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PURINERGIC VS PEPTIDERGIC STIMULATION OF LIPOLYSIS AND PROSTAGLANDIN GENERATION IN THE PERFUSED RABBIT KIDNEY*

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Abstract—Intact perfused rabbit kidney contains three different systems for generation of arachidonate oxygenated products. Each of these systems is associated with its specific type of activating agonist; the agonists are the vasoactive peptide hormones bradykinin and angiotensin II, the adenine nucleotides ATP and ADP, and exogenous arachidonic acid. The three systems are clearly distinguished by several biochemical parameters which include the type of lipolysis induced and the extent of coupling between the hydrolyzed arachidonic and its conversion to specific oxygenated products. The highest degree of selectivity in the lipolytic process and in the coupling to PGE_2 generation is seen following stimulation with bradykinin or angiotensin II. These hormones induce the hydrolysis of only arachidonic and PGE_2 is the major product. Furthermore, arachidonate hydrolysis is from a unique lipid pool which is characterized by a slow turnover of arachidonic. The entire process of lipolysis and prostaglandin E_2 synthesis is terminated within 1 min after stimulation and is followed by a hormone-induced re-acylation process in which excess released arachidonate is re-esterified into cellular lipids. The adenine nucleotides ATP and ADP induce a less selective lipolytic reaction which results in the hydrolysis of arachidonic and linoleic acids. This lipolytic process is less coupled to arachidonic oxygenation as evident from the 1 min delay between arachidonate release and prostaglandin generation. Arachidonate released by the nucleotides orginates from a lipid pool which has a higher turnover and readily incorporates exogenous acid. Generation of oxygenated products from administered exogenous acid is the least coupled process with apparent conversion of only 1–4% to prostaglandin products, amongst which 6-keto-$PGF_{1\alpha}$ predominates.

The peptide hormones bradykinin and angiotensin II and the adenine nucleotides ATP and ADP stimulate prostaglandin generation when administered to the perfused kidney. Recent studies by us [1, 2] and others [3] have shown that the peptide hormones activate a highly selective lipolytic process which deacylates only arachidonate from a distinct lipid pool. This lipolytic process is tightly coupled to a prostaglandin synthase system so that a major portion (30–50%) of the released arachidonate is converted to prostaglandin E_2. The hormones also induce a reacylation process so that excess released arachidonate is re-esterified into renal lipids.

Recently, we described the presence of different P_2-purinergic receptors for ATP and ADP in rabbit kidney and heart [4]. The present study was designed to contrast the mechanism for prostaglandin generation by several agonists—the peptides hormones bradykinin and angiotensin II, the adenine nucleotides ATP and ADP and exogenous arachidonic acid, with regard to the following parameters.

(a) The properties of the agonist-induced lipolytic process.
(b) The presence of a time-delayed activated reacylation following agonist-induced deacylation of fatty acid(s) from renal lipids.
(c) The nature and the extent of enzymatic coupling between the agonist-induced arachidonate release and its subsequent conversion to agonist-specific prostaglandin products.

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MATERIALS AND METHODS

Isolated perfused kidney system. Male rabbits, 2.5–3 kg (local strain derived from New Zealand White) were used. Ureter-obstructed kidneys were prepared and perfused with Krebs–Henseleit buffer (pH 7.4, 37°) at the rate of 15 ml/min as described previously [1]. Angiotensin II (10 μg/ml), bradykinin (10 μg/ml) ATP and ADP (50 mM) were dissolved in saline (pH adjusted to 7.4) and injected as a bolus (0.1 ml) into the perfusing media just as it enters the cannulated renal artery. In some experiments, arachidonic acid (AA) dissolved in saline (25 μg/ml, pH adjusted to 9.0) was infused into the kidney for 5 min at the rate of 0.2 ml/min (total amount infused, 25 μg). Kidney effluents were collected before and after agonist administration or during AA infusion. The effluents were extracted and assayed for AA and prostoglandin products (see below). Agonist administration or infusions were repeated several times, at various times during the perfusion.

