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P_2-, but not P_1-purinoceptors mediate formation of 1,4,5-inositol trisphosphate and its metabolites via a pertussis toxin-insensitive pathway in the rat renal cortex

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1 The adenosine receptor (P_1-purinoceptor) agonists N^6-cyclopentyladenosine and N-5'-ethylcarboxamidoadenosine at concentrations up to 10 μmol l^{-1} affected neither basal, nor noradrenaline- and angiotensin II-stimulated formation of inositol-1-phosphate, inositol-1,4-bisphosphate, and inositol-1,4,5-trisphosphate in slices of rat renal cortex.

2 In contrast, adenine nucleotides (P_2-purinoceptor agonists) markedly stimulated inositol phosphate formation. The observed rank order of potency adenosine-5'-O-(2-thiodiphosphate) (EC_{50} 39 μmol l^{-1}) > adenosine-5'-O-(3-thiotriphosphate) (587) $\geq$ 5'-adenylylimidodiphosphate (App(NH)p, 899) > adenylyl-(β,γ-methylene)-diphosphonate (4,181) was consistent with the interaction of the compounds with the P_{2y}-subtype of P_2-purinoceptors. AMP and the ADP analogue (α,β-methylene)-adenosine-5'-diphosphate were ineffective. ATP and ADP ($\leq$ 10 mmol l^{-1}) did not produce a consistent increase, owing to their hydrolytic degradation in the incubation medium.

3 Whereas the inositol phosphate response to App(NH)p was linear only up to 5 min incubation, the time-dependent stimulation of noradrenaline declined at a slower rate. Following pre-exposure of the renal cortical slices to App(NH)p, renewed addition of App(NH)p caused no further enhancement in the accumulation of inositol phosphates, whilst noradrenaline was still capable of eliciting a response. This suggests that the apparent loss of responsiveness to App(NH)p is not due to substrate depletion or enzymatic inactivation, but most likely attributable to homologous desensitization of the purinoceptor.

4 Pretreatment of the animals with pertussis toxin caused a substantial reduction of functional G_i-protein, as indicated by the lack of [^{32}P]-NAD incorporation in a membrane preparation of the renal cortex. Nevertheless, the increase in inositol phosphate formation induced by noradrenaline, angiotensin II, and App(NH)p was not significantly impaired.

5 We conclude that P_{2y}-purinoceptors are present in the renal cortex; these receptors stimulate formation of inositol phosphates via a pertussis toxin-insensitive pathway and undergo homologous desensitization. On the other hand, our results suggest that renal A_1-adenosine receptors do not use stimulation of phosphoinositide breakdown as a transmembrane signalling system.

Introduction

Adenosine acts on four major structures in the kidney, namely the vasculature, renin-containing cells, nerve endings and tubular epithelium, and causes arteriolar vasoconstriction, a decrease in renin release, a decrease in noradrenaline release, and stimulation of electrogenic chloride secretion, respectively (for reviews see Spielman *et al.*, 1987; Osswald, 1988). However, the subtype of receptor and the cellular signalling mechanism mediating each particular effect are obscure at present. We have previously shown that A_1- and A_2-adenosine receptors are located on both renal glomeruli and microvessels (Freissmuth *et al.*, 1987a), and also demonstrated the presence of A_2-receptors on renal tubules (Freissmuth *et al.*, 1987b). Whereas activation of the A_2-receptor was shown to stimulate adenosine 3':5'-cyclic monophosphate (cyclic AMP) formation, no coupling to the adenylyl cyclase system was demonstrated with the A_1-receptor, which would mediate inhibition of the enzyme.

Since A_1-receptor-mediated renal vasoconstriction and suppression of renin release have been found to be associated with a rise in intracellular Ca^{2+} in rat renal slices (Churchill & Churchill, 1985; Rossi *et al.*, 1988), which might be due to increased inositol phosphate formation (Abdel-Latif, 1987), it was the aim of the present study to investigate adenosine-stimulated changes in the phosphoinositide turnover as a signal-transduction pathway alternative to adenylyl cyclase inhibition. Further evidence that constriction of renal arteries by adenosine is probably mediated via a Ca^{2+}-dependent mechanism is provided by comparison of the mode of action of adenosine and angiotensin II (Hackenthal & Taugner 1986; Imagawa *et al.*, 1986; Rossi *et al.*, 1988). Adenosine also provides a unique synergism for angiotensin II, which, in the absence of the nucleoside, is not able to constrict afferent arterioles (Hall *et al.*, 1985). Thus, adenosine may stimulate the formation of inositol phosphates in a manner similar to angiotensin II, thereby increasing the Ca^{2+} concentration in vascular smooth muscle and juxtaglomerular cells.

