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BRADYKININ-STIMULATED DIFFERENTIAL INCORPORATION OF ARACHIDONIC ACID INTO LIPIDS OF KIDNEY CORTEX AND MEDULLA*

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Abstract—We investigated bradykinin-induced changes in the turnover of arachidonate in renal lipids of the perfused rabbit kidney. Upon hormone stimulation, this cellular system undergoes only transient dynamic changes in arachidonic acid metabolism; no loss of bradykinin effect on arachidonate release and prostaglandin generation is shown upon repeated hormone administrations durilng 8–9 hr of perfusion. Ureter-obstructed rabbit kidneys were perfused for 5–6 hr and then saline or bradykinin in saline was administered, followed after 10 sec by pulse labelling with [^{14}C]arachidonate. The pattern of distribution of [^{14}C]arachidonate in lipid fractions of the cortex showed that bradykinin caused a 2 to 2.5-fold increase in the relative incorporation of arachidonic acid into phosphatidylinositol (PI), phosphatidic acid (PA), diglyceride (DG) and triglyceride (TG) fractions and a concomitant decrease in its incorporation into phosphatidylcholine (PC) and phosphatidylethanolamine (PE). In contrast, in the medulla hormone administration caused a marked increase of arachidonate incorporation into PI and PC, and a decrease in incorporation into PE, PA, DG and TG. This differential arachidonate labelling of cortical vs medullary lipids following bradykinin stimulation suggests that the hormone activates different lipolytic processes in cortex and medulla, and promotes hydrolysis of arachidonic acid from different phospholipid pools.

The first step in the biochemical sequence for generation of prostaglandins and other oxygenated products from endogenous, lipid-esterified arachidonic acid is the release of the acid from cellular lipids. The amounts of arachidonate-derived oxygenated products produced by mammalian tissues and cells in response to various stimuli are very small (1% or less) compared to the total amount of tissue arachidonate. This suggests that arachidonic acid deacylation following specific agonist stimulation is a selective lipolytic process in which only certain lipids in specific agonist-sensitive arachidonic acid pool(s) contribute the acid for oxygenated products synthesis. The unique properties and chemical identity of this pool have been the subject of recent investigations. Studies with mouse tumour cells [1], several transformed fibroblasts [2] and platelets [3–6] suggested that PI, PC and, possibly, PE serve as lipid donors from which arachidonic acid is released following stimulation with bradykinin, thrombin and other agonists. However, these studies have employed high doses of the agonists, causing substantial deacylation of total tissue arachidonate and irreversible structural and/or metabolic changes in the cells affected.

Recent studies by us in the perfused rabbit kidney [7–9] have demonstrated the presence of a hormone-sensitive lipid pool which contributes arachidonate for prostaglandin generation. In this experimental system, the kidney can be stimulated repeatedly with physiological doses of peptide hormones (bradykinin, angiotensin II) without any loss of organ responsiveness to the release of arachidonate and prostaglandin E_2. The main properties of the renal, hormone-sensitive system are (1) selective release of arachidonic acid in response to peptide hormone stimulation; (2) slow incorporation of exogenously-added arachidonate to the hormone-sensitive pool, dependent on prior hormone-stimulated depletion of arachidonate from this pool; and (3) a hormone-induced lipolytic process coupled to a prostaglandin synthase system, with a major portion of the released acid being converted into oxygenated products. These properties indicate that a more selective labelling of the hormone-sensitive pool with radioactive arachidonic acid could be achieved by administration of the labelled acid immediately after hormone stimulation. We employed this technique to identify the hormone-induced changes in arachidonic acid turnover in renal lipids, and from it deduced the lipolytic mechanisms activated by bradykinin and the nature of the lipids which contribute arachidonate for prostaglandin generation.

MATERIALS AND METHODS

Isolated perfused kidney. Male rabbits, 2.5–3 kg (local strain derived from New Zealand White), were used. Ureter-obstructed kidneys were prepared and perfused via the renal artery with Krebs–Henseleit buffer (pH 7.4, 37°) at the rate of 15 ml/min as described previously [7].

Pulse labelling of kidney lipids with radioactive arachidonic acid. Ureter-obstructed kidneys were

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perfused for 5–6 hr with periodic administrations of bradykinin (1 μg in 0.1 ml saline) to verify development of enhanced bradykinin stimulation of prostaglandin E_2 generation during the perfusion time course. The experiment was then begun by injecting saline or bradykinin into the kidney (10 μg in 0.1 ml saline). Ten seconds later, [^{14}C]arachidonate (1 μCi dissolved in 0.5 ml of Tris–HCl buffer, pH 8.2) was gradually injected into the kidney-perfusing media over a 30-sec period. The kidney effluent obtained during the arachidonate injection (approximately 7–8 ml) was recirculated through the kidney for a 60-sec period and the kidney was then removed from the perfusion apparatus and quick-frozen in liquid air.

