H]inositol 1,3,4-trisphosphate (17 Ci/mmol), myo-[1-$^3$H]inositol 1,3,4,5-tetrakisphosphate (17 Ci/mmol), myo-[2-$^3$H]inositol hexakisphosphate (12 Ci/mmol) from New England Nuclear, Boston, MA. The standard of [$^3$H]$Ins(1,3,4,5,6)P_5$ was generously given by Dr P.T. Hawkins, Department of Cellular Pharmacology, Smith Kline and French Research, The Frythe, Welwyn, Herts, England.

## 3. RESULTS AND DISCUSSION

[$^3$H]$Ins(1,4,5)P_3$ and [$^3$H]$Ins(1,3,4)P_3$ were eluted at 0.3 M ammonium phosphate (fig.1) under the condi-



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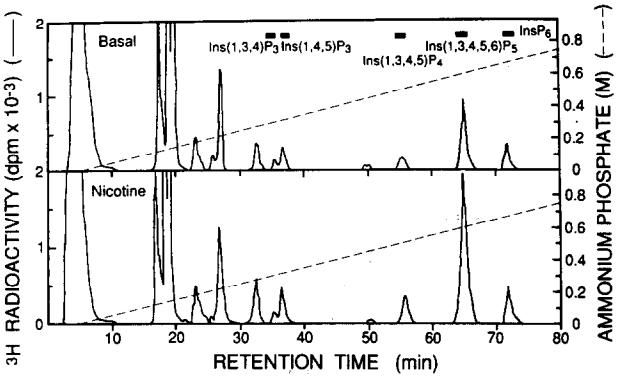


Fig.1. Elution profile of [$^3$H]inositol phosphates produced in intact adrenal chromaffin cells and separated by high-performance anion exchange chromatography. Upper panel, basal levels. Lower panel, stimulation by 10 $\mu$M nicotine. Each [$^3$H]inositol phosphate standard was eluted from the column as one major peak at indicated positions (▬). Typical data obtained from 5 different cell preparations are presented.

tions we used, consistent with the results of Balla et al. [18], who used a similar gradient. The peak at 0.5 M ammonium phosphate was considered as [$^3$H]InsP$_4$, since the standard, [$^3$H]Ins(1,3,4,5)P$_4$, was eluted at the same retention time and Balla et al. also observed [$^3$H]InsP$_4$ in the identical position [18]. The peak at 0.6 M ammonium phosphate was considered as [$^3$H]InsP$_5$; first, the retention time was identical with

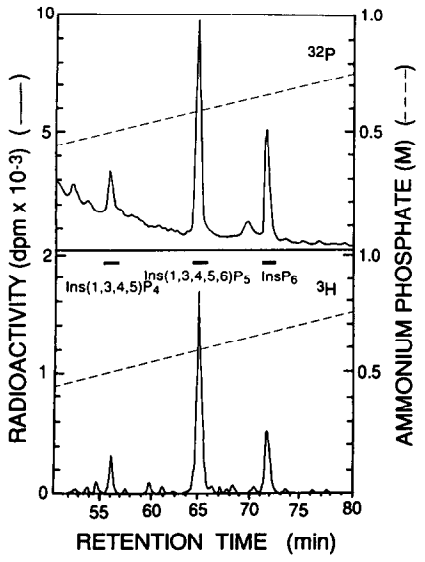


Fig.2. Elution profile of [$^3$H]InsP$_4$, [$^3$H]InsP$_5$ and [$^3$H]InsP$_6$ labeled with $^{32}$P and produced in intact adrenal chromaffin cells. Intact chromaffin cells were labeled with 50 $\mu$Ci/ml myo-[2-$^3$H]inositol and 50 $\mu$Ci/ml [$^{32}$P]$H_3PO_4$ for 2 days. Under these conditions, $^{32}$P counts with the retention time corresponded to Ins(1,3,4)P$_3$ and Ins(1,4,5)P$_3$ contained many peaks and could not be assigned to each InsP$_3$ peak. Data from unstimulated cells are shown. Each [$^3$H]inositol phosphate standard was eluted from the column as one major peak at indicated positions (▬). Upper panel, $^{32}$P radioactivity. Lower panel, $^3$H radioactivity. Typical data obtained from two different cell preparations are presented.

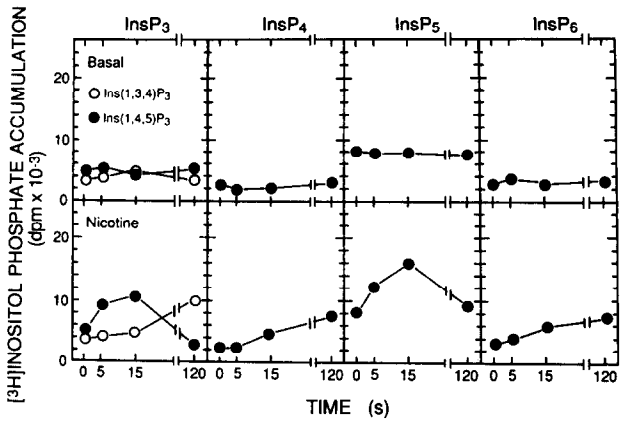


Fig.3. Time course of [$^3$H]InsP$_3$, [$^3$H]InsP$_4$, [$^3$H]InsP$_5$ and [$^3$H]InsP$_6$ accumulation in intact adrenal chromaffin cells. Upper panels, basal levels. Lower panels, stimulation by 10 $\mu$M nicotine. The experiments were repeated twice and gave similar results.

that of a standard, [$^3$H]Ins(1,3,4,5,6)P$_5$. Second, this $^3$H peak coincided with that of $^{32}$P (fig.2). The more polar [$^3$H]inositol- and $^{32}$P-containing material may be [$^3$H]InsP$_6$, since the standard, [$^3$H]InsP$_6$, was eluted at the same position.

Stimulation with nicotine increased InsP$_3$ in the chromaffin cells (fig.3), consistent with previous reports [14,17,19]. Nicotine induced a large and rapid increase in InsP$_5$, which peaked at 15 s after stimulation. This suggests the presence of active degradative pathways as well as the stimuli-sensitive productive ones. InsP$_4$ and InsP$_6$ were also increased with a slower time course and lesser magnitude than InsP$_5$. Recently, Pittet et al. [21] have reported that InsP$_5$ is accumulated by chemotactic peptides in HL60 cells. However, in HL60 cells, the InsP$_5$ accumulation occurs more slowly than Ins(1,4,5)P$_3$ does. In chromaffin cells, the agonist-stimulated increase in Ins(1,4,5)P$_3$ is small [14,15,17]. In fact, InsP$_5$ is the most pronounced inositol phosphate identified so far in the chromaffin cells. Furthermore, the time course was comparable to Ins(1,4,5)P$_3$. A current hypothesis proposes that InsP$_5$ is formed by phosphorylation of InsP$_4$ [22,23]. Alternatively, it is possible that InsP$_5$ is generated independently of InsP$_4$ formation, although more rigorous research is necessary for establishing this possibility. Although inositol pentakisphospate-containing phospholipids have not been shown, it is possible that such phospholipids exist in mammalian plasma membranes, as Heslop et al. [9] discussed.

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