According to a different nomenclature, adenosine receptors are also called P_1-purinoceptors and distinguished from P_2-purinoceptors, which are preferentially activated by adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP), and their derivatives (for review see Gordon, 1986). In contrast to adenosine receptor agonists, P_2-purinoceptor agonists have been repeatedly shown to stimulate phospholipase C, the enzyme catalyzing phosphoinositide breakdown, in various cells including hepatocytes (Okajima *et al.*, 1987), Ehrlich ascites tumour cells (Dubyak, 1986), thymocytes (El-Moatassim *et al.*, 1987), endothelial cells (Pirotton *et al.*, 1987), FRTL-5 thyroid cells (Okajima *et al.*, 1989), and turkey erythrocytes (Boyer *et al.*, 1989; Cooper *et al.*, 1989). Hence, a further aim of the present study was to establish whether P_2-receptor-mediated phospholipase C activation is also demonstrable in rat renal cortex slices and, if this proves to be the case, whether this intracellular signal is triggered via a pertussis toxin-sensitive G-protein.

Methods

Assay of inositol phosphate formation

One male Sprague-Dawley rat (200–250 g) was killed for each experiment. The kidneys were removed, decapsulated and

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transferred to freshly oxygenated modified Krebs-Ringer bicarbonate medium containing (mmol l^{-1}): NaCl 115, KCl 5, Na_2HPO_4 1.2, CH_3COONa 10, $NaHCO_3$ 25, $CaCl_2$ 1 and glucose 5.5. Slices of renal cortex (0.3 × 0.3 × 0.9 mm) were prepared with a McIlwain tissue chopper, washed and then incubated with 20 μCi ml^{-1} of *myo*-[^{3}H]-inositol. The Krebs-Ringer medium was kept at 37°C and was continuously gassed with 95% O_2/5% CO_2 mixture. Following 2 h of labelling and subsequent washing, aliquots of gravity-packed slices (20–25 mg wet weight) were dispensed into vials containing incubation buffer in a final volume of 300 μl and preincubated with LiCl (10 mmol l^{-1}) for 5 min.

The reaction was started by addition of test compounds (quadruplicate determinations) and terminated by freezing in liquid nitrogen. The chosen incubation times are indicated in the individual figures and tables. Determination of inositol phosphates was carried out as described by Scholz *et al.* (1988). After addition of 1 ml chloroform/methanol/HCl (333:666:1) frozen samples were thawed and immediately homogenized with a glass potter. Following addition of 600 μl water and 800 μl chloroform the mixture was vigorously shaken for 15 min and the phases were separated by centrifugation (1,000 *g* for 10 min). An aliquot of the aqueous phase (1.7 ml) was removed, diluted with water and incubated in a shaking water bath at 45°C to expel remnants of chloroform. A volume of 5 ml was loaded onto Dowex AG 1-X8 anion exchange columns. Inositol was removed with water and glycerophosphoinositol with 5 mmol l^{-1} sodium tetraborate in 60 mmol l^{-1} sodium formate. Inositol phosphates were then sequentially eluted with increasing concentrations (0.2–1.0 mol l^{-1}) of ammonium formate in 0.1 mol l^{-1} formic acid (Berridge, 1983). Aliquots (3 ml) of the eluate were mixed with scintillation fluid (Ready Value) and the radioactivity in the inositol, inositol-1-monophosphate ($InsP_1$), inositol-1,4-bisphosphate ($InsP_2$) and inositol-1,4,5-trisphosphate fraction ($InsP_3$) was counted in a Beckman Minaxi β-counter at an efficiency of 51%. The radioactivity of the chloroform phase (0.9 ml aliquots) containing the phosphoinosites was measured *in toto*.

In order to compensate for the variability associated with handling slice suspensions, the radioactivity in each inositol phosphate fraction was normalised for each 10,000 c.p.m. appearing together in the aqueous and the chloroform phase. The amount of *myo*-[^{3}H]-inositol incorporated into inositol phosphates in the control samples was 3–5% of the total ^{3}H-activity added.

Analysis of degradation of adenine nucleotides

The amount of adenine nucleotides still present in the reaction medium after 15 min incubation with renal cortex slices was determined in a 30 μl aliquot, withdrawn before the termination of the reactions in separate experiments. Separation of adenine nucleotides by ion-exchange chromatography (Spherisorb 5-SAX h.p.l.c.-column) was performed with a linear gradient of 0–0.5 mol l^{-1} KCl in a 50 mmol l^{-1} K_2HPO_4 elution medium (pH 5.0) for 11 min at a flow rate of 2 ml min^{-1}.