Labelling of perfused kidney with radioactive arachidonic acid. [1-^{14}C]Arachadonic acid (2–2.5 × 10^7 cpm) was dissolved in 3 ml saline (pH

Table 1. Profile of prostaglandin products released in response to agonist stimulation

Prostaglandin products*	Agonist		
	Bradykinin or angiotensin II	ATP or ADP	Arachidonic acid
6-Keto $PGF_{1\alpha}$	3 ± 2	20 ± 5	50 ± 12
$PGF_{2\alpha}$	<1	14 ± 4	<1
PGE_2	90 ± 5	66 ± 8	35 ± 7
PGD_2	<1	<1	9 ± 3
TxB_2	2 ± 1	<1	6 ± 3
PGE_2 (μg)	3.5 ± 0.5	1.5 ± 0.4	0.5 ± 0.2

* % of total radioactivity in prostaglandin products.

Perfused kidneys were prelabeled with [^{14}C]arachidonic acid as described in Materials and Methods. The kidneys were then perfused for an additional 4 hr in order to allow the development of enhanced responsiveness to the agonists. Following that, bradykinin or angiotensin II (1 μg), ATP or ADP (5 μmoles) or infused arachidonic acid was administered into the perfused kidney. Effluent samples (approx. 4 min, 60 ml collection) were obtained before (control) and after agonist administration or during AA infusion and extracted for prostaglandin products. The radioactivity in each prostaglandin zone and the total prostaglandin E_2 content were determined. Values are means ± S.E. (n = 6).

adjusted to pH 9.0–9.5 with NaOH) and infused into the kidney at 0.1 ml/min as described previously [2].

Analysis of kidney effluents. Renal effluent samples were acidified to pH 3.5 with 2 M citric acid and extracted twice with 2 vol. ethyl acetate. The extracts were dried over Na_2SO_4, concentrated *in vacuo* and dissolved in 2 ml chloroform–methanol (2:1). One tenth of the extract was subjected to thin layer chromatography (TLC) and the PGE_2 content determined by bioassay on rat stomach strip [5]. 45% of the extract was analyzed by TLC using system AIX of Hamberg and Samuelsson [6] for separation of various arachidonic acid oxygenated products and the radioactivity in each zone was determined by liquid scintillation spectrometry (Prias, Packard, Downers Grove, IL). The remainder (45% of the extract) was subjected to TLC to separate neutral lipids, and the fatty acid composition of the free fatty acid fraction determined by gas–liquid chromatography [1]. Recoveries of PGE_2 and of free fatty acids were determined by addition to each effluent sample of [^{3}H]PGE_2 (10^5 cpm) and 20 μg 7, 10, 13, 16 docosatetraenoic acid, respectively. The overall recovery for PGE_2 was 50–60% and for fatty acid methyl esters, 30–45%.

Materials. Bradykinin, angiotensin II, ATP and ADP were obtained from Sigma (St. Louis, MO). [1-^{14}C]Arachidonic acid (sp. act., 55 mCi/mmole) and [5,6,8,11,12,14,15 (n) – ^{3}H]prostaglandin E_2 (sp. act. 120 Ci/mmole) were purchased from the Radiochemical Centre (Amersham, U.K.). Arachidonic acid and 7,10,13,16-docosatetraenoic acid were from Supelco (PA). Fatty acid poor bovine serum albumin was from Calbiochem (Los Angeles, CA).

RESULTS

Differential generation of prostaglandin products by bradykinin, angiotensin II, adenine nucleotides and arachidonic acid. During infusion of [^{14}C]arachidonic acid into the perfused rabbit kidney approximately 80–85% of the radioactive acid is incorporated in renal lipids, mostly into phospholipids. Approximately 3–6% of the infused acid appear unchanged in the perfusate and 8–15% found in three main prostaglandin products: 6 keto $PGF_{1\alpha}$ (50%), PGE_2 (35%) and PGD_2 (9%) (Table 1). In contrast, administration of the adenine nucleotides ATP or ADP to the perfused kidney which was prelabeled with [^{14}C]arachidonic acid elicited the release of mainly radioactive PGE_2 (66%) with smaller amounts of $PGF_{2\alpha}$ (14%) and 6-keto $PGE_{1\alpha}$ (20%). The peptide hormone bradykinin or angiotensin II are highly selective agonists for the release of PGE_2.

