Method for Simultaneous Determination of Creatine Phosphate and Adenine Nucleotides in the Intestinal Smooth Muscle of Guinea-pig Taenia Caeci using High-performance Liquid Chromatography

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Abstract

Endo, I., Utsugi, M., Suzuki, T. and Karaki, H. *Method for simultaneous determination of creatine phosphate and adenine nucleotides in the intestinal smooth muscle of guinea-pig taenia caeci using high-performance liquid chromatography.* Japanese Journal of Smooth Muscle Research 24 (2) 127-130, 1988———A method for simultaneous determination of adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP) and creatine phosphate (PCr) by high-performance liquid chromatography is described. This method was applied to the isolated intestinal smooth muscle tissue of guinea pig taenia caeci weighing approximately 30 mg. It was found that one g of the muscle tissue contained 3.55 μmol PCr, 2.40 μmol ATP and 0.477 μmol ADP.

Introduction

Muscle contraction is dependent on the source of free energy driving contractile filaments and other active processes. This source includes adenosine 5'-triphosphate (ATP) and other so-called high-energy phosphates such as creatine phosphate (PCr). In smooth muscle, this source is so small that, even under resting conditions, the preformed energy source could suffice for only a few minutes in the absence of ATP synthesis via intermediary metabolism. Thus, the contractility of smooth muscle is strongly related to the amount of available energy and it is quite important to know the changes in energy metabolism to understand the smooth muscle function (for review see Hellstrand and Paul, 1982).

ATP and PCr contents of smooth muscle have been separately determined utilizing the luciferine-luciferase luminescence (Jabs *et al.*, 1977; Karaki *et al.*, 1982). Recently, methods for simultaneous determination of PCr and adenosine nucleotides were reported using high-

Accepted for publication, March 22, 1988

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performance liquid chromatography (HPLC) in cardiac muscle (Harmsen *et al.*, 1982; Bedford and Chiong, 1984). In the present report, a sensitive, simple and reliable method is described for simultaneous determination of PCr and adenine nucleotides in the intestinal smooth muscle of guinea-pig taenia caeci.

Methods

Equipment

The HPLC system was a Trirotor III (Japan Spectroscopic Co. Ltd., Tokyo) consisting of a pump, a system controller, a variable loop injector (VL 613), and a wave length-selectable detector (Univec 100 III) set at 230 nm.

Chromatography was performed by an anion-exchange column (4 mm internal diameter and 50 mm length) packed with Shim-pack WAX-I (Shimazu, Tokyo) using mobile phases of 2 mM KH_2PO_4 (pH 5.0, buffer A) and 750 mM KH_2PO_4 (pH 5.0, buffer B). Flow rate was 1.0 ml/min and the elution started with 100% buffer A. In the first 20 min, buffer B increased at a rate of 0.5%/min, followed by an increase of 18% buffer B/min during the next 5 min. This was followed by elution with 100% buffer B for 10 min and then with 100% buffer A for 40 min. These procedures were programmed with system controller. The sensitivity of the detector was usually set at 0.16 a.u.f.s. and the oven temperature at 45°C.

Reagents, standards and procedure

PCr (disodium salt hydrate), ATP (disodium salt hydrate), adenosine 5'-diphosphate (ADP, potassium salt hydrate), and adenosine 5'-monophosphate (AMP, sodium salt hydrate) were purchased from Sigma Chemical Co. Ltd., Dorset, U.K.

Male, Hartley guinea-pigs, weighing approximately 250 g, were stunned and bled and taenia strips of approximately 30 mg weight were dissected from caecum. The muscle strips were equilibrated for 60 min in a physiological salt solution of the following composition (in mM): NaCl, 136.9; KCl, 5.4; CaCl_2 , 2.5; MgCl_2 , 1.0; NaHCO_3 , 24.0; and glucose, 11.1. This solution was continuously bubbled with a mixture of 95% O_2 and 5% CO_2 at 37°C and pH 7.4. After a 60 min incubation period, muscle strips were quickly frozen in acetone dry ice at -78°C and stored at -80°C until analysis.

Frozen muscle strips were homogenized with Tenbroeck tissue grinder (Wheaton, USA) in 2.0 ml of 0.9 M perchloric acid at 4°C for approximately 5 min. The homogenate was centrifuged at 2,000 g for 5 min at -10°C and supernatant was neutralized with ice-cold 3.75 M K_2CO_3 . The neutralized extract was centrifuged at 2,000 g for 5 min at -10°C to remove insoluble potassium perchlorate and a 200 μl aliquot was applied to the HPLC.

Results

Fig. 1A shows the separation and sharpness of the peaks in a chromatogram of a standard solution containing 20.0 nM/200 μl PCr, 10.0 nM/200 μl ATP, 2.5 nM/200 μl ADP and 2.0 nM/200 μl AMP. The duration of the run was 55 min. The limits of detection, based on the signal-to-noise ratio of 3, were 0.136 nmol for PCr, 0.029 nmol for ATP, 0.011 nmol for ADP and 0.014 nmol for AMP.

Figure 1. Chromatogram of standards (A) and an extract of intestinal smooth muscle (B). The duration of run time was approximately 55 min. PCr : creatine phosphate. ATP, ADP and AMP : adenosine 5'-tri-, di- and monophosphate, respectively.

A typical chromatogram of an extract of the intestinal smooth muscle of the guinea-pig taenia is shown in Fig. 1B. In this preparation, peak for AMP was not identified because of the interference by impurities in the extract. Averaging the results of similar experiments, it was found that the taenia strips contained $3.55 \pm 0.40 \mu\text{mol PCr/g wet weight}$ ($n=6$), $2.40 \pm 0.09 \mu\text{mol ATP/g wet weight}$ ($n=6$) and $0.477 \pm 0.023 \mu\text{mol ADP/g wet weight}$ ($n=6$).

Discussion

In the present chromatographic method, PCr, ATP and ADP were simultaneously determined with a sensitivity which is high enough to detect these compounds contained in a small piece of the isolated intestinal smooth muscle tissue of the guinea-pig taenia caeci. The values obtained with this method were similar to those measured by other methods (Butler and Davies, 1980 ; Karaki *et al.*, 1982). Although slight changes in base line were regularly observed, the magnitude of which was almost the same in each run and this change did not interfere with the determination of these compounds. By shortening the run time and by looking for the conditions that simultaneously determine creatine as well and that separate AMP from other impurities, the use of HPLC may provide a more convenient method to examine the energy metabolism in smooth muscle and other tissues.

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