Pertussis toxin treatment of rats and pertussis toxin-catalyzed [^{32}P]-NAD-ribosylation of renal cortical membranes

Pertussis toxin (160 μg kg^{-1}) was administered by the intravenous route (tail vein) 40 h before the animals were killed. Slices of renal cortex were prepared as described above. Approximately 1/5 of each slice was removed after it had been labelled with *myo*-[^{3}H]-inositol and washed, put on ice, and homogenized in a medium containing (mmol l^{-1}) Tris-HCl 20 (pH 8), EDTA 2, EGTA 1, sucrose 250 by means of an Ultra-Turrax (2× at half-maximal speed for 15 s, 1× at maximal speed for 3 s). The homogenate was sedimented at 40,000 *g* (15 min) and washed twice in sucrose-free buffer. The resulting pellet was resuspended in assay buffer and stored under liquid nitrogen at a concentration of 15 mg ml^{-1}. Pertussis toxin catalyzed ADP-ribosylation was assayed as described by Bokoch *et al.* (1983) in a 40 μl reaction mixture containing (mmol l^{-1}): Tris-HCl 100 (pH 8), EDTA 1, dithiothreitol 1, $MgCl_2$ 2.5, thymidine 10, ATP 1, GTP 0.1, [^{32}P]-NAD (specific activity 1,000 c.p.m. pmol $^{-1}$) 0.01, 10 to 50 μg of membrane protein and 2 μg pre-activated pertussis toxin. After 1 h at 30°C, the reaction was terminated by precipitation with trichloroacetic acid (15% final concentration). Trichloroacetic acid was removed by extraction with cold acetone; the samples were dissolved in Laemmli's sample buffer supplemented with 40 mmol l^{-1} dithiothreitol and subjected to SDS-PAGE (5% stacking gel, 10% running gel). Autoradiography of the dried gel was performed with Kodak XAR-5 films with one intensifying screen over 1 to 6 days at −80°C. A bovine G_i/G_o fraction, used as reference protein, was prepared according to Sternweis & Robishaw (1984).

Materials

Myo-[2′-^{3}H]-inositol (15.2 Ci mmol $^{-1}$) and [^{32}P]-nicotinamide adenine dinucleotide ([adenylate-^{32}P]-NAD, 1,000 Ci mmol $^{-1}$) were from NEN, Boston, MA; pertussis toxin was obtained as a lyophilized powder from Peninsula Labs., St. Helens, U.K., *myo*-inositol, (−)-noradrenaline-HCl, (±)-propranolol-HCl, angiotensin II (acetate salt), N^6-cyclopentyladenosine (CPA), ATP.2Na, α,β-methylen-adenosine-5′-diphosphate (AMP-CP), and 5′-adenylylimidodiphosphate, tetralithium salt (App(NH)p), from Sigma, St. Louis, MO; ADP.2Na, AMP.2Na, NAD, adenosine-5′-O-(3-thiotriphosphate), tetralithium salt (ATPγS), adenosine-5′-O-(2-thiodiphosphate), trilithium salt (ADPβS), adenylyl-(β,γ-methylene)-diphosphonate, tetralithium salt (App(CH_2)p), from Boehringer-Mannheim, FRG; theophylline and LiCl from Merck, Darmstadt, FRG; Dowex AG 1 × 8 anion exchange resin, formate form, was from Bio-Rad, Richmond, CA, U.S.A.; and Ready-Solv EP scintillation fluid from Beckman, Palo Alto, CA, U.S.A. All other chemicals were analytical grade or best grade commercially available. Phentolamine-HCl (Ciba-Geigy, Basle, Switzerland), 5′-N-ethylcarboxamidoadenosine (NECA, Byk Gulden Lomberg, Konstanz, FRG), and 8-cyclopentyl-1,3-dipropyl-xanthine (DPCPX, Gödecke, Berlin (West)) were generous gifts from the sources indicated.