Differential stimulation of fatty acids release and PGE_2 generation by bradykinin/angiotensin II and by adenine nucleotides. Bradykinin and angiotensin II administered to the perfused kidney induce a selective lipolytic process in which arachidonic acid is hydrolyzed from a specific hormone-sensitive lipid pool [2]. In the perfused kidney system, this hydrolysis of arachidonic acid is tightly coupled to subsequent transformation of the acid to PGE_2, the entire coupled process occurring within 1 min or less (Fig. 1A). Furthermore, arachidonate thus released but not converted to PGE_2 is re-esterified into cellular lipids by a hormone-induced, timely coupled, reacylation process [7]. The adenine nucleotides induce prostaglandin generation by stimulation of a different lipolytic process. This process is characterized by: (a) A selective release of both arachidonic and linoleic acids (Fig. 1B). (b) Compared to the peptide hormone stimulation, there is a longer time lag between nucleotide-induced fatty acid release and PGE_2 generation (Fig. 1B) and (c) subsequent to nucleotide administration, no enhancement in fatty acid reacylation is seen.

Perfusion-dependent increase of PGE_2 release to different agonists. Ureter-obstructed kidneys develop, during perfusion, an increased release of PGE_2 in response to administration of a fixed amount of the peptide hormones bradykinin or angiotensin II. This increase is the result of new, perfusion-

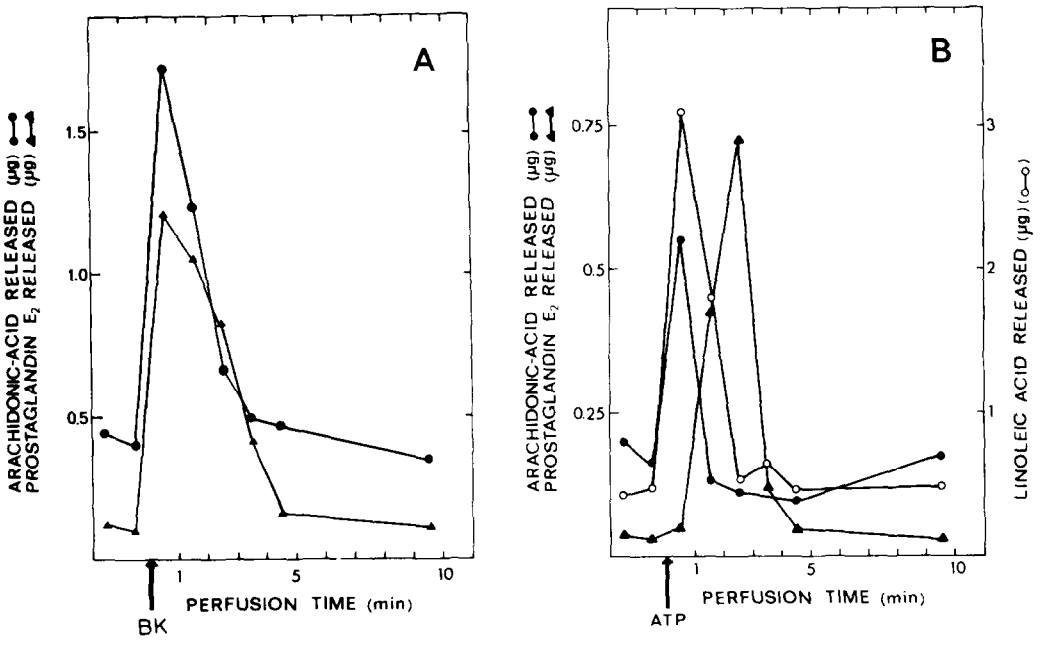

Fig. 1. Differential release of fatty acids and prostaglandin E_2 in response to bradykinin (A) and ATP (B). Ureter-obstructed kidneys were perfused as described in Materials and Methods for 4 hr to allow development of enhanced PGE_2 release in response to the agonists. Bradykinin (BK, 1 μg) or ATP (5 μmoles) was administered as a bolus injection. One minute effluent samples were collected before and after agonist administration. Values of prostaglandin E_2 and fatty acids in each effluent sample were plotted for the middle of each collection period, i.e. at 0.5 min for the period 0–1 min, etc. The values are from a representative experiment. Similar results were obtained in five more experiments.