Analysis of lipid composition. The frozen kidneys were quickly halved and the cortex and medulla separated, homogenized in 20 ml Tris–HCl buffer (0.1 M, pH 8.0) and extracted with chloroform–methanol (2:1) as described previously [10]. The extracts were subjected to thin-layer chromatography on silica gel G plates (petroleum ether–diethyl ether–acetic acid, 70:30:2, v/v/v) to separate the neutral lipid fractions from the phospholipid fraction. The phospholipid zone (origin of plate) was extracted with chloroform–methanol (2:1, v/v) and the concentrated extract subjected to thin-layer chromatography on silica gel G plates (chloroform–methanol–acetic acid–H_2O, 100:20:12.5:5, v/v/v/v). PA was clearly separated from PE. PI and phosphatidyl serine were separated by additonal chromatography on silica gel G, chloroform–acetone–methanol–acetic acid–H_2O (10:4:2:2:1, v/v/v/v/v). The radioactivity in each zone was determined by liquid scintillation spectrometry.

Materials. [1-^{14}C]Arachidonic acid (specific activity 55 mCi/mole) was obtained from the Radiochemical Centre (Amersham, U.K.). Lipid standards for thin-layer chromatography (various phospholipids; tripalmitin; 1,2-dipalmitin; 1-monopalmitin) were obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical grade.

RESULTS AND DISCUSSION

Slow infusion of [^{14}C]arachidonate into the perfused ureter-obstructed rabbit kidney results in 80–85% incorporation of the radioactive acid into renal lipids, mainly into PE (44%), PC (25%), PI (15%) and TG (7%) fractions. During subsequent perfusion for 6 hr, the ureter-obstructed kidney becomes super-sensitive to bradykinin or angiotensin II stimulated PGE_2 release. Under these conditions, a dose of 2–5 μg of either hormone causes deacylation of approximately 20–50 μg arachidonate from renal lipids [8]. Furthermore, in a kidney initially prelabelled with radioactive arachidonic acid and then periodically stimulated with bradykinin or angiotensin II during the perfusion, the specific activity of the released PGE_2 increases, indicating a hormone-dependent transfer of the radioactive acid from hormone-insensitive to hormone-sensitive pool(s) [9].

We investigated several experimental approaches in an attempt to (1) identify the renal lipid fraction(s) which serves as precursor pool(s) for arachidonate released following specific hormone stimulation, and (2) examine the lipolytic reactions activated by the hormones. In previous studies of other cellular systems, most notably platelets [3–6], significant changes in the amounts of cellular phospholipids and acylglycerols were demonstrated following stimulation with specific agonists (thrombin, Ca^{2+}-ionophore A23187), suggesting agonist activation of specific lipolytic enzymes. These studies, however, involved the use of high agonist doses, causing drastic or even irreversible metabolic and structural changes in the cells. In the perfused kidney model which we employed, the organ undergoes only transient dynamic changes in arachidonate metabolism upon stimulation with physiological amounts of bradykinin, thus reflecting more closely the *in vivo* homeostatic conditions. However, under these conditions, the hormone-induced increased turnover of phospholipid arachidonate is very small compared to the total tissue arachidonate. Thus, the possible significant changes in the composition of specific hormone-sensitive lipids were not manifested when we compared the total amounts of cortical or medullary lipids (PC, PI, PE, PA, lysoPA, DG, TG) before and after bradykinin administration (data not shown).

The experimental approach which we finally adopted is based on the fact that the turnover of arachidonic acid in the hormone-sensitive pool is selectively and drastically enhanced during the 10–60 sec following hormone administration [7,9,11] and that the incresed incorporation of free arachidonate to replenish the just-depleted lipid pool is part of the overall hormone-induced event [8,12]. The experimental protocol thus involved pulse-labelling of the kidney lipids with radioactive arachidonate immediately following stimulation with the hormone, as detailed in Materials and Methods. Control kidneys were given saline injections and then similarly labelled with radioactive arachidonate and frozen.

The distribution of esterified [^{14}C]arachidonate among the lipid classes of the cortex and medulla is given in Fig. 1. In the cortex of control kidneys, arachidonic acid was incorporated mainly into PE, PC, PI, DG and TG. Bradykinin administration prior to arachidonate labelling produced marked changes in the cortical lipid distribution, producing a 2 to 3-fold increase in arachidonate's incorporation into PA, PI, DG and TG, and a concomitant decrease in its incorporation into PC and PE. The hormone-induced changes in arachidonate incorporation into medullary lipids were quite different from those observed for cortical lipids. In medulla from control kidneys, arachidonate was incorporated mainly into PE, DG, TG, PI, PA and PC. Bradykinin administration caused a dramatic increase in arachidonate's incorporation into medullary PI and PC, and a significant decrease in its incorporation into PE, PA and DG.