Results

Accumulation of inositol phosphates in response to noradrenaline, angiotensin II and adenosine receptor agonists (Table 1)

In rat renal cortical slices the A_1-adenosine receptor agonist CPA did not affect the basal accumulation of inositol phosphates up to a concentration of 10 μmol l^{-1} during an incubation period of 15 min. Neither was there any effect when the non-selective agonist NECA (10 μmol l^{-1}) or when the original compound adenosine (5 mmol l^{-1}) was used instead (data not shown). As expected, noradrenaline and angiotensin II markedly stimulated the accumulation of inositol phosphates in renal cortex, with almost maximal effects occurring at concentrations of 5 and 10 μmol l^{-1}, respectively. In the case of noradrenaline, a further increase was observed in the $InsP_3$ fraction only at a concentration of 100 μmol l^{-1}. On the other hand, both potentiation of the noradrenaline effect by angiotensin II, as well as reversal of the noradrenaline-induced stimulation by phentolamine were observed in experiments in which the adenosine analogue CPA failed to alter pre-stimulated inositol phosphate production; enhancing or inhibitory effects of CPA were also undetectable at an earlier time point (5 min). Likewise, the inclusion of the selective A_1-adenosine receptor antagonist DPCPX (1 μmol l^{-1}) or the

Table 1 Effect of various compounds on the accumulation of inositol phosphates in rat renal cortical slices

Compound (μmol l^{-1})	C.p.m. normalized $InsP_1$	$InsP_2$	$InsP_3$
Basal values	283.5 ± 13.4	41.8 ± 2.7	15.0 ± 1.6
CPA (10)	277.0 ± 11.1	43.8 ± 2.0	15.4 ± 1.3
NA (10)	488.8 ± 27.1*	117.1 ± 10.3*	21.9 ± 2.2*
NA (10) + Phentolamine (10)	308.3 ± 16.7	47.3 ± 4.5	11.3 ± 0.5
NA (100)	441.9 ± 16.1*	94.8 ± 6.4*	36.9 ± 4.2*
NA (100) + CPA (10)	374.9 ± 29.5*	90.5 ± 18.0*	27.7 ± 3.8*
AII (5)	346.9 ± 10.9	80.5 ± 5.0*	28.8 ± 3.1*
AII (100)	393.4 ± 10.9*	70.6 ± 1.8*	23.1 ± 2.0*
NA (100) + AII (100)	696.8 ± 28.8*	239.5 ± 21.0*	64.5 ± 6.0*

The slices had been labelled with *myo*-[^{3}H]-inositol (20 μCi ml^{-1}) and washed. Incubation lasted for 15 min and was carried out at 37°C and in the presence of 10 mmol l^{-1} LiCl. In the experiments with phentolamine, the drug was added 5 min before noradrenaline (NA). Experiments where noradrenaline and N^6-cyclopentyladenosine (CPA) and noradrenaline and angiotensin II (AII), respectively, were added together were started by concomitant addition of both drugs. InsP values are normalized for each 10,000 c.p.m. of the total amount of sample radioactivity. The data are presented as means ± s.e.mean of four different experiments. *$P < 0.05$ compared with the control values (*t* test for unpaired data).

presence of the non-selective antagonist theophylline (100 μmol l^{-1}) did not affect the level of inositol phosphate accumulation, when slices were exposed concomitantly to noradrenaline and angiotensin II (data not shown).

Time- and concentration-dependent effects of adenine nucleotides on inositol phosphate accumulation

In contrast to adenosine and its analogues, adenine nucleotides markedly increased the formation of inositol phosphates. Figure 1 shows the time course of basal and agonist-induced accumulation of inositol phosphates. Under basal conditions, the turnover of $InsP_2$ and $InsP_3$ remained steady for 30 min, whereas $InsP_1$ accumulated linearly with time. In the presence of the ATP analogue App(NH)p, the formation of $InsP_2$ and $InsP_3$ was stimulated more than 3 fold by 15 min at a continuously decreasing rate; at 30 min, no further increase was observed, whereas the $InsP_1$ component accumulated further, probably due to the inhibition of $InsP_1$ degradation by LiCl. Whereas the $InsP_2$ and $InsP_3$ response to App(NH)p increased linearly only up to 5 min, the time-dependent stimulation induced by noradrenaline declined at a much slower rate.

When renal cortical slices were pre-exposed to App(NH)p for 10 min, renewed addition of App(NH)p caused no further enhancement in the accumulation of inositol phosphates (Figure 2). In contrast, noradrenaline was still capable of eliciting a response in slices pretreated with App(NH)p, suggesting that the apparent loss of responsiveness to App(NH)p was not attributable to depletion of labelled substrate. On the other hand, if the renal cortical slices were incubated only for a period of linear increment of the App(NH)p-induced effect (5 min, see Figure 2), the simultaneous presence of noradrenaline did not cause any further stimulation of inositol phosphate accumulation (data not shown), indicating that the α-adrenoceptor and the P_2-purinoceptor are coupled to a common pool of phospholipase C.