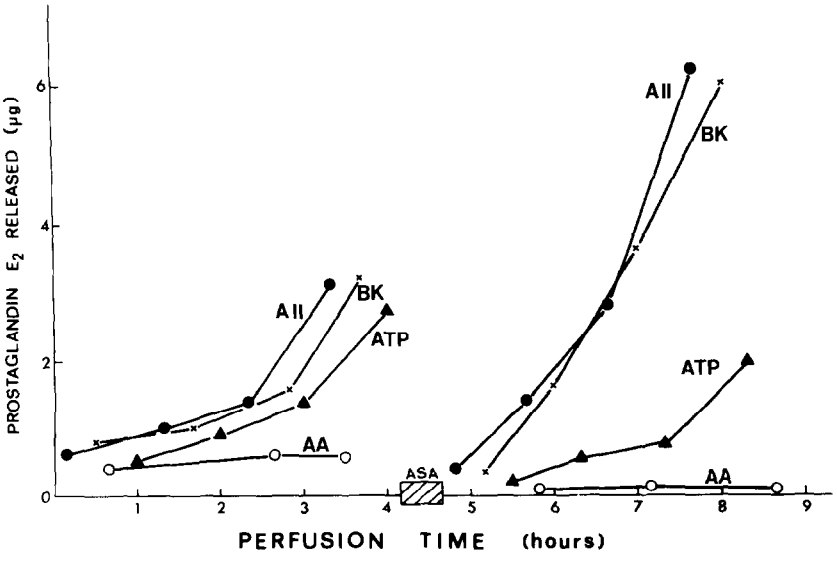

Fig. 2. Time-dependent increase in prostaglandin E_2 release in response to agonist stimulation before and after aspirin administration. Ureter-obstructed kidneys were prepared and perfused. At various times during the perfusion, kidney effluent samples (approx. 4 min, 60 ml collection) were taken, prior to and after bolus administration of bradykinin or angiotensin II (BK and A II, 1 μg), ATP (5 μmoles) or arachidonic acid (AA) infusion. Aspirin (ASA) was infused into the kidney (0.5 mg/ml final concn) during a 20 min period as indicated in the figure. The values given are from a representative experiment. Similar results were obtained in 5 additional experiments. Results with ADP were similar to those with ATP.

dependent synthesis of prostaglandin synthase and acylhydrolase enzymes [3, 8] and is therefore unaffected by aspirin pretreatment before perfusion. Enhanced PGE_2 release is also observed with ATP and ADP as agonists. This enhanced responsiveness to the adenine nucleotides is apparently also due to synthesis of new prostaglandin synthetic enzymes since it is not abolished by pretreatment with aspirin (Fig. 2), but is blocked by cycloheximide treatment (data not shown). In contrast, PGE_2 release in response to exogenous arachidonate administration does not increase during perfusion and does not return following aspirin treatment (Fig. 2).

Activation of arachidonate deacylation from agonist-specific lipid pools of esterified arachidonic acid. We have previously shown that bradykinin and angiotensin II stimulate the selective release of arachidonic acid from a unique hormone sensitive lipid pool of esterified arachidonate. This pool is characterized by a slow incorporation of exogenous radioactive arachidonic acid. Furthermore, such incorporation is dependent on prior deacylation of esterified arachidonic in this pool following hormone stimulation [2]. For this reason, the specific activity (cpm/ng) of radioactive PGE_2 released in response to hormone stimulation at the beginning of the perfusion experiments is lower than the control value (Fig. 3). With repeated bolus administration of the hormones during the perfusion course, this value increases, indicating transfer of radioactive arachidonate from the hormone insensitive pool to the hormone sensitive pool. In contrast, adenine nucleotides or exogenous arachidonate administered to the prelabeled perfused kidney induce release of PGE_2 with initial specific activity higher than that of the control value (Fig. 3). On repeated stimulations with the nucleotides or infusions of arachidonic acid, the specific activity of the released PGE_2 is gradually decreasing, reaching after 6–8 hr perfusion a value similar to that of the control samples. These experiments clearly show the existence of at least 2 distinct pools of arachidonic acid which serve as a precursor for prostaglandins. The first pool of esterified arachidonate is characterized by a slow incorporation of exogenous arachidonate into the pool and by the fact that arachidonate deacylation from this pool is activated by the peptide hormones bradykinin and angiotensin II. In contrast, the second pool (or pools) shows rapid equilibration of esterified arachidonate with added exogenous arachidonate. Deacylation of esterified arachidonate from this pool can be triggered by infusion of exogenous acid or by administration of ATP or ADP.