Several lipolytic activities have been previously suggested to participate in the agonist-mediated deacylation of arachidonate in various cells. These lipolytic systems can be divided into two distinct types:

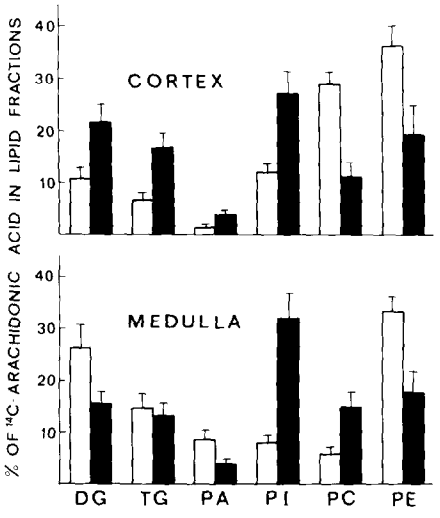

Fig. 1. Pulse incorporation of [^{14}C]arachidonic acid into the perfused rabbit kidney lipids. Pulse infusion of sodium [^{14}C]arachidonate solution (1 μCi, approximately 5.5 μg arachidonic acid) was administered into the kidney immediately after bradykinin injection (10 μg, dark columns) or saline injection (open columns). The kidney was then quick-frozen and analysed for the distribution of [^{14}C]arachidonic acid in the different renal lipids. For further details see Materials and Methods. Results are means ± S.E. of three kidneys in each treatment.

(1) a lipolytic activity which hydrdolyses arachidonate from the 2-position of phospholipids without affecting the phosphate bonding in the phospholipid molecule, and (2) lipolytic activities which release arachidonic acid following prior hydrolysis of the phospho-ester bond on either side of the phosphate group. Lipolytic activity of the first type is exemplified by phospholipase A_2. Action of this enzyme on arachidonic-rich phospholipids (e.g. PI) could account for the selective release of arachidonic acid as seen in the perfused kidney treated with bradykinin or angiotensin II [7]. Lipolytic activities of the second type are those of (i) phospholipase c, followed by diglyceride lipase [6], (ii) phospholipase D to yield PA, followed by phospholipase A_2 [4,13] and (iii) sequential action of phospholipase A_2/C, DG kinase and phospholipase A_2 [14,15]. Common to all the lipolytic sequences of the second type, regardless of the initial phospholipase activity stimulated, is the incorporation of arachidonate into newly formed PA which serves either as precursor for the resynthesis of the depleted phospholipids (e.g. PI) or as precursor for di- and triglycerides. Thus the metabolic indication for enhanced synthesis of PA containing arachidonic acid is the concomitant increase in arachidonic acid incorporation into DG and TG fractions.

Our results show that in the cortex, (1) PI is the major phospholipid into which exogenous arachidonic acid is preferentially incorporated following hormone stimulation, suggesting that this lipid is the chief arachidonic acid donor for hormone-induced prostaglandin generation; and (2) following hormone stimulation, there is a parallel increase in the incorporation of [^{14}C]arachidonic acid into PI, PA, DG and TG, this being supporting evidence for a mechanism which involves the resynthesis of PA, containing newly incorporated arachidonic acid. In contrast, the results of arachidonate incorporation into medullary lipids show that (1) both PI and PC are the main phospholipids into which arachidonate is preferentially incorporated after bradykinin stimulation; and (2) the hormone induces a decrease in the incorporation of arachidonate into PA and DG, supporting a mechanism which does not involve formation of newly synthesized PA, but rather the direct action of phospholipase A_2 on PI or PC.

As the cortex and medulla show considerable difference in the make-up of their cellular populations and in their capacity for synthesis of different prostaglandins (for review see [16]) these varied effects of bradykinin on arachidonate's product generation in cortex vs medulla undoubtedly reflect activation of different lipolytic enzymes in the different types of renal cells which are coupled to subsequent arachidonate oxygenation to cell-specific products. The concept of cell-specific coupled phospholipase–arachidonate oxygenation systems has recently gained support from several laboratories. Studies with the isolated perfused rabbit kidney in our laboratory [7,9] and in that of Needleman and co-workers [17] have demonstrated the presence of a unique lipolytic activity which is selectively sensitive to the peptide hormones, bradykinin and angiotensin II, with release of arachidonic acid tightly coupled to its conversion into PGE_2. Recent studies by Humes and co-workers in mouse peritoneal macrophages [18] have indicated the presence of two distinct processes for agonist-activated generation of cyclooxygenase and lipoxygenase products. These investigators concluded that in macrophages, different lipolytic activities yield arachidonic acid which is derived from different precursor phospholipid pools and which is subsequently coupled to specific oxygenation reactions. The results presented in this work suggest that in the cortex and medulla, a single hormone, bradykinin, may concurrently activate different lipolytic activities which are probably coupled to particular oxygenation enzymes, producing specific biochemical products with unique physio-pharmacological properties.

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