Several ATP and ADP-analogues, which act as P_2-purinoceptor agonists, were tested for stimulating activity on inositol phosphate formation in renal cortical slices. The analogues listed in Table 2, which are mostly stable to enzymatic and chemical hydrolysis (Welford *et al.*, 1986), gave essentially similar maximal responses. The ADP-analogue AMP-CP and AMP were ineffective at concentrations up to 5 mmol l^{-1} (data not shown). ATP and ADP, however, did not produce a consistent increase in concentrations up to 10 mmol l^{-1}. Their stability to enzymatic degradation was

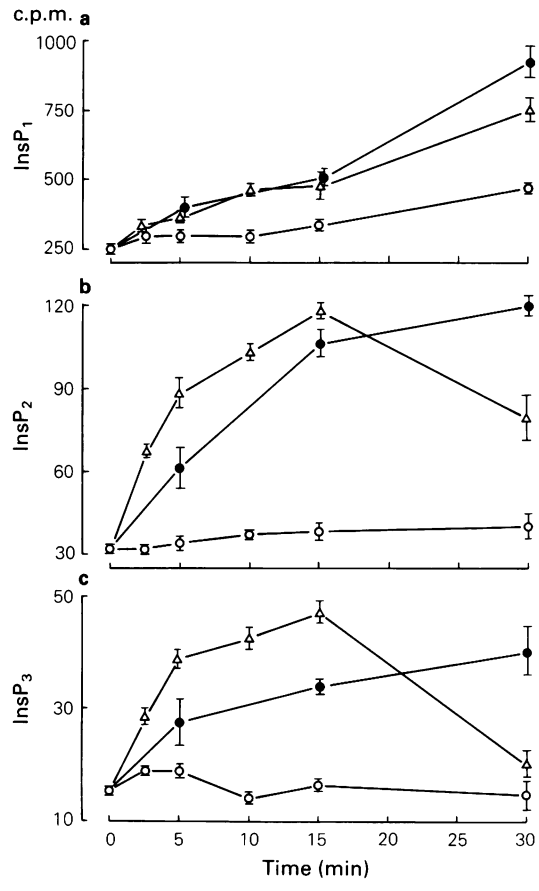

Figure 1 Time course of the accumulation of inositol phosphates (a, $InsP_1$; b, $InsP_2$; c, $InsP_3$) in response to 0.1 mmol l^{-1} noradrenaline (●) and 5 mmol l^{-1} 5'-adenylylimidodiphosphate (App(NH)p) (△) on rat renal cortical slices prelabelled with *myo*-[^{3}H]-inositol. (○) Basal accumulation. Means of 3–4 different experiments are presented; vertical lines show s.e.mean.

Table 2 Effect of P_2-purinoceptor agonists on the accumulation of inositol phosphates in rat renal cortical slices

	EC_{50} (μmol l^{-1})	% of basal value $InsP_1$	$InsP_2$	$InsP_3$
ADPβS	39	160 (142–202)	424 (237–756)	290 (227–370)
ATPγS	587	154 (104–227)	405 (266–617)	322 (237–438)
App(NH)p	899	128 (111–147)	342 (250–467)	283 (197–407)
App(CH_2)p	4,181	172 (122–241)	393 (283–545)	284 (192–419)

The maximal response is expressed as percentage increase over basal values. Prelabelled slices were incubated with each agonist at a maximally active concentration for 15 min. Data are given as geometric means and 95% confidence intervals from three different experiments. The EC_{50} values were estimated through non-linear least squares curve fitting of the experimental points to an equation describing monophasic enzyme stimulation and expressed as mean values of two separate experiments. ADPβS = adenosine-5'-O-(2-thiodiphosphate); ATPγS = adenosine-5'-O-(3-thiotriphosphate); App(NH)p = 5'-adenylylimidodiphosphate; App(CH_2)p = adenylyl-(β,γ-methylene)-diphosphonate.