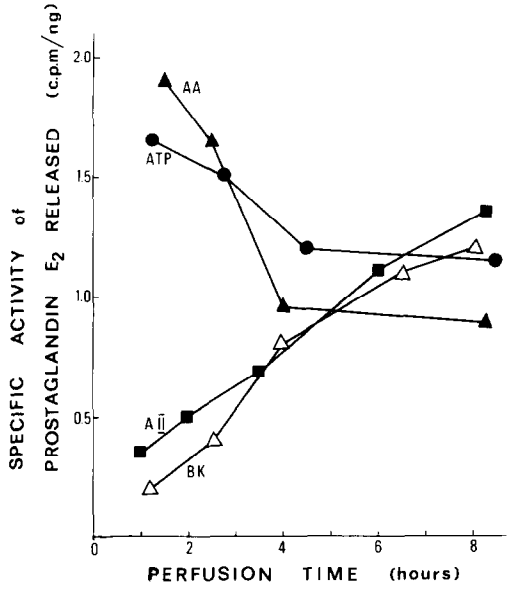

Fig. 3. Time-dependent changes in the specific activity of prostaglandin E_2 released by different agonists. Perfused kidneys were prelabeled with [^{14}C]arachidonic acid (2–2.5 × 10^7 cpm). Following that, the experimental protocol (effluent collection, agonist administration, etc.) was as described in the legend to Fig. 2. Radioactive and non-radioactive prostaglandin E_2 content in each effluent sample were determined as described in Materials and Methods, and the ratio of cpm/ng for PGE_2 in each sample was calculated. Values given are from a representative experiment. Similar results were obtained in 4 additional experiments. Values of specific activity for PGE_2 released under control (basal) conditions were 1.0–1.1. Results with ADP were similar to those with ATP.

DISCUSSION

Endogenous prostaglandin biosynthesis is a process composed of a set of reactions which involve lipolysis and arachidonic acid transformations. Control of this process can be at several key steps. These include: (a) the cellular site for agonist recognition and interaction; (b) the lipolytic step; (c) the extent of metabolic coupling between the released arachidonate and its transformation to oxygenated products; (d) the types of arachidonate metabolizing enzymes in the particular tissue or cell affected; (e) other related reactions. Recent studies with the isolated rabbit kidney perfused *ex vivo* have characterized some of these steps during prostaglandin generation induced by the peptide hormones bradykinin and angiotensin II. This report characterizes prostaglandin generation induced by the adenine nucleotides ATP and ADP and by exogenous arachidonic acid administration. The results presented here and those obtained in previous studies indicate the presence of at least three unique renal systems for generation of arachidonate oxygenated products, each system being associated with its own unique type of agonist—the vasoactive peptide hormones bradykinin or angiotensin II, the adenine nucleotides ATP or ADP, and exogenous arachidonic acid.

Several biochemical properties which clearly distinguish the three systems are depicted in the working model we propose (Fig. 4). These include: (a) the type of lipolysis induced; (b) the profile of prostaglandin products generated; (c) the time delay between arachidonate release and generation of prostaglandins; (d) perfusion-dependent induction of new agonist-sensitive prostaglandin biosynthetic activity and (e) agonist-dependent enhanced acylation of fatty-acids into cellular lipids. For each agonist-specific lipase–prostaglandin synthase system, the overall balance of its biochemical properties as outlined is an expression of a basic characteristic