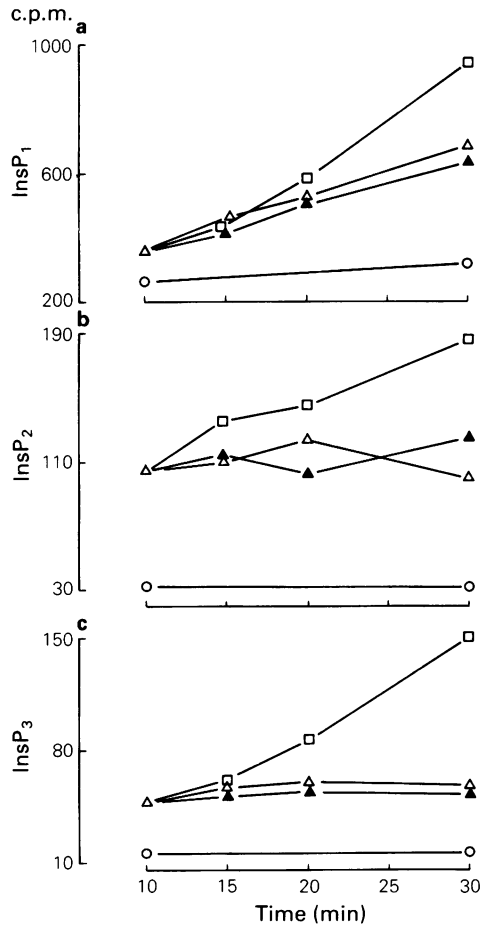

Figure 2 Rat renal cortical slices prelabelled with *myo*-[^{3}H]-inositol were preincubated with 5 mmol l^{-1} 5'-adenylylimidodiphosphate (App(NH)p) for 10 min. Following renewed addition of App(NH)p (▲) or noradrenaline (□) to the pretreated slices, inositol phosphates were measured at the indicated points of time. (○) Control without preincubation, (△) control following preincubation with App(NH)p. The data are mean values from quadruplicate determinations from one experiment. (a) $InsP_1$, (b) $InsP_2$ and (c) $InsP_3$.

therefore monitored by ion-exchange chromatography. After an incubation time of 15 min, ATP and ADP were almost completely degraded in an aliquot withdrawn from the reaction mixture; e.g., 1.5 μmol ATP added to renal cortex slices

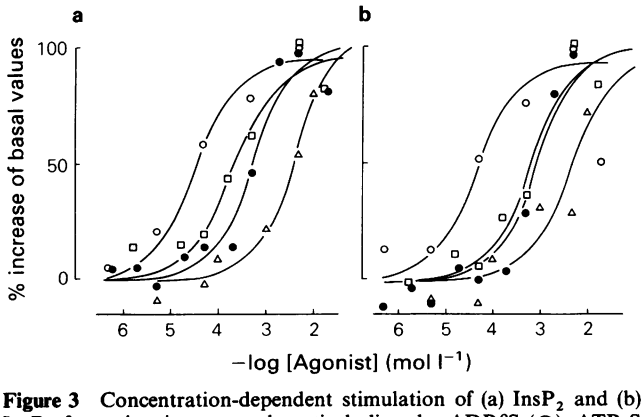

Figure 3 Concentration-dependent stimulation of (a) $InsP_2$ and (b) $InsP_3$ formation in rat renal cortical slices by ADPβS (○), ATPγS (□), App(NH)p (●) and App(CH_2)p (△). The slices, which had been pre-labelled with *myo*-[^{3}H]-inositol, were incubated with the adenine nucleotides for 15 min. The individual concentration-response curves, computed as the best fits of the values shown, are from a single experiment conducted in quadruplicate. Two further experiments gave similar results (see Table 2).

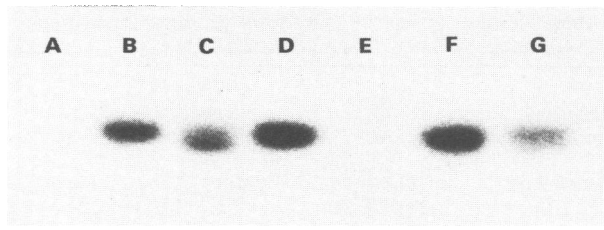

Figure 4 Pertussis toxin-catalyzed [^{32}P]-NAD labelling of rat renal cortical membranes derived from three pertussis toxin (160 μg kg^{-1} i.v.)-treated (lane A, E, G) and three control animals (lane B, D, F) receiving vehicle (sterile NaCl-solution) only. The samples containing 25 μg membrane protein were then subjected to SDS-PAGE and autoradiography. The protein in lane C represents a mixture of a purified bovine brain G-protein fraction (a mixture of G_o/G_i, but containing predominantly G_o). Only the 40 kDa region of the autoradiogram is shown. Four separate assays gave similar results.

(20–25 mg per tube) was hydrolyzed to essentially undetectable levels, with AMP forming about 80% of the original amount of ATP and ADP being present only in negligible quantities. The elution profiles of App(NH)p and ATPγS were not significantly changed after incubation with the slices.

In order to identify the P_2-purinoceptor subtype mediating the stimulation of inositol phosphate production, concentration-response curves for the effective adenine nucleotides were performed. The rank order of potency ADPβS > ATPγS ≥ App(NH)p > App(CH_2)p (Table 2, Figure 3) is consistent with the P_{2y}-purinoceptor proposed by Burnstock & Kennedy (1985).

The effects of individual adenine nucleotides were not additive. Moreover, CPA showed no detectable effect on the extent of inositol phosphate accumulation induced by App(NH)p (data not shown).

Effect of pertussis toxin-treatment on the inositol phosphate response

In order to evaluate whether a G-protein of the G_i/G_o-group is involved in the agonist-induced stimulation of inositol phosphate formation in the renal cortex, rats were treated with pertussis toxin and the inositol phosphate response was determined in the presence of noradrenaline, angiotensin II and App(NH)p. The effectiveness of the treatment was verified by quantifying the reduction of pertussis toxin-substrate in renal cortical membranes (Figure 4). In renal cortical membranes prepared from untreated control animals, the pertussis-toxin substrate migrates as a single species with a slightly lower mobility than the bovine brain G_o-standard, indicating that the predominant pertussis-toxin sensitive G-protein is a $G_{i\alpha}$-subtype (Sternweis & Robishaw, 1984). Pertussis toxin-treatment resulted in a loss of available pertussis toxin substrate in 2 out of 3 animals, which is evident from a complete lack of [^{32}P]-NAD incorporation in lane A and E. Labelling

Table 3 Effect of pertussis toxin treatment (160 μg kg^{-1} i.v.) on the agonist-induced formation of $InsP_3$ in rat renal cortical membranes

	$InsP_3$ (% of basal value)	
Agonist (μmol l^{-1})	*Control rats*	*Pertussis toxin-treated rats*
NA (100)	227 (149–345)	217 (144–327)
AII (100)	145 (101–207)	141 (107–185)
App(NH)p (5,000)	283 (197–407)	265 (190–369)

The data, presented as percentage increase over basal values, are geometric means with 95% confidence limits from pertussis toxin-treated animals, as well as from control animals receiving vehicle (sterile NaCl-solution) only ($n = 3$). NA, noradrenaline; AII, angiotensin II; App(NH)p, 5'-adenylylimidodiphosphate.

remained undetectable, even after prolonged exposure. In one treated animal, however, a faint band could still be visualized (lane G). Incorporation amounted to less than 20% of the control samples (lanes B, D, F).

In spite of the substantial reduction of functional G_i-protein, the agonist-induced increase in the accumulation of $InsP_3$ (as well as of $InsP_2$ and $InsP_1$) is not significantly impaired, much less actually reversed by pertussis toxin treatment (Table 3). Likewise, no differences were observed if the inositol phosphate response was determined in slices from the animals with complete abolition of pertussis toxin substrate and the data from the one rat with partial reduction of $G_{i\alpha}$ were evaluated separately.

Discussion

Although we have previously demonstrated on isolated renal glomeruli and microvessels that, due to GTP-dependence of agonist binding, A_1-adenosine receptors are coupled to a G-protein, the transmembraneous signalling pathway linking these receptors to their effect, e.g. vasoconstriction, inhibition of renin release, remains elusive. The results of the present study suggest that A_1-adenosine receptor activation does not affect inositol phosphate formation in the renal cortex. In particular, although we were able to demonstrate both potentiation and inhibition of noradrenaline-induced phospholipase C activation with angiotensin II and phentolamine, respectively, adenosine and its analogues had no detectable effect. Recently, A_1-receptor agonists have been shown to stimulate slightly (30%) phosphoinositide hydrolysis in a cell line of cortical collecting tubules (Arend *et al.*, 1989). Hence, identification of the A_1-receptor-activated transmembranous signalling cascade that results in vasoconstriction and inhibition of renin release would presumably require fractionation of the renal cortical tissue. Unfortunately, our attempts to measure inositol phosphate formation in a glomerular and microvessel fraction of the kidney, as described elsewhere (Freissmuth *et al.*, 1987a), were unsuccessful. Evidence is accumulating that an increase in cytosolic Ca^{2+} is responsible for the A_1-mediated effect on renal vasoconstriction and renin release, and that a pertussis toxin-sensitive G-protein intervenes between receptor occupation and an increased cellular influx of Ca^{2+} (Churchill & Churchill 1985; Rossi *et al.*, 1987; 1988).