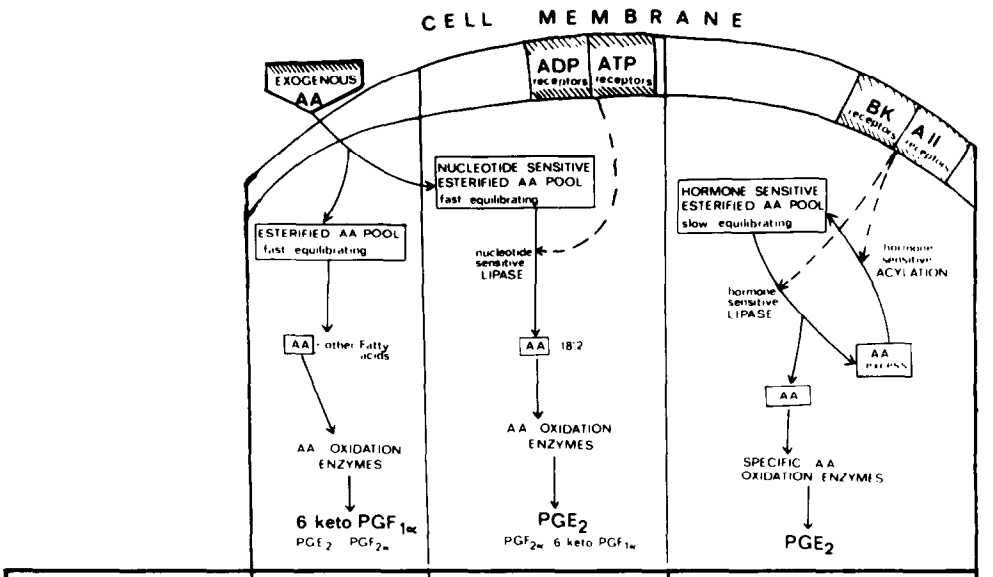

Fig. 4. Comparative properties of lipolytic and PG-biosynthetic processes induced by the peptide hormones bradykinin and angiotensin II, the adenine nucleotides ATP and ADP, and exogenous arachidonic acid. Abbreviations: PG, prostaglandin; BK, bradykinin; AII, angiotensin II; AA, arachidonic; 18:2, linoleic acid.

of the system—the metabolic coupling between the agonist-induced lipolysis and subsequent conversion of arachidonic to specific oxygenated products. Exogenous arachidonate administered into the perfused kidney is incorporated into a 'fast equilibrating' esterified arachidonate pool, causing hydrolysis and release of pre-existing acid from this pool. This release is only loosely coupled to arachidonate transformation by cyclooxygenase or other oxygenation enzymes, resulting in a very small conversion (1–4%) of arachidonate to oxygenated products, the major one being 6-keto $PGF_{1\alpha}$ (the stable metabolite of prostacyclin). Rapid equilibration of exogenous arachidonate is also obtained into an adenine nucleotide-sensitive pool. ATP or ADP-activated arachidonate release from this pool is however more coupled to subsequent conversion to oxygenated products; 10–20% of released acid is converted to several prostaglandin products, the major one being PGE_2. The lipolytic process induced by the adenine nucleotides shows a certain degree of fatty acid selectively causing the release of only arachidonic and linoleic acids, this in comparison to the non-selective release of all cellular fatty acids observed after 1–2 min of renal ischemia (data not shown).

The highest degree of selectivity in the lipolytic process and in the tight coupling to subsequent PGE_2 biosynthesis is seen during the process activated by bradykinin or angiotensin II. Firstly, these vasoactive agents induce the release of only arachidonic acid. Secondly, the entire hormone-induced event process is a highly concerted event, both in terms of the reaction time-sequence and in utilization of the released acid. Release of synthesized PGE_2 is seen at the same time as that of arachidonate indicating no detectable time delay between the lipolytic and synthetic processes under the experimental conditions we used. This observation is significant in comparison to the less coupled process induced by the adenine nucleotides where a 1 min delay was seen between the efflux of hydrolyzed arachidonic and the synthesized PGE_2. Moreover, the peptide hormones process is unique in that: (a) a major portion (30–50%) of released arachidonic is converted to PGE_2; (b) excess acid not converted to prostaglandins is re-esterified into cellular lipids in a process that is sequentially activated approximately 1 min after activation of lipolysis; (c) the esterified arachidonate pool from which the acid is released is unique in that it incorporates exogenous arachidonate very slowly, this incorporation being increased mainly following depletion of pre-existing acid from this pool by repeated hormone administration.

The role of the three agonist-specific, lipase-syn-

thase systems described here in the control of physiological and pathophysiological renal homeostasis is not yet known. Certain pathophysiological conditions with impaired renal function, like hydronephrosis or renal venous constriction, are accompanied by a markedly enhanced responsiveness to bradykinin or angiotensin II. Under these conditions, the perfused rabbit kidney shows enhanced formation of both PGE_2 (a vasodilator) and TxA_2 (a powerful vasoconstrictor), the two compounds affecting renal resistance and possibly other hemodynamic parameters. Development of increased PGE_2 synthesis in response to the adenine nucleotides is also observed (Fig. 2) although, as shown here, the peptide hormones and the nucleotides activate distinctly different lipase–synthase systems. Pretreatment of the perfused kidney with aspirin does not prevent the subsequent appearance of perfusion-dependent, newly synthesized, lipase–synthase systems associated with both the peptide hormones and the adenine nucleotides. Yet, these two enzyme systems are apparently not synthesized at the same rate. Thus, following aspirin pretreatment, the newly synthesized, hormone-sensitive system appears within 1 hr whereas the activity of the nucleotide-sensitive system appears only after 3 hr (Fig. 2). This difference in time of induction of the two agonist-sensitive systems may reflect independent rates of synthesis for each system within a single renal cell type, or perhaps synthesis of each system within different cellular populations in the kidney.

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