On the other hand, the marked increase in inositol phosphate levels evoked by ATP- and ADP-analogues suggests a physiological role for adenine nucleotides in the regulation of renal function via activation of phospholipase C, which is clearly not mediated by their degradation product, adenosine. Using several types of smooth muscles, Burnstock & Kennedy (1985) have proposed that the P_2-purinoceptors, representing the site of action of adenine nucleotides, can be subdivided into an excitatory P_{2x}- and an inhibitory P_{2y}-subtype. The rank order of potency, in particular the high potency of ADPβS and the low potency of App(CH_2)p observed in the present study is similar to that recently found in the case of P_{2y}-receptor-mediated stimulation of phospholipase C in turkey erythrocyte ghosts (Boyer *et al.*, 1989). In the present experiments on renal cortical slices, the ATP- and ADP-analogues have to be used at much higher concentrations than required for activation of turkey erythrocyte-phospholipase C. A review of the literature shows that the concentrations of adenine nucleotides required to elicit a half-maximal response vary greatly (Westfall *et al.*, 1978; Burnstock *et al.*, 1985; Fedan *et al.*, 1986; Burnstock & Warland 1987; Satchell, 1988) and the low apparent affinity observed in the present study is not unprecedented in experiments performed on isolated tissues and organs. These discrepancies may, in part, be attributable to diffusion barriers for the hydrophilic agonists used, which limit their access under these conditions.

The response to adenine nucleotides was compared with the noradrenaline-induced α-adrenoceptor-mediated effect, revealing distinct differences in the time course of inositol phosphate accumulation. The data obtained in the presence of App(NH)p and noradrenaline suggest that the P_2-purinoceptor in the renal cortex undergoes homologous desensitization. This interpretation is supported by the following observations: firstly, the P_2-purinoceptors and the α-adrenoceptors appear to be coupled to a common pool of inositol phosphate generating enzymes, but noradrenaline is still capable of eliciting a response when App(NH)p has become ineffective. This argues against substrate depletion or progressive enzyme inactivation as possible explanations for the rapid loss of responsiveness. Similarly, depletion of App(NH)p can be ruled out since renewed addition of App(NH)p does not produce any stimulation in pre-exposed slices. In addition, h.p.l.c. analysis of the incubation medium did not reveal metabolism of App(NH)p to any significant degree. Rapid desensitization of P_2-purinoceptor-mediated responses has repeatedly been observed in isolated organ and tissue preparations (for review see Burnstock & Kennedy, 1985). The homologous type of desensitization observed in the present study indicates that the loss of responsiveness results from alterations at the level of the receptor, or at the level of interaction between the receptor and a putative G-protein.

Receptor-mediated stimulation of phosphoinositide hydrolysis by phospholipase C is generally believed to be controlled by a G-protein in a manner analogous to the transmembrane signalling pathways that lead to activation of adenylyl cyclase or the retinal cyclic GMP-phosphodiesterase (Freissmuth *et al.*, 1989). In many cells and tissues (e.g. liver, cardiac myocytes), activation of phospholipase C is not disrupted by pertussis toxin treatment. There are, however, several examples (e.g. granulocytes, mast cells) where pertussis-toxin catalyzed ADP-ribosylation abolishes the phospholipase C response to agonists suggesting that more than one G-protein is involved in receptor-mediated regulation of phospholipase C (Cockcroft, 1987). In this study, we demonstrate that stimulation of inositol phosphate generation by α-adrenoceptors, P_2-purinoceptors and angiotensin II receptors is not mediated by a pertussis-toxin substrate in the renal cortex. Activation of phospholipase C via P_{2y}-purinoceptors is dependent on guanine nucleotides in turkey erythrocytes (Boyer *et al.*, 1989) and binding of ADPβS to these receptors has recently been demonstrated to be heterotropically modulated by guanine nucleotide (Cooper *et al.*, 1989), thus establishing that the P_{2y}-purinoceptors belong to the family of G-protein coupled receptors. The nature of the G-protein that interacts with the P_2-purinoceptor of the renal cortex remains to be identified.

To our knowledge, the physiological role of P_2-purinoceptors in the kidney has not been investigated. However, the concept that ATP released from sympathetic nerve endings acts as a co-transmitter on postsynaptic sites is well established (Gordon, 1986). In the kidney, both the juxtaglomerular apparatus and the proximal tubules are densely innervated by adrenergic fibres (Barajas *et al.*, 1984). An increase in efferent renal sympathetic nerve activity enhances sodium and water reabsorption, which is unaffected by α_2-adrenoceptor antagonists and is only partially abolished by non-selective or α_1-selective adrenoceptor blockade (DiBona, 1985). On the other hand, the antinatriuretic effect of exogenous noradrenaline, as well as its stimulating effect on $InsP_3$ formation in rat renal slices, is fully antagonized by prazosin (Plevin *et al.*, 1988). As demonstrated in the present study, adenine nucleotides and noradrenaline are apparently coupled to the same pool of inositol phosphate generating enzymes in the renal cortex. Thus, it is attractive to speculate that ATP released as cotransmitter during renal sympathetic nerve stimulation may directly affect tubular electrolyte and water transport.

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